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Risk assessment for Birds and Mammals

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Abstract

The European Commission asked EFSA to revise the Guidance on the risk assessment for birds and mammals. That guidance described how to perform risk assessment for birds and mammals from plant protection products, containing pesticide active substances, in accordance with Regulation (EU) 1107/2009. The current guidance document is an update of EFSA's existing guidance document titled 'Risk assessment for Birds and Mammals' which was published in 2009. It outlines a tiered risk assessment scheme covering dietary exposure, exposure via secondary poisoning and exposure via intake of contaminated water.

Keywords: hematothermal vertebrates, pesticides, feeding guilds, oral exposure, tiered approach

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Summary

The European Commission mandated EFSA to update the guidance document on risk assessment for birds and mammals to consider (i) the new regulatory framework, (ii) any new scientific developments; (iii) feedback received by Member States and stakeholders on the existing guidance in view of clarifying and correcting it, where needed; (iv) possible harmonisation of higher tier methodologies and (v) the development of a calculator tool. The working group was also prepared to develop options for specific protection goals (SPGs) in line with EFSA methodology. However, that activity was not finalised considering the ongoing activities on SPG derivation by the European Commission, as requested by the updated mandate. Therefore, for the time being the operational protection goals, as adopted in EFSA (2009) were retained and presented in line with SPGs for vertebrates in EFSA PPR Panel (2013).

The terms of reference were interpreted to be a request for an update and a repair action and therefore new approaches such as risk assessment schemes for additional routes of exposure (other than oral exposure) or for specific groups of birds or mammals (e.g. bats), and for the coverage of indirect effects due to treatment-related shifts in food availability, were not developed. This implies that the principles of the existing guidance were still followed, and the risk assessment methodologies were clarified, updated and complemented, as needed.

The guidance, in line with EFSA (2009), focuses on the three main methods of application for pesticide products, i.e. spray application, seed treatment and granules. For other types of application methods, it is the applicant's responsibility in consultation with Member State competent authorities to develop a fit-for-purpose exposure assessment.

In particular, compared to EFSA (2009), the different tiers for exposure and effect assessment were clearly indicated and explained in greater detail. The terminology was also clarified: Differences between 'indicator model species', 'generic model species' and 'focal species', which are used in the different exposure assessment tiers, were described. With regard to the Tier 1 effect assessment, clear recommendations on the selection of relevant reproductive endpoints, especially for mammals, were included. The use of *in silico* models, e.g. (Q)SAR for predicting toxicity endpoints for birds and mammals, was included as a possible option for metabolites. Additional crops are now covered in the guidance and the crop groups used for the Tier 1 exposure assessment were harmonised with those in the EPPO global database (EPPO, online[1]), as far as possible.

The working group refined the parameters related to the indicator model species and generic model species in the screening and the Tier 1 exposure assessment for spray applications and seed treatments. In particular, (i) relevant feeding guilds (herbivorous, insectivorous, granivorous and omnivorous) for the various scenarios, (ii) the range of body weights for the species identified as relevant model species for each feeding guild and (iii) the different proportions of food items in the diet for each generic model species were considered. For many feeding guilds, and in line with EFSA (2009), an in-field risk assessment is considered sufficiently protective for possible direct toxic effects due to exposure outside of the treated area. However, for small mammals, the WG considered that these direct effects caused by exposure outside of the treated area should not be ignored. Due to the need for plant cover, in some cases (crop growth stages) and in higher tier assessments, their presence may be excluded in-field; however, their presence in the immediate surrounding area (e.g. exposed via spray drift) is fully expected. Moreover, default residue unit dose values (RUD) were revised considering the outcome of an EFSA external report and data provided by Croplife Europe on residues in fruits and fruiting vegetables. Guidelines for the use of the time-weighted average approach in the reproductive exposure assessment were developed.

Options for Tier 2 effects assessment were provided, noting that the WG discourages the performance of additional vertebrate testing. Therefore, Tier 2 options for effect assessment should only be considered when additional toxicity data are available to comply with overseas regulatory jurisdictions or when retrieved from publicly available literature. Toxicokinetic–toxicodynamic (TKTD) models were also described as a potential useful tool for the Tier 2 effect refinement, but these models require more development for regulatory ERA of birds and mammals. For the time being, the evaluation of TK and TKTD modelling exercises will depend on expert judgement. Tier 2 options for exposure assessment for spray applications include refined residue values and a refinement considering substance-specific dissipation data, and further guidance was provided on evaluating these data, when submitted.

[1] https://gd.eppo.int/PPPUse/3CRGK

Tier 3 options for exposure assessment include field studies for the selection of focal species and studies for refining ecological data used in the exposure estimates, i.e. the proportion of food obtained in the treated area (PT) and the different proportions of dietary items obtained in the treated area (PD) for the identified focal species. The WG tried to harmonise the higher tier approaches by clarifying methodologies and requirements, as far as possible. Recommendations were also provided regarding refinements, like dehusking and avoidance, although the WG acknowledged that these data are difficult to interpret and to extrapolate to other species and test situations. For sake of harmonisation and transparency in the evaluation of higher tier studies, evaluation tools were developed for those refinements which are most commonly available (i.e. residue decline, effect field studies and ecological field studies for the identification of focal species, PT and PD). The WG has also recommended an approach for considering the implications of other available data to refined risk assessment (e.g. literature data or data which are not owned by the applicant).

Higher tier integrated exposure and effect assessments for spray applications and seed treatments, and based on focal species, include effect field studies and population models which are considered to integrate effect and exposure. In the absence of technical guidance, the regulatory use of these approaches will be based on a case-by-case expert judgement.

The risk assessment for granular application was revised first by considering the relevance of the routes of exposure considered so far and by updating default parameters, when additional information was retrieved.

The risk assessment for metabolites, for contaminated water and for additional routes like secondary poisoning was clarified, as needed. A Tier 1 risk assessment for benthic invertebrate-eating birds and mammals was included, in addition to the one related to piscivorous and vermivorous birds and mammals.

Formulation risk assessment and how to perform a risk assessment in case of formulations which contain more than one active substance were also updated in line with the latest developments and methods.

An approach on how to consider and communicate uncertainty in the risk assessment and on how to reach a final conclusion by considering all the available evidence in a weight of evidence approach is proposed.

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

EFSA published a guidance document for performing risk assessments for birds and mammals, under the former legislation, Council Directive 91/414/EEC[2], in 2009 (EFSA, 2009). The EFSA (2009) guidance document was developed on the basis of an Opinion of the Scientific Panel on Plant Protection Products and their Residues (PPR Panel) published in 2008 (EFSA, 2008). EFSA (2009) has been implemented since July 2010.

EFSA received a mandate from the European Commission in October 2016 requesting an update of the guidance document considering the following Terms of Reference (ToRs):

- Update the EFSA (2009) guidance document in view of the PPR Panel Opinion on protection goals (EFSA PPR Panel, 2010).
- Update the EFSA (2009) guidance document in view of the Regulation (EC) No. 1107/2009[3], Regulation (EU) No 283/2013[4] and Regulation (EU) 284/2013[5].
- Update the risk assessment methodology in light of scientific research and developments.
- Update the EFSA (2009) guidance document in view of feedback from Member States and other stakeholders.
- Provide clarifications on the current methodology where needed.
- Consider the possibility of further harmonisation of higher tier risk assessment within EU Regulatory zones.
- Consider the possibility of providing a calculator tool to accompany the updated guidance document.

EFSA received a revised mandate from the European Commission in July 2018. In the revised mandate, the first bullet mentioned above, 'update the EFSA (2009) guidance document in view of the PPR Panel Opinion on protection goals', was amended to:

> Update the EFSA (2009) guidance document taking into account planned and on-going discussions initiated by the Commission on defining specific environmental protection goals and review the risk assessment guidance based on relevant specific protection goals agreed during this process.

The revision was triggered by a project initiated by DG SANTE to involve Member States and stakeholders in defining Specific Protect Goals (SPG) for non-target organisms.

1.1. Interpretation of the Terms of Reference

The ToRs were interpreted to be a request for an update (or repair) of the existing guidance document methodology rather than a complete revision.

The WG addressed the agreed Terms of Reference as follows:

Specific protection goals

Based on the updated mandate and the ongoing activity initiated by the DG SANTE, SPG options in accordance with the methodology of EFSA PPR Panel (2010) were not finalised. The current guidance document was therefore updated considering the operational protection goals as described in chapter 3.

New Regulations

This guidance document is intended to provide guidance to applicants and risk assessors in the context of the evaluation of plant protection products (PPPs) and their active substances under Regulation (EC) 1107/2009[2] for authorisation process at Member State level the approval at EU level, respectively. As such, the scope of the risk assessment is limited to single PPPs and the active substances they contain. The screening and Tier 1 exposure and effect assessment methodology have

[2] Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC).
[3] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
[4] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.
[5] Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

made use only of data requested with Regulations (EU) No. 283/2013[4] and 284/2013[5]. The assessment factors (AF) referred to in this guidance document are defined in the Uniform Principles (Regulation EU No. 546/2011[6]). Regulation 1107/2009[2] contains specific approval criteria for substances with endocrine-disrupting properties. ECHA/EFSA (2018) provides guidance to perform regulatory assessment for these criteria. Consequently, no specific consideration of risk assessments for birds and mammals from substances with endocrine-disrupting properties was needed in this revision of the guidance document.

- **Update the risk assessment methodology in light of scientific research and developments**

This ToR was interpreted to request an update of the existing methodology considering experience and using new knowledge to consolidate best practice in terms of performing risk assessment for birds and mammals from PPP. Furthermore, the WG aimed to ensure that existing errors or points needing clarification were suitably addressed. To address this ToR, several tasks were done.

  - Information gathered by Lahr et al. (2018) and several narrative literature reviews were used to update the characteristics of the model species used in the risk assessment. This task was to ensure that the assumptions in the risk assessment are sufficiently protective of all species, within the specific feeding guild, which feed in the area of PPP use.
  - Residue data gathered under Lahr et al. (2018) and also submitted by Crop Life Europe were used to update the default residue values on food items taken by birds and mammals.
  - Guidance on risk assessment methodology was updated on the basis of the comments received from stakeholders in the public consultation together with knowledge and experience from the WG members.

- **Update in view of feedback from stakeholders and to provide clarifications**

The public was consulted on the existing guidance document from November 2017 to December 2017 and comments and opinions submitted by stakeholders were considered during the process (EFSA, 2020).

- **Further harmonisation of higher tier risk assessment within EU Regulatory zones;**

To address this ToR, several tasks were done:

  - The on-line calculator tool referred to in the next paragraph will be able to perform several higher tier refinements thus ensuring a harmonised application of the higher tier methodology.
  - Higher tier risk assessment methodology was clarified as far as practicable.
  - Critical appraisal tools (CAT) were developed to aid harmonised assessments for several higher tier studies.

- **Providing a calculator tool to accompany the updated guidance document**

An accompany calculator tool was developed to implement the screening, Tier 1, Tier 2 and Tier 3 exposure and risk assessments. The calculator was tested and discussed with WG and stakeholders from industry and Member States in October 2022. The tool is available online in the EFSA Platform, R4EU, at the following web address: https://r4eu.efsa.europa.eu/app/birds-mammals.

2. Introduction

2.1. Legislative background

In accordance with Regulation (EC) No. 1107/2009[2], a pesticide active substance shall only be approved if a risk assessment demonstrates the risks to non-target organisms, to be acceptable in accordance with the criteria laid down in the Uniform Principles (Regulation EC No. 546/2011[6]) under realistic conditions of use of a plant protection product (PPP). This means that a risk assessment for birds and mammals is needed according to good agricultural practices (GAP) which defines how the PPP will be used.

[6] Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products.

The data that applicants must provide for pesticide active substances, and (formulated) plant protection products, are defined in the accompanying Commission Regulation (EU) No 283/2013[4] and 284/2013[5], respectively.

2.2. Scope

The risk assessment methodology developed for this guidance document is specifically to address the needs for the approval and authorisation process under Regulation 1107/2009[2]. As such, the scope of the risk assessment is limited to single PPPs and the active substances they contain. Guidance is given for performing additive risk assessments for PPPs containing multiple active substances (see chapter 12). Furthermore, as is the standard practice for environmental risk assessments performed under Regulation 1107/2009[2], the risk assessment methodology considers each use (e.g. the crop) independently. Cumulative exposure to birds and mammals due to the application of multiple PPPs is not covered. Cumulative exposure due to repeated application of the same PPP in a crop, as recommended in the GAP, however, is covered. The risk assessment methodology has been developed to assess chemical active substances and plant protection products (PPP). The WG recommends that assessment methodology for microbial pesticides is addressed in a specific guidance document.

As the current guidance document was interpreted to be an update of existing risk assessment and was developed considering the currently agreed protection goals for vertebrate wildlife, some potentially relevant topics are not covered in this document. These are described in the following paragraphs.

The risk assessment methodology covers dietary exposure via direct residues on food items, dietary exposure via residues on food items following bioaccumulation (secondary poisoning) and exposure from consumption of contaminated water. Dermal and inhalation exposure is not covered. The exception is when field effect studies are used as part of the higher integrated exposure and effect assessment tier as, potentially, these could cover all routes of exposure. It is recognised that for some substances, and depending on their use, other exposure routes may contribute to the overall systemic toxicity. The WG recommends that a holistic approach is taken to develop harmonised and robust exposure models to cover additional exposure routes covering all terrestrial non-target organisms.

In 2019, the EFSA PPR Panel was asked to consider whether bat species were adequately addressed by the risk assessment methodology in EFSA (2009) (EFSA PPR Panel, 2019). The PPR Panel concluded that in some cases, oral exposure was not sufficiently covered considering the assumed parameters in the Tier 1 risk model species used in EFSA (2009). In this updated guidance document, the WG carefully checked the assumed characteristics of the model species used in the Exposure Assessment Screening tier and Exposure Assessment Tier 1. Furthermore, the residue values were updated with a larger and more robust data set. Consequently, with the revised assumptions, the lower tier oral exposure assessment for bat species is assumed to be covered by the Exposure Assessment Screening tier and Exposure Assessment Tier 1 calculations for other insectivorous mammals. Regarding other routes of exposure, dermal exposure was considered by EFSA PPR Panel (2019) to be greater than oral and inhalation exposure for bat species, if applications of PPPs are made during the foraging time of bats. The panel also considered that exposure of pups via milk was of particular importance and concluded that any risk assessment scheme for bats should consider the total body burden from all exposure routes (oral, dermal and inhalation). As already discussed in the previous paragraph, the WG recommends developing dermal and inhalation exposure models for all terrestrial non-target organisms. Once available, risk assessments considering the total body burden to bat species can be further developed.

The WG acknowledged that indirect effects of pesticides on birds and mammals are likely to be important. It was recognised that it is extremely challenging to differentiate indirect effects caused by the use of pesticides and those caused by other farming/growing practices or other forms of pest control. Nevertheless, it is noted that some pesticides are applied to remove insect and weed pests, and often cause transient effects on the abundance of non-target organisms, all of which contribute to terrestrial food webs and habitats. It was recognised that developing a meaningful methodology for the assessment of indirect effects is challenging. Furthermore, the WG considered a unified approach under several EU Regulations would be valuable (i.e. Directive 2009/128/EC,[7] the Common Agricultural Policy, etc.) to assess indirect effects of agriculture, including the uses of pesticides, in terrestrial

[7] Directive 2009/128/EC of the European parliament and of the council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides.

environments. At the time of writing, the European Commission was developing the Farm to Fork strategy[8] under the European Green Deal[9] which aims to achieve such unity.

It was further noted by the WG that, within the existing regulatory framework for the prospective assessment of PPPs, it would be most logical to address indirect effects from reduced food availability when developing the risk assessment guidance documents for non-target terrestrial plants, non-target arthropods and soil organisms. In addition, guidance development on how to address indirect effects in prospective ERA for stressors like PPPs is considered by the WG an important overarching EFSA activity. This, however, falls outside the mandate of the ToRs given in Section 1.1.

Rice is the only European crop cultivated in fields regularly flooded for long periods. This means that the attractiveness of rice fields to birds and mammals and exposure routes to terrestrial vertebrates differ considerably to other crops grown in Europe. The WG recognised that rice fields are an attractive foraging habitat for terrestrial vertebrates but considered that it would be logical the exposure models for all non-target organisms exposed from the use of PPPs in rice to be developed in a harmonised and holistic manner. The existing guidance document for products used in rice (European Commission, 2003) refers to a previous guidance document for birds and mammals which is no longer used for risk assessment. The International Centre for Pesticides and Health Risk Prevention (ICPS, Italy) has drafted an updated guidance document on harmonised exposure and risk assessment for rice crops. However, at the time of writing, the document was still under discussion and not implemented. The WG recommends that the draft guidance is further developed ensuring that terrestrial vertebrates are properly addressed.

2.3. Terminology used for pesticides, active substance and plant protection products

It is important that the terminology used in the regulatory assessment is clear and consistent. Therefore, the following information is adapted from the Commission website for pesticides.[10]

A **'pesticide'** is a substance/agent that prevents, destroys or controls a harmful organism ('pest') or disease, or protects plants or plant products during production, storage and transport. The term includes, amongst others: herbicides, fungicides, insecticides, acaricides, nematicides, molluscicides, growth regulators, repellents, rodenticides and biocides.

Plant protection products (PPP) are 'pesticides' that protect crops or desirable or useful plants. They are primarily used in the agricultural sector but also in forestry, horticulture, amenity areas and in home gardens. They are formulations that contain at least one active substance and have one of the following functions:

- protect plants or plant products against pests/diseases, before or after harvest;
- influence the life processes of plants (such as substances influencing their growth, excluding nutrients);
- preserve plant products;
- destroy or prevent growth of undesired plants or parts of plants.

They may also contain other components (co-formulants) including safeners and synergists.

An **active substance (a.s.)** is any chemical, plant extract, pheromone or microorganism (including virus), that has action against 'pests' or on plants, parts of plants or plant products.

The most common use of pesticides is in the form of plant protection products (PPPs). The term 'pesticide' is often used interchangeably with 'plant protection product'; however, pesticide is a broader term that also covers non-plant/crop uses, e.g. biocides. In this guidance document, the word 'pesticide' is used as it is commonly used term used in the regulatory community for PPPs. However, it is important to stress that that the guidance document is developed specifically for the assessment of active substances and plant protection products under Regulation (EC) 1107/2009[2].

[8] https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en#Strategy
[9] https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en
[10] https://ec.europa.eu/food/plants/pesticides_en

3. Risk assessment background information

3.1. Protection goals for birds and mammals

General protection goals underlying prospective environmental risk assessment (ERA) for plant protection products in Europe are described in several EU Regulations. These general protection goals, however, need to be translated into specific protection goals that can be linked in a transparent way to selected risk assessment schemes in guidance documents.

The specific protection goal (SPG) is the operationalised general protection goal described in legislation and is defined in terms of ecological entity and attribute of non-target organisms to protect, the magnitude of tolerable impact and temporal and spatial scale of the tolerable impact (EFSA PPR Panel, 2010; EFSA Scientific Committee, 2016a). Ideally, different SPG options for birds and mammals that are based on the general protection goals in legislation need to be proposed by risk assessors, together with their scientific basis. This allows a proper dialogue with, and a final selection by, risk managers that have the democratic mandate to take policy decisions.

The EFSA procedure to derive SPGs was not yet in place when the EFSA Guidance Document for Birds and Mammals (EFSA, 2009) was published. Nevertheless, the following operational protection goals were adopted in EFSA (2009):

- The procedures for lower tier assessment are designed to achieve a 'surrogate' protection goal of making any treatment-related mortality or reproductive effects unlikely. At higher tiers, assessments may be either at the 'surrogate' protection goal or at the 'actual' protection goal of clearly establishing that there will be no visible mortality and no long-term repercussions for abundance and biodiversity due to the treatment of the PPP in the crop.

The use of the phrase 'no visible mortality' in EFSA (2009) suggests that the individual has to be selected as an ecological entity[11] in acute ERA, while the phrase 'no long-term repercussions for abundance and biodiversity' in EFSA (2009) suggests that populations are the ecological entity of concern in long-term ERA and that some short-term impacts might be tolerated as long as the sustainability[12] of populations of wild birds and mammals, as well as their overall biodiversity, are not at stake in agricultural landscapes.

The Aquatic Guidance Document (EFSA PPR Panel, 2013) used the EFSA PPR Panel (2010) procedure (based on the ecosystem services concept) to derive SPGs. After a dialogue between risk assessors and risk managers, for aquatic vertebrates (fish and amphibians), the ecological threshold option (ETO)[13] only was selected as SPG. In this ETO option for aquatic vertebrates, the ecological entity and their attribute to be protected were, respectively, individuals and survival in acute ERA and populations and abundance/biomass in chronic ERA. For both the acute and the chronic ERA, the magnitude of tolerable effects in edge-of-field surface waters was selected as negligible. In line with the SPGs adopted for aquatic vertebrates in EFSA PPR Panel (2013), it is logical to select 'negligible' as the magnitude of tolerable effects for birds and mammals. Within the context of this updated guidance document, a negligible effect on birds and mammals typical for agricultural landscapes can be defined as follows:

- It should not exceed the response of the assessment endpoint under baseline conditions (= non-exposed field conditions) within a relevant time period.
- This requires a risk assessment with an appropriate statistical power and should be protective of vulnerable and susceptible species from both toxicological and ecological perspective.

The updated mandate requested to consider the ongoing activities by the European Commission, aiming to define SPGs involving Member States (MSs) and stakeholders. When drafting the final version of this updated guidance document, SPG options for birds and mammals based on these activities were not yet provided by the European Commission. Consequently, the operational protection goals as reported in EFSA (2009) were retained and presented in line with SPGs for vertebrates in EFSA PPR Panel (2013) (see Table 1, below).

[11] The ecological entity is one of the dimensions for the definition of Specific Protection goals as indicated in EFSA PPR Panel (2010). It refers to the level of biological organisation of the key driver or service providing unit that should be protected.

[12] Sustainability of populations, may be interpreted in terms of the attributes abundance and genetic diversity of the entity that must be protected. The attribute is another dimension in the definition of SPGs according to EFSA PPR Panel (2010).

[13] Ecological Threshold Option (ETO) allows only for negligible effects at the population level whereas recovery is not considered an option for vertebrates.

Table 1: Operational protection goals for birds and mammals as in line with EFSA (2009) and EFSA PPR Panel (2013)

Organism group	Ecological entity	Attribute	Magnitude	Time	Space
Birds and mammals typical for agricultural landscapes in the EU	Individuals of potentially sensitive species (in acute risk assessment)	Survival (no visible mortality)	Negligible effect	Not applicable (no recovery option)	Local agricultural field (and surrounding agricultural area where individuals of the population dwell)
	Populations of potentially vulnerable species (in reproductive risk assessment)	Abundance			

These operational PGs assume that a sufficient level of protection is achieved by avoiding the risk to potentially sensitive/vulnerable species of birds and mammals from direct toxicity of individual (formulated) PPPs at the local scale. Consequently, possible impacts of indirect effects by pesticide-mediated shifts in food-web interactions and that result in reduced food for birds and mammals, are not considered in an explicit way in this guidance document. To avoid these impacts at least requires that future EFSA guidance documents for pesticide risks on non-target terrestrial plants, non-target arthropods and soil organisms sufficiently address the supporting ecosystem service 'food-web support' for birds and mammals. In addition, possible cumulative stress by tank mixes and/or the sequential use of different PPPs in the crop, or the use of PPPs in nearby other crops, are not considered in this (updated) guidance document. Note that according to current legislation (see e.g. Regulation (EC) No 1107/2009[2]), mixture toxicity in prospective ERA for pesticides is only considered for formulated PPPs that contain more than one active substance (see chapter 12). The operational PGs also imply that possible emerging landscape-level risks for birds and mammals are not yet considered.

The focus of this (updated) guidance document is on:

- Birds and mammal species typical for agricultural landscapes in the EU,
- the dietary exposure route,
- direct toxic effects,
- the protection of individuals of potentially sensitive bird and mammal species in the acute risk assessment,
- populations of vulnerable bird and mammal species in the long-term risk assessment.

Considering the above, also endangered bird and mammal species typical for agricultural landscapes in the EU are assumed to be covered when addressing populations of vulnerable species in the long-term risk assessment. While the vulnerable species concept takes account of probability of exposure, toxicological sensitivity and potential for recovery (EFSA Scientific Committee, 2016b), it does not usually consider that the local conservational status of a species can already be unfavourable. If so, this requires Member State-specific local mitigation measures as well. For further information on the coverage of endangered species in ERAs at EFSA, see EFSA Scientific Committee (2016c). Also note that, the regulatory data requirements with respect to toxicity tests mainly concern the dietary exposure route (particularly for birds). Although other routes, like dermal and inhalation exposure to pesticides, may contribute to potential risks to birds and mammals, it is assumed in this guidance document that dietary exposure is the main exposure route. This assumption is surrounded by some uncertainty. An important research need is to generate proper data for a better understanding of the potential impact of the different exposure routes (in isolation and in combination) on birds and mammals in the agricultural landscape. It is anticipated that the protection of the biodiversity of birds and mammals will improve when in future guidance documents mentioned above the supporting ecosystem service 'food-web support' for birds and mammals is guaranteed. The claim to sufficiently protect the biodiversity of birds and mammals in agricultural landscapes requires that the impacts of indirect effects by a pesticide-mediated decline in food availability (e.g. in the form of earthworms, insects and weeds) and cumulative risks of pesticide use at the landscape level are ecologically negligible.

3.2. Tiered approach

3.2.1. Generalised tiered approach in prospective ERA for pesticides

In a tiered risk assessment scheme, there are several stages of the assessment (see Figure 1). A fundamental aspect of the tiered approach is that every level of the assessment should be able to address the SPG.

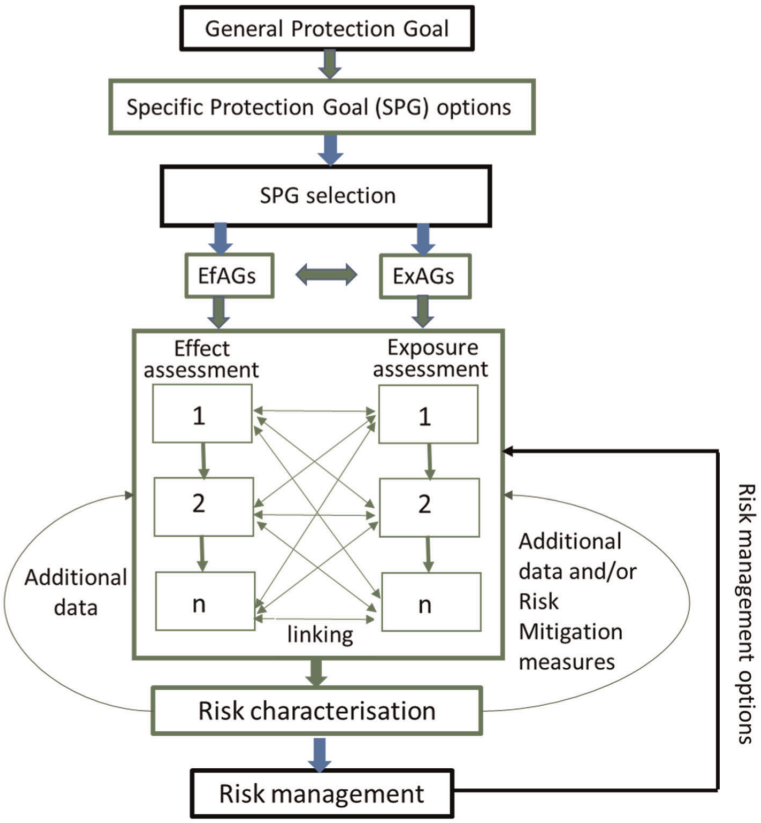


Figure 1: Generalised overview of the parallel tiered effect and exposure assessments as part of the prospective risk assessment and management procedure for pesticides and non-target organisms. EfAG is the abbreviation of effect assessment goal and ExAG that of exposure assessment goal. The responsibilities of risk assessors and risk managers are indicated by, respectively, green and black boxes and arrows. The blue arrows indicate that a dialogue between risk assessors and risk managers may be required before final decision-making. Risk mitigation measures to refine the exposure assessment can be proposed during the risk assessment process

Important components of the effect assessment goals (EfAGs) that underlie the SPG, amongst others, comprise (i) the definition of the ecotoxicologically relevant exposure quantity (or concentration/dose) that may differ between different organism groups, (ii) the incorporation of agreed upon toxicity estimates and extrapolation factors for relevant standard test species in the lower tier (usually prescribed in legislation) and (iii) criteria for the selection of additional test species and/or vulnerable focal species in higher tiers, including associated measurement endpoints, extrapolation tools (e.g. assessment factors, models) and ecological scenarios (in case of effect modelling). Important components of the exposure assessment goals (ExAGs) that underlie the SPG, amongst others, are (i) the definition of the spatial–temporal properties of the ecotoxicologically relevant exposure quantity (or concentration/dose) in the receiving environment of concern (e.g. soil, water, sediment, organisms), (ii) the selection of scenarios for exposure modelling in different tiers and (iii) the choice of a percentile of the (modelled) spatial–temporal exposure quantity that needs to be used in the exposure assessment. Note that in sophisticated higher tiers based on modelling approaches, the ecological and exposure scenarios may be combined in environmental scenarios (EFSA PPR Panel, 2014; EFSA Scientific Committee, 2016b).

With increasing tiers, the realism and complexity of the risk assessment should increase, which means that the protectiveness of lower tiers may be calibrated with higher tiers if deemed sufficiently robust. Note that, in ERA decision schemes for certain groups of non-target organisms, a screening tier (in some guidance referred to as Tier 0) may be used in the exposure and/or effect assessment. Such a screening tier aims to identify uses of substances that pose low risk to all non-target organisms of concern by using worst-case assumptions and scenarios to obtain conservative exposure and/or effect estimates.

In the effect assessment, an increase in the tier should result in a more realistic effect estimate (exposure–response relationship) for the vulnerable field populations and communities typical for agricultural landscapes in the EU (and in the acute risk assessment for individual vertebrates) actually at risk and not in conflict with the agreed EfAGs that underlie the specific protection goal. In the exposure assessment, an increase in the tier should result in a predicted exposure estimate which is environmentally more realistic, less conservative and not in conflict with the agreed ecotoxicologically relevant exposure quantity (or concentration/dose) and ExAGs that underlie the specific protection goal. In theory, it is possible to enter the tiered scheme at any level, to link different effect assessment tiers to different exposure assessment tiers in the risk assessment. However, often, it is common practice to start at the lower tiers and only move on to the higher tiers if necessary (Figure 1).

Risk mitigation can be integrated into an assessment at any tier and, in the majority of cases, concerns a reduction in exposure. In some cases, this will need the provision of refined exposure data whilst in other cases, default values are available for risk-reducing measures (e.g. spray drift reduction). What is essential is that any suggested mitigation is demonstrated to reduce the risk sufficiently so that the SPG is met (i.e. a risk assessment indicating a low risk is needed).

An appreciation of the tiered risk assessment scheme is needed in order to contextualise the outcome of any assessment. Appendix A presents a generalised description of the tiered approach in prospective ERA for pesticides and may be consulted for this purpose.

3.2.2. Birds and mammals and specific features of the tiered approach in prospective ERA for pesticides

3.2.2.1. Effect assessment tiers

In different effect assessment tiers for birds and mammals, different types of species are defined, such as standard test species, additional test species and focal species. Standard test species and additional test species are species that can be easily held and reared under laboratory conditions to conduct acute and/or long-term toxicity tests. Although the natural habitat of selected standard and additional bird and mammal test species may include agricultural fields, they may not be typical for crops in the EU where pesticides are applied. It is assumed, however, that the toxicity data for standard and additional test species can be used as surrogate for EU bird and mammal species that actually occur in crops where pesticides are used. In this guidance document, the representative subset of real bird and mammal species that actually occur in the crop and/or nearby off-crop habitats, such as in-field vegetated buffer strips, when the plant protection product is being used, is defined as focal species.

Toxicity tests with standard test species concern Effect Assessment Tier 1 data requirements prescribed in legislation (Commission Regulation (EU) 283/2013[4] and 284/2013[5]) and standardised test protocols are implemented for these species (e.g. OECD guidelines; see Section 5.2). In the EU, additional toxicity tests with vertebrates are not allowed to be generated for ethical reasons, unless there is an appropriate justification (see article 7(d) of Regulation (EC) No 1107/2009[2]; Commission Regulation (EU) 283/2013[4]). Nevertheless, reliable toxicity data for additional bird and mammal species may be found in literature or generated as part of past and current legislative data requirements within and outside the EU. These data may be used as refinement in Tier 2 effect assessment approaches. For additional test species, standardised test protocols may have been used, and if not, the test guidelines for Tier 1 standard test species may have been followed as much as possible. Higher tier effect assessment options based on (semi-)field studies and/or modelling approaches (higher integrated exposure and effect assessment tier) aim to directly assess treatment-related effects on focal species. Consequently, this tier concerns an integrated exposure and effect assessment.

The sparsity of laboratory toxicity data for birds and mammals implies that Tier 2 effect assessment options, based on laboratory toxicity data for both standard and additional test species, are limited. Nevertheless, in principle sophisticated (semi-)field studies and/or novel modelling approaches with

focal species can be made available as a higher tier option in the effect assessment, although harmonised EU-level guidance for the conduct and interpretation of these tests is lacking.

In Figure 2 and Table 2, an overview is provided of (possible) effect assessment tiers in prospective ERA for pesticides and birds and mammals.

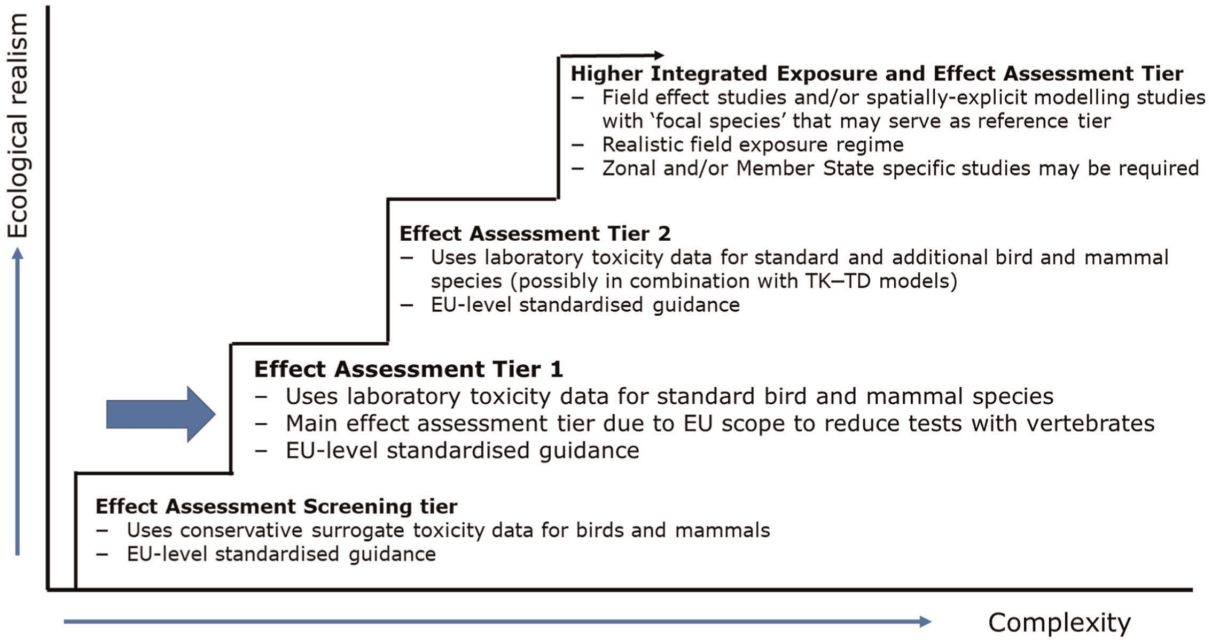


Figure 2: Overview of possible effect assessment tiers in the risk assessment for birds and mammals. The blue arrow indicates that in daily practise, the effect assessment usually will depend on Tier 1

Table 2: Overview of tiers in the acute and reproductive effect assessment for birds and mammals

Effect Assessment Tier	Acute effect assessment	Reproductive effect assessment
Screening tier = anticipated to be conservative due to the use of worst-case assumptions for toxicity estimates in case real toxicity data are missing. Generic EU-level assessment For more detailed guidance, see Section 5.1 and chapter 9 on metabolites	If valid acute LD_{50} (= Lethal Dose to 50% of test individuals) values for metabolites are missing, it is by default assumed that a metabolite is acutely 10 times more toxic than the active substance. Surrogate LD_{50} for the most sensitive standard test species is selected in risk assessment. Toxicity exposure ratio (= TER) = (LD_{50}/DD) should be ≥ 10 to conclude low acute risk (DD = Dietary Dose; see Section 3.2.2.2).	If for metabolites valid EL_{10}* (= effect level to 10% of test individuals) and/or NOAEL (= no observed adverse effect level) values of standard test species are missing and a reproductive risk assessment is triggered, it is by default assumed that for reproduction endpoints a metabolite is 10 times more toxic than the active substance. Surrogate EL_{10}* (or NOAEL) for the most sensitive standard test species is selected. TER (EL_{10}*/DDD) should be ≥ 5 to conclude low reproductive risk (DDD = Daily Dietary Dose; see Section 3.2.2.2).
Tier 1 = based on laboratory toxicity data for standard test species as part of data requirements. Generic EU-level assessment	*Standard test species approach* Technical guidance available and ready to use Basic set of acute toxicity data should always be available for active substances (*and formulated products if toxicity cannot be*	*Standard test species approach* Technical guidance available and ready to use Basic set of toxicity data for reproductive endpoints should always be available for active substances (*and formulated products if toxicity*

Effect Assessment Tier	Acute effect assessment	Reproductive effect assessment
For more detailed guidance, see Section 5.2	*predicted on the basis of the data for the active substance, or where results from mammalian testing give evidence of higher toxicity of the plant protection product compared to the active substance, unless the applicant shows that it is not likely that birds are exposed to the plant protection product itself*) LD_{50} for the most sensitive standard test species is selected. TER (LD_{50}/DD) should be ≥ 10 to conclude low acute risk.	*cannot be predicted on the basis of the data for the active substance, or where results from mammalian testing give evidence of higher toxicity of the plant protection product compared to the active substance, unless the applicant shows that it is not likely that birds are exposed to the plant protection product itself*) EL_{10}* or NOAEL for the most sensitive standard test species is selected. TER (EL_{10}*/DDD) should be ≥ 5 to conclude low reproductive risk. When the avian LD_{50}/10 is lower than the EL_{10}* or NOAEL, the LD_{50}/10 should be used as effect assessment endpoint in the TER.
Tier 2 = based on laboratory toxicity data for standard and additional test species Generic EU-level assessment For more detailed guidance, see Sections 5.3 and 5.4	*Geometric mean approach* Technical guidance is in place and can be used if valid LD_{50} values are available for more species than required in Tier 1. Geometric mean LD_{50} for all tested birds or mammals is selected. TER (LD_{50}/DD) should be ≥ 10 to conclude low acute risk, unless the LD_{50} of most sensitive species is lower than the TER (see Section 5.3). *Species Sensitivity Distribution (SSD) approach* Might be used if for at least 5 bird or 5 mammal species, a valid LD_{50} value is available. This will be seldom the case when considering the EU scope for reducing toxicity tests with vertebrates. The hazardous dose to 5% (HD_5) of the species tested derived from the SSD constructed with LD_{50} values is selected. TER (HD_5/DD) should be ≥ 3 to conclude low acute risk. *TKTD modelling* Refined acute toxicity estimation for time-variable exposure of laboratory test species by means of validated Toxicokinetic-Toxicodynamic (TKTD) modelling approaches. Technical guidance not yet developed for birds and mammals.	Since valid reference tier data (e.g. field effect studies) are not (yet) available for a sufficient number of pesticides to allow calibration of the protectiveness of lower tiers for reproductive effect assessment, a precautionary approach is followed. a) *Default approach* The geometric mean approach is not used in the reproductive effect assessment. As a default approach, the lowest EL_{10}* (or NOAEL) for the most sensitive species tested (or avian LD_{50}/10 value if lower) is selected as relevant endpoint. TER (relevant endpoint/DDD) should be ≥ 5 to conclude low reproductive risk. b) *Species Sensitivity Distribution (SSD) approach* In the exceptional case that for at least 5 bird or 5 mammal species valid EL_{10}* values are available for a relevant reproductive endpoint (most sensitive one for each species is selected), the SSD approach can be explored and a HD_5 calculated. This will be seldomly possible when considering the EU scope for reducing toxicity tests with vertebrates. If based on expert judgement this HD_5 can be used as effect estimate, then the TER (HD_5/DDD) should be ≥ 3 to conclude low reproductive risks.

Effect Assessment Tier	Acute effect assessment	Reproductive effect assessment
	Case-by-case evaluation based on expert judgement.	c) *TKTD modelling* Refined reproductive toxicity estimation for time-variable exposure of laboratory test species by means of validated Toxicokinetic-Toxicodynamic (TKTD) modelling approaches Technical guidance not yet developed for birds and mammals. Case-by-case evaluation based on expert judgement.
Higher Integrated Exposure and Effect Assessment Tier = based on experimentally observed or monitored treatment-related (toxic) effects on (vulnerable) focal bird and mammal species under realistic field conditions and/or on integrated exposure and effect modelling of treatment-related (toxic) responses on these focal species (also see highest exposure assessment tier (Table 4) Region/EU Member State specific assessment For guidance recommendations, see Sections 7.1 and 7.2.	Field effect studies on treatment-related individual- and population-level effects of focal species subject to realistic application rates of the plant protection product under evaluation and/or predicting treatment-related individual- and population-level effects of these focal species with spatially explicit models Field effect studies: – Studies should have sufficient statistical power. – Spatial–temporal extrapolation is required to address remaining uncertainties (might be done in combination with modelling). – Research activity to date and harmonised technical guidance not yet developed. – Case-by-case evaluation based on expert judgement. Spatially explicit modelling: – Requires definition of environmental scenario's as well as extrapolation to other vulnerable focal species in the treated crop or crop group of concern. – Studies should consider good modelling practice (EFSA PPR Panel, 2014). – Predictions of modelling approaches can supplement results of field effect studies, if available, but should not be in conflict with these results. – Research activity to date and harmonised technical guidance not yet developed. – Case-by-case evaluation based on expert judgement.	

*: operationalised in Section 5.2.7.1 as BMD_{10}.

3.2.2.2. Exposure assessment tiers

In different exposure assessment tiers for birds and mammals, exposure scenarios are used based on the consumption of pesticide-contaminated food items foraged in crops by indicator model species (IMS), generic model species (GMS) and focal species (FS). A detailed description of IMS, GMS and FS is given below. The exposure scenario based on IMS is intended to be conservative, that based on GMS worst case and that based on FS realistic worst case. A generalised description of the parameters considered in the oral exposure assessment is presented in Equation 1 below but note that the FIR may change if the PD also changes. For a more detailed description, see chapter 6.

$$(D)DD = ((FIR \times C \times PD)/BW) \times PT \quad (1)$$

Table 3: Explanation of the different parameters considered in the oral exposure assessment

Symbol	Definition	Unit
(D)DD	(Daily) Dietary dose for the species in question	mg a.s./kg bw per day
FIR	Food intake rate of the species in question	g fresh weight per day
BW	Body weight of species in question	g
C	Concentration of active substance per fresh diet fraction	mg/kg

Symbol	Definition	Unit
PD	Fraction of a particular potentially contaminated food type in diet (PD = 1 for single diet; between 0 and 1 for mixed diet)	[-]
PT	Fraction of diet obtained within treated area (number between 0 and 1).	[-]

The food intake rate (FIR) depends on the daily energy expenditure (DEE) of the species, which is again related to the body weight. FIR (g) is calculated by dividing DEE (kJ) by the energy content in 1 g of diet. Each time that the diet is changed (i.e. via a refined PD), the FIR must also be recalculated, as the energy content in the food will change (see Appendix G).

The concentration (C) is directly available in the special case of treated seeds, but in all other cases, C must be calculated from the residue per unit dose (RUD in mg a.s./kg food item), application rate (kg a.s./ha), number of applications (expressed as a multiple application factor (MAF)) and the half-life (DT_{50}) of the compound, incorporated via a factor accounting for the time-weighted average decline (fTWA) (the latter only applicable for reproductive exposure assessments and where the fTWA criteria are met). In the acute exposure assessment, the 90th percentile RUD is selected, while the 50th percentile (geometric mean) RUD is selected in reproductive exposure assessment (Section 6.2.4). In Tier 1 and higher tiers, crop interception of the pesticide may be considered to refine the RUD, where appropriate (see Section 6.2.6).

For a mixed diet (see Equation 2), (C × PD) must be calculated separately for each food type (PD_i) with respective residue concentration (C_i), and the resulting total (D)DD is the sum of the contributions from each food type in the diet (sum of different PD = 1):

$$(D)DD_I = FIR \times \sum_i (C_i \times PD_i)/BW) \times PT \quad (2)$$

Indicator model species (IMS) of birds and mammals are used in the screening tier of the exposure assessment. An indicator model species is not a real species, but by virtue of its size and feeding habits is considered to have a higher internal exposure than (i.e. to be protective of) all (focal) species with different feeding guilds that may occur in a particular crop (group) at a particular time. It is a composed species belonging to the feeding guild with the highest oral exposure and that consumes a single type of food (PD = 1) and has a high food intake rate and relatively low biomass. In addition, all individuals find their food in the treated area (PT = 1). The screening tier is anticipated to be conservative and applicable for large groups of crops, using worst-case assumptions for oral exposure of the indicator model species. For example, the crop groups are broad and differentiation between crop growth stages is not taken into account. Furthermore, it is assumed that oral exposure of the indicator model species will be the same within the grouped crops. The exposure assessment screening tier has the added benefit of allowing a risk assessor to screen out low-risk substances and simplifies the exposure assessment when multiple crops are under evaluation. It allows a risk assessor to reach the conclusion of 'low risk'. However, a screening assessment indicating potential risks should not be considered to indicate a 'high risk' per se, because of the underlying conservative assumptions in the exposure scenario. An exception to this may be endangered species, which the WG cannot ensure will be covered by Tier 1 and higher tier exposure assessment assumptions, but which are regulated at the Member State level. Should the screening tier be breached MS may consider whether endangered species should be investigated further.

The Tier 1 exposure assessment is based on oral exposure of generic model species (GMS). A generic model species is not a real species, but a composite 'realistic worst-case' species with a high food intake rate (and consequently low biomass) and representing a specific feeding guild (e.g. herbivorous, insectivorous, granivorous, frugivorous, omnivorous) and stratum where it feeds in the crop (e.g. foliar or ground). The set of generic model species characterised by different feeding guilds is considered to be representative for all those (focal) bird or mammal species potentially at risk in a specific crop group characterised by a similar growth pattern (see Box 1). For crop grouping, the EPPO classification scheme is used[14] (EPPO, online), and it is assumed that exposure of the generic model species is the same within Tier 1 crop groups. In the case of generic model species with mixed diets, the FIR is determined accounting for the proportions of the different food items in the diet. For each generic model species with a mixed diet, a fixed generic diet is selected for each crop and/or growth

[14] https://gd.eppo.int/PPPUse/

stage of the crop. Furthermore, it is assumed that a generic model species finds all its food in the treated area (PT = 1). In addition, the crop group-specific generic model species are considered to be representative across EU Member States.

In the Tier 1 exposure assessment, the assumptions for the proportions of the mixed diet of the generic model species are considered to be reasonably worst case. Nevertheless, it is acknowledged that not all birds and mammals feeding in treated fields with the crop under evaluation will take the same fixed proportion of food items. However, the impact of variable proportions of the diet on the (daily) dietary dose (= (D)DD) of the generic model species can be easily calculated with the calculation tool that is developed for this guidance document. Such additional (D)DD values are not part of the standard Tier 1 exposure assessment but may be used to inform risk managers on possible uncertainties in the exposure assessment. For example, and as done by some Member States already, calculating a (D)DD for a generic model species with a mixed diet on basis of a worst-case single diet might be used as a proxy for endangered bird and mammal species belonging to the same feeding guild and of which the spatial distribution locally overlaps with that of the treated crop under evaluation.

Box 1: A note on feeding guild nomenclature in this guidance document

In the context of this guidance, the generic model species of birds and mammals are divided into dietary classes of herbivore, omnivore, granivore, insectivore and frugivore. It should be noted that these classes do not represent the diet of the species at all times of the year or throughout the lifetime of the animal. Nor, as discussed previously, do they represent a specific 'real' species. These terms are used to reflect the presumed diet of the model species in the crop in question during the time frame in question. An example would be a European starling (*Sturnus vulgaris*) in orchards, where during the time frame of interest in the risk assessment, ripening, these birds can be assumed to have a virtually 100% fruit diet, though this is not possible for other periods of the year, when they may not be a focal species, or may be a focal species with a different diet. In Europe, generally, all species are to some degree omnivorous at least at some life stages and seasons. Thus, the feeding guilds mentioned in this guidance per crop and BBCH stage are not necessarily the feeding guilds to which a bird might belong for classification purposes. The diets chosen in the Tier 1 exposure assessment have therefore been chosen based on a feeding guild classification specific to the crop in question, at the BBCH stage in question (for more detail on the mixed diet considerations, see Appendix F).

This has a clear bearing on the higher tier focal species studies, as the actual feeding guild of each species should be determined depending on the crop in question, the time of year in question and the species in question. A species which is considered to be an omnivore at many times of the year may be an insectivore during the brooding season, e.g. which may coincide with the use in question. Thus, in the circumstances of the failure of a particular feeding guild in the Tier 1 exposure assessment, particular attention should be paid to the classification (per feeding guild) of the focal species in any focal species study presented in the higher tier. Attention should also be given to the study design, as to the size and expected diet of any species that might be trapped (netting size, bait type, etc.). More details on higher tier studies can be found in Section 6.5.1.

Furthermore, it is noted that in this guidance, all species which, based on their ecology and diet, were likely to be present in the crops in question, were considered for the 'building' of the model species. While, agriculturally, some of these species may be classified as 'pest species' by farmers, they nevertheless form an important basis for many food webs and Regulation (EC) No 1107/2009[2] does not indicate that species which are considered undesirable by farmers are exempt from the risk assessment. In addition, a number of these species are covered under EU and National species protection legislation. Furthermore, the 'model' species are merely fictitious species whose body weight and diet are considered to be protective of several other species likely present in the crops in question, but who do not necessarily share the same feeding guild.

Higher tier exposure assessments usually use focal species (FS), although in the Tier 2 exposure assessment based on refining residue data in the crop (group) of concern (see Table 4) 'generic model species' can also be used for the (D)DD calculation. Focal species are defined as the representative subset of real bird and mammal species that actually occur in the crop when the pesticide is being used. These focal species are differentiated in accordance with their feeding guild and also may obtain part of their food outside the treated field (PT < 1). If focal species have a mixed diet whilst in the treated area (e.g. omnivores or feeding insects from different strata), this is reflected in the PD value. Note, however, that the feeding guild may be different for different life stages of the same species (e.g. a bird may be herbivorous as adult but feeds its chicks predominantly with insects). Furthermore,

note that the 'focal species' – crop association may be different in different regions of the EU. Consequently, it may be that different EU Member States select different focal species for the same crop where the pesticide is used. This notion is important in Tier 3 and other higher tier exposure assessments that are based on focal species studies.

Note that the exposure assessment tiers described above and in Figure 3 and Table 4, generally, are applicable for spray applications and seed treatments of pesticides, but different rules may apply for granules. For details on the risk assessment for products applied as granules, see chapter 8.

In Figure 3 and Table 4, an overview is provided of (possible) exposure assessment tiers in prospective ERA for pesticides and birds and mammals.

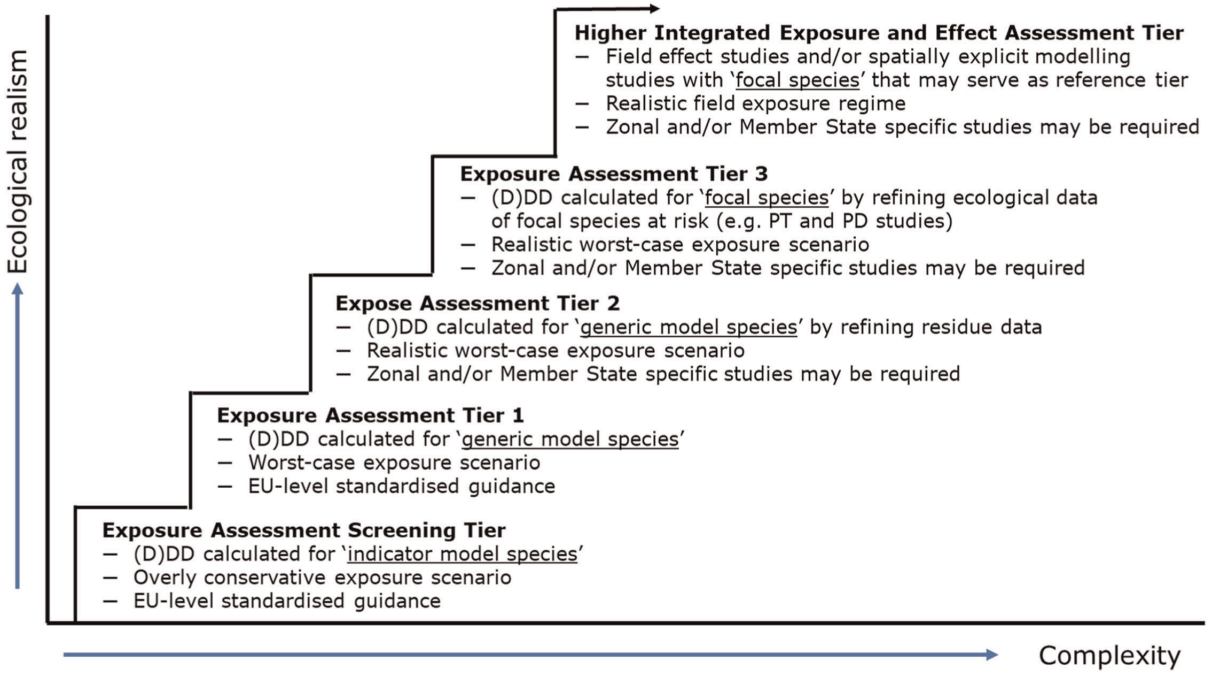


Figure 3: Overview of possible exposure assessment tiers in the risk assessment for birds and mammals

Table 4: Overview of tiers in the exposure assessment for birds and mammals

Exposure Assessment Tier	Specific features of exposure scenario
Screening tier = based on acute DD or long-term DDD calculated for indicator model species Generic EU-level assessment For more detailed guidance see Sections 6.2.2 (spray applications) and 6.3.1 (seed treatments)	Uses highest application rate within broadly grouped crops No differentiation between crop growth stages Single food type in diet of indicator model species (PD = 1) Individuals of the indicator model species find all their food in the treated area (PT = 1)
Tier 1 = based on acute DD or long-term DDD calculated for set of generic model species that differ in feeding guild Generic EU-wide assessment For more detailed guidance, see Sections 6.2.3 (spray applications) and 6.3.2 (seed treatments)	EPPO crop grouping on basis of crops with similar growth pattern Crop interception of pesticide may be considered Exposure of generic model species is the same within Tier 1 crop groups More realistic PD for generic model species with a mixed diet The PD of each food item is fixed. Individuals of the generic model species find all their food in the treated area (PT = 1)

Exposure Assessment Tier	Specific features of exposure scenario
Tier 2 = based on acute DD or long-term DDD calculated for generic model species of the feeding guild(s) potentially at risk by refining residue data in the crop (group) of concern Region/EU Member State specific assessment based on expert judgement For more detailed guidance, see Section 6.4.	Refinement may include: More realistic crop interception data at different growth stages of the crop Refinement of concentration of active substance per fresh diet fraction – Availability of treated seeds as food source – Potentially, refined residue values – DT_{50} of a.s. in food items for MAF and/or TWA calculation (note that DT_{50} values cannot easily be extrapolated between matrices and that the refinement does not exclude that another component of the diet may then become the most critical)
Tier 3 = based on acute DD or long-term DDD calculated for focal species of the feeding guild(s) potentially at risk by refining biological and ecological parameters of relevant focal species. Region/EU Member State-specific assessment For more detailed guidance, see Section 6.5.	Refinement may include: Lower concentration due to dehusking Refinement of PD (crop and growth stage specific) – Only for reproductive risk assessment – Refined selection of faeces in concert with radio-tracking – Novel studies on diet of focal species by analysing faeces with DNA barcoding Refinement of PT – Only for reproductive risk assessment – Studies on individuals' foraging behaviour – Home-range and habitat use by radio telemetry studies
Higher Integrated Exposure and Effect Assessment Tier = based on experimentally observed or monitored treatment-related (toxic) effects on (vulnerable) focal bird and mammal species under realistic field conditions and/or on integrated exposure and effect modelling of treatment-related (toxic) responses on these focal species (also see Table 2) Region/EU Member State-specific assessment For guidance recommendations, see Sections 7.1 and 7.2.	Field effect studies on treatment-related individual- and population-level effects of focal species subject to realistic application rates of the plant protection product under evaluation and/or predicting treatment-related individual- and population-level effects of these focal species with spatially explicit models Field effect studies: – Studies should have sufficient statistical power. – Spatial–temporal extrapolation is required to address remaining uncertainties (might be done in combination with modelling). – Research activity to date and harmonised technical guidance not yet developed. – Case-by-case evaluation based on expert judgement. Spatially explicit modelling: – Requires definition of environmental scenario's as well as extrapolation to other vulnerable focal species in the treated crop or crop group of concern. – Studies should consider good modelling practice (EFSA PPR Panel, 2014). – Predictions of modelling approaches can supplement results of field effect studies, if available, but should not be in conflict with these results. – Research activity to date and harmonised technical guidance not yet developed. – Case-by-case evaluation based on expert judgement.

3.2.3. Risk assessment of oral exposure to pesticides in prospective ERA for birds and mammals

In this section, the oral exposure risk assessment for individual active substances and spray applications and seed treatments is described in general terms. Specific features of risk assessment for granules, metabolites, secondary poisoning, contaminated water and additive risks of different active

substances in formulated products are presented in separate chapters (for granules, see chapter 8; for metabolites, see chapter 9; for secondary poisoning, see chapter 10; for contaminated water, see chapter 11; for additive risks, see chapter 12).

3.2.3.1. Risk assessment based on indicator model species, generic model species and Tier 3 exposure studies with focal species for spray applications and seed treatments

For all the substances under assessment which are applied with a spray application method, or seed treatments, an acute and reproductive risk assessment should be conducted for both birds and mammals in the vast majority of the cases (see chapter 4 on problem formulation for possible exceptions and Section 5.2.1). The risk assessment based on indicator model species, generic model species and Tier 3 exposure studies with focal species is performed by calculating a toxicity exposure ratio (TER) and by comparing it to the trigger values included in Regulation 546/2011[6] for acute and reproductive assessments (although a lower trigger value is possible if a Tier 2 effect assessment based on a sufficient number of additional test species is conducted; for guidance, see Table 2 and Section 5.3).

The overall features of different effect assessment and exposure assessment tiers are explained in general terms above in Section 3.2.2. More detailed information on effects assessment tiers based on standard and additional test species (Tiers 1 and 2) and that are used in the risk assessment by applying the TER approach can be found in chapter 5. Similarly, more detailed information on exposure assessment tiers based on indicator model species (screening tier), generic model species (Tier 1 and 2) and focal species (Tier 3) and that are used in the risk assessment by applying the TER approach can be found in chapter 6. The risk assessment based on the higher integrated exposure and effect assessment tier (see Figures 2 and 3) is described in greater detail in chapter 7.

The risk assessment as given below by the TER approach can be calculated, using the designated calculation tool (EFSA, online[15]).

Acute dietary risk assessment on basis of the TER approach

The acute TER is given by the following equation:

$$\mathbf{TER} = \frac{\mathbf{LD_{50}}}{\mathbf{DD}} \tag{3}$$

For further information on acute toxicity studies for birds and mammals, see Section 5.2.2. If the acute effect assessment is based on toxicity tests with standard and additional test species (see Section 5.3.3 for details), the LD_{50} of the most sensitive standard bird or mammal species in Equation 3 may be substituted by the geometric mean LD_{50} value for bird or mammal species (Tier 2 geometric mean approach) or the HD_5 (Tier 2 species sensitivity distribution approach).

For the information on the estimation of the exposure, i.e. DD, see Section 3.2.2 and chapter 6.

In case the effect assessment is based on the Screening tier, Tier 1 or the Tier 2 geometric mean approach, the risk assessment is as follows:

TER ≥ 10	**Low risk is concluded, and the assessment may stop**
TER < 10	**Either refine the risks by means of a more sophisticated exposure and/or effect assessment tier or a risk mitigation measure**

In the exceptional case that in the effect assessment, the Tier 2 species sensitivity approach is applied (calculation of HD_5), the TER trigger value of 10 may be reduced to 3.

Reproductive dietary risk assessment on basis of the TER approach

The reproductive TER is given by the following equation:

$$\mathbf{TER} = \frac{\mathbf{relevant\ endpoint}}{\mathbf{DDD}} \tag{4}$$

For further information on the reproductive toxicity studies, see Section 5.2.4. It is recommended in Section 5.2.7 to use as relevant reproductive endpoint the BMD_{10}. If this estimate is not considered suitable, an NOAEL may be selected, if appropriate (see Section 5.2.7). When the avian $LD_{50}/10$ is

[15] https://r4eu.efsa.europa.eu/app/birds-mammals

lower than the relevant reproductive endpoint, the $LD_{50}/10$ should be used, as explained in Section 5.2.8.

For the information on the estimation of the exposure, i.e. DDD, see Section 3.2.2.2 and chapter 6.

TER ≥ 5 **Low risk is concluded, and the assessment may stop**
TER < 5 **Either refine the risks by means of a more sophisticated exposure and/or effect assessment tier or a risk mitigation measure**

In the exceptional case that in the effect assessment, the Tier 2 species sensitivity approach is applied (calculation of HD_5), the TER trigger value of 5 may be reduced to 3.

3.2.3.2. Risk assessment based on field effect studies and/or spatially explicit population models with focal species as part of the higher integrated exposure and effect assessment tier

These higher tier integrated exposure and effect studies should focus on vulnerable focal species of vertebrate wildlife. The results of well-conducted studies might be directly compared with the operational protection goal in a weight-of-evidence approach to conclude low or regulatory unacceptable risks (for details, see chapter 7). In this assessment, an Exposure Multiplication Factor of 2–5 may need to be applied to address remaining uncertainties.

4. Problem formulation

4.1. Introduction

Problem formulation is a process for generating and evaluating preliminary hypotheses about why ecological effects have occurred, or may occur, from human activities (USEPA, 1998; Devos et al., 2019).

Problem formulation is considered one of the key pillars of risk assessment (see Figure 4). A proper problem formulation will set the boundaries for the assessment, with the goal of making it 'fit for purpose'. Interpreting the questions which need to be answered by a risk assessment as well as identifying its scope and objectives are key elements when starting a risk assessment. The definition of specific (operational) protection goals is pivotal for targeting the problem formulation step (see Section 3.1). Although problem formulation and risk assessment are presented sequentially, it is important to stress that risk assessments are frequently iterative.

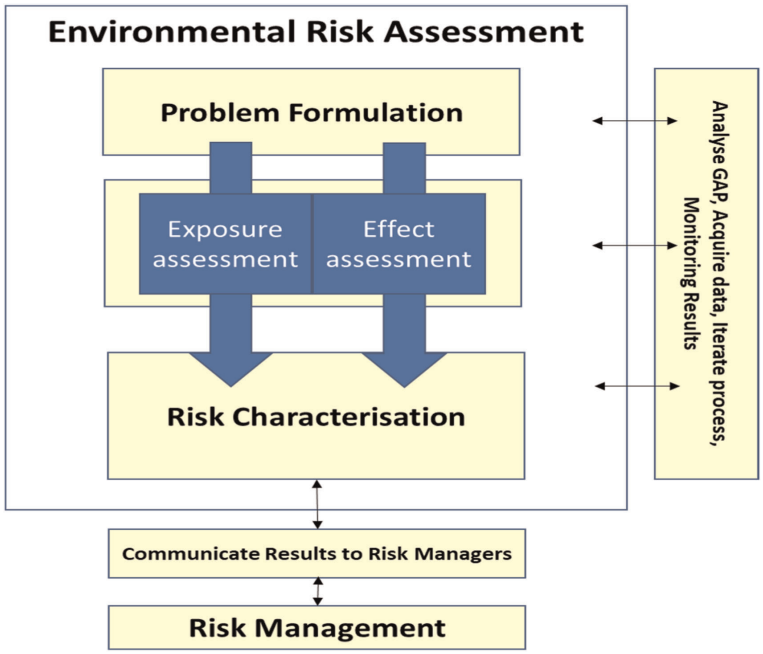


Figure 4: Illustration of the problem formulation when conducting an environmental risk assessment (from USEPA, 1998 with modifications). The box on the right side of the figure highlights steps such as GAP analysis, data acquisition, iteration and monitoring. Monitoring data provide important input to all phases of a risk assessment. They can be used to evaluate a risk assessment's predictions, to further assess the effectiveness of mitigation measures or determine the extent and nature of ecological recovery (USEPA, 1998). However, it is difficult to use monitoring data directly in risk assessment due to the fact that there are many influential parameters in the monitoring data that are generally unknown (pesticide exposure, climatic conditions, presence of disease, farming practices, etc.). Furthermore, a link between exposure and observed effects cannot be established in monitoring data (i.e. causality) (EFSA, 2013)

4.2. Aspects to consider in problem formulation

4.2.1. Routes of exposure

For a risk to occur, birds and mammals should be exposed directly or indirectly to PPPs. In the context of this guidance, only direct effects via a dietary route of exposure are considered, see Sections 2.2 and 3.1. This includes also assessment of effects through secondary poisoning and effects through consumption of contaminated water. According to Regulations 283/2013[4] and 284/2013[5], laying down the data requirements for active substances and formulated products, respectively, effects of a pesticide active substance and/or PPPs shall be investigated except where the substance is intended to be used in enclosed spaces and wound healing treatments. In those circumstances, birds and mammals will experience neither direct nor secondary exposure. Therefore, when exposure of birds and mammals is not anticipated, toxicity data and a full risk assessment are not needed and low risk can be concluded based on the lack of exposure. Based on the legal requirements, therefore, and in order to formulate the problem to address, the first step to follow is to carefully consider the proposed representative uses of a PPP as indicated in the good agricultural practice (GAP) table.

4.2.2. Agricultural practices

A GAP table defines the way a PPP is proposed for use. For national assessments, some Member States may use the proposed product label rather than a GAP table. The following information should be included (please note this list is not exhaustive):

- Information on the PPP including the product type (e.g. a water dispersible granule), which active substance(s) is included and at what concentration.

- The intended crops (or plants) and the growth stage of the crop (usually using the BBCH growth stage criteria). Sometimes the time of year when applications will be made may also be specified.
- The Member States or regulatory zones where it is intended that the PPP will be used.
- The intended application rate in terms of amount of active substance(s) per hectare must always be stated. The application rate in terms of amount of product per hectare may also be given.
- The number of applications and the application interval.
- Limited information on the method of application (i.e. broadcast air-assisted sprayer, seed treatment, etc.). The EPPO global database for PPP treatments is a useful source of application methodology (EPPO, online[16]).
- Whether the PPP will be used indoors, in outdoor fields or in protected structures. According to EFSA (2015), the type of protected structure should be defined (i.e. greenhouse or plastic tunnel). Please refer to EFSA (2014a) for more information on the types of protected structures.
- Risk mitigation measures proposed by the applicant.
- Other restrictions proposed by the applicant (e.g. applications are only allowed every 3 years).
- Sometimes other information on how the PPP is intended for use may be stated (e.g. band application or spot application).
- Whether the PPP will be used by amateur users (i.e. uses in home gardens).

There are numerous other agronomic or growing practices which will influence the presence and exposure, of birds and mammals in areas where the PPP will be used. If details of such practices are specified, it allows a risk assessment to account for them and thus be more specific. This would be of particular benefit for refinements on basis of the higher integrated exposure and effect assessment tier where information on real species occurring in the crop is used. Where information is lacking, a risk assessment should encompass (as far as practicable) all the conditions of where and how the PPP will be used. The following list includes the types of agronomic or growing practices which are expected to have an influence on the presence and/or exposure of birds and mammals (please note the list is not exhaustive):

- Specific application machinery (e.g. the quality of the sprayer)
- Tillage practices
- Weed control (whether mechanical or using a PPP)
- Use of insecticides or other PPP impacting on the occurrence of invertebrates
- Field size
- Diversity of crops in the landscape
- Sowing density of plants
- Non-cropped areas in proximity to the treated area (e.g. field margins, set-aside or compensatory areas)
- Fruit netting
- Orchard type (e.g. modern spindle, traditional)
- Presence and type of between-row vegetation.

4.2.3. Physical–Chemical properties

It is recommended to also consider the physical–chemical properties of the substance. In some cases, although direct exposure might be excluded, exposure through secondary poisoning cannot be ignored and a risk assessment should be conducted (see Table 5). This is the case when the substance and related metabolites have a logP $\geq$ 3 (see also chapter 10). Additionally, there may be situations where the generation of toxicity data is not considered justified, due to the nature of the substance. This may be the case of substances that are already present in the environment, provided that it is demonstrated that the exposure following application is not quantitatively above the background level.

4.2.4. Spatial scale in the risk assessment for birds and mammals

The risk assessment for birds and mammals in EFSA (2009) considered the exposure resulting from residues in the treated area. Considering the relative residue levels, it is implicit that possible risks due

[16] https://gd.eppo.int/PPPUse/3TREAK

to exposure outside of the treated area are covered by such an assessment. The same approach has been followed for this version of the guidance document with the exception of some small mammals who are reliant on crop coverage to be found within the crop/treated area. As these may not be present in the treated area, but will be present immediately beside it, a risk assessment for small mammals outside of treated area has been introduced. Harmonised terminology to describe the area outside of the treated area is not available and the terms 'off-crop' and 'off-field' have been interpreted variously. For this reason, for the purposes of this guidance document, and to avoid any misinterpretation, a new term is proposed: the **'Terrestrial Area of Interest' (TAI)**. Essentially the TAI is used to describe the area where mammals are located and for which the risk assessment is performed (i.e. the location of the mammals the risk assessment is protecting). It is up to Member State risk managers to consider the location of the TAI in their Member State considering, amongst others, field structures and agronomic conditions. For the purposes of performing a quantitative exposure assessment, a TAI can also be considered as the area at a set distance from the sprayer. This is in line with the current approach for other non-target organisms such as non-target arthropods and non-target terrestrial plants.

To summarise, to calculate the exposure to small mammals in the TAI, the application rate (kg a.s./ha) is adjusted by the spray drift % at a minimum distance from the spray equipment:

i) For standard horizontal sprayers, the default distance at Tier 1 is 1 m
ii) For sideward sprayers and air blast equipment, the default distance at Tier 1 is 3 m
iii) To mitigate the risk to mammals using no-spray buffer zones, the no-spray zone must be between the TAI and the sprayer.

Unless specified in the GAP, the spray drift equipment that should be assumed in the Tier 1 exposure assessment, for each of the crop groups used in this guidance document are given in Appendix I.

4.3. Problem formulation in prospective ERA for birds and mammals

The following steps and elements of a GAP table should be analysed to understand if a full risk assessment is needed and how this should be conducted, preferably in the order indicated below.

1) **Is the a.s./PPP under assessment intended to be applied in greenhouses or indoors?** In the context of this guidance, the terminology as indicated in EFSA Guidance (EFSA, 2014a) and EPPO (EPPO, online[17]) is used:

   Uses indoor and/or in greenhouses: For all these uses, exposure to birds and mammals is limited[18] and a risk assessment through dietary exposure is not needed, with the exception of the risk through secondary poisoning for the substance under assessment and its related metabolites, if any (see chapter 10) (i.e. fish-, benthic invertebrate- and earthworm-eating birds and mammals, in cases where the exposure assessment indicates exposure to soil, sediment and aquatic organisms), when triggered, i.e. logP $\geq$ 3. Furthermore, if exposure to the soil is anticipated, then the need for a risk assessment through contaminated water (via puddles) should be checked. It is crucial that it is clarified in the GAP that the use is limited to greenhouse as defined in EFSA (2014a). If this is not clarified, it cannot be excluded that the substance might be applied in semi-open protected structures.
   Uses in semi-open protected structures: Crops/plants grown in low mini tunnels, plastic shelters, net shelter/shade house and walk-in tunnels. For all these uses exposure to birds and mammals may be equivalent to outdoor field uses. See EFSA (2014a) for the definition of the types of protected crops.
   Outdoor uses: Crops/plants grown in the open field without any form of protection (includes orchards, hops, arable field crops, etc.).

[17] https://gd.eppo.int/PPPUse/3CROLK
[18] Exposure to birds and mammals from foliar spray applications and soil treatments made in (permanent) greenhouses cannot be completely excluded (e.g. birds and mammals entering the permanent greenhouse). However, in many cases, exposure to birds and mammal population is likely to be negligible.

Yes: Direct exposure to birds and mammals is not expected. Full dietary risk assessment not needed. Low risk through dietary can be concluded for the use assessed. Please note that, if exposure to sediment, aquatic or soil organisms is anticipated, and the logP of the substance under assessment and/or its related metabolites is ≥ 3, a risk assessment through secondary poisoning should be conducted. In the case exposure to the soil is expected, a risk assessment for exposure via contaminated water (puddles) should be conducted (see Table 5**).**

No: Go to 2.

Special attention should be paid to those cases where plants are transplanted to open field after application indoors or in greenhouses. In those cases, unless it is demonstrated that the residues in the crop (or other relevant matrix) are insignificant, and therefore, exposure is not expected, a risk assessment should be conducted (see 3).

2) Is the a.s./PPP under assessment intended to be applied using a method (see Table 5 **for the methods of application, other than foliar application, seed treatments and granular applications) for which direct exposure may be excluded?**

Yes: Exposure to birds and mammals is not expected. Full risk assessment not needed. Low risk can be concluded for the use assessed. Please note that, if exposure to sediment, aquatic or soil organisms is anticipated, and the logP of the substance under assessment and/or its related metabolites is ≥ 3, a risk assessment through secondary poisoning should be conducted. In the case exposure to the soil is expected, a risk assessment for exposure via contaminated water (puddles) should be conducted (see Table 5**).**

No: Go to 3.

3) Does the application methodology of the spray/seed treatment/granule result in specificities that should be considered for the exposure or risk assessment?

Points to consider:

a) Pelleted seed is the seed treated before the pellet applied? Or is the PPP contained in the pellet casing? (See Section 6.5.6).
b) As part of the 'directions for use', is it a requirement to dissolve the granule via irrigation shortly after application? (See Section 8.7)
c) What is the drilling/sowing/granule application methodology that will be used? Unless it is specified that applications will be restricted to a certain type of application machinery, then a risk assessment assuming the methodology leading to the highest exposure (e.g. broadcast) should be performed. Applicants may wish to present several risk assessments considering multiple types of application machinery. (See Section 6.5.7 and Appendix P)
d) Is the spray/granule intended to be applied 'in-furrow at the time of sowing'? If this is the case, the applicant may wish to provide more appropriate RUD values or data to demonstrate the number of granules available to birds and mammals (See Section 6.5.7 and Appendix P).
e) Is the PPP restricted to amateur users in home and gardens? If this is the case, then the applicant may wish to provide details on how the product will be used and on what scale. Unless exposure can be excluded, then it would be expected that the applicant provides a fit-for-purposes problem formulation considering the way the PPP will be used and the likely level of exposure to birds and mammals.

Yes: Consider providing data to support a specific exposure assessment for the GAP and PPP. Go to 4

No: Go to 4

4) Conduct a full dietary risk assessment as outlined in Section 3.2.3. Please note that, if substance has a logP ≥ 3, a risk assessment through secondary poisoning should be conducted (chapter 10). Additionally, a risk assessment through contaminated water (chapter 11) should also be considered (see Table 5**).**

When exposure cannot be excluded, a full risk assessment should be conducted (see Section 3.2.3). For the toxicity data to be provided and for the selection of the appropriate effect assessment endpoint, see chapter 5. For the crop groups, see Appendix E. In these cases, as part of the problem formulation, physical–chemical properties, crop(s) where the substance is intended to be applied and BBCH should be further analysed. Those aspects will structure the risk assessment by (i) selecting the most appropriate routes of exposure, including exposure via secondary poisoning, when triggered, (ii) considering whether the crop may be consumed by birds and mammals, etc. For example, depending on the crop and BBCH where a substance is intended to be used, it might be the case that the crop foliage is not consumed by birds and/or mammals as part of the diet (e.g. late growth stages of maize). Nevertheless, exposure through residues in insects, weed seeds on the ground and weeds should still be considered. Similarly, although for most generic model species, the relative exposure from residues in-field is vastly in excess of those obtained in the surrounding area and there is no need for a separate assessment, there may be cases where an exposure outside of the treated area cannot be neglected as it is not covered by the in-field risk assessment normally performed (see Section 4.2.4 and chapter 6). Risk assessments considering exposure outside of the treated area may be needed for certain application methods which expressly exclude birds and mammals from the treated area, but do not eliminate the possibility of exposure elsewhere, such as soil fumigation with the application of a soil cover. When a PPP is intended to be applied using an application method which is not covered by this guidance, including new technologies, it is considered the applicant's responsibility in consultation with Member State competent authorities to develop a proper exposure and risk assessment.

For birds and mammals, the main route of exposure is considered to be via diet. While it is recognised that dietary, dermal and inhalation routes of exposure have the potential to contribute to systemic exposure, there is good reason to believe that the relative importance of the different routes can vary widely when considering the type of PPP, its mode of action, the type and methods of application, etc. Contribution of non-dietary exposure is considered less relevant in many cases. Therefore, this revised Guidance, in line with the previous version (EFSA, 2009) focuses on dietary exposure. Although this is relevant for the majority of the PPPs, there may be situations in which the contribution of non-dietary exposure cannot be neglected (e.g. when the PPP is a volatile soil fumigant). In those situations, it is the applicant's responsibility, in consultation with Member State competent authorities, to formulate a proper problem formulation leading to a fit-for-purpose risk assessment which will need to be assessed on a case-by-case basis. It should be further noted that the Tier 1 default exposure parameters are based on data sets for a limited set of conditions (e.g. standard spray applications). In the case of a GAP/PPP which is not covered by the data used to derive the Tier 1 default values, it is the responsibility of the applicant to justify the use of the standard default values, or to provide fit-for-purpose data (e.g. RUD values in the case of a soil fumigant).

The present revised guidance covers uses as spray application, seed treatment and granular applications. Those are the most common methods of application of PPPs and for which exposure methodologies are available and consolidated. There are many other methods of application which are not specifically covered by the present guidance. Table 5 gives an overview, which may not be exhaustive, of other possible methods of application indicating when exposure to birds and mammals following that method is expected.

Table 5: Potential for exposure to birds and mammals for methods of applications other than foliar spray (professional non-foliar spray application techniques, outdoor uses), seed treatment and granular application. Definitions of the different methods are given in the EPPO harmonised classification and coding of the uses of plant protection products[19]

Method of Application	Direct exposure through diet (Yes/No)	Exposure though secondary poisoning* (Yes/No)	Exposure to contaminated water (Yes/No)
Aerial spraying	Y	Y	Y
Brushing	N (unless treatments are done to palatable parts of the plant)	N	N
Circulating water application	N	Y	N
Dipping	N (for post-harvest treatment) Y (for objects that cause in-field exposure)	N	N
Drenching	Y (if poured over soil or substrate around roots) N (if used as post-harvest treatment)	Y	Y (if poured over soil or substrate around roots) N (if used as post-harvest treatment)
Dripping	Y (exposure through ground-dwelling and soil invertebrates and germinating seedlings)	Y	N[20]
Dusting	Y	Y	Y
Fogging	Y (if applied to semi open-protected structures) N (if applied to greenhouses and indoor storage rooms unless the fate and behaviour assessment concludes the potential for deposition)	Y	Y (if applied to semi-open structures)
Fumigating	Y (unless exposure is excluded, e.g. mitigation)	Y	Y (unless exposure is excluded, e.g. mitigation)
Impregnating	N (unless the impregnated object can lead to exposure)	N (unless the impregnated object can lead to exposure)	N (unless the impregnated object can lead to exposure)
Incorporating	Y (unless it is excluded that the substance translocates)	Y	N
Injecting	N (unless there is evidence to suggest translocation to palatable parts of the plants)	Y	N
Placing	N (unless the impregnated object can lead to exposure)	N	N
Individual plant treatment	Y	Y	Y
Spot application	Y	Y	Y

[19] https://gd.eppo.int/PPPUse/3TREAK

[20] In general, it is considered that for dripping application, exposure though contaminated water is not relevant. However, in particularly dry conditions and when the irrigation tubes are not properly buried, drip irrigation system may be used by birds as water source.

Method of Application	Direct exposure through diet (Yes/No)	Exposure though secondary poisoning* (Yes/No)	Exposure to contaminated water (Yes/No)
Treatment between rows, in the row and of the row	Y	Y	Y

*: The need for a risk assessment though secondary poisoning is informed by exposure assessment in surface water, sediment or soil, i.e. if there is exposure though surface water, sediment or soil and the substance has a log Kow ≥ 3, a risk assessment through secondary poisoning should be considered. Therefore, the indications in the table may, in some cases, be only theoretical and meant to guide in the problem formulation.

5. Effect assessment tiers used in the risk assessment by applying the TER approach

5.1. Screening tier effect assessment

The screening tier effect assessment might be carried out when toxicity data are missing and is mainly applicable to metabolites. Due to the lack of actual toxicity data and the use of worst-case assumptions for toxicity estimates (metabolites are considered 10 times more toxic than the parent compound), this screening tier is anticipated to be conservative. For active substances, the basic set of laboratory toxicity data for birds and mammals should be available due to regulatory data requirements (see Section 5.2.1). See chapter 9 for a more detailed description of possible effect assessment approaches for metabolites.

5.2. Tier 1 effect assessment based on laboratory toxicity tests for standard test species

5.2.1. Data requirements

The specific data requirements for Regulation (EC) No 1107/2009[2] concerning the placing of PPPs on the market are laid down in Commission Regulation (EU) 283/2013[4] for the dossier to be submitted for the approval of a.s. contained in PPPs and in the Commission Regulation (EU) 284/2013[5] for the authorisation of PPPs. Regulation 283/2013[4] and 284/2013[5] specify that risk to birds and mammals should be always investigated except when exposure to birds and mammals can be excluded. More information on the conditions under which exposure to birds and mammals can be excluded, and thus, toxicity data can be waived, and a risk assessment is not needed, is presented in chapter 4. Similarly, reproductive toxicity data may be waived if a justification is provided that there is no exposure during breeding season and no delayed effects will occur affecting reproduction or development. Providing sufficient information for excluding exposure of adults during breeding season may be difficult, especially for evaluation at EU level, as this should consider the breeding season for many different bird and mammal species (e.g. specific focal species but also those species which are covered by such focal species), in different EU Member States characterised by specific environmental conditions, etc. Similarly, it is not straightforward to demonstrate that delayed effects are unlikely, as this might require a specific test design and therefore repetition of vertebrate testing and would only be possible for the tested species. Overall, a reproductive risk assessment is considered mandatory for all substances under assessment, as the WG considers it difficult to demonstrate that effects will not occur during the breeding season (either through a direct exposure or through delayed effects of previous exposure).

Commissions' Communications on the implementation of Regulation 283/2013[4] and 284/2013[5] illustrate the list of relevant test guidelines and guidance which are relevant for the hazard characterisation and risk assessment of birds and mammals (see Table 6).

Table 6: Ecotoxicity studies required for active substances and formulated products under certain circumstances (for metabolites, see chapter 9)

	Acute toxicity test to one bird species	Acute toxicity study with more bird species	Short-term dietary toxicity to birds	Sub-chronic and reproductive toxicity to one bird species	Sub-chronic and reproductive toxicity to a second bird species	Acute toxicity to mammals	28-day toxicity study with mammals	90-day toxicity study in rodents and non-rodents	Carcinogenicity studies with mammals (rats and mice)	Reproductive studies with rodents (rats)	Developmental studies with mammals (rats and rabbits)	Neurotoxicity studies in rodents
Every active substance[21]	x			x[22]		x		x	x	x	x	
Formulated product, if the toxicity cannot be predicted on the basis of the data for the active substance	x[23]					x						
Information available on a specific MoA or information from mammals available indicating short-term toxicity can be higher than acute toxicity, or when already available[24]			x									
When already available[25]		x			x		x					
Information available on MoA or structure similarity to substances inducing neurotoxicity												x

[21] Acute toxicity data should be always submitted for birds and mammals unless it is clearly demonstrated that birds will not be exposed e.g. the test substance is meant to be used in enclosed spaces and/or wound healing treatments.

[22] Reproductive/long-term toxicity data with birds and mammals should be always submitted unless the applicant is able to demonstrate that adults are not exposed during the breeding season and delayed effects are not expected (see Sections 8.1.1.3 and 8.1.2.2 of the Regulation 283/2013[4]).

[23] Vertebrate studies should not be performed unless absolutely necessary. Therefore, the approach outlined in chapter 12 should be followed.

[24] All short-term studies data on mammals should be checked, i.e. 28-d and 90-d studies.

[25] In some cases, additional studies than requested by the current legislation may be available in the case of e.g. renewal of approval as conducted according to previous legislations or because done for complying with non-EU legislations.

5.2.2. Standard acute toxicity studies for birds and mammals

5.2.2.1. Birds

Regulations 283/2013[4] and 284/2013[5] recommend determining the acute oral toxicity of an active substance to a quail species (Japanese quail, *Coturnix coturnix japonica* or bobwhite quail, *Colinus virginianus*). Acute toxicity studies with birds are conducted following OECD test guideline 223 (OECD, 2016a). Birds are dosed once by gavage and observed for 14 days. The highest dose used in tests should not normally exceed 2,000 mg/kg body weight. Due to issues of regurgitation, it is recommended not to use the mallard duck (EFSA, 2007), although studies with mallard duck may be available for active substances under renewal processes. Where regurgitation or emesis occurs at doses used for risk assessment, all the available evidence should be considered, and additional information is essential to complete the risk assessment and to determine the most suitable endpoint. The amount of regurgitated material should be assessed for determination of the ingested dose. In the absence of this information, the lowest overall no observed effect level (NOEL) must be used for risk assessment purposes. Where more than one study has been submitted, the study/studies where no regurgitation has occurred should be used. If, however, mortalities appear in the study in which regurgitation has occurred (at dose levels at or around the LD_{50} value for the non-regurgitation study), then it is proposed to use the NOEL (for regurgitation or mortality, whichever is lower) from the study where regurgitation has occurred. In some cases, data on additional species, such as *Serinus canaria*, may be available as requested by other legislative frameworks. The OECD test Guideline 223 describes three types of tests: the limit dose test, the LD_{50}-slope test and the LD_{50}-only test. The LD_{50}-slope test is generally recommended as it is expected that the LD_{50} together with the slope and confidence interval should be available. However, in some cases, e.g. substances with very low toxicity or metabolites, the limit dose test may be sufficient.

The test chemical may be administered in a capsule or dissolved or suspended in a suitable vehicle and then administered by gavage. However, the use of capsule or other solid carrier without a prior dissolution of the substance might, especially for some species, reduce the bioavailability of the substance and thus reduce exposure to the test substance. Available data and endpoints should be considered and compared to understand any possible underestimation of endpoints due to the use of a solid carrier. Although dietary toxicity studies may be perceived as more relevant for birds and wild mammals as they mimic a route of exposure relevant for a field situation, gavage studies should not be dismissed. Although a gavage study may often result in a lower endpoint, and thus a more conservative assessment, this is considered to counterbalance the other uncertainties linked to the hazard characterisation (data on one or few species under lab conditions with no/low feeding pressure).

The results of the test should include mortality allowing for the estimation of an LD_{50}, onset and cessation of clinical signs, gross pathological findings, individual body weight data and food consumption data.

5.2.2.2. Mammals

One or more of the following acute oral toxicity test methods with mammals may be available (LD_{50} mg/kg bw):

OECD Test 420 (OECD, 2001a): Acute oral toxicity – fixed dose procedure
OECD Test 423 (OECD, 2001b): Acute oral toxicity – acute toxic class method
OECD Test 425 (OECD, 2008a): Acute oral toxicity – up-and-down procedure

The fine details of the above studies vary, but the underlying principles are the same. Animals (normally rats, but data from studies with other mammals such as mice are also relevant) are dosed once by oral gavage and observed for 14 days. Observations include body weight, clinical signs, death and necropsy findings. A limit dose of 2,000 mg/kg bw or 5,000 mg/kg bw (depending on study) should not be exceeded.

The fixed dose procedure (OECD 420) and the acute toxic class (OECD 423) method are range estimators and are useful for mammalian wildlife risk assessment only in cases where they can be used as a limit test (e.g. > 2,000 mg/kg bw), or to provide a conservative surrogate for the LD_{50} (i.e. lowest value of range). Annex 2 of each guideline includes detailed decision trees which can be used to estimate an endpoint for use in the risk assessment.

In the up-and-down procedure (OECD 425), animals are dosed, one at a time, at a minimum of 48-h intervals, with a single-ordered dose progression. The first animal receives a dose a step below the level of the best estimate of the LD_{50}. Based on the observation, mortality or survival, the dose for the next animal is decreased or increased by 3.2 times the original dose. OECD guideline 425 gives recommendations on how to estimate the LD_{50} which should not be reported as a range, when possible.

An acute neurotoxicity study based on a USEPA procedure (USEPA, 1998) may also provide useful information. The basic design is that of the OECD Test 424 (OECD, 1997) i.e. animals (normally rats; 5/sex/group) are dosed once, normally by oral gavage and observed for up to 14 days, but in addition, observations for neurological function (a functional observation battery, FOB) are taken pre-dosing and at the time of peak effect (up to 8 h post dose), day 7 and day 14. Other observations include body weight and specific histopathological investigation of nervous tissue.

Recently, in the interest of minimising animal testing, acute toxicity studies with the formulation may be foregone in favour of a dose addition methodology for calculating formulation toxicity. In this instance, similar to the tests above, it should be possible to estimate an endpoint for use in the risk assessment. However, this will have consequences for the consideration of potential for synergism, as discussed in Section 12.3.

5.2.3. Short-term toxicity studies to birds

The following short-term dietary test method is not available for all the substances under evaluation in accordance with Regulation 283/2013[4]. This latter recommends that such a test may be requested when the mode of action or results from mammalian studies (see Table 6) indicate a potential for the dietary LC_{50} from the short-term dietary toxicity study to be lower than the LD_{50} based on an acute oral study.

- OECD Test 205 (OECD, 1984a): Avian dietary toxicity test

Information from the dietary toxicity test could be requested and used on a case-by-case basis in higher tier assessments when appropriate, e.g. in particular for TKTD modelling (Section 5.4). It can also provide an indication of food avoidance but is not sufficient on its own to demonstrate that avoidance will prevent mortality (see Section 6.5.5). Avian dietary toxicity test data (OECD 205, OCSPP 850.2200) are still requirements in other regions of the world (e.g. Latin America and Asia) and as many products are for global registration these data may be available. However, in general, new dietary LC_{50} studies should not be conducted for registration purposes in the EU, mainly due to their scientific limitations and animal welfare issues (EFSA, 2007, 2009). In cases where short-term dietary studies are available and indicate a lower endpoint (Lethal Dietary Dose, LDD_{50}) compared to the acute LD_{50} studies then the short-term LDD_{50} value should be used for the acute risk assessment.

To convert the endpoint from a dietary concentration (LC_{50}) to a daily dose (LDD_{50}), the guidance of the European Commission (2002c) should be followed and is repeated below, in Section 5.2.7.2, for ease of reference.

5.2.4. Long-term and reproductive studies for birds and mammals

In the section below, an overview of the studies available to determine a suitable endpoint that can be used in the reproductive risk assessment for birds and mammals is presented. In addition, for each study, there is an outline of the key endpoints and their relevance to the ecotoxicological risk assessment, as well as the relevance of the effect(s) observed.

It should be noted that reproductive toxicity studies are meant to cover all potentially sensitive life stages irrespective of the expected actual exposure to a substance. The endpoint derived from the test will be based on the effects seen in the test unless sufficient data are available to show with a high degree of certainty that the effect(s) seen was/were as a direct result of prolonged or repeated exposure (including the length of exposure required for the effect to manifest) rather than exposure at a relevant life stage. It should be borne-in-mind that additional testing with vertebrate species should be avoided. In fact, Article 8 of 1107/2009[2] states that a justification for the steps taken to avoid such testing would need to be provided. As a result, additional toxicity studies using shorter or more targeted exposure periods are not recommended.

5.2.4.1. Birds

A test for effects on reproduction in birds is currently requested if birds are likely to be exposed during the breeding season (see Section 5.2.1). There are currently two standard studies[26] listed in the Commission Notices accompanying Regulation 1107/2009[2], OECD 206 (OECD, 1984b) and USEPA OCSPP 850.2300[27] (USEPA, 2012a). The USEPA protocol recommends that tests be carried out on first-time breeders of an upland game species, preferably the northern bobwhite quail (*Colinus virginianus)* and a wild waterfowl species, preferably the mallard duck (*Anas platyrhynchos*). The OECD version states that the Japanese quail (*Coturnix coturnix japonica*), preferably experienced breeders, is also acceptable. However, there are concerns regarding the appropriateness of this species due to its greater sensitivity and ability to attain breeding readiness under short daylight conditions. Since the USEPA OCSPP 850.2300 has been updated more recently and clarified the type of statistical analysis to be performed and the parameters which should be analysed statistically, it is generally recommended to follow OCSPP 850.2300 when performing a new reproduction study in birds. It should be noted that neither OECD 206 nor OCSPP 850.2300 foresee the calculation of a benchmark dose level. Information on the calculation of the benchmark dose can be found in Section 5.2.7. In cases of replicates not performing as expected, please see Section 5.2.6.1 on biological relevance. On occasion, additional endpoints may be derived which are not specifically mentioned in the Guideline (e.g. % viable eggs of eggs set), and it is recommended that these are included in the table summarising the study endpoints. It is also recommended that their significance is discussed alongside all other endpoints when deriving the overall endpoint from the study.

Appendix B provides a description of OECD 206 and USEPA OCSPP 850.2300.

Other reproductive studies

In addition to the above standard guideline studies, other study protocols are available and included for completeness. Those are:

- The avian study with a reduced exposure duration (6–8 weeks): Studies with a reduced exposure duration may be available when conducted according to a draft protocol pre-dating the OECD guideline. Such studies are not considered suitable for risk assessment since all relevant reproductive phases are not exposed. In very exceptional cases, based on an overall weight of evidence (e.g. toxicity profile of the substance assessed and margin of safety in the risk assessment when using the 6-week endpoint) and to avoid repeating a vertebrate study, an endpoint derived from a 6-week endpoint may be considered. In other cases, studies with a reduced exposure duration may be conducted to mimic a more realistic exposure period, i.e. a refinement. However, in line with Article 62 of Regulation 1107/2009[2], unnecessary vertebrate studies should be avoided, and therefore, the WG does not recommend this as a refinement option.
- The avian two-generation study (OCSPP 890.2100 (USEPA, 2009)): It is recommended to never request this kind of study, given the issues experienced in developing the test guideline (e.g. the logistical complexity, the numerous sources of possible failure of the test and the large animal number used in the test to achieve statistical power) and the fact that EU Commission Regulation 1107/2009[2] states that 'animal testing for the purposes of this Regulation should be minimised and tests on vertebrates should be undertaken as a last resort'. Although a USEPA guideline is available, this test is not commonly required by other jurisdictions as data obtained from avian reproduction studies (OCSPP 850.2300; OECD 206) are considered sufficient for evaluating potential reproductive effects to birds. The test is included here for the sake of completeness, only.

[26] Whilst there are currently two guidelines, many studies will have been to older guidelines, e.g. USEPA OPPTS 850.2300 or US EPA 71-4 or in fact an amalgam of the two. When these older studies are evaluated, it is important to use the latest guideline when assessing their acceptability. It is appreciated that this could lead to issues, e.g. where the study passed the old validity criteria but fails the validity criteria in the latest guideline. The significance of this deviation should be considered in detail when determining the acceptability of the study and the overall endpoint.

[27] There is a known typo in the 2012 (most recent at the time of writing of this Guidance) version of this guideline, in Table 3 (validity requirements). The information on page 6 of the guideline about the controls is correct for normal percentages of live embryos (i.e. Live 18-d or 21-d northern bobwhite and mallard embryos as a percentage of viable embryos: 94–100% for mallard duck and 97–100% for bobwhite quail). Note that the control requirement for viable embryos is 80–100% of eggs set (correctly indicated on page 6 and Table 6). It would be impossible to achieve 94%+ live embryos/eggs set if viable embryos/eggs set was as low as 80%, which is allowable.

5.2.4.2. Mammals

A number of reproductive/long-term toxicological studies in mammals are available in line with sections 5.3, 5.5, 5.6 and 5.7 of the Regulation 283/2013[4]. These can be loosely grouped into short-term, chronic/carcinogenicity, subchronic and repeated dose and reproduction and (neuro)development tests. Endpoints relevant for the maintenance of mammalian populations in the wild might be derived from any of these tests. A brief review of the types of tests available and the endpoints that are derived from them (generally) is presented in Appendix C, together with a discussion of those endpoints which are most relevant for the wild mammalian risk assessment.

5.2.5. Data from publicly available literature

According to the Regulations 283/2013[4] and 284/2013[5], relevant data from the scientific peer-reviewed open literature on the active substance and formulated products shall be submitted together with the standard studies. Thus, additional laboratory toxicity data on relevant standard and additional species of birds and mammals may be available. When conducting a systematic literature search, the search strategy can be conducted following two general search approaches as recommended by EFSA (2011): a single concept search strategy (i.e. using terms related to the name of substances and its transformation products and synonyms) and a targeted search strategy for individual endpoints. In the context of this guidance, it is recommended to perform the literature search by using the single concept approach as a preferred methodology, since it is considered to be highly sensitive, to introduce less bias and to be less time consuming than the targeted search strategy. If a large number of hits are retrieved, the search can be further refined by running a search targeted on the information requirements not only for ecotoxicology but more generally on environmentally relevant information. In such cases, it is recommended that the search should not only focus on the standard species which are already covered by the data requirements (i.e. a reasonable number of common European bird and mammal species should be included).

Further guidance on how to conduct and evaluate literature data is provided in EFSA (2010) and EFSA (2011). Those guidance documents, however, give only general recommendations with regard to the assessment of relevance (or external validity) and reliability (or internal validity) of the literature data. Relevance criteria should not be too restrictive and the identification of relevance criteria should be considered an iterative process that starts with a clear analysis of the different components of the data requirements and risk assessment in question to set the main characteristics of a relevant study.

The reliability of the information from public literature should be comparable to those submitted for the data requirements. When assessing reliability, some general considerations could be taken into account, such as statistical power, verification of measurement methods and data, control of experimental variables that could affect measurements, etc. Studies retrieved through the literature review may be conducted according to standardised protocols. Therefore, studies providing information on toxicity endpoints should be assessed according to the standard test guidelines where possible (i.e. by taking into consideration species-specific differences). This, however, does not mean that studies not conducted according to standard protocol should be rejected. It must also be emphasised that compliance with good laboratory practice (GLP) standards should not be considered as a guarantee of reliability. Therefore, studies that are not conducted under GLP should not by default be considered as less reliable. Moreover, literature studies may be relevant, although not useful for endpoints derivation, e.g. a publication indicating farmland bird decline following application of the substance, or PPP, under assessment. It is recommended that such studies should be retained and assessed further for reliability. Although it is unlikely that such information will be attributable to a single use, or PPP, the information obtained could nevertheless be communicated to the risk managers and included in the Weight of evidence/Conclusion table (see Secion 13.4), when sufficiently reliable.

5.2.6. Selection of the endpoint for effect assessment

5.2.6.1. Biological relevance considerations and historical control data for endpoint setting

Biological relevance

According to EFSA Scientific Committee (EFSA Scientific Committee, 2011; EFSA Scientific Committee, 2017a), *a biologically relevant effect can be defined as 'an effect considered by expert judgement as important and meaningful for human, animal, plant or environmental health…'*

When assessing the results of a toxicity study, it is important to take into consideration not only the statistical significance of the results but also their biological relevance. The statistical significance and biological relevance of results, however, are not necessarily linked. In some cases, an effect, although statistically significant, may not be biologically relevant (e.g. < 10% effect in the case of birds and mammals). Similarly, a lack of statistical significance should also not be the sole rationale for concluding a lack of treatment-related effect, though in the absence of a clear statistical difference from the controls, additional consideration is necessary before concluding any change observed above a certain threshold to be biologically relevant. Simple visual inspection of the means may seem to show an effect, but if the power of the test is not high enough to detect an effect level, it cannot per se be assumed that an effect of any level was detected (or that any effect did or did not occur). Some common considerations are provided below:

1) When compared to the normal range reported in the validation criteria or annexes of the respective OECD guidelines (or potentially historical control data), the treatment mean may be clearly within the range of a 'normal' response.
2) If the variation is particularly high for an endpoint, even outside the normal range, it is advisable to investigate whether an outlier was present amongst the replicates, which would allow a better understanding of the reason for the appearance of an effect when comparing means, but the lack of statistical significance. If outliers are present, it is necessary to investigate each outlier in order to determine the appropriateness of its exclusion from the statistical analysis.
3) A general consideration of a dose–response relationship may allow extrapolation from a dose at which statistical significance was achieved to a lower dose where there was an apparent response but no statistical significance. However, an apparent dose–response with no statistical significance at any dose should still be further investigated to determine why no difference from control was achieved for any dose. This could be related to the performance of the controls, or to the variation within the treatment groups, or both, but in few cases would it be possible to declare this as a 'real' dose response (in other words that the perceived effect when simply visually comparing the means is actually an effect).

To comply with the Regulation, in the first instance, it is recommended to always consider the 10% as the effect level which is biologically relevant for all the different parameters assessed for both birds and mammals (see also Section 5.2.7).

If, for specific parameters, an effect $\geq$ 10% is proposed as not being biologically relevant, a sufficient argument should be made to justify the deviation. In supporting such an argument, it is recommended to:

- Perform a systematic review of the literature for relevance of the endpoint (following the principles of EFSA, 2011);
- Transparently report results (as well as search criteria, relevance and reliability assessment, etc.);
- Provide a consideration of the quantity and quality of the data suggesting a deviation from the standard endpoints (has it been properly studied?);
- Provide a consideration of the relevance of the evidence for the substance under assessment (e.g. was all the literature information on organochlorines or does it cover a wide range of modes of action?);
- Provide a consideration of interspecies variability for the endpoint (although this is partially addressed by the AF of 5);
- On the basis of the evidence, provide reasoning for a revised threshold value (even a range);
- Ensure that the revised endpoint is accounted for in the final uncertainty analysis (see Section 13.4).

Historical control data

In EFSA (2009), guidance was provided on how to use historical control data (HCD). Concerns and questions have been raised regarding the appropriateness of this guidance during both the peer review process and the public consultation on updating the guidance. These concerns can be briefly summarised as follows:

i) Need for more guidance on evaluation and use of HCD.
ii) Need for the consideration of the biological relevance of the HCD.

iii) Consistency with mammalian toxicology, e.g. the time frame to be considered.
iv) Relevance of HCD compared to the concurrent control, e.g. should one take precedence over the other?

Recently, an EFSA external report has been published reporting issues around the use of HCD for (eco)toxicology and recommendations for future considerations (Coja et al., 2022).

There is reference to HCD in paragraph 3 of Section 5 (Toxicology and Metabolism studies), Sections 5.5 and 5.6 of Commission Regulation (EU) No 283/2013[4]; the following is stated in the latter two sections:

Where submitted, historical control data shall be from the same species and strain, maintained under similar conditions in the same laboratory and shall be from contemporaneous studies. Additional historical control data from other laboratories may be reported separately as supplementary information.

The information on historical control data provided shall include:

- *identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location*;
- *name of the laboratory and the dates when the study was performed*;
- *description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed*;
- *approximate age, in days, and weight of the control animals at the beginning of the study and at the time of killing or death*;
- *description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (such as diseases, infections)*;
- *name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study*;
- *a statement of the nature of the tumours that may have been combined to produce any of the incidence data*.

The historical control data shall be presented on a study-by-study basis giving absolute values plus percentage and relative or transformed values where these are helpful in the evaluation. If combined or summary data are submitted, these shall contain information on the range of values, the mean, median and, if applicable, standard deviation.

Despite the above requirements, there is currently no published mammalian toxicology guidance regarding how to interpret and use such information.

The issue of the use of HCD has been debated for decades, but still there is no general consensus of the use of such data (Valverde-Garcia et al., 2018; Brooks et al., 2019; Temple et al., 2020).

Valverde-Garcia et al. (2018) collected historical control data for avian reproduction toxicity studies undertaken over a 32-year period at a contract research organisation (CRO), comprising 301 bobwhite quail and 292 mallard duck studies. For each reproductive endpoint, confidence and prediction limits around the mean of the control data were calculated and those values were then compared to the treatment mean(s) in the study under consideration. Two case studies were presented in Valverde-Garcia et al. (2018) that illustrated the interpretive value of HCD. Whilst the approach seems logical, ultimately this approach means potential effects could be excluded so long as they are within the range of the HCD. Whilst Valverde-Garcia et al. (2018) provide a useful starting point, it does not provide a complete way forward as to how to interpret and use HCD.

It is appreciated that HCD has the potential to make the most use of existing data and hence possibly reduce the likelihood of repeating vertebrate studies; however, it is currently not possible to recommend a way forward regarding how to interpret or use HCD. Until agreed guidance is available, comparing data from treated animals with data from concurrent study control is preferred. HCD may be used to determine if concurrent control animals are performing within the margins of normal variability for the species and strain (OECD, 2011a). If it is considered that HCD could help in either determining an endpoint or understanding a study, then it is proposed that the following information is considered:

Data on the same species, from the same laboratory and study guideline;
Dates of when the study was performed;
Details of the key parameter(s) including range and mean values;
Indication as to whether the studies had met the relevant validity criteria and whether they had been accepted by a regulatory authority.

The above data should cover a 5-year period, centred as closely as possible on the date of the index study. When more guidance becomes available, that should be considered to complement the information reported here and the latest developments should be considered (EFSA ongoing activity under EFSA-Q-2021-00274).

5.2.6.2. Selection of acute effect endpoints

Birds

In accordance with Regulation EU No. 546/2011[6], the acute LD_{50} value should be used for the risk assessment for birds unless there is evidence the short-term dietary LC_{50} to be lower (see Section 5.2.3). In the acute oral LD_{50} study with birds, males and females normally are not tested separately, hence the endpoint is a combined one for both sexes. In the unlikely event that separate data for males and females are available then separate LD_{50} values should be calculated and the LD_{50} for the most sensitive sex should be used for risk assessment. If more than one acute study is available on the same species and the studies follow the same methodology, the geometric mean of the available LD_{50} can be used.

Mammals

In accordance with Regulation EU No. 546/2011[6], the acute LD_{50} value should be used for the risk assessment for mammals. The current OECD guidelines 420, 423 and 425 (OECD, 2001a, 2001b, 2008a) for acute mammalian oral toxicity states that only females should be tested except where there is evidence that males are likely to be more sensitive. When the test is conducted in males, adequate justification should have been provided (OECD, 2001a). When separate data for males and females are available, then separate LD_{50} values should be calculated and the LD_{50} for the most sensitive sex should be used for risk assessment. If more than one acute study is available on the same species and the studies follow the same methodology, the geometric mean of the available LD_{50} can be used.

5.2.6.3. Extrapolated LD_{50} values

It is permissible to extrapolate an LD_{50} value upwards in cases where there is no mortality or a single mortality at a limit (highest tested) dose in an acute avian or mammalian toxicity study. The proposed extrapolation factors assume an average probit slope (5.43 for birds and 8.93 for mammals, respectively – log dose against probit-transformed mortality) generated from a large sample of pesticides tested in the bobwhite quail and mallard duck (see Appendix 5 of EFSA, 2008) and in mice and rats (see Appendix C). The extrapolation is carried out assuming a 50% binomial probability bound that mortality could have occurred but had simply been missed by chance in the test. The extrapolation may therefore be underprotective, especially in the case of pesticides having steeper than average slopes of the dose–response curve, and this extrapolation should therefore not be used in cases where clear signs of toxicity are observed in the surviving individuals.

Table 7: Extrapolation factors for avian LD_{50} values based on the number of individuals tested at limit/highest dose

Sample size	Extrapolation factor for no mortality at limit/highest tested dose	Extrapolation factor for one mortality at limit/highest tested dose
5	1.614	1.228
10	1.888	1.518
15	2.051	1.685
20	2.167	1.802

Table 8: Extrapolation factors for mammalian LD_{50} based on the number of individuals tested at the limit/highest dose

Sample size	Extrapolation factor for no mortality at limit/highest tested dose	Extrapolation for one mortality at the limit/highest tested dose
3	1.24	1.00
5	1.34	1.13
6	1.37	1.18
10	1.47	1.29

After choosing an extrapolation factor from Table 7 or Table 8, the extrapolated LD_{50} value is calculated by multiplying the limit dose with the extrapolation factor:

$$\mathbf{LD_{50} = limit/highest\ tested\ dose \times extrapolation\ factor} \quad (5)$$

5.2.6.4. Selection of the reproductive endpoint for birds

As reported in Section 5.2.4.1, reproductive studies with birds may be conducted according to either the OECD 206 or USEPA 850.2300 guideline. Many endpoints are derived from those studies, and it is important to determine which one(s) are relevant for risk assessment.

It should be noted that calculated response variables (e.g. proportion of normal hatchlings of eggs laid per pen) may sometimes considered more relevant than measured response variables e.g. number of eggs set. This is due to the fact that non-normalised parameters may be misleading as downstream effects can be magnified and this should be taken into account in the evaluation and interpretation of data from these studies.

5.2.6.5. Selection of the reproductive endpoint for mammals

Relevant endpoints for the effect assessment of wild mammals are endpoints that show an expectation of adverse effects on the population in the field. In mammalian toxicology, the entity to be protected is the individual, whereas in ecotoxicology, the entity to be protected in reproductive risk assessment is the population (see Table 1 of chapter 3). This means that although the same tests are considered as are used in the mammalian toxicology section, each effect and endpoint must be considered from a different perspective. In many cases, this may mean that an endpoint set by the mammalian toxicology section is not relevant for the ecotoxicology section. The WG is aware that significant discussion is typically devoted to this point and that as a result of the wide variety of tests and expertise available, it is a point which may be sensitive to inconsistent decision-making (Brooks et al., 2017; Brooks, 2020). As a result, we have endeavoured to provide more clear guidance for consideration of the ecological relevance of endpoints available in the mammalian toxicology section of the dossier in the section below, as well as providing a short summary of the available tests, for the ecotoxicology assessor unfamiliar with the mammalian toxicology section.

In order to adequately consider the ecological relevance of the available endpoints in a manageable way, it is recommended to list all relevant endpoints in a tabular format, as shown below in Table 9. Examples have been included for clarity (the reasoning behind the considerations of ecological relevance is discussed in the following subsections). Please note that on some occasions, it may be useful to also list effects at doses above the LOAEL to contextualise effects which are seen in more studies.

Table 9: Example of how to present available mammalian endpoints

Study	Species (Life stage/sex; if relevant)	NOAEL	LOAEL	Effects at LOAEL	Ecological relevance of LOAEL effects
Chronic toxicity					
90-day studies	Mice, rats Adult	–	–	Clinical chemistry (liver) and blood calcium effects in mice and rats at highest tested doses.	Not directly related to impaired function (survival, reproduction) thus ecological relevance is low.
18 months	Mice (CD-1) Adult, female	12 mg/kg bw per day	124 mg/kg bw per day	77% ↓ ALT in females; 8% ↓ in bw gain	Liver is a target organ. Liver enzyme effects with chronic exposure not directly linked to reproduction or survival to reproduction. BW gain effects < 10% without accompanying significant overall body weight reduction. Population relevance is low.

Study	Species (Life stage/sex; if relevant)	NOAEL	LOAEL	Effects at LOAEL	Ecological relevance of LOAEL effects
2-year chronic/carci	Rat (Wistar) Adult, female	25 mg/kg bw per day	150 mg/kg bw per day	> 10% reduced bw (not linked to decreased food intake or palatability of the food + a.s.)	Primary significant and relevant effect on body weight. Potentially relevant but more consideration of the length of exposure and pattern of effect is needed.
Reproduction and developmental toxicity					
2-generation	Rat Offspring, females/both sexes	4 mg/kg bw per day	43 mg/kg bw per day	Reduced thymus weight (6%) reduced absolute thymus weight (6%). **No histopathological changes**. @ LOEAL in F2 gen only; @ 435 mg/kg bw per day in F1 & F2	Ecological relevance doubtful: thymus important in immune function but no histopathologic/structural changes seen so not considered adverse. Furthermore, there is no direct link between thymus weight change and population effects.
Developmental	Rat Offspring, both	200 mg/kg bw	400 mg/kg bw	Statistically significant delayed ossification in the high-dose group outside HCD, in the absence of maternal toxicity	Generally speaking, delayed ossification is not an ecologically relevant effect as ossification is eventually achieved and pup survival is not impacted. But it must be considered case by case.
	Rabbit Offspring, both	25 mg/kg bw	100 mg/kg bw	Increase in rib cartilage alterations, thickened 7th rib and dilatation of lateral brain ventricles	Dilation of lateral brain ventricles can indicate neural development/ functional issues which would be relevant to survival and reproduction.
Neurotoxicity					
Acute neurotoxicity studies did not note any significant effects or effects that could be long-lasting/effect survival or reproduction. No DNT test is triggered or available.					

Reproductive and developmental endpoints

Direct effects on reproduction or development are considered relevant for wild mammals. These include particularly effects on male or female reproductive parameters, any aspect of pregnancy and birth up until weaning and pup development, including to the second generation. Please note that effects in the second generation of reproductive tests should also be considered relevant, as potentially being the result of exposure during a critical developmental phase, unless extensive data are provided to support a claim that these effects result from the duration of exposure in the test.

Endpoints from developmental toxicity tests are also considered relevant and are mainly related to the developmental phase. Effects on pregnancy and development of offspring in the absence of significant maternal toxicities will be considered relevant, and special attention should be paid to the conclusions of the mammalian toxicology section regarding this determination. Effects on dams could also be considered relevant, though these should be compared with effects on dams in the reproduction tests and on adults in other tests in order to determine whether they are relevant (this is usually only possible in rats). It should be noted that developmental tests are performed with rats and rabbits, as history has shown different sensitivity to developmental toxins. Thus, effects seen in rabbits but not in rats (and vis versa) would still be considered potentially relevant. Developmental abnormalities consist, generally, of external, visceral, skeletal and materno-fetal abnormalities. More information on the types of abnormalities and a definition of each can be found in Makris et al. (2009).

Fetal abnormalities are normally divided into severe cases (malformations), i.e. those that would compromise the ability to survive or function normally, and minor cases (variations/anomalies) that would have a minimal impact on the animal. Malformations would generally be considered ecologically relevant, whereas variations/anomalies are unlikely to be ecologically relevant. Please note that the same effect (e.g. absence of vertebrae) could be classified as a malformation or as a variation/anomaly depending upon severity and location; therefore, the evaluation of the mammalian toxicologist(s) will be of key importance in determining ecological relevance (see also www.devtox.org/).

Important uncertainties to consider in reproduction and developmental tests are the type and severity of the effect. A direct effect one.g. pup survival would have a more certain link to definite effects on wild mammal populations than sublethal effects on reproduction or development; however, both can be considered relevant in the absence of data to suggest otherwise. Nevertheless, the level of certainty related to the linking to population-level effects should be considered in the uncertainty analysis (UA) and weight of evidence (WoE). In addition, aspects of the individual tests from which the endpoints are derived should be considered, as well as the frequency of the effect. An effect which was seen in more litters/dams/pups across the different available studies would be considered more certain to have a likely population-level effect than an effect which was less often present. The presence of a dose response and the severity of the effects in question should also be considered.

Body weight

Body weight, particularly of dams, may be relevant to the ability to reproduce and to the survival of pups. The body weight of pups is also relevant to pup survival. In mammalian toxicology, a significant effect on body weight or body weight change (gain) is relevant for endpoint determination. What level is significant is loosely based both on the likelihood of other effects relating to body weight changes and the power of the tests to determine effects on body weight at lower than that level (which is unlikely). The relevance of body weight in ecotoxicology has been regularly discussed, but evidence one way or the other for an overarching level at which effects are relevant for survival and reproduction is scant (Wang et al., 2019). It is clear from laboratory studies that large effects (e.g. around 20% or higher) on body weight can have an obvious effect on survival and reproduction (Young and Rasmussen, 1985). Smaller body weight changes may also have an effect on survival and reproductive parameters, but in a more nuanced fashion (Moatt et al., 2016). However, the level at which body weight changes will have an effect in the field is coupled to, amongst other factors, the species in question and the survival and reproductive traits of that species, landscape and climate factors, and other stressors. It is, thus, not possible to determine an exact level at which effects become 'relevant' ecologically. Considering the power of the tests in question to detect an effect (see Section 5.2.7) and the uncertainties surrounding the types of effects that result from body weight changes, it is considered that the use of the BMD_{10} for body weight endpoints is ecologically relevant. In cases where a BMD_{10} cannot be calculated, it is recommended that body weight effects of 10% or greater be considered relevant, in line with the use of the BMD_{10}. Regarding the specific endpoint 'body weight gain', this endpoint would be considered less ecologically relevant than the overall body weight effect, because while effects on body weight might have a clear ecological relevance, changes in body weight gain that do not have an overall effect on body weight are more difficult to link to an eventual effect. Finally, consideration must be given to whether the effects on body weight are primary or as a result of reduced food consumption (due to palatability or other effects of the active substance). If the effect seems to be as a result of reduced palatability or other effects, it should be considered whether other effects may be relevant for the ecological endpoint setting.

Important uncertainties to consider when using an endpoint based on body weight include the level of effect, the frequency of the effect, between-species concordance and the certainty in the individual test used to determine the endpoint. When considering cross-species concordance, the type of test and the life stage and species should be carefully considered, as these aspects can clearly alter body weight and body weight changes. In addition, one tested species may be more sensitive than another to a body weight effect from a particular substance, so a lack of concordance does not necessarily mean that the body weight effects observed are irrelevant.

Organ-level effects

Many effects at the organ level are generally considered less relevant for ecotoxicology, simply because they mainly relate to individual effects and are not directly linked to the survival or reproduction of populations. However, effects on organs, at a certain level, logically have the potential

to affect survival and reproduction. Some examples of things to consider when organ-level effects are present will be discussed below.

Effects on the nervous system (resulting in behavioural effects) will be discussed below (see discussion on behavioural effects 4). For other organ systems, the reproductive system can be most easily logically linked to likely population-level effects, as disruptions can have a direct influence on reproduction. For that reason, some effects on reproductive organs are mentioned in Table 9 above. Nevertheless, in considering effects on these or any other organs, it is vital to first consider the type of effects (e.g. changes in weight, histopathological changes) and that the level of effect should be taken into account when determining an endpoint. Low-level effects can be compensated for by the individual animal's homoeostatic drive and cannot be directly linked to an eventual effect on reproduction. In addition, the duration of exposure which led to the organ-level effect and the age of the animal in which the effect occurs must also be considered and weighed heavily. The duration of exposure will also be discussed further below; however, it is important to consider whether the effect is a result of a very long-term exposure or could be linked to a shorter exposure at a critical time. Finally, many organ changes (e.g. like changes in organ weight) might not be considered relevant based on other observations in the animals, and/or might be easily reversible or transient. Organ weight changes in the absence of histopathological changes are not considered to be indications of an adverse effect (this should be already stated by mammalian toxicology evaluators for the changes in question) and therefore should also not be considered relevant for ecotoxicological endpoints. Special attention should be paid to the discussions and decisions of the mammalian toxicology section when drawing conclusions on the relevance of organ-level changes, particularly noting these issues of severity, duration and reversibility. Another important consideration is the frequency with which the observation is made and the cross-species concordance. These issues are also usually discussed by the mammalian toxicology section. Generally, effects in organs other than reproductive or neurological will have less relevance for population-level effects unless they are quite severe and frequent. Effects on reproductive and neurological organs are generally considered relevant with exceptions and will be further discussed below.

The largest uncertainty that likely needs to be considered for endpoints based on organ-level effects is likelihood of the link to a population-level effect. Certainty is increased when the effect severity and frequency are higher, when the effect in question appears to be non-reversible, and when it occurs in reproductive organs (except in aged animals). As with other endpoints, concordance across species also provides more certainty that the effect is real and could have population-level effects across multiple species.

Behavioural effects

Behavioural ecology is a relatively new field, having only gained traction in the mid-20th century, when it was shown that the original paradigms which assumed that (generally) behaviours were relatively fixed per species, and mainly determined by the desire to maintain the genes (i.e. survival of the next generation(s)), did not completely hold true and that behaviours were generally more complex than could be easily explained by that concept (Tinbergen, 1963; Williams, 1966; Lack, 1968). In situations of food scarcity for example, mother birds will abandon the nest in favour of feeding themselves, rather than raise their chicks at their own (energetic) expense (Pianka and Parker, 1975). This concept was much debated at the time, as it on the surface seemed to favour the individual over the group, but since then behavioural ecology continues to grow and expand as the complex influence of behaviours on survival and reproduction of populations, and vis versa, is further researched and understood (Visser and Lessells, 2001; Dobson and Jouventin, 2007). As a result of the still growing recognition of the importance of complex behaviours in population survival, behavioural endpoints may be considered for the risk assessment of wild animals as well as humans (Ågerstrand et al., 2020).

Considering ecotoxicology, behaviour is not often measured in toxicity tests, however, if there are indications in the mammalian toxicity data set of potential effects on behaviour/neurotoxicity (e.g. tremors, altered gait, loss of balance, usually reported along with 'clinical signs') these should be considered in a weight-of-evidence manner when setting the endpoint. Furthermore, if neurotoxicity tests are available, particularly developmental neurotoxicity tests, these must be considered for potentially relevant endpoints. In particular, effects that may directly influence survival and reproductive behaviours of the animal should be considered relevant. Examples of these are given in Appendix C in Table C.1 (under DNT study description), however, due to the variation possible in developmental neurotoxicity testing, some of these may not be available in each test, and additional

endpoints may be added on a specific basis. Examples of behavioural endpoints which might have a clear effect on survival or reproduction include effects on righting reflex and navigation. Large effects on memory and learning can also be considered relevant. Effects on startle reflex are often discussed in the context of ecological relevance. The significance of startle reflex (particularly auditory startle reflex) gives an indication of changes in the brain. Similarly, habituation (also particularly auditory) has been linked to autism in people (Chang et al., 2017; Vivanti et al., 2018; Fenckova et al., 2019). These responses may, however, be relevant in and of themselves for wild mammals, simply based on the premise that these behaviours might be considered relevant for survival and mating behaviours (Eisenstein et al., 2001; Schmid et al., 2015). As with organ-level effects, the level of these effects will have to be carefully considered as to whether they are likely to be relevant to wild mammals. Generally, many of the same considerations as mentioned for organ-level effects, above, should be considered, particularly the severity and frequency of the effect. Effects of 10% or greater will be considered relevant, unless extensive literature/data is provided showing that this is not the case (See sections on 'Less relevant endpoints' 5 and 'Non-relevant Endpoints' 6, below).

Uncertainties to be considered for behavioural endpoints include the direct or indirect link to population-level effects, as well as special considerations of the tests themselves.

Less relevant endpoints

The endpoints discussed in this section are generally not ecologically relevant, though there may be some rare exceptions.

i) Carcinogenicity endpoints

Generally speaking, carcinogenicity endpoints are considered less relevant for the wild mammalian risk assessment, for several reasons. In the first case, the dosing in the carcinogenicity studies, as mentioned in the description of the studies, is intended to represent a life-long exposure scenario, and the test is specifically intended to detect any possibility of carcinogenic potential that may not be captured in the available multigeneration tests. To that end, specific strains of rodent are selected to increase the likelihood of detection oncogenic effects. In wild birds and mammals, cancer is rare, and usually due to viruses (Tasmanian devil, California Sea Lion) or genetic background (California Sea Lion), with the notable exception of the St. Lawrence estuary Beluga whale (high exposure to PAHs) (Lair et al., 2016; Norman et al., 2017). Recent papers suggest that environmental changes and increasing wildlife in urban areas could make them interesting sentinels for human cancers, but this is only speculative, with little supporting data (Sepp et al., 2019).

Thus, both the exposure duration, strain and likelihood of relevance to wild mammals are questionable. Nevertheless, if it can be shown that a non-reversible and early-onset (i.e. before reproduction) cancer in the reproductive organs could be linked to exposure to the substance in question, it might be considered whether the endpoint could be relevant for the risk assessment.

The uncertainty in using an endpoint from a carcinogenicity study in the wild mammalian risk assessment would be quite high, based on the considerations mentioned above. The link to a population-relevant effect would, in most cases, be tenuous.

ii) Biomarkers and clinical biochemistry

In mammalian toxicology, certain biomarkers may be used for endpoint setting, usually only in the context of other changes (e.g. alanine aminotransferase (ALT) changes indicative of liver toxicity, together with related changes), but in specific cases, e.g. where an adverse outcome pathway is well known, or it relates to a direct functional effect, the biomarker endpoint may be used if giving the lowest endpoint. For ecotoxicology, for direct effects because of changes in, e.g. blood chemistry, the prevalence and severity of the effect should be considered, and it should be determined whether the effect would impair the survival or reproduction of the organisms. If that is the case, it is possible that these might also be considered ecologically relevant (e.g. diflubenzuron causes severe anaemia, which was considered population relevant due to the severity and prevalence of the effect which could impair both survival and reproduction); however, it is expected that in the vast majority of cases, there is no direct connection/it is not possible to show a connection to a relevant effect.

iii) Endpoints from 28- and 90-day toxicity studies

Endpoints from 28- and 90-day studies can mainly be used to contextualise effects seen in other available mammalian toxicology studies but may rarely be directly relevant for the ecological risk assessment. The test duration and life stage studied make these tests less relevant for the

reproduction assessment, and the endpoints studied should in most cases also be available in the acute and/or chronic/reproduction tests. These types of studies can, however, provide information about the persistency of effects or the potential for delayed effects, as well as providing more information on target organs, metabolism/toxic mechanisms. They can therefore be useful in choosing an endpoint from one of the other tests. In rare circumstances, an endpoint from these studies may be the most ecologically relevant endpoint, depending on the toxic mechanism involved.

Non-relevant endpoints

i) Screening tests

Screening tests should not be used to set endpoints. This includes tests related to endocrine-disrupting properties. OECD 150 'level 3' tests, e.g. cannot be used for endpoint setting because the power of the tests to detect effects is quite low and the animals used are not intact/reproductive. These tests are only used as a quick 'screening' before more definitive tests are used for endpoint setting. The same can be said for the range-finding tests in the mammalian toxicology section, which generally do not include enough animals to be useful for endpoint setting.

Other points to consider

ii) Test duration and age

Several tests in the mammalian toxicology package are intended to mimic a life-long exposure. Particularly the chronic/carcinogenicity tests involve daily exposure for up to 2 years (depending upon the life span of the animals in the test). Due to the higher natural prevalence of cancers in lab animals, it is necessary to use large numbers of animals and very long-term exposures to determine the chances of exposure to a xenobiotic resulting in tumour formation. This is clearly relevant for the protection of individual people but has much lower relevance for wild mammals. These tests should therefore only be considered for very specific endpoints (discussed under *'i* Carcinogenicity endpoints' in section '5 Less relevant endpoints'). In addition, for effects only detected at the end of chronic/carcinogenicity tests, it must be considered whether the effects were also seen in younger animals in other tests, and whether the effects may be most likely linked to the age of the animal. Effects, e.g. on reproductive organs which occur only after or during senescence are of low ecological relevance.

iii) Incidence/prevalence of an effect

In some cases, the mammalian toxicity section may consider effects seen in a study to be severe enough to warrant consideration for the NOAEL, although they have a very low incidence (i.e. very few animals are affected). This is important because the intention in mammalian toxicology is to protect the individual. However, in most cases, these could be considered to be not relevant for population-level effects, as the level likely will not attain a high enough prevalence in the population to be relevant for population survival. An example of this might be if there is hydrocephaly observed in two pups in a developmental toxicity study. Mammalian toxicology may consider this difference relevant though it could not be detected statistically significant and would likely lie within the natural variation for this endpoint, because, when it occurs, it is an extremely severe effect and the need to protect each individual is paramount in mammalian toxicology. In ecotoxicology, however, the relevance of such a low incidence would be dubious for the survival of the population. Although the hydrocephalitic pups would not survive, the very low incidence would be unlikely to have an effect on the overall population survival.

5.2.7. Effect endpoint for use in the chronic/reproduction assessment

In the context of this guidance document, significant consideration has been given to uncertainties in the risk assessment (see chapter 13) and to the level of protection for birds and mammals (see Section 3.1). One of the areas of uncertainty is specific to the toxicity data. Particularly, extrapolation between the laboratory and the field, and between species are large areas of uncertainty. However, additional uncertainties exist, inherent to the studies themselves. These uncertainties mainly stem from the endpoints measured and the power of the test to determine an effect. Typical studies may be able to detect different levels of effects for different endpoints, dependent on the specific study in question. Nevertheless, the detectable effect level may be as high as 20% or higher (see Appendix A of EFSA Scientific Committee, 2022). As a result of this inherent variation, the use of the NOAEL introduces

additional uncertainty in the level of protection which has been reached, as it is dependent on the ability of the specific study to detect a particular effect level for the endpoint in question. Even if no statistically significant effects were seen in the study, effects of up to or greater than e.g. 20% cannot be excluded (see Appendix A of EFSA Scientific Committee, 2022). In general, NOAELs are determined using post-ANOVA tests where the relation of the value to actual biological relevance is uncertain. High variability results in higher NOAELs, which in effect 'rewards' poor data (Murrell et al., 1998; OECD, 1998a; Isnard et al., 2001; van Dam et al., 2012). EFSA Scientific Committee (2022) notes that '…in the NOAEL approach experimental uncertainties, resulting from low study power, are not adequately covered and may result in an endpoint that is too high (see also Section 2.3.1 of EFSA Scientific Committee, 2022).' The fact of the lower power of the tests to determine some endpoints (depending on the specifics of the test in question) cannot be completely addressed without significant additional vertebrate use via tests with increased replicates and animals per replicate. However, utilising a specific effect level in the risk assessment can normalise the level of effect which is 'accepted' by the risk assessment, which lowers the overall uncertainty in the risk assessment. The use of ECx values rather than NOEC values for ecotoxicological risk assessment had been adopted by other guidance documents (e.g. EFSA PPR Panel, 2013). In addition, the use of an endpoint based on a specific effect level will allow the assessor to, e.g. much better report on the eventual conclusion of the risk assessment as to the potential level of effect which might be expected in case of a breach of the trigger value. In the event that more specific protection goals are developed, these can more easily be linked with the toxicity data/endpoint and encompassed within the risk assessment methodology which must support those protection goals.

Furthermore, Regulation 283/2013[4] states that for birds $EL_{10}/_{20}$ values should be given, and only when this is not possible, an NOAEL value can be provided (see point 8.1.13 of Regulation 283/2013[4]). For mammals, all three should be provided (see Section 8.1.2.2 of Regulation 283/2013[4]). In addition, using an effect level value in the risk assessment is beneficial as it allows harmonisation of endpoints between different substances, and reduces the impact of the large spacing between concentrations which, as aforementioned, is a frequent issue in vertebrate studies.

Effect levels of 5, 10 and 20 percent were considered by the WG, however, as an effect level of 10% is specifically mentioned in the Regulation 283/2013[4], could be relatively reliably calculated and showed more correlation with the endpoints used in the current risk assessment (see Section 5.2.7.1), the WG thus considered it to be the most appropriate effect level for implementation into the reproductive risk assessment for birds and mammals. It is noted that the extensive variety of possible endpoints means that the effect level of 10% could have a different actual impact at the population level. Ideally, an analysis of the level at which each endpoint would become relevant would be performed, however, considering the number of endpoints and the available data to support such an analysis, this was not possible.

5.2.7.1. Impact on the risk assessment when using an effect level

The effect of the change from NOAEL to EL_{10} (10% effect level) on the overall endpoints used for the reproductive risk assessment should not, overall, significantly change the screening/Tier 1 of the risk assessment by e.g. in most cases resulting in a lower or higher endpoint (Azimonti et al., 2015). The data used in Azimonti et al. (2015) for the comparison of NOAEL and EL_{20}/EL_{10} values indicated that the EL_{10} would be lower than the NOAEL in 24% of cases for birds and 13% of cases for mammals.[28] In general, bird and mammal tests show lower NOAELs than tests with other taxa due to the aforementioned wider dose spacing, and the presence of more possible endpoints, resulting in generally lower NOAEL values. The effect on the endpoints used for birds and mammals could therefore potentially be a lower EL_{10} endpoint than NOAEL endpoint in 24% or 13% of cases and an equivalent or higher EL_{10} endpoint than NOAEL endpoint in 76 or 87% of cases, respectively. See Figure 5 which reproduces data for Birds and Mammals from Azimonti et al. (2015).

[28] Although in the EFSA external report, Azimonti et al. (2015) compared NOEC values and ECx estimates, in the context of this guidance NOAEL is always used.

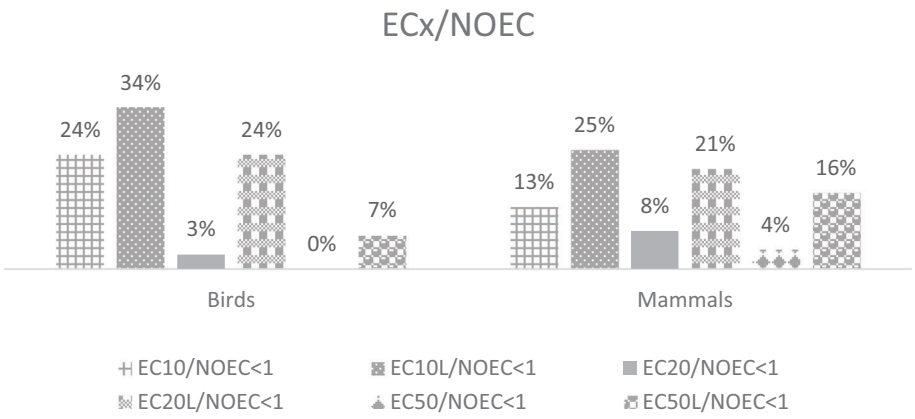


Figure 5: Comparison of ECx and NOEC for birds and mammals from Azimonti et al. (2015); EC_{10L}, EC_{20L} and EC_{50L} concern the lower bound of the credible interval of, respectively, the EC_{10}, EC_{20} and EC_{50} estimates

The WG recommends using the median 10% effect level (= EL_{10}) for the risk assessment, when such an endpoint can be estimated in a reliable way (see sections below). The use of the median 10% effect level is in line with other guidance (EFSA PPR Panel, 2013; EFSA, 2019b). The lower limit of the EL_{10} (EL_{10L}), may then be considered when there is additional uncertainty regarding the effect in question or the available data (see, e.g. Section 5.2.9). If the estimated endpoint is not considered reliable and cannot be used for risk assessment, then the NOAEL should be retained (see Section 5.2.7.2).

Calculating the EL_{10}

In order to calculate an effect level for birds and mammals, benchmark dose modelling should be used, following the general recommendations of the most recent EFSA Scientific Committee Guidance document on benchmark dose modelling (BMD) (EFSA Scientific Committee, 2022). According to EFSA Scientific Committee (2022), '[*the advantage of the BMD approach over the NOAEL approach relates to the fact that the selection of the [endpoint] takes into account the complete set of BMD confidence intervals for the [endpoint] considered and combines the information on uncertainties in the data* (see Section 2.6.5 of EFSA Scientific Committee, 2022)].' The authors further note that, unlike the NOAEL approach, the BMD approach uses the entire data set rather than only making pairwise comparisons using subsets of the data (i.e. between control groups and dose groups). '*In addition, the BMD approach can interpolate between applied doses, while the NOAEL approach is restricted to preselected doses from the study design. A BMD[L] is always associated with a predefined effect size (the BMR) for which the corresponding dose has been calculated, while a NOAEL represents a predefined dose and the corresponding potential effect size is mostly not calculated*.'

The EFSA Platform for BMD analysis (EFSA, online[29]) can be used for the modelling, although other existing software (USEPA, online; RIVM, online) can also be used, so long as the recommendations of EFSA Scientific Committee (2022) are followed. The EFSA Scientific Committee guidance (2022) outlines the methodology for determining a benchmark dose (Section 2.5 of the EFSA SC, 2022), as well as for determining the quality of the BMD analysis in question (Section 2.6.3 of the EFSA SC, 2022). The most recent version of this guidance should always be used when calculating and evaluating the BMD. It should be mentioned that the current draft and platform allow the use of 'priors' (i.e. prior information from other related studies) in the BMD calculation.[30] This is intended to improve the ability of the models to predict an acceptable BMD; however, priors should only be used in calculating a BMD when discussed and agreed upon between the applicant and RMS.

Effect modelling ideally includes a larger number of doses; however, the number of doses in most chronic/reproduction studies has been minimised due to animal welfare concerns. If the outcome of the BMD analysis remains unsuitable for calculating an effect level, in the context of the risk assessment for birds and mammals, it is recommended that the NOAEL be used, with additional

[29] https://r4eu.efsa.europa.eu/app/bmd

[30] The use of 'priors' is integral to the Bayesian approach. The results of the analysis without the use of priors will show little difference from a frequentist approach.

consideration of the resulting uncertainty in the level of protection (see section on the use of the EL_{10} vs. NOAEL).

The WG considers it appropriate to use a benchmark dose/effect level of 10% in the chronic/reproduction risk assessment for birds and mammals, in line with the requirements of the regulation (Regulation 283/2013[4] and 284/2013[5]). The recommended benchmark response is 10 for both quantal and continuous data and the median BMD_{10} of the 'averaged' model will be used as reference point. In cases where the uncertainty around the BMD is too large (see decision scheme(s)), then a case-by-case assessment of the appropriateness and reliability of alternative endpoints should be done. It is not possible to provide guidance to cover all possible circumstances. The use of an NOAEL is recommended (see section on biological relevance) in cases where the BMD_{10} is unsuitable, with additional consideration of the resulting uncertainty, particularly related to understanding the meaning of a breach of the trigger value in the risk assessment where the endpoint is used.

For mammalian endpoints, the EFSA BMD guidance (EFSA Scientific Committee, 2022) can be followed, except that the (median) BMD shall be used rather than the lower bound of its credible interval (BMDL).

A decision scheme in line with the EFSA BMD guidance is included below to interpret the quality of the BMD_{10} modelling and how to follow up in case the BMD_{10} is not reliable. This decision scheme is followed by additional guidance for birds, based on Green et al. (2022), to assist in BMD_{10}/NOAEL calculation for bird reproduction studies.

1: Is the spread of the BMD_{10} credible interval (upper bound/lower bound, $BMDU_{10}/BMDL_{10}$) of the most sensitive relevant endpoint > 50 or the ratio $BMD_{10}/BMDL_{10}$ > 20 or the BMD_{10} 10 times lower than the lowest non-zero dose

   Yes: Go directly to 2 or check whether the effect endpoint based on the BMD_{10} is much higher that the exposure estimate (DDD) so that the high uncertainty in the effect assessment endpoint has no consequence for the risk characterisation (*Note that in a tiered approach an exposure estimate is not a fixed value*)

   No: Use BMD_{10} of this endpoint as effect estimate

2: The spread of the BMD_{10} credible interval ($BMDU_{10}/BMDL_{10}$) of a related relevant endpoint, with similar biological consequences based on expert judgement, is > 50 or the ratio $BMD_{10}/BMDL_{10}$ of this endpoint is > 20 or its BMD_{10} is 10 times lower than the lowest non-zero dose. (*Note that in this case, it is the risk assessor's responsibility to make substantiated decision whether this alternative endpoint will be used in the BMD assessment and the rationale for this needs to be documented*).

   Yes: Go to 3

   No: Use BMD_{10} of the most relevant analogous endpoint as effect estimate

3: The effect in the toxicity study for the most sensitive and/or the related relevant endpoint (with similar biological consequences based on expert judgement) in the highest dose tested is smaller than 10% or, the experimental data show a monotonic concentration-response pattern with significant effects at several doses and the NOAEL of the most sensitive relevant endpoint (or that of the most relevant alternative endpoint) is lower than its corresponding BMD_{20}

   Yes: Use of the NOAEL as effect estimate from reproductive studies.

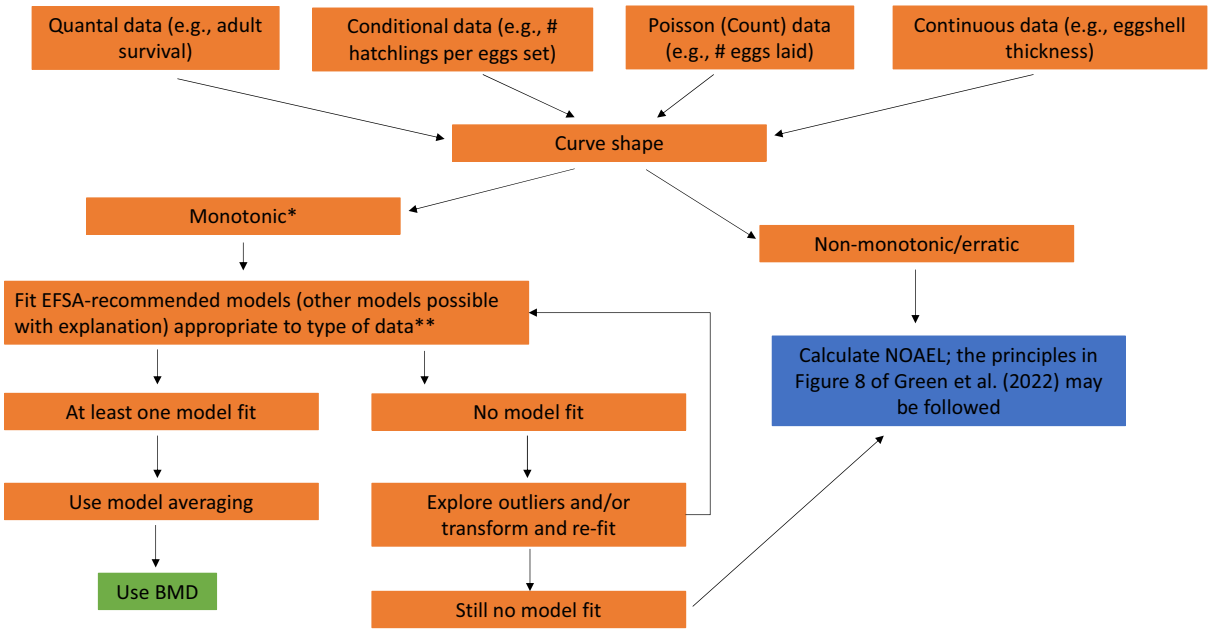
   No: Additional data may be required, or additional methods explored (e.g. DEBtox modelling) to predict a reliable reference point for the reproductive effect assessment.

The EFSA Scientific Committee Guidance (2022) notes that *the above quantitative categorisation depends on several cut-off values inspired by those proposed by the US EPA as 'default logic assumptions'. Although plausible, they lack a theoretical statistical basis and have not been tested empirically (e.g. a systematic review of risk assessment practices)*. They therefore note that these should be considered as 'indicators', pending the expert decisions on the suitability of the BMDL as a reference point. For further details, please see Section 2.6.5 of the EFSA Scientific Committee Guidance (2022).

For birds, some additional guidance is useful, as, compared to mammal studies, different endpoints and a different study type are used. Green et al. (2022) have published some useful guidance on endpoint determination in bird reproduction studies. One important thing to note from that publication is that it should be possible to alter the current OECD protocol to include fewer birds per treatment and more treatments without significant loss of power to detect effects for most endpoints. Similar to

the current OECD protocol except for this change, the modified protocol would be able to detect effects at varying levels, depending on the endpoint and the natural variation around that endpoint and would also provide a better understanding of the dose–response. The WG recommends consideration of an adapted protocol for bird reproduction studies to allow better measurement of effect levels/dose responses in the future.

Furthermore, Green et al. (2022) propose a decision scheme for determining endpoints in bird reproduction studies. The WG generally agrees with the proposed scheme, with some notable slight alterations (see Figure 6, below).



* If hormesis is detected, a hormesis model can/should be attempted. Hormetic models are not specifically included in the models in the EFSA BMD Guidance nor calculator tool.
** See discussion of data types in Green et al., 2022 and EFSA, 2022.

Figure 6: Decision chart for BMD_{10}/NOAEL estimation of avian reproduction studies (adapted after Green et al., 2022)

What Green et al. (2022) call 'conditionally quantal' data could be considered as adding covariates to the analysis, and 'count data' can be dichotomised or, if the counts are large enough, approximated by continuous distributions. Nevertheless, since these are simply ways of noting the various types of data available in bird reproduction studies, and they all lead to the same first step, the manner in which they are considered in either Green et al. (2022), or by the WG, is not especially divergent. Green et al. (2022) propose specific errors to be considered based on the type of data being modelled. It should be noted that in the Bayesian approach recommended by EFSA Scientific Committee (2022), these are not applicable, however, in such cases where priors cannot be determined/used, this advice may be followed (when modelling under frequentist conditions).

If it is not possible to determine a BMD_{10}, the WG recommends the use of the NOAEL. Green et al. (2022) propose specific considerations for determining the NOAEL(C) in bird reproduction studies (see Steps 3–5 of figure 8 in Green et al., 2022). In principle, the WG considers these proposals appropriate and that they may be used for NOAEL determinations.

5.2.7.2. Conversion of the endpoint from a dietary concentration to a daily dose

It is necessary to have toxicity endpoints in mg a.s./kg bw per day, i.e. in a daily dose format to be consistent with the units used in the exposure assessment. Most studies already provide endpoints in terms of mg a.s./kg bw per day. However, some avian reproduction studies may be reported in terms of parts per million (ppm) or mg a.s./kg diet, and therefore, their endpoints need to be converted into daily dose.

The general rule for the conversion is:

$$\textbf{Daily dose} = \textbf{C} \times \textbf{daily food consumption}/\textbf{bw} \quad (6)$$

Parameter	Explanation	Unit
Daily Dose	–	mg/kg bw per day
C(food)	Concentration in food	mg/kg
Daily food consumption	–	g per bird per day
bw	body weight	g

The guidance from European Commission (2002c) is still relevant in this regard and is repeated below for ease of reference:

- Food consumption:

  For the short-term dietary toxicity test, usually group consumption rates (expressed as g per bird per day) are given in the report for the 5-day exposure period and the 3-day post-exposure period. The group consumption rates for the 5-day exposure period should be used.
  For reproductive studies, data are reported on a weekly basis for pairs or groups. Food consumption usually is higher during egg-laying (to be attributed to the females), however, for the purpose here the average consumption over the entire exposure period is taken.

- Body weight:

  For the short-term dietary toxicity test, group means for day 0, 5 and 8 are reported. For the purpose here take the average of day 0 and day 5.
  For the reproductive studies, take average body weight for both sexes over exposure period.

- Conversion

  For the short-term dietary toxicity test, the conversion from concentration to daily dose is not appropriate for those treatment groups where a strong food avoidance is obvious (in that case, the average dose over 5 days is misleading) as well as for treatment groups with a high mortality (in that case data for the body weight at day 5 and for the food consumption have a poor quality or are missing at all).

  Case 1: LC_{50} is above top concentration

  Convert each treatment group separately (however, only the top level is needed for the risk assessment).

  Case 2: LC_{50} is below top concentration, food consumption not affected. There are two possibilities:

  a) convert concentration into achieved dose for each treatment group, and conduct a new probit analysis, this time using the daily dose data
  b) take the overall mean value for food consumption and body weight (mean from all dose groups where calculation is possible) and use these figures to convert the LC_{50} (this option is sensitive against concentration-dependent food avoidance). If food consumption is slightly affected, expert judgement is required to decide whether procedures according to case 3 should be followed better.

  Case 3: LC_{50} below top concentration, distinct food avoidance well below the LC_{50}:

  Conversion from concentration to daily dose may be unreliable due to the low number of survivors on which food consumption is based; furthermore, food consumption may change markedly from day to day. These problems alone should be no reason to repeat the study. Rather assessment should be conducted on a case-by-case basis (e.g. if the study delivers an NOEL, then this could be a starting point as the converted LC_{50} must be well above this level); applicants should seek advice from the competent authority.

  For the reproductive studies, convert each treatment group separately.

5.2.8. Use of a surrogate endpoint for avian reproductive risk assessment

Before conducting the avian reproductive risk assessment with the relevant reproductive endpoint as derived from reproductive studies, it is recommended to check whether there may be evidence of sublethal effects which may lead to reproductive impairment. Clinical signs and behavioural (neurotoxicity) studies can be considered for mammals (see also Section 5.2.6.5); however, this is not always possible for birds, since short-term studies are not required, and the available acute and reproduction studies typically include less extensive reporting of sublethal effects. As a result, this check should be done by comparing the acute LD_{50} divided by an assessment factor of 10 which allows extrapolation between the lethal effects and the relevant reproductive endpoint (BMD_{10} or NOAEL). If the LD_{50}/10 is lower than the relevant avian reproductive endpoint, it is recommended to use the LD_{50}/10 in the avian reproductive risk assessment. The use of the LD_{50}/10 is a conservative approach that ensures that the toxicity of substances with specific mode of action are appropriately covered in the risk assessment (see Section 5.2.9). Moreover, the use of the LD_{50}/10 accounts for parental toxicities which might result in pair-forming/nesting/brooding impairment.

5.2.9. Special consideration for migratory birds

Pre-migratory fattening and excessive food consumption (hyperphagia) are typical behaviours of migratory birds in order to accumulate fuel for sustained and very intensive migratory flights (Vincze et al., 2019). Migratory birds may use farmland habitats as a stopover and refuelling source. It has been demonstrated that migratory birds may be particularly sensitive when exposed to neurotoxic insecticides. One of the possible explanations is that this class of compounds, by acting as nicotinic acetylcholine receptor (nAChR) agonists or by inhibiting acetylcholinesterase (AChE) could have subtoxic effects on cognitive and motor functions. These functions are essential for refuelling, orientation and navigation and are thus crucial for successful migration (Eng et al., 2017, 2019).

Based on the available knowledge and in order to consider possible effects on migratory birds and to assess whether the risk assessment is also protective for those species, it should first be checked whether, based on the mode of action of the substance (AChE inhibitors, nicotinic acetylcholine receptor (nAChR) agonists, other neurotoxic mechanisms), it may be expected that migratory birds could be more sensitive than other birds.

If, from available information, the substance is known to have a neurotoxic MoA, it should next be checked whether data on passerine species are available. As specified above (see Sections 5.2.2.1 and 5.3.2), studies on passerine species may sometimes be available as required by other jurisdictions. Additionally, toxicity data with passerine species may be available from publicly available literature or information on the potential neurotoxic MoA may be available from screening zebrafish assays (EFSA PPR Panel, 2021a). If acute data on passerine are available, the difference in sensitivity between passerine and the other standard species may be compared to see whether the reproductive risk assessment (based on data on quail and/or mallard) with the use of a standard assessment factor of 5 is assumed to adequately cover possible effects on migratory birds.

When (specific) data on passerine are not available, the mammalian data package should be considered with particular attention to endpoint(s) from neurotoxicity studies and observed primary effects on body weight in the available long-term studies. Generally speaking, it should be possible to determine from the mammalian data set (1) if there is a primary neurotoxic effect; (2) the range of doses where primary neurotoxic or body weight effects are seen across mammalian species. Comparison of the range of doses where similar endpoints in mammal vs. bird studies (e.g. body weight, food consumption, clinical signs) are seen may give general information to extrapolate other endpoints to birds, thereby informing as to whether the proposed chronic endpoint from the available study(ies) with birds can be assumed to be adequately protective of migratory birds. Should this not be the case, the possibility of an assessment factor or additional conservativeness in the risk assessment e.g. lower endpoint such as the lower limit of the BMD_{10} ($BMDL_{10}$) can be explored, or at least the potential risks to migratory birds can be communicated in the conclusions/to the risk managers.

Under no circumstances should additional bird (vertebrate) testing be performed.

5.3. Tier 2 effect assessment: Incorporation of laboratory toxicity data for additional test species

5.3.1. Introduction

If reliable and relevant oral toxicity data are available for additional birds and mammals besides the minimum data requirements, the effect assessment might be refined by using the geometric mean approach (in acute ERA only), the weight-of-evidence (WoE) approach or the species sensitivity distribution (SSD) approach. Note, however, that the generation of additional toxicity data for birds and mammals conflicts with the statement in Commission Regulation (EU) 283/2013[4] that for ethical reasons tests on vertebrates shall be undertaken only where no other validated methods are available and by taking into account the scope for reduction, refinement and replacement of animal tests. Consequently, additional toxicity data for birds and mammals should not routinely be generated to refine the toxicity estimate for the TER. In daily practice, this means that only for a few active substances already placed on the market valid toxicity data for additional bird and mammal species will be available. Nevertheless, due to data requirements outside the EU, and toxicity data already available in the open literature, for active substances to be (re)placed on the European market, oral toxicity data for additional species might have been generated.

5.3.2. Toxicity data for additional test species from former EU requirements, dossier requirements outside the EU and publicly available literature

According to Commission Regulation (EU) 283/2013[4], the minimum data requirement is an acute and reproductive oral toxicity study for one species of bird (preferably Japanese quail or Bobwhite quail). In addition, besides for a quail species also toxicity data for mallard duck was a data requirement in the past, so that for many substances already registered reliable toxicity data for at least two bird species may be available. Furthermore, toxicity data for the canary may be available as part of the dossier requirements outside the EU (e.g. USA). For mammals and environmental hazard characterisation, the data from mammalian toxicology are used, but in principle for mammals the minimum data requirement is the same from the environmental hazard characterisation perspective: one acute oral test and one reproduction test. However, there are many other data requirements for mammalian toxicology, also depending on the 'if' clause in the regulation, based upon exposure or mode of action (MoA) of the substance under evaluation or the results of previous ecotoxicity tests (see Section 5.2.1). For this reason, relevant toxicity data for up to four to five mammal species may be available in the standard dossier. In addition, according to current regulation, relevant data from the scientific peer-reviewed open literature on PPPs, shall be submitted together with the standard studies. Thus, additional laboratory toxicity data on additional bird and mammal species exceeding the regulatory requirements (Commission Regulation (EU) No 283/2013[4] and No 284/2013[5]) may be available. These data can be used in the effect assessment if considered relevant and reliable (see Section 5.2.5 on data from publicly available literature).

5.3.3. Acute effect assessment based on toxicity tests with standard and additional test species

If valid acute oral LD_{50} values are available for several species of birds and/or mammals, the previous Birds & Mammals GD (EFSA, 2009) already proposed using the geometric mean LD_{50} in the effect assessment, calculated separately for bird and mammal species and using a single valid LD_{50} value for each species group as input. This geometric acute oral LD_{50} value was then used as toxicity estimate in the acute TER and this TER should be greater than 10 to consider the acute risk as 'low'. Using the geometric mean acute oral LD_{50} of multiple species is stated by EFSA (2008, 2009) to be sufficiently conservative for use as toxicity estimate in the risk assessment, provided that the standard TER of 10 includes sufficient allowance for between-species variation in intrinsic acute oral toxicity.

In this updated guidance, the use of the geometric mean LD_{50} value for multiple species in the effect assessment is not challenged, but we propose a weight-of-evidence (WoE) approach if the LD_{50} for the most sensitive bird or mammal species is lower than the geometric mean LD_{50} for multiple bird or mammal species divided by the AF of 10. In this WoE approach, the lowest available endpoint (LD_{50}) of multiple bird or mammal species is then used in the TER while the AF may be reduced. In analogy with the proposed WoE approach in acute ERA for aquatic vertebrates (EFSA, 2019b), this

refined acute TER should be greater than 3 to consider the acute risks as 'low'. However, if for the most sensitive species, the acute LD_{50}/LD_{10} ratio is larger than 3, selecting a higher TER may be justified to consider the acute risks as 'low'.

Below a decision scheme is presented to facilitate the use of the geometric mean approach in the acute effect assessment for birds and mammals. Note where for the same species several valid acute LD_{50}'s are reported, the geometric mean LD_{50} for this species is used as input. The decision scheme is also applicable in the case that one, or more, of the LD_{50} values are extrapolated values (Section 5.2.6.3).

1: For at least two bird or mammal species valid acute LD_{50} values are available?

- **Yes**: Calculate the group-specific geometric mean LD_{50} using the LD_{50} for each species within the group and go to 2.
- **No**: Use the LD_{50} of the available Tier 1 bird or mammal species as input for the TER. If the TER (acute LD_{50}/DD) $\geq$ 10, the acute risks are considered 'low'.

2: Is the (group-specific geometric mean acute LD_{50})/10 lower than the acute LD_{50} of the most sensitive species within the group?

- **Yes**: use the group-specific geometric mean acute LD_{50} as input for the TER. If the TER (geometric mean acute LD_{50}/DD) $\geq$ 10, the acute risks are considered 'low'.
- **No**: Calculate the LD_{50}/LD_{10} for the most sensitive species and go to 3.

3: Is the LD_{50}/LD_{10} ratio for the most sensitive species $\leq$ 3

- **Yes**: Use the LD_{50} of the most sensitive species as an effect estimate. If the TER (lowest acute LD_{50}/DD) > 3, the acute risks are considered to be 'low'.
- **No**: Use the LD_{50} of the most sensitive species as effect estimate, but, based on expert judgement, select a trigger value for the TER in line with the actual LD_{50}/LD_{10} ratio to consider the acute risks to be 'low'.

In analogy with the acute effect assessment for other non-target vertebrates than birds and mammals (e.g. fish), the species sensitivity distribution (SSD) approach might also be used in a refined effect assessment, if for at least five bird and five mammal species valid acute oral LD_{50} values are available. This will be seldom the case, certainly when considering the EU requirements for reduction, refinement and replacement of toxicity tests with vertebrates. The hazardous dose to 5% of the species tested (HD_5) derived from the SSD constructed with the acute oral LD_{50} values for different species might then be used as toxicity estimate in the refined acute TER and this refined TER (HD_5/DD) should be $\geq$ 3 to consider the acute risks to be 'low'.

5.3.4. Reproductive effect assessment based on toxicity tests with standard and additional test species

For mammals in particular, for up to four to five species, long-term studies may be available in the dossier. Nevertheless, the use of the geometric mean NOAEL or BMD_{10} for multiple species as toxicity estimate in the TER in many cases is not justified due to differences in test design (e.g. test duration) and measurement endpoints between tests conducted with different species. Consequently, if for less than five species of birds or five species of mammal's valid toxicity data are available, the reproductive endpoint (BMD_{10} or NOAEL, or the avian LD_{50}/10 if lower) from the most sensitive standard and additional species tested (separately for birds and mammals) should be used as toxicity estimate in the TER (toxicity estimate/DDD). If the TER is greater than 5, then the reproductive risk to birds or mammals is considered to be 'low'. In the exceptional case that for at least five bird or five mammal species valid BMD_{10} values (or a valid NOAEL if a trustful BMD_{10} cannot be estimated) are available for a specific relevant reproductive endpoint, the SSD approach can be explored and a HD_5 calculated. The SSD must be constructed with the lowest valid and relevant reproductive endpoint if several are available for the same species. If, based on expert judgement, this HD_5 can be used as effect estimate, then the TER (HD_5/DDD) should be $\geq$ 3 to conclude low reproductive risks.

5.4. Tier 2 effect assessment: TK and TKTD models

5.4.1. Introduction to TK and TKTD models

Toxicokinetics (TK) essentially concerns how a toxic substance gets into the organism and how the individual organism deals with the substance. In other words, it refers to all processes that influence the dynamics in internal exposure to the toxicant.

Toxicodynamics (TD) essentially concerns how this internal exposure affects biological processes. It refers to processes that lead to toxicant-induced mortality or sublethal damage of the individual, including possible repair from sublethal damage.

The TK processes absorption, distribution, metabolism and excretion (ADME) of active substances (and their metabolites), as well as the TD process of repair of the damage within the organism, may be potentially important characteristics in the refined risk assessment for birds and mammals. An early example for this is the refined acute risk assessment of the carbamate insecticide pirimicarb for birds feeding on insects in cereal fields (EFSA, 2005). It could be made plausible, amongst others based on available standard experimental ADME data for mammals and information on feeding rates of wild birds, that animals recover quickly from the cholinergic syndrome due to rapid toxicokinetics and the relatively rapid reactivation of inhibited acetylcholinesterase. Furthermore, it was concluded that the framework developed to refine the acute risks of pirimicarb for birds may be applied to other compounds and situations, on a case-by-case basis (EFSA, 2005). The proposed framework, however, is not yet considered formalised TK or TKTD modelling approaches.

Although largely a research objective for birds and mammals to date, the development of TK and TKTD models as higher tier tools in the ERA of chemicals is developing quickly. These models either consider an organism as a whole (one compartment) or consider several compartments that may represent internal entities such as the digestive system or a set of organs. In physiologically based TK (PBTK) models, chemical fluxes between compartments (specific target tissues or organs) are deciphered by physiological fluids (e.g. blood). These PBTK models are equivalent to physiologically based pharmacokinetic (PBPK) models, which is the more common nomenclature for such models in mammals. Currently, these PBTK models are mainly used to perform simulations that serve to extrapolate parameters between related species or from mammal species to humans (for more details, see Larras et al. (2022) and Astuto et al. (2022)). The EFSA TKPlate open-source platform provides further information for PBTK modelling in risk assessments related to the food and feed chain.

Metabolism at the enzyme level is well-conserved across vertebrates (Peregrin-Alvarez et al., 2021), although uncertainty exists regarding the similarity in other TK processes between related taxa. Comparative *in vitro* metabolism studies have been developed using specific mammalian cell types, e.g. hepatocytes (EFSA PPR Panel Opinion, 2021b). Similarly, OECD guidance on the determination of clearance of chemicals in rainbow trout cell lines is also available (OECD, 2018a). These types of *in vitro* studies are considered to be useful as an alternative to *in vivo* testing to better understand the metabolic pathway (TK properties) and further support extrapolation and the development of TK models and/or the TK module of TKTD models. For the time being, considering the data package normally available, the need to reduce vertebrate testing in accordance with Directive 2010/63/EU[31] and the conservation of metabolism at the enzyme level across vertebrates, a certain degree of extrapolation of TK properties between vertebrate species is considered possible in the refined risk assessment for birds and mammals on a case-by-case basis.

TKTD models can translate constant or more realistic time-variable exposure into expected effects on life-history traits of living organisms by connecting external exposure dynamics to internal exposure dynamics, and to the prediction of effects over time. Consequently, TKTD models allow extrapolation of effects under a tested exposure pattern to other untested ones and calculation of any x% effect at different points in time (Baudrot and Charles, 2019). For a general description of TKTD models and their use in prospective ERA for pesticides, reference is made to the 'Scientific Opinion on the state of the art of toxicokinetic/toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms' (EFSA PPR Panel, 2018) and to Astuto et al. (2022).

Generally, TKTD models are both species- and substance-specific, but parameters can usually be interpreted in a physical and biological manner. For calibration of TKTD models, more or less standardised toxicity data can be used; however, an independent toxicity data set or a subset of the

[31] Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. OJ L 276, 20.10.2010, pp. 33–79.

study data not used for calibration is required for validation. Note, however, that the standard tests with bird and mammal species as part of the data requirements, have not been designed for TK and TKTD modelling purposes. Therefore, the working group advises investigation of the possibility of adapting these tests so that test results (e.g. additional ADME information and pesticide dynamics in blood samples) can be optimally used for TK and/or TKTD modelling purposes in the near future, while keeping the number of test animals at a minimum.

In the context of the risk assessment for birds and mammals, TKTD models might be used to predict mortality (acute effect assessment scheme) and sublethal effects (long-term reproductive effect assessment scheme) under more realistic time-variable exposure conditions, by explicitly considering the relation between external and internal exposure dynamics. TK models can also be used to better estimate total body burdens and risks due to bioaccumulation.

According to Ashauer and Brown (2013) and Ducrot et al. (2015), TK and/or TKTD modelling can bring more realism to the risk assessment for birds and mammals when:

- There is a strong variation of exposure concentration in time;
- Behavioural responses may modify exposure dynamics (e.g. polyphasic activity patterns, avoidance, dehusking);
- Compounds have a particular TK, e.g. internal concentration changes more slowly than external concentration or when substances are absorbed and eliminated in a short time;
- Risks through bioaccumulation must be refined;
- A reduction of animal testing is needed, e.g. by optimally extracting the information from available test data for a limited number of test animals by means of interpolation (between tested concentrations) and extrapolation (to non-tested concentrations) purposes;
- The influence of different exposure routes (oral, dermal and inhalation) needs to be assessed;
- Long-term risk predictions are needed, but only acute test data are available;
- Complex exposure patterns might lead to delayed or carry-over toxicity.

Note that the latter three bullet points in particular are important research areas to data and cannot readily be applied in prospective ERA for birds and mammals.

Detailed guidance on how to properly conduct TK and TKTD modelling, and how to interpret the results in a regulatory context, is not given in the previous Bird and Mammal Guidance document (EFSA, 2009). In this update, complete guidance still cannot be given because of lack of published examples on the use of TK and TKTD models in the refined risk assessment for vertebrate wildlife, and birds in particular. Nevertheless, recent developments allow the provision of greater detail regarding the conditions under which TK and TKTD modelling approaches can be used as a Tier 2 refinement for birds and mammals.

5.4.2. Use of TK and TKTD models in acute effect assessment for birds and mammals

Using laboratory data on ADME, such as reported in the Toxicology section of the active substance registration dossier, body burden (TK) modelling was already identified in EFSA (2009), as a relevant refinement option. Data from ADME studies are often available for rats, livestock or hens. Based on expert judgement, it may be decided that extrapolation of TK properties between vertebrate species is considered possible (see e.g. EFSA, 2005).

In recent years, a few examples of the application of TK models in the risk assessment of rats in particular have been published in the open literature (e.g. Bednarska et al., 2013; Ducrot et al., 2015). These studies demonstrate that TK modelling together with information on feeding pattern in risk assessment may help to reduce uncertainties associated with acute studies, relating to dosing and internal exposure (substance bioavailability at the target site). Bednarska et al. (2013) and Ducrot et al. (2015) argue that ADME information that is available in the registration dossier for e.g. rats and hen can be used to parameterise and calibrate a TK model for these standard test species under the condition that (i) the kinetics of the active substance is first order and (ii) the concentration of the active substance in blood represents the concentration in other tissues correctly. As mentioned above, based on expert judgement, a certain degree of extrapolation of TK properties between related vertebrate species is considered possible, since metabolism at the enzyme level is well conserved across vertebrates (Peregrin-Alvarez et al., 2021). A possible limitation of ADME studies on the basis of 14C-radioactivity is that the fraction of parental active substance and its possible metabolites are not characterised separately.

In its simplest form, a one-compartment model with first-order kinetics includes the processes of absorption and elimination and aims to track the total body burden of the organism. Multi-compartment models, such as physiologically based pharmacokinetics (PBPK), usually have many variable biochemical and physico-chemical determinants, and these complex models are able to estimate residue levels in specific organs. Ideally, the simplest model that is fit for purpose in regulatory ERA should be selected.

Toxicokinetics (TK) only addresses part of the potential risk due to pesticide exposure, as toxicodynamics (TD) will also affect the eventual effects. Although a sufficiently calibrated and validated TK model may suffice to estimate critical body burdens to refine the risks due to bioaccumulation, it may not suffice to estimate toxic effects caused by time-variable exposure, particularly if physiological recovery is not immediate once the toxicant leaves the system. Depending on the physico-chemical properties and the pesticides' mode of action in the bird or mammal species of concern, a full TKTD model needs to be used.

Within ERA, the general unified threshold model of survival (GUTS; Jager et al., 2011) has been recognised by EFSA as a suitable TKTD modelling approach to extrapolate lethality in laboratory tests to predict the lethal effects resulting from realistic time-variable exposure profiles, at least for aquatic organisms (EFSA PPR Panel, 2018). Unfortunately, sufficiently calibrated and validated GUTS-TKTD models currently are not yet available for terrestrial vertebrate wildlife. Nevertheless, a GUTS-TKTD type of model may have potential in prospective acute ERA for birds and mammals. For example, in acute laboratory tests with standard bird species, exposure by gavage (administration of the pesticide dose in a single short-term procedure) is common practice. This exposure scenario may be unrealistic for a 24-h feeding regime in the field that is used in acute exposure assessment. If this more realistic 24-h exposure pattern is made available for representative focal species (e.g. via appropriate Tier 3 PT studies), calibrated and validated GUTS-TKTD models might be used to calculate an exposure pattern-specific LD_{50} to be used in the risk assessment (TER).

As mentioned above, GUTS-TKTD models must be calibrated based on experimental data for the specific species–pesticide combination, and subsequently validated on an independent data set with a different exposure profile to prove that the model is able to extrapolate mortality estimates across different exposure conditions. For birds, a standard acute toxicity test dosed once by gavage (OECD test guideline 223) is a data requirement in the EU (see Section 5.2.2.1). The results of this test might be used to calibrate the GUTS-TKTD model. If available for the same test species, avian dietary toxicity data (OECD test guideline 205) might be used to validate the GUTS-TKTD model (see Section 5.2.3).

The EFSA PPR Panel (2018) defined quality requirements on the data sets and on the goodness of fit in the calibration and validation procedures of the models. Please refer directly to chapter 7 'Evaluation of models' and Annex A (checklist for GUTS models) of the EFSA scientific opinion on TKTD models (EFSA PPR Panel, 2018) for detailed information. This checklist developed for GUTS models and aquatic organisms needs to be adapted to evaluate the GUTS-TKTD models developed for vertebrate wildlife, once sufficient published examples become available. Until then, the evaluation of GUTS-TKTD models as an appropriate Tier 2 refinement tool depends on expert judgement.

Sufficiently validated, substance-specific TK and GUTS-TKTD models for standard test species of birds and mammals can be used in the Tier 2 risk assessment to calculate a refined LD_{50} based on a more realistic time-variable exposure pattern and the same duration as the acute standard toxicity test (= TKTD-LD_{50}). The refined acute risk can be assessed as follows:

> If the TKTD-LD_{50}/DD $\geq$ 10, a low risk due to more realistic internal exposure is identified. The TKTD-LD_{50} value is derived for the standard test species on basis of its TK or GUTS-TKTD model (substance and species-specific) and the realistic dietary dose (DD) is derived for the generic model species or focal species of concern. It is assumed that the assessment factor of 10 addresses the species-to-species and lab to field extrapolation (separately for birds and mammals).

Alternatively, based on valid TK or GUTS-TKTD models for standard test species, the dose profile causing 50% mortality (= $DP_{lethal\text{-}50}$) might be calculated by using multiplication factors applied to the realistic time-variable exposure profile. The concept of $DP_{lethal\text{-}50}$ resembles that of the LP_{50} (Exposure profile causing 50% mortality) as used in the refined risk assessment for aquatic organisms on basis of GUTS-TKTD models (see chapter 8 'Use of TKTD models in Tier-2C risk assessment' of EFSA PPR Panel, 2018). The refined acute risk can be assessed as follows:

If the $DP_{lethal\text{-}50} \geq 10$, a low risk due to more realistic time-variable exposure is identified. The $DP_{lethal\text{-}50}$ value is derived for the standard test species on basis of its TK or GUTS-TKTD model (subcutane and species-specific) and the realistic time-variable dose profile for the generic model species or focal species of concern. It is assumed that the assessment factor of 10 addresses the species-to-species and lab to field extrapolation (separately for birds and mammals).

5.4.3. Use of TKTD models in long-term reproductive effect assessment for birds and mammals

Since sublethal endpoints are the most critical in the long-term reproductive risk assessment for birds and mammals, the dynamic energy budget (DEB) modelling framework combined with a TKTD part (DEB-TKTD, formerly also referred to as DEBtox) would be the appropriate approach to select in the refined risk assessment. For a general description of the DEB-TKTD modelling framework and its potential use in pesticide risk assessment is referred to EFSA PPR Panel (2018) and Jager (2020). As was the case for GUTS-TKTD models, DEB-TKTD models are species and substance specific, but parameters can usually be interpreted in a physical or biological manner. Again, for their regulatory use, these models need to be calibrated to (standard) data, and ideally validated against independent data.

Overall, a DEB model for an individual organism describes the rates at which the organism assimilates and utilises energy for maintenance, growth and reproduction, as function of the organism's key life cycle parameters and its environment (Nisbet et al., 2000; Kooijman, 2001). Currently, the 'add-my-pet' database holds DEB information for more than 2000 species, with extensive data on many of the standard species used in ERA, including those for birds and mammals (Marques et al., 2018). These data facilitate using primary parameters for the DEB part of the DEB-TKTD models without requiring vast quantities of species-specific physiological data to be gathered in every experiment. The curator board of the add-my-pet database is checking the technical appropriateness of the parametrisation of the DEB part of the model. Based on the submitted data for the species entry, improved parametrisations on additional data are stored in a new version. So, a version control system is in place for the add-my-pet database, the used data and the code are transparently documented. What needs to be checked is if the submitted data are sufficient so that the model is considered appropriate for regulatory use. This check could be coordinated by EFSA (e.g. within an AIR submission by an expert group or a version control group). In other words, the species-specific DEB part of the model, once considered appropriate for regulatory use, needs not be re-evaluated when developing DEB-TKTD models for the same species but different active substances. For each active substance, however, the TKTD part of the DEB-TKTD model needs to be evaluated for regulatory use.

The DEB-TKTD modelling approaches described in literature vary from relatively complex to simple modelling approaches. Sherborne et al. (2020) describe the major model variants of different DEB-TKTD models to facilitate communication. However, there is currently no consensus as to which variant of the model is best suited to regulatory demands and data availability.

At present, DEB-TKTD modelling is limited to research applications as it is not yet regarded ready for regulatory use in the prospective risk assessment for pesticides due to the lack of published examples (see EFSA PPR Panel, 2018). The available DEB-TKTD models for pesticides and vertebrate wildlife in the open literature are limited. Recently, Martin et al. (2019) described a DEB-TKTD modelling approach for effects of pesticides on growth of laboratory rats from existing regulatory studies and argue that the modelling approach used may also be adapted to address effects on reproduction. A similar DEB-TKTD study that addresses the effects of a pesticide on birds could not be found in the open literature.

Despite the fact that DEB-TKTD modelling is largely a research activity to date, EFSA PPR Panel (2018) concluded that these models have great potential for future use in prospective ERA for pesticides. EFSA has recently initiated a project to develop DEB-TKTD models for birds aiming at predicting effects from time-variable exposure. The project is still in its embryonic phase but once available the model can be used for risk assessment.

In the prospective risk assessment for birds and mammals, a specific advantage of TKTD models is that they may facilitate an overall reduction in animal testing if e.g. methods are developed that allow the incorporation of information from *in vitro* assays. Although developed for aquatic organisms, the checklist for DEB-TKTD models as reported in Annex B of EFSA PPR Panel (2018), may be a good

starting point to consider when further developing a DEB-TKTD model as a Tier 2 approach in the long-term reproductive risk assessment for birds and mammals.

Once sufficiently validated substance-specific DEB-TKTD models for standard test species of birds and mammals are made available, they can be used in the Tier 2 risk assessment to calculate a refined long-term (reproductive) endpoint based on TKTD modelling by considering a more realistic time-variable exposure pattern.

Besides DEB-TKTD models, physiologically based TKTD (PBTKTD) models might in future be used in environmental ERA for birds and mammals. As with PBTK models, also PBTKTD models use the body of an organisms as a set of interconnected compartments and mathematical equations describing ADME of a specific chemical, but then they connect the internal dose to the dose-response of the adverse dynamic effects based on in vivo, and more recently, in vitro studies. However, so far, no generic PBTKTD models are available for refined environmental risk assessment of birds and mammals, since data requirements are high (Astuto et al., 2022).

5.4.4. Concluding remarks on regulatory use of TKTD models for vertebrate wildlife

It can be concluded that TKTD models are still mainly an academic research activity and require more development before they can be used in a standardised way in regulatory ERA to assess risks of pesticide exposure to birds and mammals. Currently, no general accepted methods are available for how to calibrate/validate TKTD models without animal testing. To reduce animal testing, a tailored calibration–validation strategy needs to be developed either based on all acute, subchronic and chronic toxicity tests already available, or by the incorporation of information from *in vitro* assays. For the time being, the interpretation of results of TK and TKTD modelling exercises and their use in regulatory ERA for birds and mammals, needs to be done on a case-by-case basis and expert judgement. It is important that TKTD models and their documentation develop to a high level, following the comprehensive suggestions of EFSA PPR Panel (2018). This will enable the realisation of the full potential of TKTD models in successful use cases for regulatory purposes in the risk assessment for birds and mammals, allowing all parties to increase their experience with model development, application and evaluation.

6. Exposure assessment tiers

6.1. Introduction to the exposure assessment for substances applied as sprays or as seed treatments

In different exposure assessment tiers for birds and mammals, exposure scenarios are used based on the consumption of pesticide-contaminated food items foraged in treated/contaminated areas by indicator model species, generic model species and focal species. Consequently, the focus of the exposure assessment is on dietary exposure, acknowledging that dermal and inhalation exposure routes are not covered in the decision schemes of this guidance document. Furthermore, in the acute risk assessment, the exposure estimate used is by default the highest dietary dose (DD) of the exposure profile. In the reproductive risk assessment, before estimating the exposure of the active substance, it should be assessed whether the eco(toxicological) data indicate that reproductive effects are likely from acute/short-term exposure (criteria are given in Section 6.1.2 below). If this is not the case, a 21-day time-weighted average approach is used to estimate the daily dietary dose (DDD).

Outlined in the following sections are the principles of how to estimate the screening level and Tier 1 exposure to birds and mammals for spray applications and seed treatments. Several of the underlying parameters and assumptions are relevant for both types of application method whereas some principles are specific to the application method. Section 6.1.1 provides information on the common principles and considerations. Section 6.2 is specific for spray applications and Section 6.3 for seed treatment applications.

6.1.1. Indicator Model Species and Generic Model Species

The screening exposure assessment is only done for spray applications and is performed for indicator model species (IMS). Indicator model species are not a real species but a model species with assumed characteristics meaning that the risk assessment performed for an indicator model species is protective of all other exposed species. They are determined by taking the worst case (most exposed)

of all the generic model species used in the Tier 1 exposure assessment for a broad group of crops. For the screening assessment, the crop growth stage is not accounted for and only a single indicator model species is assessed. These are summarised in Table 11 (Section 6.2.2). Indicator model species are assumed to take a single type of food, which leads to the highest level of exposure.

The Tier 1 exposure assessment should be performed for both spray and seed treatment applications and should be determined for the relevant generic model species (GMS). GMS are representative of different feeding guilds, but it is important to note that they are not identical e.g. there are no truly frugivorous mammals in the EU, but there are some which are heavily reliant on fruit when it is available. This may seem semantical where the Tier 1 exposure assessment is concerned, but since Tier 1 is used to inform the Tier 3 exposure assessment, it is important to acknowledge this fact. Again, GMS are not real species, but model species, with assumed characteristics, meaning that the risk assessment for this model is protective of all other species of the same feeding guild exposed. The GMS are triggered, depending on the crop and growth stage under assessment. The GMS needed to be considered for each crop group are summarised in Annex B whilst the background information on the GMS parameters is discussed in Appendix F. It should be noted that the GMS defined in Annex B depend on the growth stage of the crop. For the majority of crops, this has been done according to BBCH growth stages (Meier, 2001). However, some (less common) crops are not included in Meier (2001). The GMS selection for these crops has been defined for the BBCH principal growth stages with the assumption that the crop growth will follow a similar pattern.

The most efficient way to select the correct GMS for the Tier 1 exposure assessment is to use the online tool (EFSA, online[32]). This tool will also select the correct exposure parameters and perform the Tier 1 calculation.

6.1.2. Body weight of the IMS and GMS

In the screening and Tier 1 level assessment, the body weight (BW) (g) is a fixed value. The values are summarised in Table 11 (Section 6.2.2) for each IMS and in Annex B for each of the GMS. Background information to the selection of the BW values is given in Appendix F.

6.1.3. Food Intake Rate

A key parameter in the estimation of the dietary exposure of birds and mammals to residues of pesticides substances is the food intake rate (FIR) by the indicator or generic model species. The estimates of food intake are based on means of daily energy expenditure for free-ranging animals and the energy and moisture content and assimilation efficiencies for each food item. For Tier 1 GMS taking a mixed diet, the FIR should be calculated considering the proportion of each food item in the diet. Values for the energy and moisture content and assimilation efficiencies needed for the calculation of FIR are provided in Appendix G. FIR is calculated according to Equation 7.

$$\mathbf{FIR} = \mathbf{DEE} / \sum_{\mathbf{i}} \left(\mathbf{PD_i} \times \mathbf{FE_i} \times (\mathbf{1} - \mathbf{MC_i}) \times \mathbf{AE_i}\right) \quad (7)$$

Where:
FIR: Food Intake Rate [g fresh weight/day]
DEE: Daily Energy Expenditure [kJ/day] of the IMS or GMS
PD_i: Proportion of food item i in the diet
FE_i: Food energy of food item i [kJ/dry g]
MC_i: Moisture content of food item i (obtained by moisture content in % divided by 100)
AE_i: Assimilation Energy for food item i by the IMS or GMS (obtained by assimilation energy in % divided by 100)

6.1.4. Criteria to assess whether a time-weighted average factor (fTWA) can be used in the reproductive exposure assessment

In the exposure calculation for reproductive assessments, it may be possible to take in to account the degradation of a substance in the form of a time-weighted average factor (fTWA). The use of an fTWA in the exposure calculations for the reproductive risk assessment assumes that the toxicity follows the rule of linear reciprocity because of continuous or variable exposure over an extended period of time (in this case 21 days, see Appendix H for discussions on the selected averaging period).

[32] https://r4eu.efsa.europa.eu/app/birds-mammals

However, it is the nature of toxicity testing that it is not typically possible to differentiate when a specific toxicity is because of an extended exposure (e.g. as in reproductive toxicity testing) or if it is the result of an exposure at a critical or sensitive life stage. In other words, many toxicities are as a result of long-term exposure; however, others may be because of short-term exposure at a critically sensitive developmental or reproductive phase. With that in mind, the fTWA is suitable to assess the reproductive risk in many cases (cases based, for example, upon body weight effects[33]). In cases where the critical effect upon which the endpoint is based is a toxicity that is possibly a result of short-term exposure at a critical life stage, it is likely that the fTWA should not be used in the risk assessment, as it is not certain that the critical effect was because of a long-term exposure. Examples of this would be cases of clear developmental effects which were not correlated with maternal effects, cases of decreased fertility or fecundity as a primary effect (i.e. not because of other (systemic) toxicities), and cases where there is an indication that the adverse effects may be caused by an endocrine mode of action. This includes such effects observed in the second generation of two-generation toxicity tests, as the second generation covers developmental phases not exposed in the first generation. In these cases, it is difficult to determine whether one short-term exposure at a critical phase or several exposures over time were required for the manifestation of the effect. Experts should therefore consider whether to employ the fTWA on a case-by-case basis for these types of effects. Some data may be extracted from existing toxicity studies to support time-to-effect considerations. (Note that no additional vertebrate testing should be performed.) Finally, many non-genotoxic tumours could be considered to be as a result of multiple/chronic exposures. However, some tumours may also be potentially as a result of an endocrine system alteration from a short-term/acute exposure. These pathways to tumorogenesis are complex and will have been discussed in the mammalian toxicology section. On the rare occasions when tumour formation would be considered a relevant endpoint (see ecologically relevant endpoint), the ecotoxicology assessor should consider the determination of mammalian toxicology as to the potential pathway for tumorogenesis before determining whether the use of the fTWA is appropriate (see Table 10).

Where birds are concerned, the same concepts apply, except that a true delineation of effects is more difficult primarily owing to the limited data requested for birds. As a conservative assumption, if the lowest relevant endpoint is based upon effects on eggshell thickness in the absence of systemic toxicity (e.g. significant effects on adult body weight, activity, clinical signs such as feather loss), no fTWA should be used. Due to the fewer data and endpoints available for birds compared to mammals, if the fTWA is assessed not to be justified for mammals, the fTWA should also not be used for the bird risk assessment. A rare exception may be in the case that sufficient data on birds are available to allow the applicant to clearly justify use of the fTWA for birds. Some data may be extracted from the studies in order to support argumentation relating to time-to-effect considerations. Effects on chick development which are not linked to parental toxicity should be considered carefully and unless significant evidence is provided to indicate that these effects are definitely linked to a long-term exposure, no fTWA should be used. Please also note that any of these effects could be as a result of exposure of parents during development, meaning that no consideration of whether application during the breeding season should be considered (see also Section 5.2.1). Finally, in EFSA (2009), the LD_{50}/10 was considered as a surrogate for parental toxicities which might result in reproductive impairment due to sublethal effects on pair formation and breeding site selection, incubation, parental care of nestlings and survival of fledgling birds. It was advised that the value which was lowest (relevant reproductive endpoint (BMD_{10} or NOAEL) or LD_{50}/10) be used in the long-term risk assessment. However, it should also be considered that effects occurring in the parents prior to egg laying could also indicate problems with pair-forming or nesting behaviours as a result of short-term exposures. Therefore, also in these cases (where the LD_{50}/10 is used or where there is evidence of parental effects prior to egg laying), it is not acceptable to use an fTWA. When there are no effects observed in the reproductive toxicity test, the use of the fTWA is justified.

If the lowest ecologically relevant endpoint allows the use of the fTWA, it should also be checked whether a higher ecologically relevant endpoint (within 3x of the lowest endpoint) would not be appropriate with the fTWA, and if so, in that case a risk assessment should be presented using each endpoint.

[33] Bodyweight effects, specifically reduction, in the absences of other clear toxicities, are typically found in toxicity studies as a result of longer term exposure. The physiological mechanisms for body weight loss are mainly as a result of long-term changes to metabolism, see Section 5.2.6.5.

Please note that in some cases, based on the available data, it may be possible to use the fTWA for either birds or mammals and not for the other (e.g. it is possible to use it for birds but not for mammals).

Table 10 provides examples of types and endpoints and a consideration as to whether an fTWA can be used for the reproductive risk assessment. Following the table, flow diagrams (Figures 7 and 8) are also provided.

Table 10: Examples of endpoints for which the fTWA is or is not appropriate*

	Mammals	Birds
Effects for which the fTWA is appropriate	Body weight and body weight change	Effects other than those specified in the following rows of this column
	Food intake	
	Liver and kidney effects	
	Other organ-level effects (See Section 5.2.6.5)	
Effects for which case-by-case expert judgement should be employed	Effects on reproduction/development with some (parental) body weight or slight toxic effects	Effects on chick development which may be primary effects (i.e. unless a clear pattern of maternal toxicity is proven)
	Some tumours (see explanatory text above)	Effects on parental birds as represented by body weight changes in females prior to egg laying (see explanatory text above)
Effects for which the fTWA should not be used	Primary reproductive effects (consult with mammalian toxicology and see Glossary and abbreviations); i.e. effects on fertility and fecundity not as a result of systemic toxicity	Effects on eggshell thickness not correlated maternal bw/bw change/ systemic toxicity
	Primary developmental effects (already judged sufficient for endpoint setting see Section 5.2.6.5); e.g. developmental effects in the absence of parental or systemic toxicity	When the LD_{50}/10 is lower than the endpoints from the avian reproduction study

*: The list is not exhaustive and is intended to provide guidance to the assessor and better guide harmonised decisions.

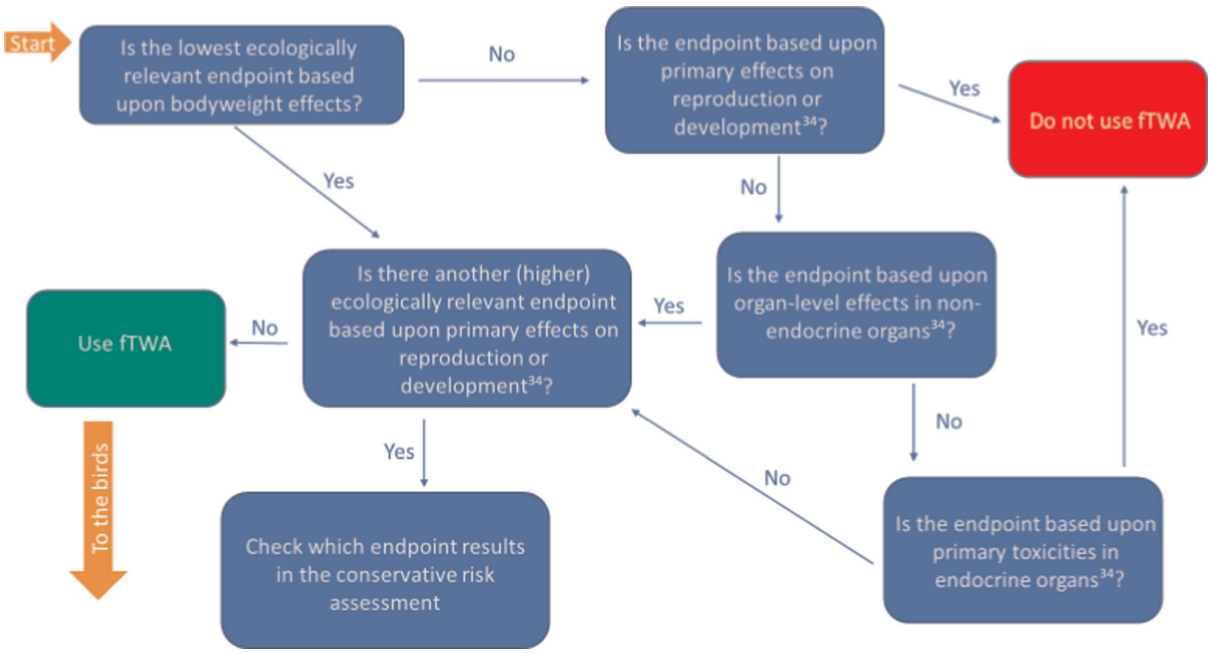
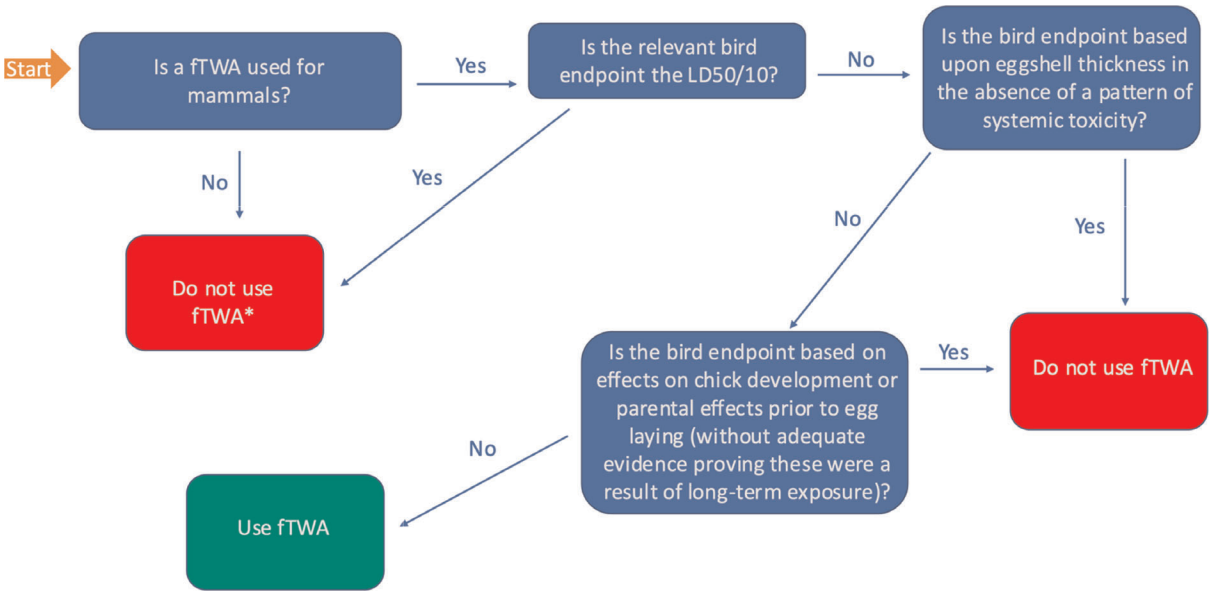


Figure 7: Flowchart for deciding if fTWA can be used in the risk assessment for mammals



An exception may be in the case that sufficient data on birds are available to allow the applicant to clearly justify use of the fTWA for birds. Some data may be extracted from the studies in order to support argumentation relating to time-to-effect considerations.

Figure 8: Flowchart for deciding if fTWA can be used should be used in the risk assessment for birds

[34] See Table 10.

6.2. Specific considerations for spray applications

6.2.1. Scenarios for inside and outside of the treated area

Birds and mammal species are exposed both to residues in the treated area, and those resulting from drift on to food items in adjacent areas. Considering the relative residue levels, for most GMS, the potential direct effects due to exposure outside of the treated area are covered by the more worst-case exposure in in-crop assessment. The exception is for some small mammals who are reliant on (crop) coverage to be found within the crop/treated area. As such, a risk assessment for small mammals outside of treated area but within the 'Terrestrial Area of Interest' (TAI, see Section 4.2.4) has been introduced (this is reflected in the Tier 1 scenarios but may also be relevant for Tier 3, where focal species studies are required).

It is acknowledged that to accurately assess the residues on food items in the TAI is complex, partially owing to the enormous variability in structures of field margins and application machinery used in the EU. To balance the complexity and effort needed to perform the in-crop and TAI exposure assessments and considering the relative exposure to birds and mammals in general, the WG agreed to take a simplistic approach to estimating exposure in the TAI. Consequently, it is recommended just to simply correct the in-crop application rate by the appropriate spray drift value (see Appendix I). For food items on the soil surface, interception by vegetation can be accounted for by use of deposition values[35] (See Section 6.2.6 and Appendix L). Note that given the requirements for coverage, it is always assumed that the plants within the TAI are at BBCH 40, the minimum growth stage to allow appropriate coverage for small mammals. Given that the Tier 1 exposure assessment provides information to the Tier 3 (if required), it was agreed that it would be preferable to perform such assessment for all growth stages in the Tier 1 assessment to provide clarity on whether any higher tier assessment should consider exposure in the TAI. It is emphasised that a risk assessment in the TAI is only needed for small mammals (herbivorous, granivorous and insectivorous), in all crops, as the in-crop risk assessment for all other generic model species covers exposure from the TAI. Regarding spray drift mitigation, please refer to Chapter 14.

6.2.2. Screening tier exposure assessment for spray applications

The conceptual model for dietary exposure to each of the screening indicator model species (IMS) for spray applications is a simplified version of that of the Tier 1 exposure assessment outlined in the following section. The following equations calculate the (daily) dietary dose (D(DD)) for IMS assuming a diet containing a single food item (PD = 1) all obtained from the treated area (PT = 1).

$$\textbf{Acute DD} = \textbf{FIR} \times \sum_{\textbf{i}} (\textbf{AR} \times \textbf{RUD}_{\textbf{i}} \times \textbf{MAF}_{\textbf{acute}}) / \textbf{BW} \quad (8)$$

In this equation, the concentration (C) (see Section 3.2.2.2) of the a.s. in the single food item is given by ($AR \times RUD \times MAF_{acute}$)

$$\textbf{Reproductive DDD} = \textbf{FIR} \times \sum_{\textbf{i}} (\textbf{AR} \times \textbf{RUD}_{\textbf{i}} \times \textbf{MAF}_{\textbf{repro,i}} \times \textbf{fTWA}_{\textbf{i}}) / \textbf{BW} \quad (9)$$

In this equation, the concentration (C) (see Section 3.2.2.2) of the a.s. in the single food item is given by ($AR \times RUD \times fTWA \times MAF_{repro}$).

where:

Acronym	Explanation	Unit	Further information
Acute DD	Acute dietary dose for the IMS	mg a.s./kg bw	–
Reproductive DDD	Reproductive daily dietary dose for the IMS	mg a.s./kg bw per day	–
Bw	Body weight of the IMS	g	Table 11

[35] It is acknowledged that the interception by plants in the TAI area will vary significantly. However, since plant coverage is key for the small mammal GMS for which the risk assessment is being performed, the WG considered that it was reasonable to assume plant interception in the TAI.

Acronym	Explanation	Unit	Further information
AR	Application rate of the pesticide active substance per hectare.	Kg a.s./ha	Taken from the GAP table of the pesticide product under assessment
FIR	Food intake rate for food item by the IMS (Equation 7)	g fresh weight per day	Table 11
RUD	Residue per unit dose for the food item and is defined as the initial residue on the food item. For an acute assessment, the 90th percentile value should be used. For reproductive assessments, the geometric mean value should be used.	mg a.s./kg food item	Table 11
fTWA	Time-weighted average factor which accounts for degradation on food item during a 21-day period. See note 1.	[–]	Table 11 and Section 6.2.5
MAF_{acute} MAF_{repro}	Acute or repro Multiple Application Factor (MAF) for the food item which depends on the number of applications and the interval between applications (defined in the GAP table of the product under assessment). See note 2.	[–]	Table 11 and Section 6.2.5

Note 1: fTWA is only applicable for the reproductive assessments for pesticide active substances which meet the criteria defined in Section 6.1.4.
Note 2: A moving window fTWA × MAF should be calculated. The methodology for this is given in Section 6.2.5 and will be automatically calculated by the online tool (EFSA, online[36]).

In the screening exposure tier, the (daily) dietary dose should be calculated for the IMS listed in Table 11.

Table 11: Crop groups and parameters for screening tier exposure estimation, indicator model species and spray applications

Crop group	Risk assessment category	Indicator model species	Diet	Parameters			
				BW	FIR	RUD	MAF × fTWA
Crop group 1 All orchard and field crops listed in Appendix E	Acute bird	Small omnivorous bird	Dicot. foliage	27	62.06	84.8	(a)
	Reproductive bird	Small omnivorous bird	Monocot. foliage	27	31.65	47.2	(b)
	Acute mammal	Small omnivorous mammal	Monocot. foliage	23	31.41	117.8	(a)
	Reproductive mammal	Small omnivorous mammal	Monocot. foliage	23	31.41	47.2	(b)
Crop group 2 • Ornamental cactuses and succulents • Ornamental herbaceous plants	Acute bird	Small omnivorous bird	Dicot. foliage	27	62.06	84.8	(a)
	Reproductive bird	Small omnivorous bird	Monocot. foliage	27	31.65	47.2	(b)

[36] https://r4eu.efsa.europa.eu/app/birds-mammals

Crop group	Risk assessment category	Indicator model species	Diet	Parameters			
				BW	FIR	RUD	MAF × fTWA
• Ornamental herbaceous plants excluding bulbs	Acute mammal	Small omnivorous mammal	Monocot. foliage	23	31.41	117.8	(a)
	Reproductive mammal	Small omnivorous mammal	Monocot. foliage	23	31.41	47.2	(b)
Crop group 3 • Broadleaf forest trees • Coniferous forest trees • Biomass trees • Ornamental conifers • Ornamental woody monocotyledonous plants • Ornamental woody plants • Ornamental plants (unspecified) • Ornamental broad-leaved trees, shrubs and climbing plants	Acute bird	Small omnivorous bird	Dicot. foliage	11	33.82	84.8	(a)
	Reproductive bird	Small omnivorous bird	Monocot. foliage	11	17.25	47.2	(b)
	Acute mammal	Small omnivorous mammal	Monocot. foliage	23	31.41	117.8	(a)
	Reproductive mammal	Small omnivorous mammal	Monocot. foliage	23	31.41	47.2	(b)

(a): MAF_{acute} calculated according to the equations given in Section 6.2.5.2. No fTWA is applied to acute assessments.
(b): Moving window $MAF_{repro} \times$ fTWA calculated according to the equations given in Section 6.2.5.2. fTWA is only applicable for the long-term assessment for pesticide active substances which meet the criteria defined in Section 6.1.2.

6.2.3. Tier 1 exposure assessment for spray applications

The conceptual model for dietary exposure to each of the generic model species (GMS), for spray application, is already defined in EFSA (2009), and is summarised below. In EFSA (2009), some exposure parameters (namely the food intake rate, body weight, residue per unit dose and deposition value) were combined into shortcut values to make performing the risk assessment easier. However, this step has not been taken for this version of the guidance document as the online calculator tool will mean that risk assessors do not need to 'look-up' the exposure parameters for every risk assessment. The following equations calculate the daily dietary dose (D(DD)) for GMS assuming a mixed diet containing 'n' food items. The proportion of time spent foraging in the treated area (PT) is fixed in Tier 1 and is assumed to be 1. The proportions of food items in the diet (referred to as the dietary proportions, PD) are fixed for the Tier 1 GMS. In the following equations, PD is represented by the sum of the exposure coming from each of the dietary components (i).

$$\mathbf{Acute\ DD} = \mathbf{FIR} \times \sum_{\mathbf{i}} (\mathbf{AR} \times \mathbf{RUD_i} \times \mathbf{MAF_{acute}} \times \mathbf{DV_i} \times \mathbf{PD_i}) / \mathbf{BW} \quad (10)$$

In this equation, the concentration (C_i) (see Section 3.2.2.2) of the a.s. in food item i is given by ($MAF_{acute} \times AR \times RUD_i \times DV_i$).

$$\mathbf{Reproductive\ DDD} = \mathbf{FIR} \times \sum_{\mathbf{i}} (\mathbf{AR} \times \mathbf{RUD_i} \times \mathbf{MAF_{repro,i}} \times \mathbf{fTWA_i} \times \mathbf{DV_i} \times \mathbf{PD_i}) / \mathbf{BW} \times \mathbf{PT} \quad (11)$$

In this equation, the concentration (C_i) (see Section 3.2.2.2) of the a.s. in food item *i* is given by ($MAF_{repro} \times AR \times RUD_i \times DV_i \times fTWA_i$). Note that PT is fixed in Tier 1 and assumed to be 1.

where:

Acronym	Explanation	Unit	Further information
Acute DD	Acute dietary dose for the Tier 1 GMS	mg a.s./kg bw	–
Reproductive DDD	Reproductive daily dietary dose for the Tier 1 GMS	mg a.s./kg bw per day	–
Bw	Body weight of the Tier 1 GMS	g	Section 6.1.2

Acronym	Explanation	Unit	Further information
AR	Application rate of the pesticide active substance per hectare. In the case of TAI exposure assessments, this should be corrected for the appropriate spray drift value (see note 1 below).	Kg a.s./ha	Taken from the GAP table of the pesticide product under assessment
FIR_i	Food intake rate for food item i by the Tier 1 GMS	g fresh weight per day	Section 6.1.3, Appendix G
RUD_i	Residue per unit dose for food item i and is defined as the initial residue on food item i For an acute assessment, the 90th percentile value should be used. For reproductive assessments, the geometric mean value should be used.	mg a.s./kg food item	Section 6.2.4, Appendix J
DV_i	Deposition value is the proportion of substance reaching the food item i accounting for interception by the crop.	[-]	Section 6.2.6, Appendix L
$fTWA_i$	Time-weighted average factor which accounts for degradation on the food item i during a 21-day period. See note 1.	[-]	Section 6.2.5, Appendix K
$MAF_{acute,i}$ **$MAF_{repro,i}$**	Acute or repro Multiple Application Factor (MAF) for food item i which depends on the number of applications and the interval between applications (defined in the GAP table of the product under assessment). See note 2.	[-]	Section 6.2.5
PT	Proportion of food item obtained in the treated area. At Tier 1, PT is assumed to be 1.	[-]	–
PDi	The proportion of food item i in the diet of the GMS.	[-]	Annex B

Note 1: fTWA is only applicable for the reproductive assessments for pesticide active substances which meet the criteria defined in Section 6.1.4.

Note 2: A moving window fTWA × MAF should be calculated. The methodology for this is given in Section 6.2.5 and will be automatically done by the online calculator tool (EFSA, online[37]).

In the Tier 1, (daily) dietary dose should be calculated for each of the necessary GMS defined in Annex B.

6.2.4. Residue per Unit Dose (RUDs) for spray applications

Important data in the risk assessment for birds and mammals from spray applications are the initial residue values per unit dose (RUD), which are defined as the residue concentrations on the different diet items resulting from pesticide application, standardised for a rate of 1 kg active substance per hectare. Considering that the DD for an acute assessment may be obtained in a short period of time (possibly in a single feeding bout), the WG agrees that a 90th percentile RUD value should be used. This is in line with the approach in EFSA (2009). The DDD for a reproductive risk assessment is over a longer period and consists of multiple feeding bouts. In this case, it is not likely that a bird or mammal will always feed on items contaminated with a 90th percentile residue level; therefore, a geometric mean (50th percentile) is more appropriate. The WG considered that a geometric mean value was preferable to the arithmetic mean which was used in EFSA (2009), as there is less influence of the extreme values in the distribution.

The default RUD values given in EFSA (2009) had several shortcomings (e.g. only a few data were available for some matrices). Consequentially, the previous RUD values were replaced using the database developed by Lahr et al. (2018), in which residue data were systematically collected and evaluated in the context of approval of active substances and authorisation of plant protection

[37] https://r4eu.efsa.europa.eu/app/birds-mammals

products and additional information were retrieved through a systematic literature review. For the RUD values for fruits, besides the data collected by Lahr et al. (2018), a residue data set developed by CropLife Europe[38] was available. The studies made available by CropLife Europe were evaluated according to the procedures outlined in Appendix J. The raw data were analysed and, where possible, the two data sets were combined to derive RUD values in fruits. For some relevant food items, no RUD values were available; therefore, surrogate food items were selected (Table 12). The database by Lahr et al. (2018) contained few data for weed and crop seeds; therefore, it was decided to continue to use the RUD values reported in EFSA, 2009.

For each crop category, the geometric mean and 90th percentile of the available RUD values were derived to be used in the reproductive and acute assessments, respectively (Appendix J). An overview of updated RUD values is presented in Table 12, and a more detailed description is presented in Appendix K. The RUD database containing all data used to derive the RUD values is available in Annex B.

Table 12: RUD values for different food items to be used for calculating the exposure in the screening step and first-tier assessment

Food item	Sample size	Geometric mean [mg/kg]	90th percentile [mg/kg]	Source
Arthropods				
Ground-dwelling arthropods	30	2.8	20.2	Lahr et al. (2018)
Foliar-dwelling arthropods	53	8.4	24.8	Lahr et al. (2018)
Vegetation				
Monocotyledon leaves	218	47.2	117.8	Lahr et al. (2018)
Maize foliage	120	29.7	71.3	Lahr et al. (2018)
Dicotyledon leaves	355	21.9	84.8	Lahr et al. (2018)
Fruits, vegetables, buds and seeds				
Weed seeds	0	40.2*	87.0	Surrogate, EFSA (2009)
Crop Seeds	0	40.2*	87.0	Surrogate, EFSA (2009)
Citrus fruits	53	1.34	17	CropLife + Lahr et al. (2018)
Fruit from cucurbitaceous vegetable crops	347	0.47	1.4	CropLife + Lahr et al. (2018)
Fruit from solanaceous vegetable crops	12	0.73	2.52	Lahr et al. (2018)
Grapes	324	2.28	5.33	CropLife + Lahr et al. (2018)
Pome fruits	102	0.97	2.87	CropLife + Lahr et al. (2018)
Stone fruits	238	1.22	3.61	CropLife + Lahr et al. (2018)
Fruit from small fruit crops(1)	164	3.3	8.88	CropLife
Strawberries	178	0.95	2.1	CropLife + Lahr et al. (2018)
Bananas	0	1.34	17	Surrogate from 'citrus fruits'
Figs	0	1.34	17	Surrogate from 'citrus fruits'
Flower buds	0	3.3	8.88	Surrogate from 'fruit from small fruit crops'
Fruits from forests(2)	0	3.3	8.88	Surrogate from 'fruit from small fruit crops'
Legume vegetables	0	0.73	2.52	Surrogate from 'fruiting solanaceous'
Kiwifruit	0	1.34	17	Surrogate from 'citrus fruits'
Olives	0	2.28	5.33	Surrogate from 'grapes'
Pineapples	0	0.73	2.52	Surrogate from 'fruiting solanaceous'
Tree nuts/seeds	0	1.22	3.61	Surrogate from 'stone fruits'

[38] Submitted to EFSA in March 2020.

(1): Fruit from small fruit crops = berries (See Appendix E).
(2): Fruits from forest = biomass trees, broadleaf forest trees, coniferous forest trees.
*: Mean value.

6.2.5. Residue Dynamics and the use of Multiple application factor (MAF) and time-weighted average factor (fTWA) for spray applications

6.2.5.1. Background and DT_{50}

Residue dynamics are an important consideration for predicting the concentrations of pesticides on the food items consumed by birds and mammals. Simply, a distinction can be made between accumulation of residues following multiple applications and the dissipation of residues via various processes. To account for both accumulation and dissipation in the exposure estimation, a DT_{50} value is needed. For the screening and Tier 1 assessments for spray applications, a DT_{50} value of 10 days is assumed for all food items. Appendix K describes why this value is selected for the Tier 1 assessment. It is important to remember that an fTWA is only applicable for the reproductive assessments for pesticide active substances which meet the criteria defined in Section 6.1.4.

The calculations described in the following sections (MAF_{acute}, fTWA and $MAF_{repro} \times$ fTWA) are based on the equation for first-order kinetics in its integrated form:

$$\mathbf{C_t = C_0 e^{-kt}} \tag{12}$$

with:
c_t = actual concentration at time t
c_0 = initial concentration
k = rate constant, where $k = \ln 2/DT_{50}$.

6.2.5.2. Multiple application factor (MAF)

Multiple applications may cause accumulation of residues on the food items eaten by birds and mammals and therefore must be considered in the exposure assessment. If only peak concentrations are considered in the risk assessment, residue dynamics can be expressed by the multiple application factor (MAF), which is used to consider the buildup of residue levels if there is more than one application. For the acute assessments, an MAF_{acute} should be used, and for reproductive assessments, an MAF_{repro} should be used. Please note that MAF_{acute} and MAF_{repro} are the same as MAF_{90} and MAF_{repro}, respectively, which were used EFSA (2009).

An MAF_{repro} factor can be calculated as:

$$\mathbf{MAF_{repro} = \frac{\left(1 - e^{(-nki)}\right)}{\left(1 - e^{(-ki)}\right)}} \tag{13}$$

with:
$k = \ln 2/DT_{50}$ (rate constant)
n = number of applications
i = application interval (d)
An MAF_{acute} factor can be calculated as:

$$\mathbf{MAF_{acute} = \frac{MAF_{repro} \times RUDm + f_{90} \times \sqrt{MAF_{var} \times \sigma^2}}{RUD_{90}}} \tag{14}$$

with

$$\mathbf{MAF_{var} = \frac{1 - e^{(-2nki)}}{1 - e^{(-2ki)}}} \tag{15}$$

f_{90} = 1.28 (90th percentile for standard normal distribution)
$k = \ln 2/DT_{50}$
n = number of applications
i = application interval (d)
RUD_m = geomean RUD value
RUD_{90} = 90th percentile RUD value
σ^2 = variance of RUD data set
MAF_{var} = MAF variance

6.2.5.3. Time-weighted average factor (fTWA) and moving window $MAF_{repro} \times fTWA$

To account for the dissipation of residues in a reproductive assessment, using the assumption of first-order kinetics, it is possible to calculate the time-weighted average factor (fTWA). An fTWA is used to translate residue decline following peak exposure into a long-term exposure concentration over 21-day period. However, before using an fTWA, it should be considered whether there is evidence of short-term exposure resulting in reproductive effects. Therefore, the substance should be evaluated against the criteria in Section 6.1.4.

At the screening step and Tier 1 of the risk assessment, the exposure after multiple application will be translated into time-weighted average concentrations, however this cannot be achieved by simply multiplying the $MAF_{repro} \times fTWA$, and therefore, a moving time window approach is needed to identify the maximum time-weighted average concentration, i.e. worst-case average exposure concentration. A moving time window approach should be used for all exposure estimations when there are multiple applications. If multiple applications occur in a short period, the maximum time-weighted average concentration may not be positioned after the last peak but after the last two or three exposure peaks. Details of the calculation of a moving time window $MAF_{repro} \times fTWA$ are given in Equations 16, 17, 18 and 19. However, the calculation is performed by default by the accompanying calculator tool (EFSA, online[39]).

When multiple applications do not need to be considered, using the assumption of residue dissipation via first order kinetics, the fTWA can be calculated using the following equation:

$$\mathbf{fTWA} = \frac{\left(\mathbf{1} - \mathbf{e}^{(-\mathbf{kj})}\right)}{\mathbf{kj}} \tag{16}$$

with
$k = \ln 2/DT_{50}$ (rate constant)
j = averaging interval

$MAF_{repro} \times fTWA$ for the time period *i* after the *n*th application can be described by a simple equation that is only valid if the *n*th is the last application or if the fTWA averaging interval is shorter than the application interval between the *n*th and the (*n* + 1)th application:

$$\mathbf{MAF_{repro}} \times \mathbf{fTWA} = \frac{\left(\mathbf{1} - \mathbf{e}^{(-\mathbf{nki})}\right)}{\left(\mathbf{1} - \mathbf{e}^{(-\mathbf{ki})}\right)} \times \frac{\left(\mathbf{1} - \mathbf{e}^{(-\mathbf{kj})}\right)}{\mathbf{kj}} \tag{17}$$

with
$k = \ln 2/DT_{50}$ (rate constant)
n = number of applications
i = application interval (d)
j = TWA averaging interval

In case the application interval (i) is equal to the averaging interval (j), then the equation can be simplified to:

$$\mathbf{MAF_{repro}} \times \mathbf{fTWA} = \frac{\left(\mathbf{1} - \mathbf{e}^{(-\mathbf{nki})}\right)}{\mathbf{kj}} \tag{18}$$

If the averaging interval covers several applications and ends before the (n + 1)th application (i.e. it is a multiple of the application interval), the equation has to be expanded, and $MAF_{repro} \times fTWA$ is calculated after each application event and then averaged:

$$\mathbf{MAF_{repro}} \times \mathbf{fTWA} = \frac{\mathbf{1}}{\mathbf{n}} \times \sum_{\mathbf{n}} \frac{\left(\mathbf{1} - \mathbf{e}^{(-\mathbf{nki})}\right)}{\left(\mathbf{1} - \mathbf{e}^{(-\mathbf{ki})}\right)} \times \frac{\left(\mathbf{1} - \mathbf{e}^{(-\mathbf{kj})}\right)}{\mathbf{kj}} \tag{19}$$

6.2.6. Deposition values (DV) for spray applications

Deposition values (DV) are used in the exposure calculations to account for the amount of active substances deposited on the food items consumed by the bird or mammal following a spray application.

[39] https://r4eu.efsa.europa.eu/app/birds-mammals

When the food item is the crop or is located on the crop (i.e. foliar-dwelling arthropods, crop foliage, crop seeds, fruits, flower buds), the DV is 100%. For food items located on the surface of the soil (ground) and for weeds, the DV are determined considering the crop interception values. Consequently, the DV differs for each crop, BBCH growth stage and whether the application is made to the crop plant or is directly to the ground (e.g. as would be the case of herbicide application in an orchard). Details of how the DV values were derived are discussed in Appendix L together with a summary of the available values. Annex D provides an overview of the deposition values which should be used in the exposure assessment. It is worth noting that crop interception values, hence deposition values, are only available for a limited number of crops. As is standard practice in soil exposure assessments, surrogate deposition values were assigned based on the plant structure and the type of spray equipment used. This was done for the crop groups as described in Appendix E which, in some cases, include a broad range of crops/plant structures. Therefore, in selecting the surrogate value, a worst-case approach was necessary. Should the proposed use (GAP) be limited to a single crop or subset of crops then the applicant may wish to propose to use a more appropriate crop interception/deposition value. In this case, it is then essential that the selected value is also aligned to that used for the soil exposure assessment.

6.2.7. Risk envelope approach for Tier 1 exposure assessment for spray applications

As previously discussed, shortcut values have not been included in this version of the guidance document since the online calculator tool will calculate the exposure assessment. However, Member States noted that the shortcut values were also useful for performing a so-called 'risk envelope approach' for product assessments or when there are multiple crops in the GAP. A risk envelope is where the relative exposure from one use is compared to that for another use under assessment i.e. extrapolation between crops. It is only applicable for screening and Tier 1 exposure assessments. To help perform risk envelope assessments, Annex E contains an acute DD and a reproductive DDD, for a single application of 1 kg a.s./ha, for all avian and mammalian GMS species, for all crop groups given in Appendix E. An fTWA factor was not used for the reproductive assessment.

6.3. Specific considerations for seed treatment applications

6.3.1. Screening tier exposure assessment for seed treatment applications

No screening assessment is available for seed treatment applications.

6.3.2. Tier 1 exposure assessment for seed treatments

Birds and terrestrial mammals can be exposed to pesticide active substances when plant protection products are applied as seed treatments, pelleted (treated) seed (usually a pill of clay or other inert material encasing the treated seed), a pesticide bait (e.g. those used for to target slugs) or bulb/tuber treatment. The route and level of exposure to terrestrial vertebrates will depend on the seed type, and associated agronomic practices, in addition to the chemical characteristics of the compound. In some specific cases, i.e. granules ingested as source of food, the recommendations included in this paragraph for seeds may be also followed for granules, see Section 8.3.

For substances applied as solid formulations, in the majority of cases, the exposure from the direct intake of the seed/granule/bait will be higher than the exposure through the ingestion of food contaminated with dust drift. Nevertheless, to be consistent with TAI assessment for spray applications, when harmonised and agreed dust drift values are available, then the exposure methodology should be updated to cover exposure via dust drift.

The Tier 1 exposure assessment for products applied as seed treatments should be performed for two general types of generic model species (GMS). Firstly, a GMS directly consuming the treated seed/bait/tuber/bulb is selected. The GMS parameters will depend on the size and type of treated seed/bait/tuber/bulb. For the purposes of this guidance document, small seeds are defined as seeds with a diameter of $\leq$ 0.5 cm and large seeds are defined as those with a diameter of $>$ 0.5 cm. It should be noted that the risk assessment for treated seed/bait/tuber/bulb is intended to cover both the situations where the seed/bait/tuber/bulb is left on the soil surface and those where animals seek the planted seed/bait/tuber/bulb below the soil surface. The list of seed types and generic model species to be considered are summarised in Annex B, with background information available in Appendix F. The last

group of GMS for this scenario are those which consume seedlings germinating from the treated seed/bait/tuber/bulb.

While addressing the relevance of the seedling scenario as a relevant route of dietary exposure for birds and mammals, particular consideration should be given to the systemic translocation of the active substance. For areas of risk assessment other than ERA, the definition of systemicity relies on the evaluation of metabolism studies in primary and rotational crops (e.g. as detailed in European Commission, 2018). However, this assessment may not necessarily be relevant for – or protective of – birds and mammals feeding on young seedlings from treated seeds, which may be considered as a worst-case scenario. For this reason, poor systemic translocation, *per se*, is an insufficient argument to dismiss the seedling exposure scenario. Indeed, pesticides may directly contaminate young plant tissues (e.g. the coleoptile or cotyledons) during emergence. The likelihood and magnitude of this contamination is proportional to the penetration, redistribution and translocation of pesticides into plant tissues (e.g. translaminar or systemic).

Another relevant exposure route may be the consumption of seed parts through plucking of seedlings or ingestion of the treated seed coat (i.e. the outer, protective layer of a seed), which may adhere to seedlings during germination. This exposure scenario may be considered covered by the treated seed scenario in Tier 1, but these two scenarios need to be separately considered in higher tiers.

The GMS triggered depends on the type of seed under assessment and is summarised in Annex B. The most efficient way to select the correct GMS for the Tier 1 exposure assessment for both treated seed/bait/tuber/bulb and seedling germinating from the treated seed/bait/tuber/bulb is to use the online tool (EFSA, online[40]). This tool will also select the correct exposure parameters and perform the exposure calculation.

The conceptual model for dietary exposure to GMS taking treated seed/bait/tuber/bulb and seedling germinating from the treated seed/bait/tuber/bulb is summarised below. The following equations calculate the (daily) dietary dose ((D)DD) for GMS assuming a mixed diet containing *n* food items. The proportion of time spent foraging in the treated area (PT) is fixed in Tier 1 and is assumed to be 1. The proportions of food items in the diet (referred to as the dietary proportions, PD) are fixed for the Tier 1 GMS. In the following equations, PD is represented by the sum of the exposure coming from each of the dietary components (*i*).

$$\textbf{Acute DD} = \textbf{FIR} \times \sum_{\mathbf{i}}(\mathbf{C_i} \times \mathbf{PD_i})/\mathbf{BW} \tag{20}$$

In this equation, FIR is given by Equation 6, while (C_i) = the concentration of the a.s. in food item i (see Section 3.2.2.2).

$$\textbf{Reproductive DDD} = \textbf{FIR} \times \sum_{\mathbf{i}}(\mathbf{C_i} \times \mathbf{PD_i} \times \mathbf{ftwa_i})/\mathbf{BW} \times \mathbf{PT} \tag{21}$$

In this equation, FIR is given by Equation 7, while (C_i) = the concentration of the a.s. in food item i (see Section 3.2.2.2). Note that in the Tier 1 assessment, PT is assumed to be 1.

Note, the equation for the long-term DDD indicates that both the proportion of food item in the diet (PD) and the proportion of treated food obtained in the treated area (PT; in Tier 1 fixed to be 1) can be accounted for in the exposure assessment. However, for treated seed, it is not appropriate to refine both ecological parameters (PT and PD) in the Tier 3 exposure assessment.

where

Acronym	Explanation	Unit	Further information
Acute DD	Acute dietary dose for the Tier 1 GMS	mg a.s./kg bw	–
Reproductive DDD	Reproductive daily dietary dose for the Tier 1 generic model species	mg a.s./kg bw per day	–
Bw	Body weight of model species	g	Annex B
C_i	Concentration of the pesticide active substance on the food item i.	–	–

[40] https://r4eu.efsa.europa.eu/app/birds-mammals

Acronym	Explanation	Unit	Further information
	The concentration of the pesticide active substance on the seed/bait/tuber/bulb	mg a.s./kg seed/bait/tuber/bulb	Taken from the GAP table of the pesticide product under assessment
	For the germinating seedling, it is calculated by the concentration on the seed/bait/tuber/bulb divided by a dilution factor of 5	mg a.s./kg	Taken from the GAP table of the pesticide product under assessment, Appendix M
	Other food items the concentration is zero.	mg a.s./kg	–
FIR_i	Food intake rate for food item *i* by the model species	g fresh weight per day	Section 6.1.3, Appendix G
$fTWA_i$	Time-weighted average factor which accounts for degradation on the food item i during a 21-day period. When deemed appropriate(1).	[–]	Section 6.2.5.3
	For Tier 1 assessments of treated seed, the value is assumed to be 1.	[–]	–
	For Tier 1 assessments of seedlings, the value is assumed to be 0.28	[–]	Section 6.3.5 and Appendix M
PT	Proportion of food obtained in the treated area. At Tier 1, PT is assumed to be 1.	[–]	–
PDi	The proportion of food item i in the diet of the GMS.	[–]	–

(1): fTWA is only applicable for the reproductive assessments for pesticide active substances which meet the criteria defined in Section 6.1.4.

To contextualise the risk, in line with 10.1.1 of Regulation 284/2013[5], the number of seeds required to reach the regulatory acceptable dose (RAD) should be presented as follows:

i) Calculate number of seeds needed to reach toxicity endpoint = Toxicity endpoint (mg a.s./kg bw)/concentration per seed (mg a.s./seed)[a].
ii) Divide by assessment factor (10 for acute, 5 for reproductive).
iii) Express the number of seeds in terms of body weight of Tier 1 generic model species (Annex B).

[a]The concentration per seed may be specified in the GAP or can be calculated using the thousand grain weight of the seed.

6.3.3. Residue values for seed treatment applications

The residue value on the treated seed itself (mg a.s./kg seed) is given by the GAP and can be directly used in the Tier 1 exposure estimation for GMS consuming treated seeds.

The residue in seedlings germinating from treated seed is estimated using the concentration on the seed divided by a dilution factor of 5 (see Appendix M).

6.3.4. Time weighted average factor (fTWA) for seed treatments applications

For those substances which meet the criteria in Section 6.1.4, an fTWA (Equation 16, Section 6.2.5.3), over a 21-day period, may be considered in the estimation of the DDD for the reproductive assessment. Fundamental to the calculation of the fTWA is an estimation of the dissipation of the substance on the food item (DT_{50}). Insufficient data were available to derive a default DT_{50} for active substances on treated seed. Consequently, the fTWA for treated seed in Tier 1 is 1.

For seedlings, a default fTWA of 0.28 can be applied. This value was derived considering the growth dilution of the substance over a 21-day period (Appendix M).

6.4. Tier 2 exposure assessment approaches for spray applications and seed treatments

6.4.1. Refinement of initial residues values for spray applications

For spray applications, the default RUDs in different matrices were derived from an extensive database (Lahr et al., 2018).

In general, a refinement of the risk based on measured initial residues in the crop or other matrices (weeds, insects, etc.) based on the feed items relevant for the generic model or the focal species under assessment (as indicated in the GAP) is not typically an acceptable refinement, as the number of studies that may be available in a single dossier is limited when compared to the existing database. It is anticipated that EFSA will periodically update this database to ensure that new data are appropriately incorporated.

To refine default RUDs for the matrix under assessment, two options are possible:

1) Estimation of refined RUDs by merging substance specific initial measured residues with data for that specific matrix in the existing database. It is important to note that when the number of measured residues is limited compared to what has been considered in the database for that specific matrix, RUDs refined in this manner would be unlikely to differ significantly from the default RUD. Therefore, this kind of refinement would probably have a low impact on the overall outcome of the risk assessment. Double counting of measured residue values must be avoided, and therefore, only residue studies performed after 2016 can be considered.
2) Replacement of the default RUDs with substance-specific initial measured residues for the relevant matrix. This may be possible when the number of available new studies is high and, in the database, very few data points are included for the specific matrix under assessment. For example, this may be the case for the RUDs for weed/crop seeds, flower buds and some fruits where data were lacking, and surrogate values were selected (Table 12 and discussed in Appendix J). Although it was considered that this was the best available data at the time of drafting, it was agreed that there should be more flexibility in allowing the refinement of the initial residue value for the aforementioned matrices. Guidance for the evaluation of residue studies is given in Annex A. A critical appraisal tool (CAT) for residues studies was developed in a parallel project Lahr et al. (2022).

For the refinement of RUDs for food items below the crop, where the deposition value accounts for the interception of the crop, it should be checked whether the refined RUD value already accounts for crop interception. If this is the case, then the deposition value in the Tier 2 assessment should be fixed to 100% deposition to avoid double counting of the crop interception. Following any Tier 2 refinement of exposure using refined RUD values, an uncertainty analysis should be performed according to the recommendations in Section 13.3.

6.4.2. Refinement of initial residues values for seed treatment applications

To estimate exposure from products used as seed treatments, RUD values are not used as the concentration on the seed is known from the GAP and the concentration in the germinating seedling is estimated from this value. However, it is possible to refine the concentration in germinating seedlings in a Tier 2 exposure assessment. It is important that the seedlings are sampled at an early growth stage to best capture the peak residue. Further guidance on performing and evaluating residue field studies, including criteria for the number of required studies, can be found in Annex A. A Critical Appraisal Tool (CAT) for residues studies was developed in a parallel project Lahr et al., 2022.[41] Following any Tier 2 refinement of exposure considering measured residue values, an uncertainty analysis should be performed according to the recommendations in Section 13.3.

6.4.3. Refinements considering dissipation

Residues in food items generally decrease over time. In fact, dissipation and degradation of residues from plant material, seeds and arthropods may be more rapid than in other environmental media. For substances meeting the fTWA criteria defined in Section 6.1.4, a reasonable worst-case

[41] Were these both to be used in the exposure assessment equation, the resulting amount of treated diet would only be 56% (0.75*0.75), although neither data support a value that low.

assumption (i.e. a half-life of 10 days) is used at Tier 1 of the exposure assessment (for spray applications) as a basis for calculating the MAF and the fTWA. However, this default value can be adjusted if sufficient reliable substance-specific data are available. This is also true for treated seeds and seedlings from treated seed. Annex A gives detailed guidance on the refinement of DT_{50} and how to use refined values in a Tier 2 refined exposure assessment. A critical appraisal tool (CAT) for residues studies was developed in a parallel project Lahr et al. (2022).

Where a suitable DT_{50} value is obtained, this can also be used to refine the MAF_{acute} value used for the acute assessment and/or the moving window $MAF_{repro} \times$ fTWA for the reproductive assessment (see Sections 6.2.5.2 and 6.2.5.3). It should be noted that for seedlings from treated seed the default fTWA from tier 1 (based on growth dilution, Section 6.3.4) should be replaced with the refined value covering both growth dilution and dissipation. The available calculator tool can perform such refinements (EFSA, online[42]). For single applications, seed treatments and seedlings from treated seed, no MAF value is used and only the fTWA value can be refined (see Section 6.2.5.2). Following any Tier 2 refinement of degradation, an uncertainty analysis should be performed according to the recommendations in Section 13.3.

6.5. Tier 3 refinement options in exposure assessment

There are several options for Tier 3 studies that can refine the exposure assessment. As explained in chapter 3, Tier 3 options are based on studies with focal species. Since Tier 3 assessments usually are based on field studies, Section 6.5.1 presents general considerations for study protocols and reporting requirements of field studies. The identification of relevant focal species for the crop of concern is considered in Section 6.5.2. Guidance for Tier 3 field studies to refine PT and PD is presented in Sections 6.5.3 and 6.5.4, respectively. Finally, in Sections 6.5.5 and 6.5.6, avoidance studies and dehusking studies are discussed, respectively. Following any Tier 3 refinement of exposure, an uncertainty analysis should be performed according to the recommendations in Section 13.3. Furthermore, as part of any Tier 3 refinement there must be consideration of whether there is additional Tier 3 data available, according to the recommendations in Section 6.5.8.

Box 2: Clarification of the definition of PT and PD

In EFSA (2009) PD was defined as the 'the composition of the diet obtained from the treated area'. The exact definition of 'treated area' has been interpreted in two different ways i.e. data must be obtained for an animal foraging within the treated crop, or data must be obtained from a landscape with a sufficient coverage of the crop of interest. In reality, even for well-designed field studies where the animals are caught on crop, it cannot be guaranteed where the animal has been foraging in the relevant time period before being caught. Consequently, the practical working definition of PD is better defined to be '**the composition of the diet obtained from the area containing a sufficient coverage of the crop of interest**'. Nevertheless, as described in Section 6.5.4, it is recommended that field studies performed to measure PD still aim to capture animals in the field with the crop of interest.

In EFSA (2009), PT is defined to be 'proportion of an animal's daily diet obtained in the habitat treated with the pesticide'. For standard spray applications the working definition of 'habitat treated with the pesticide' is 'in-field'. Therefore, the working definition of PT is defined to be '**proportion of an animal's daily diet obtained in the in-field area for the crop under assessment'.**

Considering that the clarified definition of PD means that the data are not specific for the animal foraging in the treated area, it is important that there is no 'double counting' of the refinement. In the case of spray applications, all food items within the treated area will contain residues of the pesticide. Therefore, when PD is refined the impact on the outcome of the risk assessment will be limited. However, for seed treatments, only the treated seed, or seedling germinating from the treated seed, is assumed to contain residues of the pesticide. Consequently, a refinement of PD, using data obtained in the general vicinity of the crop, together with PT, would lead to a double counting of the same refinement (e.g. a bird caught in, or near, a cereal field containing 75% of cereal field in its stomach (PD) can also be assumed to have obtained approximately 75% of its diet in the treated area (PT)).[41] Therefore, for seed treatments (or any other types of application leading to only some of the food items in the diet being contaminated), **only PD *or* PT can be refined**, but not both. In those cases where data for both PT and PD are available, the evaluator will need to judge which of the two values are more reliable for use as a refinement, which will not necessarily be the most conservative of the two. Based on experience, it is likely PT will be the more reliable of the two parameters.

[42] https://r4eu.efsa.europa.eu/app/birds-mammals

6.5.1. General considerations for study protocols and reporting of field studies

The following paragraphs report some general considerations for conducting field studies. It is important to note that field studies may be used to identify focal species (Section 6.5.2), to refine the exposure assessment for focal species (Sections 6.5.3–6.5.6), or to refine the risk for focal species in an integrated exposure and effect assessment (chapter 7). Although the scope is different, these field studies have many common aspects, such as the study area and the agricultural practices which are described in Sections 6.5.2.1 and 6.5.2.2.

6.5.2. Study objective

A well-defined study objective is vital and should be reported for all field studies. It is worth noting that the study may be used to address a risk assessment question which does not completely match the original study objective. For example, the study objective may have been to identify avian focal species in an apple orchard in Germany whereas the risk assessment question is to identify key focal species in apple and pear orchards in the central zone. A difference between the study objective and the risk assessment question leads to uncertainty in the relevance of the data and will impact the outcome of the relevance evaluation of the study. This should be considered in the final uncertainty analysis (Section 13.3).

6.5.2.1. Justification of study area selected

The area selected should be representative of where the pesticide is used or is intended to be used (i.e. to address the risk assessment question). This may be across relevant geographical and climatic regions, within the MS if the pesticide is to be used in only one MS, within a zone, or, if the pesticide is used across a range of MS/zones, then it may be appropriate to have a selection of fields across the MS/zones. Depending upon the research question, the results of field studies can be used in the risk assessment at the EU, zonal or MS level. For example, PD and PT information obtained in the relevant crop for the same focal species of interest may be used in comparable areas, though it is expected that this will be somewhat limited as many factors affect the amount of time spent foraging in agricultural fields, as well as the diet at a specific time and place are quite sensitive and changeable. Field effect studies may use surrogate crops (see 7.1, below), and it may therefore be possible to extrapolate them further, assuming that the main climatic, ecologic (population) and landscape factors are adequately comparable or worst-case. The selected study site(s) should always be justified considering the spatial–temporal coverage of the research results to be used in the risk assessment. Furthermore, landscape characteristics must be well-described, with particular attention paid to the importance of these to the focal species under consideration (e.g. depending on the study objective, the following may be reported: presence of water bodies, compensatory areas, hedgerows, roosting sites, size and composition of field margins, etc.).

A justification must be provided that the study area selected for the field study is sufficiently realistic worst-case to refine default values. This is particularly important for non-commodity crops typically grown in relatively small volume and low intensity (e.g. with a coverage less than 20% of the agricultural area in the selected landscape), a high diversity of neighbouring habitats and focal species that do not prefer foraging in the (treated) crop of concern. See OECD, 2009a for further discussion of crops which are considered minor. Percentage coverage may be demonstrated by use of GIS and/or photographs. For annual commodity crops typically grown in relatively large volumes (e.g. winter cereals, potatoes, maize) the coverage of the crop in the selected study area should preferably be at least 33.3%, also considering common crop rotation practice. In some cases, there will be no crop rotation (e.g. permanent crops, not common practice). In those cases, the percentage should be considered on a case-by-case basis, considering the proposed and expected use areas, but should certainly be greater than 33.3%. Simulation studies on basis of population models for the focal species of concern (e.g. Kleinmann and Wang, 2017) may help to understand which landscape features determine the risk or can improve the field study design.

6.5.2.2. Description of agricultural practices

Pesticide use and GAP

Agricultural practices can have a significant impact on the presence of different species in the study area and therefore on the outcome of the study. However, agricultural practices, including pesticide application techniques and equipment, are not routinely specified in the GAP. As mentioned in the General Introduction to this guidance (chapter 2), this means that the lower tier risk assessments are

intended to address the most common application methods and equipment in current practice, simply as a matter of practicality. The same can be said for the higher tiers, where the WG has made recommendations focusing on the most common agricultural practices, acknowledging that other practices may require other types of study set-up. Some common variations in practice are mentioned specifically in Section 4.2.2, but the issue is not considered exhaustively, as in any case new innovations will necessitate new consideration. In addition, new legislation intended to encourage more sustainable agriculture in the EU (e.g. the EU Green Deal and the farm-to-fork strategy) will likely also have an impact on what is 'common' practice in the future. The development of 'environmental scenarios' as proposed by EFSA PPR Panel (2014) would greatly assist in defining appropriate conditions for both the higher and lower tiers of the risk assessment and would result in more specific results.

The lower tier risk assessment addresses application of pesticides to the full field (hectare) and does not, therefore, address spot application or other precision application techniques (e.g. via drones, drip irrigation, etc.). If a field study, particularly a field effect study, for example, is performed under these circumstances, it is noted that the conclusion of low risk will only be relevant for the conditions specified in the GAP table. This means that the resulting risk assessment which may indicate a high potential risk in the lower tiers, may result in no/low risk once higher tier data is submitted, but if the higher tier data are specific to precision application techniques, the eventual use will only have been shown to be low for those application techniques. This information should be communicated to risk managers, so they can consider risk management options e.g. a restriction for use to those techniques for which a low risk has been determined.

The following requirements and recommendations apply to all types of field studies:

- A justification must be provided that the area selected for the field study is sufficiently representative to cover the intended use of the PPP under investigation.
- The landscape characteristics must be well-described, particularly considering their relevance to the species under consideration (e.g. depending on the study objective, the following may be reported: weed/insect/seed abundance, presence of water bodies compensatory areas, hedgerows, roosting sites, size and composition of field margin, etc.). This can be supported by e.g. habitat mapping, photographs, GIS data, etc.
- For field effect studies in particular (as part of the Higher Integrated Exposure and Effect Assessment Tier; see chapter 7), the recent use of pesticides in the field(s) of interest, as well as other agricultural practices, such as fertiliser application and mechanical weeding, should be reported for the year preceding the start of the study. Both for field effect studies and for Tier 3 focal species, PT and PD studies, evidence needs to be provided to show that agricultural activities in the selected fields with the crop of interest did not substantially deviate from normal agricultural practice and/or may be considered as a realistic worst-case for the exposure/risk assessment.
- In order to minimise potential disturbance of the behaviour of birds and mammals during field studies, observations/assessments should not be performed during agricultural activities in the study area.
- When multiple applications of the PPP of interest are relevant to the risk assessment question, the refined field data should cover the season/period where the highest risk due to oral exposure is expected. A justification should be provided that the period selected for the field study is a sufficiently realistic worst case to refine default values. It is likely that multiple studies will be needed in order to adequately cover the period of use.
- The range of growth stages of the crop of interest during the study period must be indicated. In addition, the local meteorological conditions (e.g. rainfall, hours of sunshine, temperature) during the field study should be reported.

Tillage

No or low tillage practices will generally increase the species diversity and density in the field itself and in the vicinity of the field (Best, 1985; Heroldova et al., 2017; Faria and Morales, 2019; Roos et al., 2019; Santamaria et al., 2019). To cover both no-tillage and no/low-tillage practices in the risk assessment, the WG recommends that field studies for crops which accommodate no/low-tillage practices should generally be carried out under those circumstances. This is assumed to also be protective of full tillage circumstances, where bare soils likely support lower diversity and density of species. Only in circumstances where it can be shown that full tillage is common practice for the crop

or area in question or represents a worst-case situation for the specific risk assessment question, should studies performed exclusively in full-tillage areas be considered sufficient. This could, for example, be established using a field study with the focal species in question to determine the worst-case agricultural practice (e.g. availability of residue-free seeds for granivores in a seed treatment assessment). In general, it is not possible to eliminate a (focal) species from the risk assessment based on a restriction to full tillage practices only if low-tillage practices are common as well in the region of interest (or by other methods such as removal of hedgerows, weeds, etc.), as this would be counter to the spirit of the regulatory requirement to protect biodiversity (see Section 4.2.2).

6.5.3. Identification of focal species for Tier 3 field studies

6.5.3.1. Background information for focal species definition

If the risk assessment fails in the Tier 1 and Tier 2 effect or exposure assessment approaches, it may be possible to refine the risk assessment by considering a specific focal species (FS) to replace the generic model species (GMS). A focal species is a real species which is present in the field, or immediate off-field, at the time of the application. The fundamental concept is that the risk assessment for the selected focal species should be sufficiently protective of other animals within the particular feeding guild represented by the focal species, and as such, it should be prevalent and abundant in the field of interest. When refining the exposure or effect assessment to consider an FS to replace a GMS, care must be taken to determine whether an FS may be present from each relevant feeding guild. It is possible that, in the end, a feeding guild may not be represented in a particular crop, but the methodology used to determine a focal species should nevertheless have been sufficient and appropriate to identify focal species from all relevant feeding guilds. It is essential then, that the FS chosen based on the data is able to fully represent all species from that feeding guild present in the crop at the correlating time of year/crop growth stages and for the geographical location in the risk assessment question. In many cases, it may thus be appropriate to use the representative species within the guild with the lowest body weight. If other refinements are utilised, however, it may be necessary to determine further which species would be the most appropriate FS (i.e. worst-case/highest exposure according to the refined risk assessment) or to perform a risk assessment for several FS of the same feeding guild. Furthermore, when identifying feeding guilds, it is important to note that the feeding guild may change during the year (e.g. many bird species become insectivorous whilst brooding/rearing chicks). It should be noted that many species which are normally omnivorous may show a fully insectivorous diet during nesting/chick feeding. This underlines the need for studies to be performed at the correct time of year and also means that care should be taken when assigning observed species to feeding guilds (see Box 1). In addition, MS-specific agricultural circumstances may affect the presence of certain species and their diets and exposure levels (Section 6.5.2.2 and Appendix F).

6.5.3.2. Data used to determine focal species

Focal species can be determined using field studies, ideally involving sophisticated methodologies adapted to the various possible focal species. Transect count/observation (using night-vision where necessary), capture–mark–recapture, radio-tracking and the use of photography/videography can all be of use, depending upon the species in question. Generally speaking, birds are relatively easy to observe in field studies as they can be vocal and clear to see at certain times of the year (Gibbons and Gregory, 2006). Observation of mammals, on the other hand, can be more problematic and additional specific considerations depending on the type of animal of interest may be required for an adequate study. In any case, the observation methodology used for field studies should always be justified and the risk of bias considered.

It *may* also be possible to determine a focal species by evaluating published data. In the grey literature, data are available for which the aim has been to determine focal species in certain crops at certain times of the year. Other data that may be used to determine focal species may include survey or census information. Often studies reported in open literature lack sufficient details for use in a regulatory risk assessment as they were performed with a different objective. As a result, it is infrequent that information from the literature alone is sufficient to properly justify the focal species for the refinement of ecological parameters. Care should also be taken if data are derived from old studies which may have been performed in agricultural landscapes which are not comparable to contemporary landscapes. In all cases where literature is considered, a systematic review should have been performed to capture all relevant literature. Please note that ecological data on habitat use of birds

and mammals may be useful, where consideration is made of crop structure and the surrounding areas, season, food availability and the species in question. A list of European species can be found in Lahr et al. (2018); however, it is not exhaustive. Furthermore, regionally specific but relevant species may have to be considered by the authorities depending upon the proposed area of use. Generally, a weight of evidence would have to be considered based on the quality and quantity of the available literature, as well as specific considerations for the proposed use and area in question.

Regardless of whether literature or made-for-purpose field study data is used to determine a focal species, the studies should be evaluated according to the Critical Appraisal Tool (CAT) for ecological field studies (Appendix N and Annex F).

Finally, it is also essential to ensure that there are sufficient sites visited and that the landscape characteristics are well described. Information from one field only is unlikely to provide sufficient information on the prevalence and abundance of potential focal species, hence multiple fields/sites should be utilised (e.g. at least 20) (Benito and Dittrich, 2018) with different landscape characteristics. There is little information available as to the appropriate number of sites for focal species selection. For the time being, the WG therefore considers it pragmatic to follow the recommendations of Benito and Dittrich et al. (2018) as to this variable. Ideally, multiple studies should be available, adequately representing the proposed use area(s). If only a single bespoke FS study is available, it should at least be supported by an adequate literature review, and the uncertainty may be higher. Extrapolation between different areas should be sufficiently supported with other data, particularly referring to the presence of (and, where applicable, activity of e.g. for migrating species) the identified species and the similarity of the environmental scenario where the test was performed.

Birds

For birds, the most common methods are transect counts, point counts, scan sampling and, somewhat less often, mist netting (Benito and Dittrich, 2018). It is noted that some insectivores are aerial feeders and will only be present flying over a field. Although these do not land in the field, they should be taken into account for focal species definition as they may be consuming contaminated arthropods.

The time of day of the FS study should coincide with the time of highest activity, which for birds is generally dawn and dusk. Observations during the day may be additionally performed in order to fully cover the individual and species variations.

Mammals

For mammals, the most typical methodology for small mammals is live trapping (including capture-mark-recapture methods) but point counts and transect counts may also be utilised, often involving night-vision equipment. Camera traps are a more recent way of understanding which species visit fields. Since most mammals tend to be nocturnal or crepuscular, the observation times should in most cases be from dusk until dawn. As noted below, for different mammals, different types of traps may be required for sufficient information on the presence and density of various species. For some studies, this may mean a variety of traps should be used, depending upon the crop in question.

6.5.3.3. Evaluating focal species studies

The WG has provided a tool to assist with evaluating focal species, PD and PT studies. The tool lists specific points of consideration with these types of studies, including tips and examples (See CAT for ecological field studies, Appendix N and Annex F). For focal species studies, it should be noted that all species found in the study area may be considered focal species. The frequency of occurrence (FO) is not considered as important as feeding guild, body weight, and diet composition, and a low FO (e.g. $< 20\%$) should not eliminate a species from consideration out-of-hand. A vulnerable species which is infrequently observed but nevertheless clearly present may be considered a more appropriate focal species for the risk assessment, and more protective of similar species, than a less vulnerable species with higher occurrence. This will depend upon the PT data available (Section 6.5.3) and other species characteristics. An iterative process should be used to determine the most appropriate species amongst those observed in the focal species study. It is not uncommon that the refinement of exposure to focal species results in the species no longer being the most exposed species within the feeding guild (e.g. a low PT value). Some pragmatism should be followed of course, but applicants and risk assessors should always question whether a refined assessment is still sufficiently protective of other species within the feeding guild.

An additional aspect is that care should be taken when assigning animals to a feeding guild. As discussed previously, the majority of birds and mammals are actually somewhat omnivorous but may take a high proportion of a food type at certain times of the year (e.g. when fruit is available) or depending on food availability. Therefore, assigning animals to a feeding guild must be accompanied by a clear justification which accounts for the time of year and food availability in the study area. It may also be the case that the same species could be the representative focal species for more than one feeding guild (e.g. theoretically a European starling (*Sturnus vulgaris*) could be a focal species frugivorous or omnivorous) (see Appendix F).

Finally, it is noted that landscape and agricultural practice can have enormous impact on focal species studies. Please refer to the Introductory section for further considerations and guidance from the WG (see Section 6.5.1).

Further specific considerations are discussed below.

6.5.3.4. Specific considerations, presence of small mammals

Animals are often not evenly distributed; traps may have different detection probability relative to food source, ways of escape or proximity of the surrounding individual's home ranges (Watkins et al., 2010). To understand the dynamics of small mammal populations in and around treated fields, it is necessary to have sufficient trapping effort both in-field and in the nearby off-field. Since field borders are often more extensively utilised than mid-field areas, particularly when crop-coverage is lower, the trapping efforts should also be more focussed on edge of in-field areas, where applicable.

In addition, the detection probability of occurrence can be influenced by features of the local habitat or landscape, as well as behaviour of individuals (Gu and Swihart, 2004). Individual detectability is thus a major issue for small mammal studies, particularly for some species with special behaviour like the harvest mouse (*Micromys minutus*) which is known to be difficult to trap in the wild (Flowerdew et al., 2004). It is known that trappability of some small mammals may decline during summer months, as for the wood mouse (*Apodemus sylvaticus*) (Butet, 1994; Paillat and Butet, 1997), but it is even more pronounced for the harvest mouse (Trout, 1978; Darinot, 2020).

The harvest mouse and the hazel dormouse (*Muscardinus avellanarius*) are the only small mammals in Europe that make aerial nests in tall vegetation. It is possible to search for the nests themselves in order to determine the presence of the animals in a location, but this is not a very accurate measure and does not provide information on actual population size (Riordan et al., 2009). Nevertheless, if nests are detected it can be assumed that harvest mice or hazel dormice are likely potential focal species. For harvest mice, ground traps are generally unsuccessful, especially in the spring and summer when they have built nests in the canopy of grasses and forbs and jump between stalks rather than travelling via, or foraging on, the ground (Darinot, 2020). Harvest mice may nevertheless be trapped via ground trapping (Jensen and Hansen, 2003; Scott et al., 2008; Vogel and Gander, 2015), however, it is not known what level of accuracy as to population has been achieved in those cases and it is generally recognised that aerial traps in addition to ground traps are required in order to measure the presence of harvest mice (Darinot, 2020).

Shrews have an exceptionally high mass-specific metabolic rate (Vogel, 1976; Hanski, 1984) and small body energy reserves, which means that shrews have short starvation times and that they are consequently relatively poor dispersers, which makes trapping somewhat hit-or-miss due to distribution (Watkins et al., 2010). In addition, shrews are difficult to trap because many but the largest shrews, and in particular pygmy shrews, may be able to enter and leave traps without the door shutting (van Boekel, 2013). For this reason, similar to harvest mice, modified traps are necessary in order to appropriately monitor shrew (and especially pygmy shrew) populations and an additional quantity of bait may be necessary to do so without significant mortality. The starvation time for pygmy shrews is 1.6 times shorter than for common shrews due to lower fat reserves (Hanski, 1994).

6.5.3.5. Specific considerations, presence of bird species

It was further noted by the WG that many species which are normally omnivorous may show a fully insectivorous diet during nesting/chick feeding. This would have to be considered for any focal species studies performed in order to refine the risk assessment and is discussed further in Section 6.5.2.1. An additional point for avian focal species studies is a consideration of the migratory behaviour, and localised presence, of the birds within the feeding guild. For example, a focal species study for large herbivorous birds in the central zone could easily overlook a species such as the red-breasted goose (*Branta ruficollis*) which is only present in a specific region of Romania at certain times of the year. It is therefore recommended that, before initiating a study, the study director/applicant ensures that the

selected study site is carefully considered and justified and that this point be carefully evaluated by the assessors.

When assessing the presence of aerial feeders typical mist netting, only performed at the height of the crop, may not be sufficient to determine the presence of these species.

6.5.3.6. Diet of focal species

As explained in the preceding chapters, Tier 1 GMS are not real species but have assumed characteristics which result in worst-case exposure; this includes the assumed proportion of food items in the diet (PD). When a Tier 3 focal species has been identified it would be preferable to have high quality data to define an appropriate PD value relevant to the GAP under assessment (see Section 6.5.4 for refinement of PD). However, it is acknowledged that such data, covering all the locations covered by the GAP, are not always available. In the absence of such data, as a pragmatic solution, it is recommended that the PD values assumed for the Tier 1 GMS can also be applied to Tier 3 focal species since the PD values were selected to be worst-case. Conversely, refinement of ecological parameters (PD and PT) should never be applied to Tier 1 GMS and an appropriate focal species must first be identified.

6.5.4. Refinement of PT in reproductive RA

6.5.4.1. Definition of PT and use of refined PT values in Tier 3 exposure assessment

As explained in Box 2, PT is defined as the 'proportion of an animal's daily diet obtained in the in-field area of the crop under assessment'. Within the context of the pesticide exposure assessment for birds and mammals, it is assumed that this PT estimate is equal to the proportion of active time individuals of a relevant focal species spend in recently treated fields, with the relevant crop and crop growth stage, per day. Furthermore, it is assumed that food obtained from these treated fields is in line with the overall dietary composition known for this focal species and obtained from different habitats. If these assumptions are accepted, it can be further assumed that 50% of the daily food intake of an individual bird that spends 50% of its active time per day in a given and recently treated crop is likely to be contaminated with pesticide. Likewise, an individual that spends 70% of its active time per day will obtain 70% of its food from the treated crop (EFSA, 2009).

In the screening step, as well as at the generic model species step (Tier 1 exposure assessment), it is assumed that individuals find all their food in the treated area, therefore PT = 1. In reality, birds and mammals in the agricultural landscape may visit a variety of habitats within a single day and may obtain their food from a variety of fields. Therefore, in Tier 3 exposure assessment, it may be possible to use more realistic estimates of PT.

Since it cannot be excluded that mortality of individual animals is caused by a short-term foraging event in a pesticide-treated field, currently a refinement of PT in the acute exposure assessment is not accepted. In the longer term reproductive risk assessment, however, refined PT values may be used.

6.5.4.2. Tracking and PT estimation

PT can be estimated indirectly by radio/thermal imaging/GPS-tracking of individuals of the relevant focal species by assuming that time spent foraging is represented by 'active' tracking records. For a manual on radio-tracking is referred to Kenward (2000) but note that automatic acquisition of detailed animal movement data via GPS-tags has evolved (Singh and Bais, 2018; Gottwald et al., 2019; Katzner and Atlettaz, 2020).

According to Prosser (2010), the observational information obtained in crops whilst tracking generally supports the assumption that foraging is the predominant 'active' behaviour. However, observed instances where the animal is performing some other activity (e.g. singing, nest building) should be excluded from the 'active time' data. In addition, 'inactive time' data (e.g. resting, hiding) should be excluded from the data set used to calculate PT, since by definition individuals cannot forage while they are inactive. A justification should be provided for how the activity of the animal was determined. By comparing for each radio/thermal imaging/GPS-tracked individual, the daily 'active time' in treated fields of a certain crop (and relevant crop growth stage) with the total recorded 'active time' in all habitats present in the area of farmland monitored, the proportion of total daily 'active time' spent in the treated crop is obtained, considered equivalent to the PT in that crop.

Available tracking studies obtained for specific focal species indicate that PT values may show high inter- and intra-individual variation (e.g. Prosser, 2010; Ludwigs et al., 2017; Ludwigs et al., 2022),

while species-specific PT values also may show considerable variation between crops, agricultural landscapes and seasons (e.g. Prosser, 2010; Kleinmann and Wang, 2017).

6.5.4.3. Requirements and conditions for valid PT field studies

Studies to determine PT should be evaluated using the critical appraisal tool (CAT) for ecological field studies (Appendix N and Annex F). The following requirements and conditions for PT field studies should be considered:

- See for generic requirements of field studies with focal species Section 6.5.1.
- If possible, the site selection process for the PT study should be discussed with the Member State competent authority. In addition, several sites need to be pre-selected and the rationale for the final site selection should be described. Within a selected study site, at least five study fields with the crop (and relevant growth stage) need to be randomly selected for animal tracking. If fewer than five fields are investigated, then it needs to be appropriately justified and may be considered appropriate on a case-by-case basis.
- The proportion and spatial configuration of the crop (with the relevant growth stage) and other crops/habitats in the study area should be described, as well as the exact location of study fields and/or semi-field study sites (e.g. enclosure studies).
- As with all ecological studies, a justification must be provided for the appropriateness of the focal species selected based on a focal species study (see Section 6.5.2).
- It should always be borne-in-mind that focal species selection should be considered as an iterative process and, with this in mind, deriving a summary PT value indicating a low proportion of diet obtained in a treated area may lead to doubt as to whether the focal species selected is sufficiently protective of other species within the feeding guild (also see Sections 6.5.2 and 6.5.3.6). A low PT, however, may also be an indication that the crop is not attractive for the respective feeding guild in general.
- In the selected farmland, the individuals of the relevant focal species are preferably trapped in the crop. For certain mammal species, however, trapping may be possible only in close proximity to the crop (this should always be justified). Healthy individuals are tagged and released, preferably where they were trapped. In addition, relevant data for each tagged and released individual should be recorded and reported, such as species, date and location of (re)trapping, body weight, sex, developmental stage. The weight of tagging devices (e.g. radio transmitters) should be < 5% of the animal's body weight to ensure that the behaviour of the tagged individual is not influenced significantly (Kenward, 2000). Justification needs to be provided on the trapping and tagging strategy adopted, and potential consequences for PT derivation and the well-being of the animals. To allow animals to become accustomed to tagging devices, it is recommended to track them at least 24 h after tagging.
- Per single PT session, ideally continuous telemetry for daily activity periods of the tagged individuals should be conducted. Therefore, nocturnal species need to be tracked at night, diurnal species during daylight or, depending on the activity period of the focal species of concern, all day long. Preferably, multiple PT sessions with the same-tagged individuals are conducted for several days (not necessarily consecutive) to better cover longer term behaviour, but the required growth stage of the crop should be the same in this period. To obtain insight into the extent of intra-individual variation, including observations with a period of several days between sessions is recommended. This, because the likelihood that an individual changes its foraging behaviour increases with time. For PT sessions lasting less than one full 24-h active period, it must be justified that this information can nevertheless be used as a realistic worst-case proxy. Alternatively, it should be demonstrated that the likely bias that shorter observation may have on the estimation of PT can be estimated and corrected for. For a more detailed description of how to deal with PT sessions that are shorter than one full 24-h active period is referred to Appendix 29 of EFSA (2008).
- If, during a tracking session, it is not possible to determine an animal's location due to loss of the (radio) signal, the habitat should be recorded as 'position not assignable'. These 'position not assignable' time periods must be assigned as 'potential foraging' in the crop to obtain a realistic worst-case exposure scenario in the PT estimation. Only if proof is provided that the 'position not assignable' time periods with high certainty do not concern the crop and are less than 10% of daily activity period might they be excluded from the daily PT estimation. If the corresponding time periods are longer, the data from the animal in question must be excluded

from the session, unless proof is provided that they can be used for a realistic worst-case PT estimate.
- Study design, field methodology, location and number of trapped and tagged animals, duration and number of observation sessions, individual PT values, etc. should be reported in detail, including technical limitations of the selected radio/thermal imaging/GPS-tracking approach such as maximal distance to receive reliable telemetry signals, accuracy or battery life.
- If a valid PT value cannot be obtained for at least 10 confirmed consumer individuals (individuals actually foraging in-field during at least one tracking session) on study fields at a particular study site, either the number of study fields of the study site should be extended in the course of the study (if not in conflict with the required growth stage of the crop) or a second PT study in another farmland has to be conducted with the same focal species and in the same crop (and a similar growth stage). In the latter case, the PT values from the different studies may be combined to calculate summary PT values for the risk assessment.
- In higher tier risk assessment for small mammals in TAI habitats, refinement of PT is not considered appropriate. Mitigation measures (e.g. to reduce drift exposure) would be more appropriate here.

6.5.4.4. Tracking and inclusion of individuals in the summary estimate of PT

There are two main methods for the selection of individuals of a relevant focal species to track:

a) To focus on the crop and to track only those focal species individuals that were caught and/or observed shortly before tagging in (or in close proximity to) the treated target crop (recommended for new regulatory studies);
b) To focus on the species and to track focal species individuals captured in local farmland habitats where they are most abundant, not necessarily in (the life stage of) the crop of concern (not recommended, but older studies may have adopted this method).

Both approaches may provide useful data. However, it is necessary to consider that the estimated PT will generally be different. This will reflect the fact that they are derived from different populations. The birds or mammals studied by means of method (b) better represent the farmland population whereas the birds or mammals in (a) are a subset that spends potentially more time in the crop of concern (EFSA, 2008).

Having applied either method (a) or (b), it is necessary to further consider which individuals from this data set are used to determine a summary PT for the Tier 3 exposure assessment.

The first proposed option (the 'confirmed consumer option') is to consider only those birds or mammals that visited the crop after tagging, i.e. confirmed consumers only, so only individuals that have at least one daily activity period with a PT > 0, irrespective of method (a) or (b) described above.

The second proposed option (the 'potential consumer option') is to consider all birds or mammals, that actually visited the crop in at least one tracking session (at least one daily activity period with a PT > 0) supplemented with individuals captured and/or observed shortly before tagging in the crop and that have a PT = 0 (also in case of multiple sessions). The 'potential consumer option' can be considered a less conservative, but not necessarily less realistic, approach when compared with the 'confirmed consumer option'.

6.5.4.5. Calculation of summary PT values

As summary PT to be used in the Tier 3 exposure assessment, the daily 90th percentile PT (PT_{90}) is recommended to calculate from the PT values of all tracked individuals, if possible, separately for the 'confirmed consumer' and 'potential consumer' options and preferably with its 90% confidence intervals. As a default, in case of multiple observations on the same tracked individual, the mean 'daily' PT value for that individual is used in calculating the PT_{90}.

If proof is presented for preference of the focal species to forage in the crop of concern, this may be a reason to select the 'potential consumer option'. The difference between the mean PT (= mean over all PT values) and the median PT_{50} in combination with the difference between the mean PT and the median PT_{90} can be used to evaluate the attractiveness of the treated crop for the selected focal species. Also, the Jacobs Index (Jacobs, 1974) might be used as a measure of preference for the crop. This index ranges from −1.0 to +1.0 and values > 0, particularly those near to 1.0 are an indication for habitat preference.

Statistical techniques like parametric bootstrapping can be used to calculate PT_{90} values and its confidence intervals. As PT data are limited between 0 and 1, the Beta distribution likely provides a

good fit for these proportional data. Prosser (2010) showed that for bird and mammal PT data, the 'method of moments' performs better than the 'maximum likelihood method' for Beta distributions. Also see the detailed proposal for analysis of PT data in Appendix 29 of EFSA (2008). According to this appendix, bootstrap methods can be categorised as *parametric* or *non-parametric* bootstraps. Non-parametric bootstraps repeatedly resample from the same data set and the results of such a procedure will be critically dependent on how representative the underlying data set is. Small data sets are less likely to be representative and the confidence limits obtained by non-parametric bootstraps are likely to be underestimated. Therefore, parametric bootstrapping may be preferable for small tracking data sets. For the analysis of PT data, the following approach is proposed (for further details and references, see Appendix 29 of EFSA (2008)):

1) From field study *n* PT values are obtained, where *n* is the number of birds observed during one tracking session.
2) A beta distribution is fitted (distribution A) to all *n* PT values.
3) A random sample of sample size *n* is taken from distribution A.
4) Again, a beta distribution (B) is fitted to the new random sample.
5) From distribution B the 90th percentile (or another estimate) of PT is calculated and recorded.
6) Steps 3–5 are repeated many times (e.g. 1000 times), each time a random sample of size *n* is taken from distribution A, a new beta distribution is fitted and the 90th percentile is recorded.
7) Finally, the upper 95th (or other) one-sided confidence bound is calculated by ordering all 1000 estimates of the 90th percentile from low to high and picking the value of the **95th** place (or other) in the sequence.

When presenting summary PT values, a justification on the statistical methods used, and proof of a proper fit of the statistical model to the basic PT data, needs to be provided.

As an alternative for the default approach described above (based on the empirical 90th percentile using observed individual mean 'daily' PT values as input), the summary PT_{90} value to be used in Tier 3 reproductive exposure assessment may be refined by considering inter- and intra-individual variance in 'daily' PT values by pooling data and using Monte Carlo simulations (Ludwigs et al., 2017; UK Health and Safety Executive, 2019). In this Monte Carlo bootstrapping approach, the mean 'daily' PT values for many 'virtual' individuals (e.g. 10,000) over 21 days are derived (Ludwigs et al., 2022) and these data are used to calculate a 'virtual' 21-d PT_{90}. Note that this approach is valid only if a time-weighted average factor can be used in the reproductive exposure assessment (see Section 6.1.4). In addition, this approach requires that for enough 'confirmed consumer' or 'potential consumer' individuals (where applicable), 'daily' PT values of multiple sessions are available. Furthermore, the Monte Carlo simulation approach requires that PT values used as input are independent (see e.g. Crocker and Langton, 2019), i.e. the proof that the intra-individual variability in 'daily' PT values is comparable to the variability in 'daily' PT values between individuals. Since it usually is difficult to demonstrate this convincingly (e.g. from a statistical point of view due to limited number of repeated sessions with the same individual), the WG recommends using in first instance the observed mean PT value of each individual as input for Monte Carlo simulations.

Only if sufficient proof (based on statistics and biological and ecological knowledge) is provided that the different 'daily' PT values of repeated sessions with the same individuals can be considered as independent, the 'daily' PT values of all tracked individuals may be pooled and used as input for the Monte Carlo simulations to derive mean 'daily' PT values for a large number of 'virtual' individuals over 21 days and to calculate a 'virtual' 21-d PT_{90}. Clear guidance on what constitutes sufficient proof of independence of empirical PT data cannot be given yet, because of the limited number of available examples of 'virtual' 21-d PT_{90} derivation published in literature (e.g. Ludwigs et al., 2017, 2022) and the absence of agreed-upon statistical techniques and data sets (technical guidance) by experts from Member States and other stake holders. For the time being, regulatory decisions to use a 'virtual' 21-d PT_{90} based on independent empirical 'daily' PT values can only be accepted if the aforementioned technical guidance is developed.

Generally, to evaluate summary PT values, the fully digitalised underlying data need to be submitted allowing to check the calculations. In addition, a clear description and motivation of which session information has been used and discarded in the PT calculations is mandatory.

6.5.4.6. How to use the summary PT in Tier-3 exposure assessment

In selecting a suitable summary PT in the refinement, the required level of protection and remaining uncertainties need to be considered. For example, if for the refinement the median PT_{90} is selected, this implies that, respectively, with 50% certainty 90% of the individuals that visited the treated crop had a PT value that is lower (and consequently 10% of the individuals higher) than the selected summary PT. Also note that in PT field studies usually one focal species is selected and assumed to represent other species with a similar feeding guild. Therefore, it is important to select the species of the relevant feeding guild that most likely shows the highest PT and overall highest oral exposure. If this cannot be predicted clearly, several species might have to be tracked. In addition, a limited sample of individuals within the population of the selected focal species is assumed to represent the behaviour of the whole population in PT studies. Consequently, for a realistic worst-case assessment, the focus should be on tracking individuals that likely show a high PT (e.g. confirmed or potential consumer individuals). Furthermore, since usually a limited number of activity days in the life of an individual bird or mammal is assessed and assumed to represent its overall long-term behaviour (relevant for reproductive risk assessment), it is important to track at least a subset of individuals on several days (including observations with a period of several days between sessions) to assess intra-individual variation.

Although selecting a percentile for PT does not automatically provide the same percentile of TERs, due to the potential influence of the other parameters and factors, the WG recommends selecting the median 90th percentile PT (=PT_{90}) if for more than 10 individuals of a focal species PT values are available from a valid study. In selecting the summary PT to be used in the Tier 3 exposure assessment for birds and mammals, the following decision scheme is proposed if the general requirements of higher tier field tests (Section 6.5.2) and the specific requirements and conditions for a valid PT field studies as mentioned in Section 6.5.3.3 are met:

A: For the relevant focal species, a 'daily' PT value is available for each of more than 10 confirmed consumer individuals.

Yes: Go to B
No: Request an additional PT study, or use expert judgement to decide whether the highest empirical 'daily' PT value or the upper bound of the PT_{90} can be used in the Tier 3 exposure assessment

B: For the relevant focal species at least 20 empirical 'daily' PT values for confirmed consumers are available (including repeated sessions per individual and PT = 0 values)

Yes: Go to C
No: Select the median PT_{90} using observed individual mean 'daily' PT values of confirmed consumers as input (confirmed consumer option), irrespective whether they are obtained from single or multiple tracking sessions

C: Proof is presented for preference of the focal species to forage in the treated crop, at the relevant growth stage, and the study area is strongly representative of the area of where the PPP will be used

Yes: Go to D
No: Go to E.

D: Select potential consumer individuals for the PT assessment (potential consumer option): For > 10 individuals empirical 'daily' PT values are available for repeated sessions and the reproductive exposure assessment allows the use of the time-weighted average factor

Yes: Select the 'virtual' 21-d PT_{90}, using **observed mean 'daily' PT values** of potential consumer individuals as pooled data for Monte Carlo simulations. (*Note that regulatory decisions to use a 'virtual' 21-d PT_{90} based on independent empirical 'daily' PT values can only be accepted if the aforementioned technical guidance is developed*).
No: Select the empirical median PT_{90}, using observed individual mean 'daily' PT values of potential consumers as input.

E: Select confirmed consumer individuals for the PT assessment (confirmed consumer option): For > 10 individuals empirical 'daily' PT values are available for repeated sessions and the reproductive exposure assessment allows the use of the time-weighted average factor

Yes: Select the 'virtual' 21-d PT_{90} using **observed mean 'daily' PT values** of confirmed consumer individuals as pooled data for Monte Carlo simulations. (*Note that regulatory decisions to use a 'virtual' 21-d PT_{90} based on independent empirical 'daily' PT values can only be accepted if the aforementioned technical guidance is developed*).
No: Select the empirical median PT_{90} using observed individual mean 'daily' PT values of confirmed consumers as input.

6.5.4.7. Use of other sources of information in refining PT

Radio/thermal imaging/GPS-tracking studies will not be available for every combination of crop and focal species. In cases where these data are not available, an attempt may be made to refine PT using other types of information. However, it should be recognised that this will generally involve a much higher level of uncertainty, which must be taken into account in risk characterisation and decision-making (EFSA, 2009).

If radio/thermal imaging/GPS-tracking data are available for other species or crops, this may provide a useful starting point from which to extrapolate to the species and crop of interest. In some cases, it might be reasonable to treat the available data as a direct surrogate for the species and crop of interest, but with additional uncertainty due to the extrapolation. To address the additional uncertainty, it may be decided to select the upper limit of the PT_{90} estimate, instead of the median PT_{90} estimate as proposed in the decision scheme mentioned above in Section 6.5.3.6.

In other cases, it might be considered that some adjustment should be applied to the basic PT data set to make it more relevant to the species and crop of interest. In all cases, the extrapolation should be clearly documented and justified with reference to relevant supporting evidence, e.g. regarding the ecological similarity of the species, foraging strategy and crops involved, or from other types of data (e.g. observational studies) (EFSA, 2008).

Many types of information other than radio-tracking may contribute to the assessment of PT. The most useful are systematic visual observations (e.g. scan sampling observations) and mark–release–recapture studies, but even these are subject to substantial uncertainties. For example, visual observations of unmarked individuals cannot determine how PT varies between individuals and can estimate average PT (which may not be sufficient for risk assessment) only if the size of the local population is known. Less systematic data, such as informal or incidental observations, nest locations and general ecological or natural history knowledge can contribute to expert judgements about PT, but these are inevitably highly uncertain (EFSA, 2008).

6.5.5. Refinement of PD in Tier 3 exposure assessment

6.5.5.1. Definition of PD and use of refined PD values in Tier 3 exposure assessment

As described in Box 2, the practical working definition of PD is defined to be 'the composition of the diet obtained from the area containing a sufficient coverage of the crop of interest'.

At the screening step, it is assumed that individuals have a single diet and a dietary regime corresponding to a very worst-case exposure covering all species within the feeding guild that forage in the treated crop of concern. In exposure Tier 1, a mixed diet is used for some generic model species, but the proportion of each diet has been set based on conservative assumptions so that a dietary regime inducing a worst-case exposure is obtained. In exposure Tier 3, it is possible to refine the PD values in the reproductive risk assessment based on information on a more realistic dietary regime of the focal species, inducing a realistic worst-case exposure.

The more realistic diet should be determined based on supporting data from dedicated studies or from open literature.

6.5.5.2. How to determine a diet for birds

Several methods for measuring the composition of the diet of birds are used. Direct monitoring of the birds' food selection is often hindered by vegetation or the observation distance. Therefore, alternative methods have to be considered. Video recordings at bird nests can offer an insight into the nestlings' diet. However, the diet of nestlings may differ considerably from the diet of adults.

The investigation of faeces or that of stomach contents obtained via gastric lavage (stomach flushing) of adult birds is not subject to these constraints. For these approaches, it is essential to be able to identify food items on the basis of diminutive remains found in faeces or stomach flushing samples. A considerable difficulty is the differential digestibility of different food types. Few remains may be found either because few items were eaten or because food items were almost completely

digested. Calibration trials with captive birds can help to overcome this difficulty. Also, in some cases, it may be possible to apply correction factors taken from the literature. It should be kept in mind that correction factors can differ between the species. Therefore, the transfer of these factors from one species to another cannot be done without justification.

If radio-tracking is applied simultaneously to the collection of diet samples, the source (e.g. a specific crop) of the food items found in the sample can be identified.

It is also possible to analyse stomach contents for birds shot by hunters (partridge, pheasant, geese, etc.). However, the representativity of hunted animals for the population in question should be considered and discussed.

6.5.5.3. How to determine a diet for mammals

The method of faeces analysis outlined above in Section 6.5.4.2 can also be used for mammals. It is also possible to analyse stomach contents for mammals caught in snap-traps (mice, voles, etc.) or shot by hunters (hares, rabbits, etc.). However, the representativity of hunted animals for the population in question should be considered and discussed. However, stomach flushing is not possible for many mammals, like rodents and lagomorphs.

6.5.5.4. Trapping and sample collection

a) Bird trapping and sample collecting

To obtain an estimate of the diet of a focal species, it is necessary to trap birds using accepted methods (e.g. mist nets, whoosh nets, perch traps, spring traps), when they have access to the crop of interest. The study should also be done at the appropriate time of year. Nets and/or traps should be placed within or at least in close proximity to the target crop. The sites should be representative of where the pesticide is used or is intended to be used. This may be across relevant geographical and climatic regions, within an MS if the pesticide is to be used in one MS, within a zone, or if the pesticide is used across a range of MS, then it may be appropriate to have a selection of fields across MS.

Once caught, it is possible to obtain a diet sample of a bird by obtaining faecal and/or stomach flushing samples. Generally, faecal sampling is favoured over stomach flushing as it is not intrusive and tends to give more reliable results (see e.g. Jenni et al., 1990). Therefore, it is recommended that stomach flushing should only be used if no faeces can be obtained.

Samples could also be obtained from birds without trapping: Birds should be observed feeding in the relevant crop very carefully (e.g. by using high-quality spotting scopes). If an observed individual bird is setting a dropping and this dropping (or its exact location) can be kept in focus by the observer, a second field biologist can immediately approach this spot (guided via radio by the observer still looking on the spot) and collect the fresh droplet.

b) Mammal trapping and sample collecting

To obtain an estimate of the diet of a focal mammal species, it is necessary to trap individuals using accepted methods (e.g. live trapping methods). For mammals, more information on such methods, including for small species like harvest mouse, can be found in the recommendations for focal species. For mammals, stomach flushing is not possible and faeces analysis must be used to collect samples.

6.5.5.5. Sampling techniques and analysis

Faeces sampling

For collecting faeces of birds, trapped animals can be kept in a clean bird bag or held over a polythene sheet during handling (Sutherland, 2004). Droppings can often also be collected in the field, e.g. where birds perch, roost and at nests.

For collecting faeces of mammals, the presence of faeces in the trap can be checked. If no faeces are available in the trap, the animals can be kept in captivity until faeces become available. For species living in holes or nests, faeces should also be collected in the close vicinity of the living places.

Faeces samples should be stored separately and can be preserved with sodium chloride. It is important to keep samples separate and not to pool them. Separation of the samples serves two purposes; (1) to account for individual variability and (2) to apply correction factors to the food contents in order to take account of digestibility (see Section 6.5.4.6). Since these correction factors are derived from individual samples, proper application requires separate storage and analysis of each sample. Some suggestions to generate corrections factors are also given in Wolf et al. (2018).

Stomach sampling

A vaseline-coated narrow plastic tube is inserted into the stomach and lukewarm water is pumped in the stomach through a syringe until the contents of the oesophagus and stomach are voided (Sutherland, 2004). The obtained sample is transferred in a sample container and preserved with alcohol. As for faeces sampling, it is important not to pool the samples.

Stomach flushing is not possible for many mammals, like rodents and lagomorphs. However, it is possible to analyse stomach contents for mammals caught in snap traps (mice, voles, etc.) or shot by hunters (hares, rabbits, etc.) even if the representativity of hunted animals for the population in question should be considered and discussed.

Collection of reference material

For an accurate determination of the diet, a 'reference collection' is useful as it facilitates the identification of the taxa of the food items. Additionally, the collection of reference material or food items (such as invertebrates, seeds or plants) from the study area can help to estimate the original size of food items. As a rule, un-digestible fractions of one food item are not obtained as a whole but rather as food fragments ('remains'). To minimise the uncertainty of the size estimation of food items a regression analysis of the dimension (size) of the potential food items and parts of these food items likely to be found within the samples can be conducted. Reference material, i.e. potential food items can be collected within the crop and the assumed home range of the bird or mammal.

Sample analysis

Food items are investigated via microscopic analysis (reflected light microscopy and transmission light microscopy; see e.g. Flinks and Pfeiffer, 1988). Insect remains can often be assigned at least to the family. The remains of other invertebrates can mostly be assigned at least to the class. For the determination of the green plant material, structures of the cuticle, particularly stomata, are considered. Seeds can be identified by analysing husk remains.

The size of characteristic parts of invertebrates or plants (e.g. chitin fragments of arthropods, setae of earthworms, fragments of seeds (pericarp), plant material, i.e. area of leaves and stems) can be measured with a measuring ocular. The obtained sizes can be compared to the specimens from a reference library.

To quantify the number of food items (e.g. number of arthropods), within each sample food fragments found in the sample are counted and the minimum number of individuals required to account for the number of assigned remains is calculated (see e.g. Jenni et al., 1990). For example, two right mandibles and one left mandible of a beetle species can be attributed to (at least) two individuals. In plant material, the number of fruits and seeds can be obtained by measuring the area of the fragments and dividing this figure by the area of a reference fruit or seed. From remains of leaves, the area is measured and recorded.

The quality of the results obtained by the analysis of faeces or stomach-flushing samples depends significantly on the ability of the processor to identify the remains accurately. Trials using captive birds fed with a variety of different food items can help to quantify the recovery rate (see Section 6.5.4.6).

Some new methodologies using the identification of consumed species by characterisation of DNA present in faecal samples have been developed. In one approach, a standardised DNA region (DNA barcode) is PCR amplified, amplicons are sequenced and then compared to a reference database for identification (Wolf et al., 2018). Some potential biases still remain on these methodologies even a well-designed dietary barcoding study is likely to only provide semiquantitative data on the diet of a species (Pompanon et al., 2012). However, these methodologies would help to identify easily and rapidly digested food items by identification of DNA. They could also be used in combination with visual identification.

6.5.5.6. Data evaluation

Conversion of the number of food items in the faeces samples or stomach flushes to the number of food items actually ingested

When estimating how many food items were ingested by a bird or mammal based on the number of food items found in the faeces or stomach, to account for differing processing times of food items, correction factors (or correlation coefficients) should be applied. For each type of food, a specific correction factor has to be used, because during the digestion process, some food items may almost completely disappear while others remain almost intact. For example, earthworms or other soil

invertebrates are usually digested efficiently. In contrast, cuticle parts of many arthropods often remain unaffected and can easily be identified in the faeces. For example, correction factors for some food types can be derived from literature (e.g. Green, 1984; Jenni et al., 1990). It has also been shown that, for birds, the number of Araneida (spiders) ingested is about 3.9 times higher than the number found in the birds' faeces (100/25.5; Jenni et al., 1990).

Alternatively, focal species-specific feeding trials can be carried out in captivity to identify traces found in faeces and, only for birds, stomach-flushing samples when known food items are consumed. These data can be used to establish food item specific correction factors which compensate for differential digestion (Jordan, 2005). Feeding trials also offer the opportunity to account for the uncertainty and variability of correction factors.

Calculation of dry weight from length of food items ingested

To convert the calculated numerical proportions into mass proportions length–weight regressions derived from the literature (e.g. Rogers et al., 1976; Collins, 1992; Sample et al., 1993; Henschel et al., 1996; Klotz et al., 2002) can be applied, which are available for different invertebrate taxa and plant seeds. Hence, the approximate dry weight of food items can be calculated from their estimated length.

Quantification of percentiles of the diet

The quantification of the diet can be done by using the geometric mean from the samples. However, this method has some biases due to possible measurement errors or natural variability (e.g. body size food items).

6.5.5.7. Requirements and conditions for valid PD studies

Studies to determine PD should be evaluated using the critical appraisal tool (CAT) for ecological field studies (Appendix N and Annex F). The following requirements and conditions for PD studies should be considered:

- See for generic requirements Section 6.5.1.
- As with all ecological refinements, for PD studies, a justification must be provided for the appropriateness of the focal species selected for the study, based on a focal species study (see Section 6.5.2).
- It is therefore recommended that the food item type and abundance is assessed in the field selected for the study, as well as in a few neighbouring fields with a similar structure. This to demonstrate that the distribution, amount and type of food items available in the field study is adequately representative of the landscape.
- In the selected study area, the individuals of the relevant focal species are preferably trapped in, and/or in close proximity of the selected field.
- If several trapping sessions occurred during the study, the trapped animals should be individually marked to determine if several diet samplings coming from the same animal have been collected.
- Study design, field location and number of trapped and tagged animals, duration and number of trapping sessions, individual PD values, etc. should be reported in detail, including technical limitations of the selected approach such as trapping or sampling methods.

6.5.5.8. How to use the PD values in Tier-3 exposure assessment

In selecting the PD values to be used in the Tier 3 exposure assessment for birds and mammals, the following decision scheme is proposed if the requirements and conditions for a valid PD field study are met:

A) For the relevant focal species, valid and corrected PD values are available for ≥ 10 individuals

   a) **Yes**: Go to B
   b) **No**: Request an additional PD study, or use expert judgement to consider whether to use the PD values inducing the highest exposure from all individuals would be suitable to be used in the risk assessment

B) For the relevant focal species, PD values are available for at least 20 individuals

a) **Yes**: The geometric mean[43] of PD values from all individuals can be used in the risk assessment
b) **No**: Use the PD values inducing the highest exposure from all individuals in the risk assessment

Once refined PD values have been determined, then D(DD) should be calculated. The refined PD is included in this calculation by recalculation of the FIR based on the new PD. If the PD values are given in wet weight, then equation 6 (Section 6.1.3) can be used. However, if PD values are given in dry weight, then there is no need to correct for the moisture content of the food and the equation is given in Appendix G.

Location of the food item when PD is refined

In Tier 1 and Tier 2 exposure assessment, the GMS are assumed to consume a fixed diet and it is specified where each food item is located – either the crop/on the crop, weeds, or on the ground. For spray applications, the location of the food item has a consequence for the deposition value (DV) (see Section 6.2.6 and Annex D) used for the calculation of the residue on the food item. Consequently, when a tier 3 refinement of PD is used, the location of the food item must also be considered. For animals which consume both the crop and weeds, the refined PD values should specify the proportion of each food type, e.g. a herbivorous species may have PD values of 0.5 monocotyledon crop foliage, 0.25 monocotyledon weed foliage and 0.25 dicotyledon weed foliage.

6.5.6. Avoidance

A degree of avoidance of food contaminated with pesticides has been observed in dietary studies (5-day studies) with captive animals and in other types of studies (OECD, 2011b, 2016b). Therefore, it cannot be excluded that birds and mammals may avoid contaminated food, potentially reducing exposure and hence risk in the field. Avoidance can potentially occur independently of the method of application and may be influenced by a range of different factors like species, sex, type of treated food and prior food deprivation, colour of the treated food, etc. (OECD, 2011b). Rejection of toxic food may be due to different mechanisms:

- Avoidance, i.e. rejection due to the physical characteristics of the food such as taste, odour and colour;
- Aversion, i.e. rejection due to sublethal intoxication/illness.

Although aversion and avoidance have sometimes been used interchangeably, aversion is a different mechanism occurring only after consumption of the contaminated food, which actually implies that there is no initial avoidance at all (Mineau et al., 1994; McKay et al., 1999; Lopez-Antia et al., 2014).

It is hard to determine the precise mechanism(s) of avoidance for a given pesticide, so attention should focus on its effectiveness in reducing exposure and effects, and on how this may vary under field conditions. As highlighted above, particular attention should be paid fto differentiating between avoidance and aversion.

6.5.6.1. Avoidance as a refinement in risk assessment

An OECD draft guidance on avoidance testing for birds is available (OECD, 2011b updated 2016b). This may be consulted when designing an avoidance test for birds and adapted in the case of mammals.

Reductions in food consumption may also be measured in dietary toxicity tests (Luttik, 1998), but these normally do not ensure a high feeding rate. Various other methods exist, including some intended for testing the efficacy of avian repellents for protecting crops (see discussions OECD, 2016b) and approaches specifically developed for testing the avoidance of treated seeds by birds (e.g. Fryday et al., 1999, 2001; Esther et al., 2020). Standardised approaches may be useful for comparative assessment between substances (e.g. choice between two substances intended for the same pest). However, due to the complexity of factors affecting avoidance, interpreting data on avoidance from captive studies and assessing its implications for risk in the field is difficult and uncertain.

[43] Geometric mean is preferred but if it cannot be calculated then an arithmetic mean may be used.

Although avoidance is possible and was considered one of the possible refinements in EFSA (2009), it is currently considered challenging for a number of reasons:

- Use of vertebrate testing: The use of avoidance as a refinement in risk assessment implies that avoidance is appropriately demonstrated by performing additional vertebrate studies. In line with Directive EU 2010/63[31] and considering the uncertainties that such a type of refinement presents, the WG recommends that vertebrate testing is only considered as a last resort and other possibilities for refinement should be explored first.
- Selection of the most suitable species: In order to use avoidance to refine the risk, it should be demonstrated for the species at risk (i.e. the specific focal species). However, it is not appropriate to select a representative species for an avoidance test and extrapolate to other species within the same feeding guild. This is mainly due to the number of factors that may influence avoidance behaviour as explained above. Therefore, it is rather considered species specific, once demonstrated. Should avoidance be used in a refined risk assessment, then according to the principles of the focal species selection an iterative consideration of other potential focal species should also be done.
- Establishment of worst-case conditions mimicking field conditions: The testing conditions should resemble a likely situation in the field. The most important difference between a lab study with captive species and wild species is the feeding pressure. It is essential that lab studies use a feeding regime, including fasting of the animals, length of the feeding period allowing to achieve a realistic worst-case feeding rate. Furthermore, it is also important to consider the different energy requirements and behaviour traits in the different life stages (e.g. chicks) and physiological stages (e.g. migration). Finally, it is also challenging to predict the distribution and occurrence of treated food in the field (Lopez-Antia et al., 2014).
- Aversion cannot be considered for refinement and therefore it is important to differentiate between avoidance and aversion in any study (e.g. Mineau et al., 1994; McKay et al., 1999; Lopez-Antia et al., 2014). It should be noted that many of the avoidance studies performed in the past did not adequately distinguish between avoidance and aversion.

Overall, in acute risk assessment, where birds and mammals may reach a critical dose in one feeding bout, it is not considered suitable to consider avoidance. This is mainly explained by the fact that environmental stress (e.g. hunger, nutritional stage, competition, predation, cover) may often suppress possible avoidance effects and thereby increase the likelihood of ingesting a lethal dose in one feeding bout.

For reproductive risk assessment, avoidance may be considered if it can be underpinned by robust and valid realistic data allowing, among other factors, differentiation between avoidance and aversion, since the latter cannot be considered as a refinement option. In such a case, avoidance may be used as part of the weight of evidence. Nevertheless, as discussed above, additional vertebrate studies are strongly discouraged.

6.5.7. Dehusking/deshelling/depelleting

While Tier 1 uses GMS and worst-case assumptions to assess the exposure of birds and mammals, in the higher tier a possible option is to refine the exposure assessment by selecting a focal species (Section 6.5.2). Depending on the selected focal species, dehusking or depelleting of seeds may be considered an additional refinement option in Tier 3 exposure assessment of treated seeds (directly treated or pelleted seeds). It may also be possible to consider deshelling in the risk assessment for over-sprayed flower buds. However, several important aspects need to be considered before considering dehusking as a suitable refinement, such as, (i) the behaviour of the species within the feeding guild, (ii) the extent of dehusking and (iii) the quantity of the residues remaining in/on the dehusked seed or bud. For pelleted seed, it should be understood if the seed is treated prior to the application of the pellet or whether the treatment is contained in the pellet itself.

Pesticide residues will be mainly on the outside part of the seed/flower bud, thus, dehusking of these food items may reduce exposure in granivorous birds and mammals. On the other hand, the proportion of seeds/buds dehusked and the amount of the residue removed will depend on the species, the seed-/bud-type, the substances properties (e.g. palatability) and the handling of the food item by the species, and it may also be influenced by conditions in the field (Avery et al., 1997; Prosser, 1999; Prosser and Hart, 2005; Defra, 2010a; Brühl et al., 2011).

Further discussion on the available literature on dehusking/deshelling/depelleting behaviour is presented in Appendix O.

6.5.7.1. Performing studies to investigate dehusking/deshelling/depelleting behaviour

For animal welfare reasons, performing studies to understand dehusking behaviour of birds and mammals a non-toxic dye or substance that simulates the PPP (i.e. without toxic properties) should be used for the specific species in question. In addition, it is recommended that:

- The exposure reduction (% of traced substance in the original item that is not ingested by the animal) should be specifically calculated for each seed type and species.
- A minimum of 10 animals per experimental group should be used (balanced per sex).
- A palatable coating should be used.
- It is important to monitor and consider behavioural aspects of each species (for example, the amount of time the animals handle the seed).
- Other factors that may modulate the exposure when dehusking under field conditions should be considered and properly recorded during cage trials. Examples of these factors are food deprivation period (which for example may influence dehusking or hoarding behaviour; Defra, 2010a) or treated seed moisture (which for example may influence the exposure while handling/hoarding).

Values obtained with experiments performed with a dye may be considered as a proxy of exposure reduction but the way these values should be used in risk assessment must be considered and decided on a case-by-case basis and considering specific properties of the pesticide that may influence this parameter (e.g. cohesion, translocation).

6.5.7.2. Considering dehusking/deshelling behaviour in risk assessment

To use dehusking/deshelling behaviour in a risk assessment, the following steps should be followed:

- The first part of considering dehusking in a risk assessment is to derive an appropriate focal species according to the recommendation given in Section 6.5.2.
- Secondly, data are needed to understand the extent of the dehusking of the seed/bud by the focal species, with the correct type of seed/bud under suitable conditions as described above.
- Next it should be considered whether the dehusking behaviour of the selected focal species raises a doubt as to whether the focal species still represents the most exposed species within the feeding guild (i.e. are there other species present which may not dehusk to the same extent?).
- Finally, a suitable study to estimate the residues remaining on the dehusked seed/bud is needed. This measured residue value can then be used as an estimate of the concentration on the food item and follow the steps of a Tier 2 refined exposure assessment for the specific focal species (i.e. for treated seed it would replace the nominal concentration on the seed whereas for over-sprayed flower buds it would be used as a refined RUD value, see Section 6.4.1).

6.5.8. Maximum searching area to reach the RAD

The Tier 1 acute/reproductive exposure assessment from treated seeds and granules for birds/mammals feasibly could be refined considering the feeding area that must be exploited by an individual to obtain a dose that equals the Regulatory Acceptable Dose (RAD) i.e. the LD_{50}/AF or the 'BMD_{10} or NOEL'/AF. If it is demonstrated that an animal could not obtain the RAD in a feasible searching area, then a low risk may be concluded.

Although this refinement may be used for both acute and reproductive exposure assessment, special consideration of space–time factors (i.e. asynchronous sowing between local fields and surface seeds attractiveness) must be taken for the latter. Consequently, to address this issue, the seed availability immediately after sowing should be considered.

Moreover, additional digging after seeds of some species (e.g. Pascual and Hart, 1997; Kennedy and Connery, 2008; Curtis et al., 2019) must be also considered for acute and reproductive risk. Therefore, to consider the maximum searching area to reach the RAD as a refinement, it is first necessary to establish appropriate focal species (Section 6.5.2) considering both those that feed only from the soil surface and those which are known to dig for seeds/granules. For seeds which are buried

within the top 3 cm of soil, it is not appropriate to consider reduction in exposure to mammals based on reduced seed availability on the soil surface (Appendix P).

The maximum searching area is the maximum area that can be feasibly exploited by an individual of a certain bird or mammal species with the assumption it takes all of the sown seeds/granules available in the surface of that area. Considering the number of seeds/granules on the soil surface after sowing/application in certain crop (see below some general rules about how this should be measured), if the area that must be exploited by the animal to obtain a dose that equals the RAD (i.e. the $LD_{50}/10$ or the reproductive endpoint/5; i.e. searching area to reach the RAD) is similar to or smaller than the maximum searching area of any relevant focal species, this will indicate a cause of concern. However, there are no default reference values for a typical maximum searching area for generic or focal species and this could be highly variable depending on food abundance. The lack of reference values makes the consideration of the foraging area problematic for risk assessment. It is noted that the Northern Zone Guidance for higher tier risk assessment for Birds and Mammals (Northern Zone, 2021) proposed some values for small granivorous and omnivorous species (i.e. passerines & wood mice) and were indicated to be based on expert judgement. These values are:

- For small granivorous and omnivorous species 70 m^2 for acute and 35 m^2 for long-term exposure
- for wood pigeons and geese 140 m^2 for acute and 70 m^2 for long-term exposure

These values represent the minimum area necessary to exceed to rule out a risk (what could be considered equivalent to the maximum searching area). To properly ascertain whether these are reasonable for a range of focal species and conditions, the WG recommends that they are considered via expert elicitation (EFSA Scientific Committee, 2014) which would also elicit the uncertainty associated with such values. However, until such activity has been performed the WG considers that it would be reasonable to consider the above values for assessment.

If an applicant wishes to suggest a larger maximum searching area, then additional evidence should be provided for the selected focal species. However, it would be expected that careful consideration is given to the selected focal species used for the assessment.

If the seed density on the surface after drilling is to be considered in higher tier exposure assessment, standardised methods are needed to measure this density. Based on experience, some general recommendations can be extracted:

i) Multiple fields must be considered, the number of sampled fields should be sufficient to reflect the range of variation in soil characteristics and agronomic methods (seedbed preparation, post-drilling and drilling methods),
ii) In each field, availability of seeds in the headlands must be calculated separately from the one in-field and multiple measures must be taken in each section to reflect within-field variation.
iii) The sampling should be done within the first 24 h after sowing but maybe extended in time to measure seed disappearance.
iv) Since animals are likely to concentrate their foraging in areas of higher seed density (usually in the headlands), the area containing sufficient exposed seeds to provide a lethal dose should be calculated for the higher densities encountered (90th percentile) as well as the average. The same general rules can be applied for studies with granules.

Further information on performing seed availability studies together with possible mitigation measures are given in Appendix P. Unless defined in the GAP, or if there is reasonable certainty that only a certain type of driller will be used, then the risk assessment should consider both the worst-case (broadcast) and better-case (precision drilling) situation. It should be borne in mind that there is only a single regulatory zone for the approval of seed treatment products according to EU Regulation 1107/2009[2] and therefore information on drilling technique needs to be available for the locations where the crop is grown.

6.5.9. Consideration of additional Tier 3 data

The data collection performed by Lahr et al. (2018), aimed at systematically collecting relevant ecological data from field studies which have been performed specifically for the exposure assessment of PPPs and literature studies which provide relevant information. The search of the applicant dossiers covered the period 2010–2016, the search of the literature covered the period 1984–2016.

Furthermore, additional dossier studies were provided by Applicants (via the European Crop Protection Association, now CropLife Europe) and data were obtained from the Swiss Ministry of Agriculture. The data were evaluated for their relevance and reliability. It is important to acknowledge that the reliability criteria for ecological field studies were based on the recommendations of EFSA (2009), some of which have been updated in this version of the guidance document. It is particularly important to note that the WG has raised several concerns with the methodology used in focal species studies and their ability to detect some species e.g. very small mammals.

A further point to note is that many of the studies coming from dossiers are owned by specific Applicants and the data protection status under Regulation 1107/2009[2] has not been checked.

For these reasons, the data contained in the Lahr et al. (2018) database cannot simply be applied to regulatory risk assessments of PPPs under Regulation 1107/2009[2]. Nevertheless, the WG considered that the information in the database should not be ignored, and, in fact, it was recommended that the database is updated with any new data when it becomes available. The WG recommends that, when a refined Tier 3 exposure assessment is performed, the Tier 3 database should be consulted, and relevant information extracted for the specific purpose of providing context to the risk assessment.

Where relevant data is identified, the most appropriate approach to use the data would be for the Applicant to merge the data from their study with the one(s) in the Tier 3 database. However, it is acknowledged that this will often be unfeasible as, even if they have access to the study, they may not have the raw data required.

Therefore, it was considered that the most suitable approach was for any relevant information to be reflected as part of the uncertainty analysis for higher tier assessments. This serves several purposes:

> Although, variability between ecological field studies is inevitable, understanding whether there are other relevant studies available providing similar information may reduce uncertainty with the data in the dossier under assessment. Equally, when other relevant information is available which gives conflicting information, this may indicate a higher level of uncertainty in the data in the dossier.
>
> If relevant information is available, it may help address some of the uncertainty Member States may have regarding extrapolation of data in the dossier to their Member State/Zone.

Therefore, the WG suggests the recommendations in the proceeding paragraphs are followed when focal species and ecological (PT/PD) field studies are used for a Tier 3 exposure assessment. To recap, the information in the Tier 3 database can never be used alone for a regulatory risk assessment under Regulation 1107/2009[2]. It is the responsibility of the applicant to provide suitable data in their dossier and of the RMS to evaluate it for use in the risk assessment.

The following steps are only needed when ecological data are included in the dossier and are used for a Tier 3 exposure refinement. It is expected that Applicant will consult the Tier 3 database and present a summary of relevant information as part of their proposed refined risk assessment. The Member State risk assessors can then account for this when evaluating the Applicants' proposed refined risk assessment.

6.5.9.1. Consideration of the Tier 3 data base for data on focal species

When a focal species is identified based on the information in the dossier, the Tier 3 database (Lahr et al., 2018) should be searched (using filters) for other studies relevant for the GAP (i.e. taking in to account the location of the study, the crop and the growth stage(s)).

- All potential focal species included in the Tier 3 database should be reflected and discussed in the risk assessment.
- If a focal species with a lower BW than those identified in the studies presented in the dossier is detected, the level of uncertainty, and hence reliability, of the dossier focal species study should be questioned. Unless appropriately justified, the refined risk assessment for the larger focal species cannot be assumed to cover the smaller focal species.
- To address this additional uncertainty, and to cover the smaller focal species, the Applicant may present a risk assessment to address this lower body weight species, as well as those identified in the focal species study.
- In addition to the specific information contained in the database, a reflection of the quantity of relevant supporting data in the Tier 3 database should be included uncertainty analysis.

- It is emphasised that the Tier 3 database can only be used to reduce/contextualise uncertainty and cannot be used to identify focal species for refined assessment.

6.5.9.2. Consideration of the Tier 3 data base for data on ecological parameters PD/PT

When an ecological parameter (PT or PD) is refined with information in the dossier, the Tier 3 database (Lahr et al., 2018) should be searched (using filters) for other studies relevant for the GAP (i.e. taking in to account the location of the study, the crop and the growth stage(s)).

- If relevant ecological information is available for the focal species of interest, and the Applicant has access to the raw data, then the data should be appropriately combined.
- If this step cannot be taken, then the relevant data should be reported and discussed in the risk assessment and uncertainty analysis.
- For a multitude of reasons, some variation in ecological parameters between studies is expected.
- Values from the database should not replace those in the dossier (even if more conservative) but the range of variation should be discussed. If more conservative parameters are identified, it may affect the certainty, hence reliability, of the data included in the dossier.
- In addition to the specific information contained in the database, a reflection of the quantity of relevant supporting data in the Tier 3 database should be included in the uncertainty analysis.
- It is emphasised that the Tier 3 database can only be used to reduce/contextualise uncertainty and cannot be used to identify ecological parameters for refined assessment.

7. Integrated exposure and effect assessment tiers for birds and mammals

Field effect studies and population model studies concern integrated exposure and effect tiers and should focus on a sufficient number of vulnerable focal species of vertebrate wildlife. The results of well-conducted studies might be directly compared with the operational (specific) protection goal in a weight-of-evidence approach to conclude low or regulatory unacceptable risks. Following any integrated exposure and effect refinement, it is expected that an uncertainty analysis is performed according to the recommendations in Section 13.3.

7.1. Field effect studies

7.1.1. Introduction

This section focuses on the use of effect field studies to detect or quantify mortality or reproductive impairment of wild birds and mammals caused by exposure due to a realistic application rate of the PPP in a specific crop. Effect field studies have many features in common with field studies that aim to refine the exposure assessment for focal species. For information on general considerations for study protocols and reporting of field studies is referred to Section 6.5.1 and for the identification of focal species for the Integrated exposure and effect tier to Section 6.5.2.

Field effect studies may be considered when the refinements of effect assessment Tier 1 or 2 and/or Tier 1, Tier 2 and Tier 3 exposure assessments still cannot exclude a risk (see Figures 2 and 3 in Chapter 3). Field effect studies encompass a large number of variables, many of which cannot be controlled. Due to the high uncertainties involved, studies from only one location are unlikely to be sufficient, and they are most likely to be used in a weight of evidence consideration.

7.1.2. Advantages and disadvantages of field effect studies

Aimed at the direct measurement of the effects of concern under realistic field conditions, field studies to detect mortality and reproductive effects can take account of all routes of exposure and – depending on the number of study sites – many relevant sources of variation. In addition, field effect studies may address both direct toxic effects and indirect effects, e.g. due to treatment-related shifts in food availability. The uncertainties surrounding the sensitivity of test animals or the extrapolation from lab to field (i.e. uncertainties inherent in the effect assessment) may be addressed via direct measurement of potential effects in the field on species present in the field. However, field effect studies have large uncertainties surrounding the representativeness of the situation wherein the study was performed for the whole area of use of the substance in question, and the ability of sampling/

measurement methodology utilised to capture an effect. A main challenge in field effect studies is demonstrating that the study has been performed with an appropriately worst-case environmental scenario, which can be considered to sufficiently cover the extremes of possible species exposure and vulnerability. Furthermore, adequate monitoring/evaluation of the endpoints in question may be less feasible and there may be uncertainty as to whether it was sufficient for the particular case in question. If no effect is observed, it must be plausibly shown that these uncertainties are minimal and did not significantly factor into this result to conclude a low risk.

7.1.3. Performing and evaluating field effect studies

There is no internationally agreed standard protocol for avian and mammalian field effect studies/monitoring. The USEPA protocol (OPPTS 850.2500, USEPA, 2012b) is still current, although field studies are no longer requested by USEPA as part of higher tier assessment for pesticides risk assessment. In Europe, papers and recommendations from two workshops held in the 1980s are available (Greaves et al., 1988; Anonymous, 1990; Somerville and Walker, 1990), but no official guidance or protocol exists.

Field effect studies have not been widely used in prospective risk assessments in the EU. This is in part owing to criticism of the methodologies used and/or a reluctance of applicants to invest in such studies without knowing whether they will be accepted by regulatory authorities. Furthermore, frequently it is the potential reproductive risk that drives the risk assessment for birds and mammals and performing effect field studies to address reproduction/population effects is complex and may be challenging. Several improvements have been made to the methodologies in recent years (e.g. Dittrich et al., 2018; Fülling et al., 2018; Ruß et al., 2018) and effect field studies have the potential to provide useful information for risk assessment, preferably in a weight-of-evidence approach.

As reported in Section 6.5.1, although the scope of a field study may be different (identification of focal species, refinement of the exposure assessment for focal species or refinement of the risk for focal species in an integrated exposure and effect assessment), these field studies have many common aspects, such as the study area and the agricultural practices which are described in the Sections 6.5.2.1 and 6.5.2.2.

Before choosing and using study methods, relevant literature should be consulted. Such literature includes the USEPA guidance (OPPTS 850.2500) and workshop publications cited earlier (Greaves et al., 1988; Somerville and Walker, 1990). However, in order to address the objectives of each study, the methods described or recommended in these sources should be considered, modified and justified on a case-by-case basis.

A broad range of species (e.g. five species of the appropriate feeding guild[44]) should be studied to take account of the wide variation in toxicity between species.

If effect field studies are performed to address the risk to birds or mammals from secondary poisoning, additional considerations to the study design are needed. Specifically, it is important that the study methodology is able to cover the time period where peak residues are expected: This may be some time after application. In any case residue analysis should be performed on the food item of interest.

There are two main approaches regarding the number of sites to be investigated for effect field studies: 'extensive' and 'intensive' approaches (EFSA, 2009). The extensive approach uses simple techniques such as carcass searching and census methods but covers many study sites. The intensive approach involves more detailed investigations, but on fewer sites. Both approaches have an inevitable level of uncertainty (chapter 13). Dittrich et al. (2018) proposed that a combination of extensive and intensive approaches be utilised. Whilst this seems a logical approach, and worth considering when initiating a new study, its necessity will depend on the risk assessment question and the other available data (e.g. if focal species studies are available extensively covering the geographical landscapes and the range of growing conditions then this may be used to justify the use of intensive approach studies).

A study protocol should also be available and deviations from the protocol appropriately recorded and justified. It is essential that the species of interest are known, and that the methodology

[44] The normal minimum number required for a species sensitivity distribution (SSD) analysis of vertebrates. An exception may be for feeding guilds where less than five species can be followed/are present. Argumentation to apply this exception should be supported with high-quality data. The species to be followed can be determined based upon e.g. the available focal species study. Species excluded from the lower tier assessment due to e.g. bodyweight, would nevertheless be relevant in a field effect study.

employed accounts for their behaviour (e.g. time of day, observation methodology, etc.). The distance between control and treatment fields should be adequate to ensure that there is no possible overlap of individuals, taking into account the home range(s) of the species in question. Similarly, the number of control and treatment areas required will differ based upon the species and endpoints concerned.

An untreated control is essential in effect studies. It is, however, acknowledged that a field or a plot with no pesticide use (chemically synthesised or organically derived) is seldom available in European landscapes. Furthermore, it is questionable whether a non-agricultural or protected wildlife (i.e. pristine) area would be sufficiently comparable to the test plot to be used as an untreated control. In many cases, therefore, it may be considered whether a 'surrogate' crop is appropriate (e.g. grassland) in order to ensure observation of an established population, adequate numbers of animals, and a lack of intensive agricultural disturbances. If a surrogate is used, the vegetation height and surrounding area should be as close as possible to the actual risk assessment question, or it should be fully explained how they may be considered appropriately worst case. In addition, the comparison to the landscape of the risk assessment in question, and the possible 'best-case-ness' for population dynamics (in comparison to the actual crops/landscapes in question) should be evaluated and discussed, particularly considering the potential for recovery and presence of source vs. sink areas. Regardless, it is expected that the use of all pesticides and rodenticides, in both the control area and test area, is transparently reported. Moreover, effort should be made to limit the use of other products around the time of application of the test item and no acutely toxic substances should be used in the control fields for a sufficient period (considering the fate and behaviour of the substance) prior to application. In cases where other PPP are used around the time of application, the study author/applicant should justify the why such applications do not affect the reliability of the study (i.e. specifically considering the length of time between the use of other PPP and the one of interest). The historical use of pesticides over the previous year should also be reported.

It is recommended that residue analysis is performed on the food items likely to be consumed by the species of interest (e.g. invertebrates, weeds, weed seeds, etc.). An assessment of the food availability may also be useful to provide evidence of the potential exposure of the animals and the representativeness of the field site.

In addition to the text that follows, the WG has developed a Critical Appraisal Tool (CAT) to help harmonise the evaluation of such studies – both for their reliability (or internal validity) and relevance for the risk assessment (also referred to as external validity). This tool is presented as an accompanying spreadsheet to the guidance document (see Annex G). Moreover, in Appendix N, general instructions on the evaluation of each item of the evaluation are reported.

Key issues to be considered by risk assessors in the evaluation are discussed in Sections 7.1.4–7.1.6.

7.1.4. Use of field effect studies in the risk assessment

Considering the aforementioned uncertainties, typically a field effect study can only be used as a part of a weight of evidence consideration on the potential risk of the substance in question.

Field effect studies may be used to refine the potential for acute and/or reproductive risks identified in Tier 1 and 2 effect assessments. These studies must sufficiently show that the proposed use of the PPP will not result in mortality, nor negatively affect the population density or normal make-up or functioning in line with the operational protection goal (see Section 3.1). By nature of the differences between species, a monitoring or field effect study will need to be adequately tailored to the specific ecology of the species in question. As a result, the sections below, and the accompanying CAT (see Annex G and Appendix N), can only provide general guidance as to study set-up and evaluation. Differences between individuals, particularly where the environmental scenario is concerned, should be expected and should be evaluated considering the ecology of the species in question. Population modelling (Section 7.2) can provide important support and information for field effect study development, particularly concerning environmental scenario and species-specificity. For example, field effect studies supported by population modelling may be helpful in showing/determining a realistic worst-case environmental scenario within the scope of the proposed use (Section 7.2). It may also be used to focus on appropriate endpoints or provide further weight-of-evidence for the endpoints in question.

Assessing the power of field effect studies

Owing to the lack of an agreed Specific Protection Goal (SPG) for birds and mammals, a quantitative 'acceptable' effect or level of uncertainty for interpretation of field effect studies is not yet

set by risk managers. However, in line with the operational protection goals for birds and mammals, statistically significant differences between mortality data and population-level measurement endpoints (that are considered biologically relevant) of selected focal species observed in field effect studies should not occur between control and treated fields. To conclude this, however, the power of the field effect study for the relevant measurement endpoints should be assessed.

The most common way of calculating statistical power for field effect studies and pesticide exposure to birds and mammals is to calculate the MDD as a post-hoc test. MDD is defined as 'the difference between the means of the controls and the treatments that must exist in order to conclude that there is a significant difference'. For regulatory studies the MDD classification given in the Aquatic Guidance Document (EFSA PPR, 2013) provides a good basis, but MDD class V is added for field effect studies with birds and mammals (see Table 13).

Table 13: Proposal on classes of minimal detectable differences (MDD) due to treatment-related effects on relevant bird and mammal endpoints (adapted from EFSA PPR, 2013)

Class	MDD	Comment
0	> 100%	No effects can be determined
I	90–100%	Only large effects can be determined
II	70–90%	Large to medium effects can be determined
III	50–70%	Medium effects can be determined
IV	50–20%	Small effects can be determined
V	< 20%	Very small effects can be determined

The common approach to report the power of ecotoxicological field studies for normally distributed and closely related t-distributed data is to calculate the MDD (see Duquesne et al., 2020).

$$MDD = (\overline{x}_1 - \overline{x}_2) = t_{1-\alpha,df} + t_{1-\beta,df}\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

$\overline{x}_1$	= arithmetic mean of controls
$\overline{x}_2$	= arithmetic mean of treatment
s_1^2, s_2^2	= variance of control and treatment
n_1, n_2	= number of control and treatment samples
$t_{1-\alpha,df} + t_{1-\beta,df}$	= t value with degrees of freedom to α and β
df	= degree of freedom for α and β

The power corresponds to the value (1 – β), i.e. the probability of observing an effect in the sample if one of a specified effect size or greater exists in the population. The type II error (failing to reject the null hypothesis when it is actually false) and the type I errors are defined as described in Table 14.

Table 14: Useful definitions for power analysis (and MDD calculation)

	Accept null hypothesis	Reject null hypothesis
Null hypothesis is True	1 – α	Type I error (α)
Null hypothesis is False	Type II error (β)	Power (1-β)
Definitions:		
Type I error: α = probability of rejecting the null hypothesis of no effect when it is actually true. The wrong conclusion is that the effect has occurred;		
Type II error: β = probability of accepting the null hypothesis of no effect when it is actually false. The wrong conclusion is that the effect has not occurred;		
Power: the probability of correctly rejecting a false null hypothesis, where the null hypothesis is the hypothesis that there is no significant difference between control and treatment groups.		

For example, if β is set at 0.20, this represents a power of 0.80, i.e. 80% chance of detecting an effect of a given size. In case significant effects of 25% are detectable (e.g. decrease in a specific reproductive endpoint of species A in the pesticide-treated fields with the crop under evaluation), and a power of 80% (i.e. β value of 0.20), then the assessor will conclude with high

certainty (80 times out of 100) on a significant effect corresponding to 25% effects in the pesticide-treated field. If the power is set to 50% (i.e. β value of 0.50), meaning that the assessor will conclude a significant effect corresponding to 25% of effects of the pesticide-treatment 50 times out of 100.

In laboratory OECD toxicity tests usually a α value of 0.05 and a β value of 0.20 is selected. For the time being, the WG recommends following this approach when assessing the MDD's of field effect studies for relevant birds and mammal measurement endpoints, although clear regulatory guidance for selecting a proper value for β in complex field studies is not yet available. Alternatively, the confidence intervals (CIs) concept of Mair et al. (2020) may be considered to evaluate the power of field effect studies. In addition, the closure principle computational approach test (CPCAT) might be used to evaluate the statistical power of regulatory field effect studies (Lehmann et al., 2018).

The WG is aware that the relevant level of effect that needs to be detected in a valid field effect study is difficult to set *a priori*, since it is dependent on the normal operating range of the relevant endpoint of the focal species of bird/mammal in unstressed field conditions (for similar landscapes and periods of the year). Furthermore, it is noted that MDD values (or other statistical power estimates) of relevant population-level endpoints may differ on different post-treatment sampling dates. For the regulatory evaluation of the MDDs of aquatic mesocosm studies on consecutive sampling days Brock et al. (2015) proposed a decision scheme. The WG recommends that similar technical guidance is developed on how to evaluate statistical power estimates for field effect studies with birds and mammals.

Until such technical guidance is available, the WG proposes:

1) The data be systematically explored, analysed and reported. Zuur et al. (2010) describe a protocol for data exploration to avoid common statistical problems in applied ecological research. Zuur and Ieno (2016), suggest a streamlined but comprehensive stepwise protocol for data analysis and presentation, which assists both the researcher and the evaluator in understanding the data set completely and determining the answer to the risk assessment question posed.
2) The level of effect that can be detected in the field effect study should be clearly reported according to the categories outlined above. The assessors must then decide based upon the weight of the evidence whether this level was appropriate to reach the operational protection goal(s) of no mortality and no effect on the population, based also upon the MDD outline provided above. This information is clearly reported to risk managers in order to contextualise the likelihood of actual risk.
3) Regulatorily accepted population models can be used to determine what level of effect would result in population effects under what time frame and/or environmental scenario. These can then be used to determine the appropriate study protocol for field effect studies. Again, the information for the choices made should be clearly reported to risk managers in order to contextualise the risk assessment and likelihood of actual risk. Alternatively, regulatory accepted population models might be used to complement the interpretation of field effect studies.
4) If there is mortality that can be attributed to the application of the test item in the crop under assessment (e.g. via measurement of residues in the carcass), this would be considered a breach of the protection goal.

7.1.5. Field studies to investigate acute mortality

As mentioned above, specific recommendations for study design cannot be given due to the myriad of situations covered and difference in necessary MDD depending upon the situation, however, some general recommendations are given in the following paragraphs.

The study protocol for an acute effect study should clearly state how mortality will be assessed, together with criteria for deciding whether observed mortality is due to the application of the test item. If residue analysis is included, the level of residue required to link exposure to mortality must be determined *a priori* and agreed upon by the RMS.

A key advantage of radio-tracking in an acute effects field study is that it addresses some of the uncertainty and concerns as to whether animals leave the study area before mortality occurs, or their carcass is not detected.

As the operational protection goal is to prevent mortality due to acute exposure, the WG considers that it would be reasonable if acute effect field studies can be interpreted using a combination of radio tracking and related techniques (Dittrich et al., 2018), together with robust investigations for the cause of death on detected carcasses. Should mortality be detected which is attributable to the test item (i.e. a carcass with residues above a predefined threshold) then this should be considered as a breach of the protection goal (i.e. high risk).

As discussed in Section 6.5.1, landscape characteristics and agricultural practices have a strong influence on the presence and behaviour of birds and mammals. Therefore, landscape characteristics and agricultural practices used in the study area must be adequately reported.

The methodology for assessing mortality should be justified and the potential for bias considered, noting that interpretation of results is difficult if the animals are not individually marked.

Available methods include (but are not limited to):

- Systematic searching for dead or sub-lethally affected individuals. This should include the treated area as well as adjacent habitats where exposed individuals might go to rest, roost or take cover (see Fryday et al., 1996). Searches should be carried out at appropriate times to maximise detection of casualties, taking account of the mode of action of the substance, while minimising disturbance that could artificially reduce exposure. Pre-treatment searches on at least two occasions are advisable to remove pre-existing animal remains and assess the level of natural mortality to aid interpretation or analysis of post-treatment mortalities. Search efficiency and rate of carcass removal by scavengers should be estimated using dummy carcasses.
- Radio-tracking to monitor activity and survival of tagged individuals (e.g. Prosser et al., 2006). The number of individuals should be sufficient to measure the level of mortality with the desired level of certainty. Casualties must be recovered very promptly and in a condition that is adequate to diagnose the cause of death (Dittrich et al., 2018).
- Post-mortem examination to diagnose cause of death: this may include residue analysis, biomarker assays (e.g. enzyme inhibition) and histology. Criteria should be defined *a priori*.

Complementary methods to investigate mortality events are:

- Capture-mark-release-recapture studies.
- Monitoring of biomarkers (e.g. enzyme inhibition). Repeated sampling from the same individuals may be desirable to control for high natural variability in biomarker levels, although this must be balanced against the risk that repeated capture will alter the behaviour of the animals and hence will bias the results. When possible, exposure should be measured in the same sampled animals (i.e. the measurement of the pesticide and/or its metabolite(s) and the biomarkers should be performed in the same animals), to better link the effect biomarkers to the actual exposure (levels).
- Visual observations to monitor populations and activity of birds and large mammals.

7.1.6. Field studies to investigate effects on reproduction

The operational protection goal for reproductive risk assessment is to ensure no treatment related effect on the population. As a result, field effect studies addressing a potential risk indicated by the lower tier reproductive risk assessment must cover multiple reproduction cycles and population cycles in order to adequately capture potential effects and address the operational SPG.

As with acute effect studies, the operational protection goal for reproduction effect studies is not quantitative and thus a quantitative recommendation on a power level for each reproduction field effect study cannot be given. Nevertheless, an *a priori* power analysis may be helpful to gain insight in the number of fields and/or individuals that ideally should be assessed (see Section 7.1.4). Fülling et al. (2018) for example, present data for 26 fields (control fields only) from four vole field studies which showed that at least two trapping sessions were required before a long-term study can reliably detect 50% difference between vole populations (determined via minimum number alive, hitherto MNA). The average MDD for MNA analysed with General Linear Mixed Models achieved for the studies was 18% (see Fülling et al. (2018) for further details). They further analysed the natural variation between untreated populations in the same area and year. This natural variation between

neighbouring populations was on average ± 53% (min. ± 17%; max. ± 110%). All these populations were stable, and thus, an effect study with an MDD below 50% MNA was interpreted by Fülling et al. (2018) as capable of detecting relevant effects, which may negatively influence the population. Note, however, that the natural variation between untreated neighbouring populations will be different for different species. Furthermore, for the same species this natural variability (normal operating range) may be different in different regions/geographical locations as well. Nevertheless, this type of analysis is key to determining the level of effect which should be detectible in field studies for effects on reproduction/populations but must be performed on a species-by-species level using appropriate field studies on untreated fields, or as mentioned above, using accepted population models.

Depending upon the weight of the available evidence and the species in question, the most vulnerable/sensitive life stage may be unknown. If this information is available, this might be used as an endpoint for the field effect study. In the absence of this information, the main variables of population density (e.g. minimum-number-alive (MNA) and population composition (e.g. number of juveniles and adults, etc.)) may be considered as possible endpoints, taking into account the uncertainties and potential for inherent biases involved in MNA determinations (Pocock et al., 2004; Byrne and Do Linh San, 2016).

Also similar to acute effect studies, the 'extensive' and 'intensive' methodologies may be used for population/reproduction effect field studies. The methodology used should be tailored not only to the use pattern in question but also considering population dynamics. For example, populations that exist in relatively smaller areas may be best monitored via 'intensive' methodologies rather than extensive methodologies, if this would also be appropriate to the risk assessment question (area of proposed use). The endpoints (as mentioned in the paragraph above) should be determined statistically, and the power of the study to detect an effect on the endpoint(s) in question should be reported, using the MDD categories outlined above. The study should cover the full breeding season of the species in question and should cover more than one geographic area. While live trapping and capture-mark-recapture may be the best ways to monitor many mammal populations, it is noted that bird populations and some mammal populations may require other methodologies. In addition, the trapping caveats mentioned in the focal species study (Section 6.5.2) are of course also true for field effect monitoring. Live trapping is less useful for birds and some mammals. Population monitoring is therefore usually achieved via other means.

Nest searching and monitoring, for example, may be used. Large samples of nests are required to ensure that an adequate number are active at the time of pesticide application. 'Nest fate' may then be a way of determining the possible effects on the population level, though it is noted that there is no standard guideline for categorising different 'outcomes'. Ruß et al. (2018) propose some possible categories for nest fate, as well as ways to use the data to link them to population survival (via a standard 'nest survival' endpoint). It is noted, however, that more comprehensive nest monitoring has some advantages over this simple 'nest survival' endpoint, in that it can provide a quantitative measure of success (i.e. brood size) which, in conjunction with sampling for pesticide exposure, could be more sensitive for detection of potential effects of pesticides (Rands, 1985). The select methodology would need to account for the differences between altricial and precocial species. It cannot be assumed that a risk assessment, using nest monitoring, for an altricial species covers precocial species, or vice versa. A justification of the chosen methodology should be given taking into account the risk assessment question and the birds within the feeding guild(s) of interest (i.e. not only a single focal species). In addition, other endpoints, including some measured in laboratory reproduction studies (e.g. OECD 206 or OECD 416) can be monitored and thus allow a more direct link back to the Tier 1 effect assessment. A critical aspect of any assessment of breeding success is having sufficient properly defined control plots, though literature data on this can also be helpful, particularly since true untreated control fields may be difficult to find. It is noted that, whenever literature data is used to support the conclusions, it should have been retrieved in a systematic manner and will also need to be evaluated fully. Only then will the data analysis be able to detect effects of the test item (e.g. nest survival or brood size) and also to exclude the possibility that an increase in the number of failed nests is because of other pressures, such as predation and/or abandonment. Nest monitoring is used regularly for population monitoring in birds, and the public literature on the topic should be consulted for specific study design and evaluation, depending upon the species and area in question. Nest monitoring for aerial mammals such as harvest mice and dormice may also be useful, typically in conjunction with other methodologies (see Section 6.5.2). The WG has provided some recommendations on common methodologies, but it is noted that other methodologies are possible (e.g. camera traps) and are also considered potentially useful.

7.2. Enclosure studies

Under some circumstances, semi-field (enclosure) studies may be useful to exclude certain variables (i.e. predation, agricultural activities) and study the effects of a substance on a population.

In enclosure studies, a semi-field area is built in order to study an effect in the absence of confounding variables (Boonstra and Krebs, 1977; Gaines et al., 1979; Schauber et al., 1997; Hahne et al., 2011; Jochym and Halle, 2013; Eccard et al., 2022). Voles and other small mammals have been the most frequently studied species. The size of the enclosure required depends upon the space requirements of the species and endpoints to be studied, however the enclosures are generally walled, with a depth to exclude/discourage entry into/departure from the enclosure via digging and may include netting/fencing above to fully exclude predation. The species in question is/are then introduced into the enclosure. After a period of acclimation appropriate to the endpoints to be studied, external factors (i.e. in this case a PPP) may be introduced.

Enclosure studies may be particularly useful for studying specific endpoints related to population dynamics and behaviour more closely and in the absence of confounding variables. When considering the results of an enclosure study for use in the risk assessment, the iterative process around focal species must be considered (i.e. whether the species studied is representative of/realistic worst case for the variable in question for all focal species). Where appropriate, enclosure studies may be invaluable for understanding population effects. Furthermore, enclosure studies can be used in conjunction with modelling (see Section 7.3). Enclosures might be used to show the effect of specific assumptions on a population and similarly models might be used to extrapolate the results of an enclosure study to a 'field' situation, by including some factors excluded in the enclosure. In general, the statistical power considerations for field studies should apply to enclosure studies, as well.

7.3. Population models

7.3.1. Introduction to population models

Population models for bird and mammal species can be used to estimate the impact from exposure to pesticides. They translate lethal and sublethal endpoints as observed in individual-level toxicity tests to the population level by considering key life-history traits of the species and by simulating specific exposure conditions in pre-defined environmental scenarios typical for the agricultural landscape where the species occurs, and the pesticides are applied.

Based on the kind of variables used to characterise a population, and consequently how survival and reproduction are represented, Accolla et al. (2021) distinguish three model types:

Unstructured population models (synonymous to 'scalar' or 'ordinary differential equation' models)

In these models, the only state variable used is population size or total biomass. Any structure regarding age, size, sex or distribution in space is disregarded. All these aspects are implicitly averaged over the entire population, and in the risk assessment only the net outcome of pesticide exposure to survival and reproduction, the per capita growth rate, is considered.

Structured population models (synonymous to 'matrix' models)

In these models, the state variables capture certain aspects of population structure, such as age or developmental stage. Structured models represent the fact that demographic rates (life-history traits) depend on age or stage, and, consequently, the impact of pesticide exposure is age and stage group dependent. Demographic rates (life-history traits) are averaged accordingly within each age or stage group.

Agent-based population models (synonymous to 'individual-based' models).

In these models, each individual is represented and may differ from all other individuals, depending on its traits and behaviour. Many of these models are spatially explicit because they consider local interactions between individuals and the responses of individuals to local habitat features. These models are relatively complex and often harder to develop, parameterise, and analyse than unstructured and structured population models, but they can be more realistic and easier to validate with various aspects of real systems.

As a result of their greater flexibility, agent-based models may be more amenable to the addition of all key features that affect the responses of populations of birds and mammals to pesticide exposure in agricultural landscapes. Structured models, however, are relatively popular in ERA as well (see e.g. Forbes et al., 2016; Larras et al., 2022). For most populations of focal bird and mammal species in the field, the limitations of many unstructured models hamper their appropriate application in spatially explicit higher tier risk assessment. According to Accolla et al. (2021) there is, however, a trade-off between the need to incorporate a particular feature, data availability, and computational and/or mathematical effort that modellers have to consider when using population models to answer specific ERA questions.

Since the publication of the birds and mammal's guidance document in 2009 (EFSA, 2009), substantial advances in the development of population models for the prospective ERA of pesticides occurred, and several review papers were published (e.g. Galic et al., 2010; Forbes et al., 2016; Larras et al., 2022) and workshops organised (e.g. Forbes et al., 2015; Hommen et al., 2016; Forbes et al., 2021) on their potential regulatory use. In addition, several case studies on mechanistic effect models have been published for wild species of birds (e.g. Topping and Odderskær, 2004; Etterson and Bennett, 2013; Millot et al., 2015; Etterson et al., 2017; Topping and Luttik, 2017; Crocker and Lawrence, 2018; Moore et al., 2018) and mammals (e.g. Wang et al., 2001; Wang and Grimm, 2010; Dalkvist et al., 2013; Liu et al., 2013; Liu et al., 2014; Schmitt et al., 2016; Topping et al., 2016; Kleinmann and Wang, 2017; Wang et al., 2022) demonstrating the potential use of these models in higher tier risk assessments for pesticides.

Advantages of using population models in the ERA for pesticides are:

- They can make quantitative linkages between individual-level properties - such as survival, growth, reproduction and behaviour - and population-level properties - such as population density, age structure and growth rate.
- They can be used to assess population-level effects of focal species selected for modelling over short to long-term time periods (including multi-generation impacts) and different environmental scenarios representing different landscape properties and use patterns of pesticides at MS, zonal and EU level. In this sense, they are more flexible compared to e.g. single field effect studies.
- They add the value of mechanistic understanding by capturing the key biological mechanisms underlying the structure and dynamics of populations such as life-history traits, density dependence, behaviour, stochasticity and exposure-response relationships
- When TK and/or TKTD models are coupled to population models, they may allow a more realistic integration of exposure and effects
- They can address specific protection goals in a more realistic, consistent and transparent way, and even may be used to improve the operationalisation of the protection goals (e.g. by setting maximum levels and duration of effect than do not harm long-term population densities)
- They can be used as tools to improve site selection (e.g. realistic worst-case landscape), design and interpretation of field effect studies and to evaluate consequences of possible risk mitigation measures
- Sufficiently calibrated and validated population models might be used directly as a cost-effective higher tier risk assessment tool and in addition may allow reduction of animal testing
- Population modelling may reduce uncertainties identified at lower tiers and visualise remaining uncertainties by providing confidence bounds of modelled endpoints and show what may happen with these confidence bounds if different input values of key parameters are modelled.

Despite these advantages, some factors that hamper the regulatory use of population models for birds and mammals can be mentioned:

- Limited availability of appropriate essential input data to develop and run the population model, e.g. lack of key information on life-history traits in a realistic environment and/or sensitivity of the focal species selected. If information of surrogate taxa is used (e.g. toxicity data of standard test species) the remaining uncertainty needs to be estimated and addressed in the risk assessment (e.g. by applying a small assessment factor; see Section 7.3.3)
- Parameterisation of sensitivity to toxic effects in the population model usually is based on laboratory data, while species in the field may be more sensitive due to suboptimal conditions

- Field populations may suffer indirect effects due to a decline in food resources caused by pesticide application, while this often is not directly addressed in population modelling approaches
- The uncertainty that modelled focal species and environmental scenarios selected to run population models sufficiently address the risks of a specific pesticide in a specific crop for other species within the same feeding guild and in other regions of Europe
- Lack of consensus between regulators of different EU member states on (i) the role that population models should play in prospective ERA, (ii) different views on operational protection goals, and (iii) how to interpret uncertainty in model output
- Distrust in the 'validation statuses' of models and the notion that true validation of complex spatially explicit population models is not possible (for a discussion on this topic see Augusiak et al., 2014). Although full model validation may not be feasible because complete empirical data sets often are not available, it often is possible to verify essential components of models separately.

7.3.2. Guidance on the use of population models in regulatory ERA for birds and mammals

In 2014, EFSA published the 'Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products' (e.g. EFSA PPR Panel, 2014). The opinion identified several critical steps in order to set models within risk assessment, namely:

- Problem formulation, considering the specific protection goals for the taxa or functional groups of concern;
- Model domain of applicability, which drives the species and scenarios to model;
- Species (and life stage) selection, considering relevant life history traits and toxicological/toxicokinetics characteristics of the pesticide;
- Selection of the environmental scenario, which is defined by a combination of abiotic, biotic and agronomic parameters to provide a realistic worst-case situation.

According to EFSA PPR Panel (2014), model development should follow the modelling cycle, in which every step has to be fully documented: (i) problem definition; (ii) model formulation, i.e. design of a conceptual model; (iii) model formalisation, in which variables and parameters are linked together into mathematical equations or algorithms; (iv) model implementation, in which a computer code is produced and verified; (v) model setup, including sensitivity analysis, uncertainty analysis plan of model predictions and comparison with observed data; (vi) prior to actual use in risk assessment, the regulatory model should be evaluated for relevance to the specific protection goals; (vii) feedback from risk assessor with possible recommendations for model improvement.

EFSA PPR Panel (2014), recommended that mechanistic effect models be documented in a complete and transparent way by model developers and notifiers, and that model evaluation by regulatory authorities should consider each step of the modelling cycle. To facilitate the evaluation of mechanistic effect models, a comprehensive checklist is provided in Appendix B of EFSA PPR Panel (2014).

Amongst others, inspired by the recommendations of EFSA PPR Panel (2014), Raimondo et al. (2021), published a decision guide and modelling framework with a focus on population models, viz., Population model Guidance, Use, Interpretation, and Development for Ecological risk assessment (Pop-Guide). Pop-Guide is a taxa-independent model development approach which includes the following phases:

1) Definition of model objectives

   The overall objective of a model is to meet the intended use for the ERA by providing relevant endpoints within the constraints of the acceptable ERA uncertainty and available resources. Possible trade-offs with respect to generality, realism, and precision associated with the ERA objective are evaluated. The model required should generally be no more complex than what is required for the ERA trade-offs.

2) Data compilation

   The intention of this phase is to consistently survey, collect, and evaluate available data relevant to population models in the risk assessment. Data collection targets information

pertaining to the modelled focal species, the environment and chemical impacts. In cases for which data are lacking, taxonomic surrogacy and/or the use of trait-based information to reduce model uncertainty should be documented in this phase.

3) Decision steps to consider

The decision steps to consider concern (i) life history representation of the modelled focal species, (ii) relevant organism-level processes, such as growth and reproduction, (iii) population and spatial factors, such as density dependence, movement, behaviour and habitat characteristics, (iv) external factors such as diet and interspecific interactions, and (v) linking exposure to effects e.g. in relation to type of available toxicity data, endpoints affected and information on exposure in space and time.

4) Definition of a conceptual model

A conceptual model provides a high-level, graphical, and textual summary of the components and functions within a model and their linkages. The conceptual model makes assumptions explicit, particularly for processes that are not represented in the model due to lack of data and other considerations.

5) Model implementation and evaluation

This phase assumes the modeller extends the conceptual model to a fully parameterised computational tool following best modelling practices. The model evaluation hinges on implementation in appropriate software (i.e. coded) and includes data evaluation, sensitivity analysis, uncertainty analysis, robustness analysis, and model validation. To estimate how well the model and associated environmental scenario represents the population of the modelled focal species of interest in the non-stressed and stressed environment, independent empirical data need to be available for comparison to model outputs. However, full model validation may not be feasible because complete empirical data sets often are not available (for a discussion on this topic see Augusiak et al., 2014).

In developing and using population models as a higher tier tool in the ERA for pesticides and focal species of birds and mammals, it is recommended in this update of the Birds and Mammals Guidance Document to follow the recommendations on good modelling practice as described in EFSA PPR Panel (2014), with reference to Appendix B, the summary checklist of for model evaluation by the risk assessor, as well as Appendix C, the qualitative assessment of uncertainty in ecological modelling in this document. In addition, when using a population model to refine the risks it is recommended to consider the Pop-guide phases as described by Raimondo et al. (2021) in the documentation of the population model. Furthermore, a risk assessment based on a population model requires an in-depth motivation that the modelled focal species really is representative for the vulnerable species within the feeding guild potentially at risk. The regulatory relevance of selected modelled focal species to cover the risks within a specific feeding guild may be different for different regions and regulatory zones within the EU. Ideally, the appropriateness of the focal species selected for modelling should be discussed with and agreed upon in advance by the responsible regulatory authority. In addition, it should be motivated that the selected environmental scenario to run the population model is sufficiently realistic worst-case and that the inclusion of landscape considerations in the scenario sufficiently integrates the ecological, temporal-spatial and managerial variability in the ERA for the relevant (modelled) focal species and pesticide under evaluation. In this context, it should be realised, as shown by Wang et al. (2022), that it is not possible to define a generic worst-case landscape scenario that is fit-for-purpose for all bird and mammal species. In other words, the worst-case landscape scenario has to be determined for each modelled focal species separately.

Furthermore, the uncertainty surrounding model assumptions should be compared and correlated to the sensitivity analysis in order to determine overall uncertainty of the specific use of the model in question.

Note that both field effect studies and population modelling studies are considered in Integrated Exposure and Effect Tier approaches. If in a field effect study a population-level effect on a bird and/or mammal species is observed, and that is considered reliable and relevant, population modelling approaches might be used to put the observed effect in a broader spatial–temporal context or to estimate the possible effectiveness of risk mitigation measures. However, population modelling cannot be used to supersede the study-specific results of this field effect study as such.

7.3.3. Concluding remarks on regulatory use of output of population models in higher tier risk assessment

All tiers in a risk assessment scheme should address the same specific protection goal, but it is assumed that higher tier approaches, among which the use of population models, address the specific protection goal more realistically. In the acute risk assessment scheme for birds and mammals, the current operational protection goal is to avoid mortality due to direct toxicity of exposed individuals of birds and mammals that (temporarily) occur in treated crops, while in the long-term reproductive risk assessment scheme the current operational protection goal is defined as negligible population-level effects due to direct toxicity on pesticide-exposed bird and mammal species that (temporarily) occur in pesticide-treated crops.

Population models can make quantitative linkages between individual-level and population-level properties over short to long-term time periods and allow the simulation of impacts of different pesticide application rates. Consequently, showing that population-level effects of a specific pesticide application rate are unlikely to occur, seems to be the best operationalisation of the current protection goal in the risk assessment based on population modelling, at least under the condition that the environmental scenario used is sufficiently realistic worst-case and the selected modelled focal species are vulnerable representatives of the relevant feeding guild. A vulnerable bird or mammal species is defined here as a species with a high chance to become exposed (= relatively high DD or DDD), a high sensitivity to direct toxic effects (usually unknown for focal species so that that toxicity data of surrogate standard test species have to be used) and a high chance to suffer indirect effects. In this context, it should be mentioned that the treatment-related responses of birds and mammals in field effect studies may not only be caused by direct toxic effects but also by indirect effects (e.g. due to treatment-related shifts in food availability). Compared to field effect studies, population modelling approaches might or might not consider treatment-related indirect effects. This should be clearly described in the model documentation. Note, that lower tier assessments have their focus on direct toxic effects only, but in principle should be more conservative than in Integrated Exposure and Effect Tier assessments.

As mentioned above, whether a specific environmental scenario is sufficiently worst-case depends on the ecology of the focal species (e.g. behaviour, and habitat and food preference) and on the proportion and configuration of the treated crop, other crops, and semi-natural and natural habitats in the agricultural landscape of concern. One approach is to select a number of realistic agricultural landscapes as environmental scenarios. For example, Topping et al. (2016) used ten real Danish agricultural landscapes as a basis for population-level risk assessment of pesticide exposure to Brown Hare. Alternatively, a sensitivity analysis with the population model can be conducted to evaluate the properties of the agricultural landscape that have the largest influence of pesticide treatment in the crop on the focal species. This information can then be used to define a 'virtual' agricultural landscape that can be used as a realistic worst-case environmental scenario in the population model simulation. In all cases, a detailed motivation should be provided showing that the selected environmental scenario(s) is (are) sufficiently worst-case for the MS/region/zone covered by the population modelling approach.

As model output the (relative) density (abundance) of a critical life stage (e.g. reproduction or juveniles) or that of the total population of the modelled focal species can be used as an appropriate assessment endpoint.

Repeated model runs allow the calculation of the mean and 95% confidence limits for this endpoint in both control and pesticide-treated fields/agricultural landscapes. Note, however, that this 95% confidence interval is dependent on the number of iterations applied and the environmental scenario selected (e.g. size and structure of the landscape). Consequently, the impact of the number of iterations applied on the modelled endpoint and its 95% confidence interval should be described, as well as the 'worst-caseness' of the environmental scenario used.

A low population-level risk might be assumed if in the treatment and post-treatment periods (i) the mean of the population abundance for treatment simulations in the selected realistic worst-case scenario is always higher than that of the corresponding lower 95% confidence limit for control simulations and (ii) the lower 95% confidence limit of population abundance for treatment simulations is not consistently lower than that of control simulations (e.g. lower on a limited number of isolated data points only). The duration of the post-treatment period should at least be a full calendar year, but a multi-year assessment may be required to evaluate the potential impact of annual pesticide application in the agricultural landscape of concern, and in perennial crops in particular. Alternatively, a

biologically relevant 'unacceptable' effect size should be defined a priori in consultation with the responsible regulatory authority (and in line with the operationalised protection goal) to decide whether treatment-related effects are present or not. In this context is referred to the NOR (Normal Operating Range) of the relevant endpoint as discussed in EFSA Scientific Committee (2016b).

To address remaining uncertainties, an exposure multiplication factor (between 2 and 5) needs to be applied to the application rate of the pesticide in the crop of concern. In the assessment, the modelled risks to relevant population-level endpoints should be low after the application of the selected exposure multiplication factor. The height of this exposure multiplication factor should be based on a weight-of-evidence approach by considering remaining uncertainties of e.g. the representativeness of the modelled focal species for the other species of the critical feeding guild that may be at risk, the level of expected conservativeness of surrogate toxicity data of standard test species as input for the model and whether also indirect effects due to treatment-related declines in food for birds and mammals are considered in the population model. For example, the exposure multiplication factor may be lowered (< 5) if appropriate results are provided for several (focal) species of the critical feeding guild in the region/regulatory zone of concern. This of course under the condition that more representatives of the critical feeding guild can be expected in the crop/agricultural landscape of concern. The assessment should be based on the most critical focal species tested. It is important that population models and their documentation develop to a high level, following the comprehensive suggestions of EFSA PPR Panel (2014). This will enable the realisation of the full potential of population models in successful-use cases for regulatory purposes in the risk assessment for birds and mammals, allowing all parties to increase their experience with model development, application and evaluation.

8. Risk assessment for products applied as granules

The EFSA Guidance (2009) suggested five different scenarios for the Tier 1 risk assessment of granules:

1) Ingestion of granules as a source of food
2) Ingestion of granules as grit
3) Ingestion of granules when seeking small seeds
4) Ingestion of granules as part of soil intake
5) Ingestion of food contaminated with residues from granular application, i.e. soil invertebrates and arthropods, seedlings.

Each scenario and its relevance are further discussed in Sections 8.1–8.5.

It is recommended to follow the schemes proposed in this chapter for all granular applications except for those granules that disperse or dissolve quickly when water is added or in general to granules that are disintegrated immediately after application (see Section 8.7).

8.1. Animals ingesting granules as source of food

This type of assessment has to be performed only if granules have some calorific value and therefore, they may be taken for food, e.g. in the case of granular products formulated on corncob carrier, carriers to which oil is added or carriers having some calorific value. Additionally, the size of the granules should be similar to the size of food items, normally consumed by some bird and mammal species, e.g. weed seeds.

If exposure through this route cannot be excluded, the risk assessment should be conducted by using the relevant acute and reproductive endpoint as explained in chapter 5. For exposure estimation, the (D)DD[45] has to be calculated, as explained in Section 6.3.2 (Tier 1 exposure assessment for seed treatment). Generic model species for Tier 1 assessment are reported in Section 6.1.1 and Annex B. In particular, based on the size of the granule, the selection of the focal species should be done as recommended for seed treatments and using an appropriate surrogate, i.e. medium birds by considering maize as a surrogate for granular products formulated on corncob carrier.

[45] Dietary dose (DD) for acute risk assessment and Daily Dietary Dose (DDD) for long-term risk assessment.

8.2. Birds ingesting granules with/as grit

Grit consumption by farmland birds is an important constituent of dietary intake both for mineral content and grinding (Best and Gionfriddo, 1994). Although grit is not essential, it has been reported that grit consumption increases the digestive efficiency and the assimilation rates of food, due to its primary function as mechanical grinding of food (Best and Stafford, 2002; Amat and Varo, 2008; Bennett et al., 2011). The occurrence and the number of grits in the gizzard of bird species has been reported to be variable and influenced by the feeding guilds. Grit has been found in almost all gizzards of granivorous and omnivorous birds while the occurrence in the gizzard of insectivorous species was much less (Bennett et al., 2011).

The steps for conducting the risk assessment for this route of exposure are reported below. The risk assessment should be performed for both small and medium/large birds, unless based on the granule size, it is justified to exclude one category, knowing that small birds will likely not consume granules larger than 2 mm and large birds larger than 6 et al., 2010. All the background information regarding the proposed default values is reported in Appendix Q. It is reported that grit preference may be influenced by different factors like colour and shape (Møller and Erritzøe, 2010). Although those factors may lead to a difference in grit ingestion and consequently on granules that may be ingested as grit, it is not possible at this stage to provide clear recommendations. Evidence suggesting little use of granules because of the colour and shape may be provided to refine the risk.

Acute risk assessment

The acute risk assessment is done by calculating TER (toxicity exposure ratio) (see Equation 22) for small and large birds, as appropriate based on the granule size, knowing that small birds will not consume large granules.

$$\mathbf{TER_{acute}} = \frac{\mathbf{LD_{50}}}{\mathbf{DGritD_{acute}}} \tag{22}$$

Where:

- LD_{50} = relevant acute endpoint for birds (see Section 5.2.2.1) in mg/kg bw
- $DGritD_{acute}$ = Daily Grit Dose for small and medium/large birds, expressed as mg/kg bw per day. The $DGritD_{acute}$ should be calculated by applying Equation 23 (also see Tables 15 and 16):

$$\mathbf{DGritDacute} = \mathbf{DGritI} \times \frac{\mathbf{G_{density}}}{\left(\mathbf{SP_{surface}} + \mathbf{G_{density}}\right)} \times \mathbf{G_{loading}} \tag{23}$$

$TER_{acute} \geq 10$ Low risk identified
$TER_{acute} < 10$ Refined acute risk assessment required, see Section 8.7

Reproductive risk assessment

The reproductive risk assessment is done by calculating the TER (see Equation 24) for the relevant granule size and comparing the TER to the respective trigger value.

$$\mathbf{TER_{repro}} = \frac{\mathbf{Relevant\ endpoint}}{\mathbf{DGritD_{repro}}} \tag{24}$$

Where:

- Relevant endpoint= relevant reproductive endpoint for birds (see Sections 5.2.6.4 and 5.2.7.2) in mg/kg bw per day
- $DGritD_{repro}$= Daily Grit Dose for small and medium/large birds, expressed as mg/kg bw per day. The $DGritD_{repro}$ should be calculated with the following equation (also Tables 15 and 16):

$$\mathbf{DGritD_{repro}} = \mathbf{DGritI} \times \frac{\mathbf{G_{density}}}{\left(\mathbf{SP_{surface}} + \mathbf{G_{density}}\right)} \times \mathbf{G_{loading}} \times \mathbf{ftwa} \tag{25}$$

$TER_{repro} \geq 5$ Low risk indicated
$TER_{repro} < 5$ Refined acute risk assessment required, see Section 8.7

Table 15: Explanation of symbols and value for exposure assessment through granular application for the grit scenario

Symbol	Definition	Unit	Explanation	Reference
DGritI	Daily Grit Intake of birds normalised for body weight	N° grit per kg bw per day	This value is estimated by the grit consumption normalised by the bird body weight. For converting it to a daily dose a conversion factor was used in EFSA (2009). This conversion factor has been reviewed. A factor of 1 is recommended. For the acute risk assessment, the 90th percentile of the available data for small or medium/large birds is used while for the reproductive the geomean is recommended. See Appendix Q for further information and default values.	–
$G_{density}$	Number of granules at soil surface	N° granules/m^2	See default values reported in Appendix Q for granules that are incorporated and the approach for estimation of this value for broadcast granules.	–
$SP_{surface}$*	Number of soil particles at soil surface in the same size classes as granules per m^2	N° soil particles/m^2	Soil particles are in the same range of small and large granules.	Luttik and de Snoo (2004)
$G_{loading}$	Amount of the active substance in one granule	mg a.s./granule	Substance and GAP specific.	
ftwa[46]	Time-weighted average factor which accounts for degradation depends on the half-life of the compound and during a 21-day period. Ftwa is only applicable to reproductive assessment and if certain criteria are not met. For the criteria on when an ftwa can be applied, see Section 6.1.4. For the ftwa formula see Equation 16 in Section 6.2.5.3	[–]	No default value available	

*: It should be noted that the values are taken from the paper by Luttik and de Snoo (2004), as described in the EFSA (2009) and are related to 3 Dutch soils, 2 sandy and one clay. In case of evidence from different regions suggesting different soil properties, this information may be refined.

[46] Disappearance of granules over 21 d-period could also be account for in a Tier 2 risk assessment should suitable data be available.

Table 16: Estimation of input parameters for acute and reproductive risk assessment for birds ingesting granules when seeking grit

Exposure duration	Size of birds (See Appendix Q)	Number of grit per kg bw per day (DGritI)*	Number of soil particles on the soil surface in the same size category ($SP_{surface}$)	f_{TWA} for the active substance
Acute exposure	Large	683	71	No
	Small	9,349	15,200	No
Long-term exposure	Large	142	71	Yes
	Small	2,002	15,200	Yes

*: No conversion factor is considered anymore, see Appendix Q.

8.3. Birds and mammals ingesting granules when seeking seeds as food

Granules are often smaller than most seeds taken by birds and mammals but can be comparable to some of the smaller seeds of arable weeds e.g. *Stellaria media, Capsella bursa-pastoris, Veronica arvensis* and *Urtica dioica*. Furthermore, granule size may also be comparable to some crop seeds (e.g. rapeseed). It is therefore possible that granules, when of comparable size to seeds, may be ingested by birds and mammals searching for seeds as food. It is important to note that in EFSA (2009) consumption of granules while seeking small seeds was excluded for mammals. However, when considering the ecology of small mammalian species and the available evidence (Green, 1979; Povey et al., 1993; Hulme, 1994; Westerman et al., 2003; Fischer and Türke, 2016) the WG considered that mammals may also accidentally take granules as weed seeds.

It is assumed by default that granules size may overlap with weed seed size and therefore that granules may be mistaken for weed seeds by seed-eating birds and mammals. This means that a risk assessment using this scenario should always be performed unless differently proven, i.e. it has to be demonstrated that granules may not be mistaken for weed/crop seeds because of their size.

Acute risk assessment

$$\mathbf{TER_{acute}} = \frac{\mathbf{LD_{50}}}{\mathbf{DGD_{acute}}} \quad (26)$$

Where:

- LD_{50}= relevant acute endpoint for birds and mammals (see Section 5.2.2.1) in mg/kg bw
- DGD_{acute}= Daily Granule Dose for acute risk assessment in mg/kg bw per day should be calculated by applying Equation 27 (also see Table 17):

$$\mathbf{DGD_{acute}} = \mathbf{DGI} \times \frac{\mathbf{G_{density}}}{(\mathbf{SSS_{density}} + \mathbf{G_{density}})} \times \mathbf{G_{loading}} \quad 27$$

$TER_{acute} \geq 10$ Low risk indicated
$TER_{acute} < 10$ Refined acute risk assessment required, see Section 8.7

Reproductive Risk assessment

$$\mathbf{TER_{repro}} = \frac{\mathbf{Relevant\ endpoint}}{\mathbf{DGD_{repro}}} \quad (28)$$

Where:

- Relevant endpoint = relevant reproductive endpoint for birds and mammals (see Sections 5.2.6.4, 5.2.6.5 and 5.2.7.2) in mg/kg bw per day
- DGD_{repro} = Daily Granule Dose for the reproductive risk assessment should be calculated by applying Equation 29 (also see Table 17):

$$\mathbf{DGDrepro} = \mathbf{DGI} \times \frac{\mathbf{Gdensity}}{(\mathbf{SSSdensity} + \mathbf{Gdensity})} \times \mathbf{Gloading\ x\ ftwa} \quad (29)$$

$TER_{acute} \geq 5$ Low risk indicated
$TER_{acute} < 5$ Refined acute risk assessment required, see Section 8.7

Table 17: Explanation of symbols and value for exposure assessment through granular application for the seed scenario

Symbol	Definition	Unit	Explanation	Reference
DGI	Daily granule intake of birds normalised for body weight	N° granules per kg bw per day	It is assumed that a generic granivorous model bird (16g) and mammal (23g) will eat small seeds with an average caloric content of 21.7 kJ/g dry weight, an average water content of 9.9% and an average assimilation efficiency of 80% for birds and 84% for mammals. Based on equations for food intake rate (see Section 6.1.3, Equation 7) the number of seeds needed in terms of g/day may be estimated and this converted to the number of seeds per day by considering an average weight for *Phalaris canariensis* of 7 mg.	–
$G_{density}$	Number of granules at soil surface	N° granules/m^2	See default values reported in Appendix Q for granules that are incorporated and the approach for its estimation for broadcast granules.	–
$SSS_{density}$	Soil surface seed density per m^2	N° seeds/m^2	100. This value is based on studies on UK arable fields (summarised in EFSA, 2009). A uniform distribution of seeds is assumed in the top 20 cm, and 20,000 seeds/m^2 in the top 20 cm would correspond to about 100 seeds/m^2 in the top 1 mm. It is acknowledged that this is based on older studies. If new information becomes available, this may be considered in the risk assessment as a possible refinement.	EFSA (2009)
$G_{loading}$	The amount of the active substance in one granule	mg/granule	Product and GAP specific.	–
ftwa	Time-weighted average factor which accounts for degradation depends on the half-life of the compound and/or the half-life of the granules during a 21-day period. Ftwa is only applicable to reproductive assessment. For the criteria on when an ftwa can be applied, see Section 6.1.4. For the formula, see Equation 16 in Section 6.2.5.3.	–	No default value available	–

8.4. Ingestion of granules as part of soil intake

When considering the ecology of birds and mammals, the WG did not consider it justified to include this scenario in the risk assessment. Birds and mammals may ingest soil either involuntarily or deliberately. The unintentional consumption of soil, while consuming other food types that may have soil attached to them (e.g. soil invertebrates, seeds), does not justify the inclusion of a separate scenario as the contribution to the overall residue intake is considered minor.

Deliberate soil ingestion or geophagia has been reported for birds and mammals, although data are mainly related to species not relevant for agroecosystems. Additionally, the intentional consumption of soil is not a widespread phenomenon among animals, in general and it can be explained by minerals or microelements deficiency, needs for detoxification remedy for gastro-intestinal disturbance or stress (Beyer et al., 1994; Gilardi et al., 1999; Mahaney and Krishnamani, 2003; Abrahams, 2012; Golokhvast et al., 2014). Furthermore, this is true for any formulation type which leads to exposure of the soil (i.e. not exclusive to granules). Taking into account all available information, it is not recommended to consider the scenario for ingestion of granules as part of soil intake.

8.5. Animals consuming other food items with residues from granular applications

In EFSA (2009), no standardised scheme was available for assessing the risk of residues of granular formulations in other food items such as earthworms and plant seedlings. This was mainly due to the lack of transfer factors for calculating concentrations in the food items for birds and mammals, e.g. transferring the load of granules to a concentration in the earthworm and the seedling.

It is expected that an active substance having a log $K_{ow} \geq 3$, once applied as granule, will be taken up by the worm mainly via the pore water. Therefore, this route of exposure is considered to be covered by the risk assessment through secondary poisoning (see chapter 10). If the substance has a log $K_{ow} < 3$, the bioaccumulation potential is considered low, and therefore, no risk assessment will be triggered. This scenario is not intended to cover those situations in which the substance does have a potential for bioaccumulation but is present in the earthworm or slug stomach, e.g. an active substance with a log $K_{ow} < 3$ acting against slugs and highly toxic.

Unless it is demonstrated that substances do not translocate into seedlings then an assessment for birds and mammals consuming contaminated seedlings should be done. As a pragmatic approach the worst-case concentration in the soil pore water (commonly at 1 cm, however, pending on the type of application the worst case may not be the one at 1 cm) (see Section 10.2.1) may be considered as a proxy of the concentration that may be translocated to seedlings. This concentration is divided by the soil bulk density in order to convert to mg a.s/kg.

The calculation of the (Daily) Dietary Dose should be performed by using the Equations 20 and 21, see Section 6.3.2. The GMS are given in Annex B for germinating seedlings. The risk assessment is performed by applying Equations 3 and 4, as reported in Section 3.2.3

8.6. Conclusion Tier 1

The relevant scenarios for Tier 1 risk assessment for granular applications have been revised compared to EFSA (2009). The risk assessment should be conducted as described in Sections 8.1–8.5 except for those granules that dissolve quickly when water is added or in general to granules that disintegrate immediately after application, for which an assessment according to the methodology for spray applications can be performed. It may be considered, depending upon the speed with which the granules are likely to dissolve (including the instructions for use) whether an acute assessment according to the granules risk assessment and a reproduction assessment according to the spray methodology should be followed. This will be a case-by-case decision depending upon the particular granular formulation (see Table 18).

Table 18: Summary of the different scenarios for Tier 1 risk assessment for granules and their relevance

Routes of exposure	Relevance for birds	Relevance for mammals	Is an assessment required in all situations?
1 Ingestion of granules as a source of food	✓	✓	No. An assessment is only needed when granules have a calorific value, e.g. granular products formulated on corncob carrier, carriers to which oil is added. Additionally, the size of the granules should be in the range of those seeds that may be consumed by birds. See Section 8.1.
2 Ingestion of granules as grit	✓	✗	Yes. See Section 8.2.
3 Ingestion of granules when seeking small seeds	✓	✓	No. An assessment is only needed when the granule size may overlap with weed/crop seeds that are can be consumed by birds and mammals. See Section 8.3.
4 Ingestion of granules as part of soil intake	✗	✗	No. This route is not considered relevant, as explained in Section 8.4.
5 Ingestion of food contaminated with residues from granular application, i.e. soil invertebrates and arthropods, seedlings.	✓	✓	Yes. For earthworm-eating birds and mammals, only for substances with a log K_{ow} > 3. See Section 8.5.

8.7. Special consideration for fast dissolving granules

The schemes proposed in the section above are recommended to be followed for all granular applications. Fast dissolving granules are considered an exception. Those granules once applied, disappear rapidly as e.g. they may dissolve after irrigation. For specific types of granules, irrigation is required and reported on the label, or as part of the GAP, for efficacy reasons. However, this does not mean that the active substance will also disappear but only that it will not be present as a granule. Without experimental data, the length of time the granules will take to dissolve is not easy to predict nor to generalise between different granular products. However, depending on the time needed for applying the granules in field and subsequent irrigation, there will be a time period (of variable length) in which the granules are available to birds and mammals. Therefore, considering that the acute risk assessment is equivalent to a single feeding bout, the use of the granule risk assessment seems appropriate for acute risk assessment and Sections 8.1, 8.2 and 8.3 should be used.

For the reproductive risk assessment, due to their physico-chemical and fate properties, it is suggested that the risk assessment should consider the GMS summarised in Table 19 and calculate the residue according to the recommendations in Table 20, unless applicants provide a better, and more fit for purpose, exposure assessment. The suggested GMS and their diet are the same as for a spray application to 'all field crops with a BBCH 0–9' and should be adapted if the granules are to be used in a different location (e.g. forestry uses). The RUD for ground arthropods and ground seeds, following spray applications, may be used as a surrogate for RUD after granules have dissolved following dissolved granules. The residue in dicot and monocot foliage should be estimated in the same approach as for standard granules which uses the concentration in the soil pore water at 1 cm (see Section 10.2.1) which may be considered as a proxy of the concentration that may be translocated to seedlings.

Table 19: Suggested approach to the reproductive risk assessment for fast-dissolving granules

GMS		Body weight [g]	Diet
Bird	Small insectivorous	17	100% ground arthropods
	Small omnivorous	27	25% ground arthropods 50% ground seeds 12.5% dicot foliage 12.5% monocot foliage
	Granivorous	11	100% ground seeds
	Medium omnivorous	390	50% dicot foliage 50% monocot foliage
Mammal	Small insectivorous	4	100% ground arthropods
	Small omnivorous	23	25% ground arthropods 50% ground seeds 25% monocot foliage
	Granivorous	23	100% ground seeds
	Medium herbivorous	1500	50% dicot foliage 50% monocot foliage

Table 20: Residue estimations for use in a reproductive risk assessment for fast-dissolving granules

Matrix	Residue [mg/kg]	Deposition value [%]	fTWA × MAF
Ground arthropods	RUD = 40.2	100%	fTWA may be used assuming a DT_{50} of 10 days of the criteria in Section 6.1.4 is met. MAF_{repro} as calculated for spray applications should also be used
Weed seeds	RUD = 2.8	100%	
Monocot and dicot foliage	Residue = the concentration in the soil pore water at 1 cm (see Section 10.2.1)	n/a	Already considered in the calculation of the pore water PEC

8.8. Refinement options

If at Tier 1, high risk is identified, refinements of the exposure may be considered.
Possible refinements are the following:

- Real measurements of those parameters that are proposed based on literature studies, mainly for scenario 2;
- Selection of focal species, applicable to all scenarios, see Section 6.5.2;
- Estimation of PT value based on real data for the selected focal species, applicable to all scenarios, see Section 6.5.3;
- Measurements of residues in seedlings for scenario 5;
- Effect field studies, applicable to all scenarios, see Section 7.1.
- For scenario 5, for contaminated earthworms, see Section 10.2.3 for possible refinements.

Following any type of refinement, it is expected that an uncertainty analysis is performed according to the recommendations in Section 13.3.

9. Risk assessment for metabolites

9.1. Introduction

Birds and mammals can be exposed to metabolites that are formed in and on plant material (including fruits and nuts, buds, seed and germinating seedlings), arthropods, surface water, fish, benthic organisms, soil and earthworms.

The risk assessment scheme for metabolites proposed in this chapter focuses on residues from consumption of plants parts which are sprayed with pesticides. The risk assessment from residues on treated seed, germinating seedlings or granules is considered to be covered by the risk assessment for

the parent compound, unless the metabolite is known to be more toxic than the parent. In this case, see Section 9.4.2. for a combined risk assessment. For the risk assessment of metabolites through secondary poisoning or through contaminated water, see chapter 10 and 11. Studies are normally not available to assess whether a metabolite may be formed in arthropods, fish, benthic invertebrates and/or earthworms which may be consumed by birds and mammals. Due to lack of suitable data in these food items, it is suggested using information from plant metabolism studies as surrogate for fish, benthic invertebrates, arthropods and earthworms. In some cases, a fish metabolism study is available, which should then be used to identify relevant metabolites in fish. Metabolites that are formed in soil and are taken up by the plants may be covered considering the results of the metabolism studies in rotational crops (see Section 9.3.3).

A risk assessment for metabolites found in plants (and used as surrogate for other matrices, e.g. fish, arthropods, earthworms) is triggered when residues of metabolites from metabolism studies in primary crops are at or above 10% TRR (Total Radioactive Residue) and 0.01 mg eq/kg. Different trigger values might become relevant in the future, e.g. guidance development on rotational crop metabolism studies, revision of OECD test guidelines (OECD, 2006a) and the EFSA guidance on residue definition for risk assessment (EFSA PPR Panel, 2016). For the human health risk assessment, residue field trials are conducted investigating the presence/levels of the active substance and/or its metabolites in those plant parts which are relevant for human consumption. In the absence of such specific data for birds and mammals, guidance is given on how to use residue data from plant metabolism studies and, if feasible, from residue field trials.

The first step is to assess whether a risk assessment is triggered for any identified metabolite in the available plant metabolism studies. An overview of residue data, including recommendations on how the data should be assessed in order to get the relevant information on metabolites, are reported in Sections 9.2 and 9.3, below. Guidance on how to conduct the risk assessment is given in Section 9.4.

9.2. General considerations for residue data

Based upon the proposed GAP, some indication of the residue situation in plants can already be ascertained.

9.2.1. Application method, e.g. soil, foliar, seed treatment, post-harvest treatment

Some application methods, such as post-harvest treatment (of the edible commodity), are not relevant for the exposure to birds and mammals. For other application methods, such as soil or seed treatments, exposure through feed for livestock might become relevant when residues are taken up from soil and/or translocate in the growing plant parts, whereas for foliar application (depending on the growth stage of application) exposure to residues is in general more likely, though exceptions are possible.

9.2.2. Growth stages and season

Applications at very early growth stage and/or early in the growing season might lead to lower residues in feed for livestock and food crops at harvest/maturity. However, this is less relevant for birds and mammals, as treated plant parts might be eaten at early growth stages/immediately following application.

9.3. Sources for residue data in plants

Residues in food for humans and feed for livestock resulting from the use of the active substance on plants are addressed at different levels in the residue section:

Metabolism in plants (nature of residues)
Magnitude of residues in plants (residue field trials)

For crops that can be grown in rotation, and when the DT_{90} of the active substance and/or relevant soil metabolites is above 100 days, data on their potential uptake from soil via roots and metabolic behaviour in the rotated crops will be requested in a tiered approach: first data on the metabolism of the active substance in rotated crops (OECD, 2007a) and then, if relevant, on the magnitude of residues in rotated crops (OECD, 2007b; OECD, 2018b).

9.3.1. Metabolism in primary crops

Metabolism studies are intended to:

- elucidate the degradation pathway of the active substance;
- identify the metabolism and/or degradation products produced; and
- provide an estimate of the total residues in the various parts of food and feed crops after crop treatment, which allows determination of the distribution of residues within the crop, e.g. whether the active substance is absorbed through roots or foliage, or whether translocation occurs.

An active substance and/or metabolite is considered systemic if, based on the metabolism studies in primary and rotational crops, it can be clearly established that the parent compound and/or its metabolite(s) are present in the non-treated parts of the crops when the active substance is applied according to the critical GAPs (European Commission, 2019).

In the OECD test guideline 501 on metabolism in crops (OECD, 2007c), five crop categories (fruit, root crops, leafy crops, cereal/grass crops and pulses/oilseeds) and a miscellaneous category are established in order to investigate the metabolic pathway followed (see Table 21). For each crop on which representative use is proposed, a metabolism study is needed from that category. A maximum of three categories is adequate, provided a consistent metabolism picture is obtained from the three studies. Additional categories may need to be studied if there are differences in metabolism across groups. However, it is not uncommon that, even if not required for the ongoing assessment, metabolism studies for several categories are submitted, as they are the core studies needed to describe the residue behaviour of an active substance. It is noteworthy that one study performed with a crop of one category is acceptable for the residue assessment in all crops of this category, provided that the same type of application (e.g. foliar treatment) is used. For example, a metabolism study with tomato would be acceptable for the proposed representative use in grapes, as both crops belong to the 'fruit' category. Whenever possible, according to OECD 501 (OECD, 2007c), metabolism studies in plants should be performed with exaggerated application rates, in order to ensure that enough residues in plant parts are available to allow identification of the residues, but also in order to cover potential future uses with higher application rates.

In general, plant metabolism studies aim at identifying residues in edible plant parts and in plant parts which are used as feed for livestock. The OECD Guidance document on overview of residue chemistry studies lists raw agricultural commodities to be analysed for crop metabolism and rotational crop studies (OECD, 2009b) but this list is not comprehensive with respect to the plant parts to be analysed, nor is the growth stage at which sampling should take place indicated. However, the OECD 501 (OECD, 2007c) proposes that, in addition to sampling of mature plant parts, also immature plant parts should be sampled (a) in cases where the crop could be consumed at immature stages (e.g. baby corn or leafy salads) and (b) to facilitate identification/characterisation when residues are expected to be too low in the mature plant parts. Although samples might have been taken, these crop parts are not necessarily analysed for residues and/or metabolites identified. Furthermore, the guideline suggests that 'If applicants wish to use mature but inedible crop parts (e.g. apple leaves, potato foliage) to help identify residues on the mature raw agriculture commodity, evidence of similar chromatographic profiles for mature edible and inedible crop portions is necessary.' If analysis is performed, the results can be useful for the understanding of residue behaviour in the same plant part over the covered vegetation period. Table 21 lists plant parts which are frequently analysed in metabolism studies, together with an indication as to whether these plant parts can form part of the diet of birds and mammals. Considering that birds and mammals may consume weeds, which are only addressed in the cereal/grass crop category, or fruit buds, which are not usually included in plant metabolism studies, general advice how to deal with residues in these plant parts cannot be given, but information may be extrapolated on a case-by-case basis between crop categories for the purposes of the exposure assessment for birds and mammals. For example, if the representative use is on citrus fruit and metabolism studies are available for citrus and cereals, residues in cereal forage could potentially be used as a surrogate for weeds. In the absence of an 'in this case not mandatory' cereal metabolism study, information on residues in citrus leaves (if available) might be used as best-available surrogate.

Relevant data for individual metabolites from primary and rotational crop metabolism studies

Quantitative and qualitative information on the residues of the active substance and its metabolites in the investigated plant parts is usually available in a tabulated or structured format. The following approach is recommended for metabolites (for the active substance, the RUD values will be used):

- *Concentration threshold for consideration of the residue*
  only identified residues (including conjugates of parent or metabolites provided that their toxicity profile is covered by the respective free form) that are present individually at or above 10% TRR and 0.01 mg (parent) equivalent (eq)/kg are considered relevant, unless there are toxicological data showing the metabolite to be more acutely toxic than the parent. Usually in metabolism studies the values are normalised to the molecular weight of the parent. This is then expressed as 'eq'.
- *Relevance of plant parts and choice of the appropriate sampling time point (in case of multiple sampling time points)*
  Residues in all parts of a plant which can be consumed by birds and mammals should be considered, e.g. rapeseeds and rapeseed foliage, for the risk assessment. **An indication of which plant parts may be consumed is given in** Table 21.
  If, for a given plant part, information on the concentration of the metabolite is available for several sampling time points within the BBCH stages indicated in Table 21, the time point resulting in highest residue concentration should be considered in order to be protective. (Example: In leaves, the active substance degrades over time and a metabolite is formed with increasing concentration over time. Results for residues of the active substance and this metabolite in leaves are available at sampling time corresponding to BBCH 10, BBCH 30 and BBCH 89. The concentration for the metabolite would be highest at BBCH 89, then this result should be considered although it is more likely that leaves would be eaten by birds and mammals up to BBCH 30).
- *Extrapolation*
  If the metabolism study is performed in the same crop for which a representative use is proposed, the data from the respective relevant plant parts can be used as it is (for use from residue field trials refer to 9.3.2).
  If the metabolism study is performed in a different crop of the same plant category, a case-by-case extrapolation will need to be done. Even if a plant part is not considered as food for birds and mammals, it could serve as a proxy for another crop group within the same category.
  Example: the representative use is in strawberries but only a metabolism study in citrus is presented. Although it is not likely that B&M would consume citrus leaves, they may consume strawberry leaves, and residue values from citrus leaves could be used to extrapolate residues in strawberry leaves. The same consideration holds true for extrapolation between the five crop categories.

For the risk assessment of metabolites in Section 9.4, a factor is established by calculating the ratio of concentration of metabolite to concentration of parent (Q_{met}). In order to be protective, this factor should be derived from plant parts resulting in the highest ratio, regardless of whether the plant part is likely to be consumed by birds and mammals. For a refined risk assessment, the actual concentration of the metabolite might be considered instead of using the default RUD of the parent in combination with this factor. The aforementioned considerations on the relevance of plant parts and choice of the appropriate sampling time point should be taken into account for the choice of concentration values from plant metabolism studies.

9.3.2. Magnitude of residues in plants

Residue field trials are aimed to estimate the human exposure to all compounds included in the plant residue definition for risk assessment (i.e. active substance and/or its toxicological relevant metabolite(s)) from consumption of edible parts of treated crops and through carry over via feed to food of animal origin. Therefore, these studies generally will not provide residue data for all metabolites identified in the metabolism studies, nor will they report residues in non-edible plant parts unless those are used as feed for livestock. The trials are intended to capture the residue situation at harvest to be protective for humans. This might not be the case for birds and mammals as it is likely that no information on the residues at early growth stages will be available, except in the decline studies (see below).

As stipulated in Commission Regulation No 283/2013[4], for major crops a set of eight independent residue field trials per zone (depending on the proposed use for each of the three zone, Northern European and Southern European zone and indoor use) performed under the conditions of the critical good agricultural practice (GAP) and measuring all compounds covered by the residue definition for risk assessment is required. The highest residue (HR) levels and supervised trial median residue (STMR) are derived in the crop parts of interest for the active substance and/or its toxicologically relevant metabolites. If a significant part of the consumable crop is present at the time of application, 50% of the residue trials should be decline studies, i.e. supervised residue trials that show how the residue level changes over time. In general, each decline trial consists of five sampling stages, two of which are frequently set to coincide with the time of application and the harvest, respectively. The recommended preharvest interval (PHI), as defined in the GAP, must be declared in every case.

The measured concentration of metabolites which are included in the risk assessment residue definition from residue field trials are therefore more representative of the actual likely residues present than those derived from a metabolism study, as they consider the residue situation under different climatic conditions (NEU/SEU and indoor). Furthermore, they are conducted in compliance with the representative uses and provide insight into the degradation behaviour via decline studies. It is therefore recommended to use the concentration for the metabolite(s) from residue field trials whenever possible, rather than the levels reported in the plant metabolism studies.

Data to be used from residue field trial studies

It is recommended to use the HR (highest residue) for the acute and the STMR (supervised trial median residue) for the chronic risk assessment from the residue trials set for the zone resulting in higher residues. For human risk assessment HRs and STMRs from decline studies are taken usually at maturity. If higher residues are observed at earlier sampling stage (e.g. covering immature plant parts), it is suggested to take these higher values for the risk assessment in birds and mammals. In case no decline studies are available, the HRs and STMRs obtained at harvest should be compared to the residue data from metabolism studies to verify that exposure is not underestimated.

For other metabolites identified in metabolism studies, but which are not measured in residue field trials, data from the metabolism studies still need to be considered (see Section 9.3.1). The same applies for plant parts which are consumed by birds and mammals, but analysis is not performed in the residue field trials but only in the metabolism study.

9.3.3. Rotational crops

For crops that can be grown in rotation, and when the DT_{90} of the active substance and/or relevant soil metabolites is above 100 days, studies should be performed to allow the determination of the nature and extent of potential residue accumulation in rotational crops from soil uptake, and of the magnitude of residues in rotational crops under realistic field conditions.

If the metabolism studies in rotated crops indicate that residues of the active substance or of relevant metabolites or breakdown products either from plant or soil metabolism may occur at $>$ 0.01 mg eq/kg in food/feed crops for livestock, limited field studies and, if necessary, field trials will have been carried out for the residues section (see OECD, 2007b, 2018b).

In principle, the same approach as for primary crops is applicable, i.e. if the residues of the active substance and/or its metabolite(s) are found in plant parts of rotated crops at concentrations below 10% TRR and 0.01 mg eq/kg, they are deemed not relevant for the risk assessment unless there is a specific toxicological concern.

Table 21: Crops and crop groups for purposes of metabolism in crop studies and plant parts which are typically analysed in metabolism studies (basis of OECD 501)

Category	Crops	Plant part A	Plant part A relevant for B&M (Y/N)	BBCH stage of plant part A	Plant part B	Plant part B relevant for B&M (Y/N)	BBCH stage of plant part B	Plant part C relevant for B&M (Y/N)	Plant part D relevant for B&M (Y/N)
Fruit	Citrus fruit	Fruit (peel and pulp)	**Y**	71–89	Leaves	N	10–92 (4)	n.a.	n.a.
	Tree nuts	Fruit (=nut)	**Y**	71–89	Leaves	N	10–92 (4)	n.a.	n.a.
	Pome fruit	Fruit	**Y**	71–89	Leaves	N	10–92 (4)	n.a.	n.a.
	Stone fruit	Fruit	**Y**	71–89	Leaves	N	10–92 (4)	n.a.	n.a.
	Berries	Fruit	**Y**	71–89	Leaves	**Y**	10–92	n.a.	n.a.
	Small fruit	Fruit	**Y**	71–89	Leaves	**Y**	10–92	n.a.	n.a.
	Grapes	Fruit	**Y**	71–89	Leaves	**Y**	10–92	n.a.	n.a.
	Fruiting vegetables	Vegetable	**Y**	71–89	Leaves	**Y**	10–92	n.a.	n.a.
	Banana	Fruit (peel and pulp)	(1)	71–89 (4)	Leaves	(1)	10–92 (4)	n.a.	n.a.
	Persimmon	Fruit	**Y**	71–89	Leaves	N	10–92 (4)	n.a.	n.a.
Root crops	Root and tuber vegetables	Root/tuber	N	41–49 (4)	Leaves	**Y**	10–92	n.a.	n.a.
	Bulb vegetables	Root/tuber	N	41–49 (4)	Leaves	**Y**	10–92	n.a.	n.a.
Leafy crops	Brassica vegetables	Head	**Y**	41–49	Leaves	**Y**	10–92	n.a.	n.a.
	Leaf vegetables	Leaves immature	**Y**	10–39	Leaves mature	**40–49**	X	n.a.	n.a.
	Stem vegetables	Immature plant	**Y**	10–30 (circa)	Mature plant	> 30		n.a.	n.a.
	Hops	Cones	(1)	71–89 (4)	Leaves	(1)	10–92 (4)	n.a.	n.a.
	Tobacco	Leaves	(1)	10–90 (circa) (4)	n.a.			n.a.	n.a.
Cereal/grass crops	Cereals	Forage	**Y**	10–30	Grain	**Y**	71–99	Hay (3)	Straw (3)
	Grass and forage crops	Forage	**Y**	10–30	n.a.			n.a.	n.a.

Category	Crops	Plant part A	Plant part A relevant for B&M (Y/N)	BBCH stage of plant part A	Plant part B	Plant part B relevant for B&M (Y/N)	BBCH stage of plant part B	Plant part C relevant for B&M (Y/N)	Plant part D relevant for B&M (Y/N)
Pulses and oilseeds	Legume vegetables	Seeds	**Y**	71–89	Forage	**Y**	10–30	Pods (3)	Straw (3)
	Pulses	Seeds	**Y**	71–89	Leaves	**Y**	10–30	Hay (3)	Vines (3)
	Oilseeds	Seeds	**Y**	71–89	Leaves/whole tops	**Y**	10–30	Stalks (3)	n.a.
	Peanuts	Seeds (Nutmeal)	(1)	71–89	Leaves	(1)	10–90	Hay (3)	n.a.
	Legume fodder crops (2)	Forage	**Y**	10–30	Hay (3)	**N**		n.a.	
	Cacao beans	Cocoa bean	(1)	71–89 (4)	Aerial parts	(1)	10–99 (4)	n.a.	
	Coffee beans	Coffee bean	(1)	71–89 (4)	Aerial parts	(1)	10–99 (4)	n.a.	

(1): These crops/crop parts are not considered as diet for bird and mammals but could in the absence of other data on a case-by-case decision be used as a worst-case proxy.
(2): Example for legume fodder crops: alfalfa, clover, cowpea, vetch crown, lespedeza, pea, trefoil, lupin.
(3): Information on residues can be taken on a case-by-case basis (e.g. hay, straw and pods are usually harvested for feed purposes and not available to birds and mammals for a longer period, e.g. maximal a few days after harvest).
(4) Growth stage given for completeness and in case of extrapolation to other crops is needed.

Metabolism studies are not necessarily to be performed on the same crop for which a GAP is proposed, but on a representative crop from the same category (see Table 21). However, the critical GAP conditions should be comparable. Example: the critical GAP proposed is 3 × 300 g a.s./ha on strawberries, Interval for application is 7 days, BBCH 30–89, PHI 3 days and a metabolism study is performed with cucumbers 4 × 300 g a.s./ha, interval for application is 10 days, BBCH 81, sampling material: immature and mature cucumbers.

Several crop parts might be analysed and are named in Table 21 from A to D. For the crop parts A and B, an indication is given whether they are relevant as food for birds and mammals and the BBCH stage of interest, i.e. when the plant part might be eaten by birds and mammals. Plant part C and D are usually sampled for analysis at harvest and or are relevant for feed items for livestock and therefore not or less relevant except 'whole fruiting vegetable' which might be also sampled at immature stage and therefore are relevant as food for birds and mammals.

9.4. How to conduct the risk assessment for metabolites

When a metabolite requires further assessment based on residue data[47] (i.e. ≥ 10% TRR and 0.01 mg eq/kg,[48] unless there are toxicological data showing the metabolite to be more acutely toxic than the parent), and no toxicological data are available, it is important first to check whether the metabolite(s) is/are formed in the available rat and/or poultry metabolism studies with the active substance (parent compound) and thus whether it/they may be considered covered by the toxicity studies with the parent. This would allow avoiding requesting additional vertebrate data and performing a separate quantitative risk assessment. Goat metabolism studies may also be available. Those studies should be considered when specific studies with rat or poultry are not available since they may give indications about the level of metabolisation of an active substance. However, for assessing whether a metabolite risk assessment is needed for mammals, rat metabolism studies should be considered in first instance. The same is recommended for bird risk assessment and poultry metabolism studies.

If toxicological data are available and the metabolite requires further assessment, a quantitative risk assessment should be conducted.

Metabolites are considered covered by the toxicity studies conducted with the active substance if they contribute to 10% or more (as an individual metabolite) of the administered dose in terms of total radioactive material recovered in the urine, as detected in metabolism studies or ADME (absorption, distribution, metabolism and excretion) studies. The 10% cut-off is understood to be considering the total amount of urinary excreted material over the collection time in rat metabolism study (EFSA PPR Panel, 2016). The amount excreted in urine may not be available from poultry metabolism studies, in which case the % in excreta (including urine, faeces) should be considered. Moreover, concentration of metabolite in plasma or bile may also be taken into consideration (see EFSA PPR Panel Guidance, 2016; on-going OECD Guidance on the definition of residue).

If a metabolite occurs at < 10% in total urine (or excreta, for poultry) in the metabolism/ADME studies, it is unlikely that it was present at a level high enough to contribute to any potential effect in the toxicity studies with the parent; thus, the potential risk from that metabolite is not considered covered by the risk assessment for the parent compound. As a result, a separate risk assessment will be necessary. In determining whether the 10% trigger has been reached, sequential metabolites may be summed up, e.g. conjugates (glucuronides, sulfates, amino acids). In general, when a metabolite is not found in rat/poultry/goat metabolism studies, but it is formed in plants, this is not considered covered by the parent, unless further substantiated. This for example may be the case of glycoside plant metabolites and aglycon that have a corresponding conjugated or unconjugated metabolite found in the rat, i.e. aglycon. (EFSA PPR Panel, 2016; on-going OECD Guidance on the definition of residue)

The steps to be followed to properly assess the risk from exposure to each metabolite are outlined below.

[47] In this section reference has been made to the EFSA PPR Panel Guidance (2016), however, it should be noted that there is an on-going activity at OECD to future develop guidance for residue definitions. In future, it would be more appropriate to refer to the officially noted guidance for residue definition.

[48] The WG propose that the trigger for when plant metabolites need to be considered in the risk assessment for birds and mammals is when ≥10 % TRR **and** 0.01 mg eq/kg. It is acknowledged the 'and' may not be in line with the triggers for other groups of non-target organisms. However, the 'and 0.01 mg eq/kg' was considered sufficient when considering the relative concentrations to the tier 1 RUD values used for the assessment of the parent substance.

Step 1

Is there any metabolite formed in plant materials at ≥ 10% TRR and 0.01 mg eq/kg (including leaves, crop seeds, fruits, nuts, germinating seedlings, etc.) (used as surrogate for other relevant matrices) and/or is there any toxicity information showing the metabolite to be more toxic than the parent compound?

No: No major metabolites are formed in plants and no toxicological information is available showing any metabolite to be more toxic than the parent. No further consideration for metabolites is needed.
Yes: go to step 2 when the metabolite(s) formed at ≥ 10% TRR and 0.01 mg eq/kg.
Go to Section 9.4.1 for further guidance on how to select the surrogate endpoint for the metabolite when data are not available and 9.4.2 in case there are toxicological data showing the metabolite to be more toxic than the parent.

Step 2

Is the identified metabolite formed in the available metabolism studies in poultry and/or rat and/or goat?

No: go to step 4 and conduct a bird and/or mammal quantitative screening risk assessment for the metabolite.
Yes: go to step 3.

Step 3

Is the metabolite considered covered by the toxicity studies with the active substance (contributing to 10% or more (as an individual metabolite) of the administered dose in terms of total radioactive material recovered in the urine/plasms/bile of rat/goat metabolism study or excreta of poultry metabolism study)?

No: go to step 4 and conduct a bird and/or mammal quantitative screening risk assessment for the metabolite.
If a poultry metabolism study is not available, information from other vertebrate metabolism studies may be considered. If from those studies, it is clear that:

- the metabolite contributes to a high level (higher than 20%) of the administered dose, then a separate risk assessment for birds is not deemed necessary. The fact that the metabolite contributes to a level that is higher than the threshold of 10% is considered to cover potential inter-species difference in the metabolism. If the levels in the available vertebrate metabolism studies are close to the threshold of 10% (i.e. within 20%), and the margin of safety in the risk assessment is not considered high enough, a separate bird risk assessment is recommended.

Yes: No separate quantitative risk assessment is needed for the metabolite, as it is considered covered by the available risk assessment of the active substance.
Please note that it is suggested to consult goat metabolisms studies when rat and/poultry metabolism studies are not available.

Step 4

1. Conduct an acute screening risk assessment for birds and/or mammals

An acute risk assessment should be conducted for birds and/or mammals by applying Equation 3 (see Section 3.2.3).

i) Choice of the (surrogate) toxicological endpoint

If toxicity data are available for the identified metabolite, then the relevant LD_{50} should be used for risk assessment. If toxicity data with birds and mammals are available with the metabolite under assessment showing it to be equal or more toxic than the parent, please see Section 9.4.2 for a combined risk assessment. When no toxicity data are available for the metabolite but based upon level of occurrence in the residue's studies (see Sections 9.1–9.3) a risk assessment should be performed, it is recommended to assume the metabolite as 10 times more toxic than the parent (LD_{50}/10). However, when it is clearly demonstrated (see Section 9.4.1 below for further guidance) that the

metabolite is not expected to be more toxic than the parent, the metabolite can be assumed to be as toxic as the parent.

ii) Exposure estimate

For estimating the exposure for the acute risk assessment, the DD (daily dose) should be calculated for the indicator or generic model species (see Sections 6.2.2 and 6.2.3). Equation 8 or 9 should be used but RUD_i should be replaced by RUD_{met}.

$$\mathbf{RUD_{met} = RUD_{parent} \times Q_{met}} \quad (30)$$

where
RUD_{parent} refers to the RUD for the active substances used in the Tier 1 risk assessment for spray applications. If RUDs have been refined (see Section 6.4.1), those can be used.
Q_{met} (amount of metabolite) can be established from the metabolism studies by taking the highest concentration of metabolite (expressed as parent equivalent) and normalised by 1 kg or by considering the highest percent total recovered radioactivity ($\%TRR_{metabolite}/100$). As a conservative and pragmatic approach, it is recommended to first use the highest %TRR recovered for all the relevant food items (i.e. a single Qmet is derived and applied to all food items). Although this may seem conservative, it has to be considered that residue data specific for food items eaten by birds and mammals are in many cases not available. Therefore, the available data are often used as surrogate for food items which are part of the diet of birds and mammals. Only if, when using this approach, high risk is identified, residue data on specific food items (e.g. fruits) may be used to refine the Qmet.

iii) Retrieving information from residue data to establish Q_{met}

a) In principle, all available metabolism studies for primary and rotational crops should be considered.
b) The metabolite-to-parent factor should be calculated in the plant part where the trigger was breached at any time, regardless of whether the plant part or the growth stage at sampling are relevant or not as food for birds and mammals. This calculation must be applied for all plant parts in which the metabolite breaches the trigger. The highest factor is then taken for the risk assessment. Taking into account residues in all plant parts in which the metabolites breached the trigger, even if these are in the first instance not relevant as feed for birds and mammals, guarantees a worst-case approach.
c) If several metabolism studies are available for the same crop, the study resulting in highest Q_{met} should be considered unless duly justified, e.g. the metabolism study is not valid but supportive only, or if the representative use is foliar application and for the same crop a study with foliar application and a study with seed treatment are available, then the latter is not relevant.

iv) Outcome of the acute screening risk assessment:

TER ≥ 10	Low risk is concluded
TER < 10	High Risk due to exposure to the metabolite cannot be excluded. Go to step 5

2. Conduct a reproductive screening risk assessment for birds and/or mammals

A reproductive risk assessment for metabolites is not warranted in the majority of the cases. However, there may be situations where such an assessment is triggered. In general, a reproductive risk assessment with metabolites is deemed necessary when there is data to suggest that the metabolite may be more toxic than the parent. This could be based upon acute toxicity data on birds and/or mammals, if available, or if data for birds and mammals and the metabolite are not available, data on other taxa, e.g. preferably vertebrates, may be checked. It may also be considered whether mammalian toxicology data are available, and if so, why these data were developed. Additionally, a

reproductive risk assessment for a metabolite may also be triggered if it is known that the metabolite is persistent in the environment (i.e. P criteria met in accordance with Annex XIII of Regulation (EC) No 1907/2006[49]) and/or the metabolite is found to be relevant based on rotational crop metabolism studies.

A reproductive risk assessment should be conducted for birds and/or mammals by applying Equation 4 (see Section 3.2.3).

i) Choice of the (surrogate) relevant toxicological endpoint

If toxicity data are available for the identified metabolite, then the relevant reproductive endpoint (see Sections 5.2.6 and 5.2.7 for the selection) should be used for risk assessment. If toxicity data with birds and mammals are available with the metabolite under assessment showing it to be equal or more toxic than the parent, please see 9.4.2 for a combined risk assessment. When no toxicity data are available for the metabolite but based upon level of occurrence in the residue's studies (see Sections 9.1–9.3) a risk assessment should be performed, it is recommended to assume the metabolite as 10 times more toxic than the parent (relevant reproductive endpoint/10). However, if it is clearly demonstrated (see Section 9.4.1 below for further guidance) that the metabolite is not expected to be more toxic than the parent, the metabolite can be assumed to be equally toxic as the parent.

ii) Exposure estimate

For estimating the exposure for the reproductive risk assessment, the DDD should be calculated for the indicator or generic model species (see Sections 6.2.2 and 6.2.3). Equation 8 or 9 should be used, but RUDi should be replaced by RUD_{met} (see Equation 30 for estimating the RUD_{met}).

iii) Follow steps a–d as reported above for the acute risk assessment for getting the right information from residue data, Q_{met}

iv) Outcome of the reproductive screening risk assessment:

TER ≥ 5 Low risk is concluded

TER < 5 High risk due to exposure to the metabolite cannot be excluded. Go to step 5

Step 5

Further options for risk assessment of metabolites

If a high risk cannot be excluded based on the screening assessment, for addressing the risk of the metabolite it is recommended to first explore all those possibilities which do not require (additional) vertebrate testing, which should only be considered as a last resort. Those options may be the following:

- For birds, if the screening risk assessment based on the toxicity endpoint of the parent divided by 10 shows high risk, it can be considered whether toxicity data with mammals are available for the metabolite under assessment and if the risk assessment for mammals shows low risk with high margin of safety. In this case and if the toxicity of the parent to birds and mammals is similar, low risk to birds might also be concluded.
- Estimation of a toxicity endpoint using computational modelling, i.e. QSAR (see Section 9.4.3). This may only be possible for acute toxicity data, taking into account the availability of QSAR models.
- Estimation of a substance specific DT_{50} in the representative crop/matrix.
- Refine the concentration of metabolite in the relevant plant commodity or relevant food items by using matrix-specific initial measured residue data (see 6.4.1) (i.e. data on fruits).
- As a last resort, if low risk is not demonstrated by using one of the previous options and toxicity data with the metabolite are not available, acute and/or reproductive toxicity studies may have to be conducted.

[49] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

9.4.1. Appropriate evidence for the selection of the relevant surrogate endpoint

As described above, when a separate risk assessment for the identified metabolite is triggered and specific toxicity data are not available, it is recommended to consider the metabolite as 10 times more toxic than the parent, by default. However, there may be situations in which robust evidence is provided justifying the use of the same endpoint of the parent, i.e. the metabolite is assumed to be as toxic as the parent. The data which can be considered in order to show that the use of the factor of 10 may be too conservative are the following:

- QSAR data estimating the toxicity of the identified metabolite to birds and mammals. For further guidance on how to assess QSAR data, see Section 9.4.3.
- Toxicity data on birds and mammals extracted from existing databases, like USEPA ECOTOX Knowledgebase, ECHA website, etc. The submission of a single endpoint from a database will not be considered as sufficient evidence, as when an endpoint is extracted from a database, this cannot normally be fully validated. Therefore, it is suggested that a number of endpoints for birds and mammals and other species, preferably vertebrates, are provided, showing that overall, the assessed metabolite is not expected to be more toxic.
- Information on the molecular structure showing that the chemical group responsible for the toxicity of the parent compound (toxophore) is not present anymore. Although this may be an option, it is not always straightforward, as the chemical group leading to the toxicity observed may not be known, or its effect may be specific to some taxa.
- Read-across from other similar compounds. If the assessed metabolite is known to have a similar molecular structure to other substances for which data are available, a read-across argumentation may be substantiated by following the ECHA guidance (ECHA, 2017a).

9.4.2. Risk assessment for toxic metabolites

When a metabolite is of comparable or higher toxicity than the parent compound, a combined risk assessment considering exposure to the two (or more) compounds (parent and metabolite(s)) is considered to be more appropriate than a separate risk assessment.

A screening assessment is first proposed by considering that the sum of the parent and the toxic metabolite in the environment will unlikely exceed the maximum application rate of the parent.

9.4.2.1. Screening level assessment

An acute and reproductive risk assessment should be conducted for birds and/or mammals by applying Equation 3 and 4 (see Section 3.2.3).

In this case, the relevant endpoint to be used for the TER estimation is the lowest available acute and/or reproductive endpoint for either the parent or the metabolite.

DD(D) is the (daily) dietary dose[50] considering the maximum application rate of the parent. As this is a screening step and is intended to be worst-case, it is recommended that no ftwa factor be applied for the reproductive risk assessment.

Acute TER $\geq$ 10 and/or reproductive TER $\geq$ 5 Low risk is concluded

Acute TER $\geq$ 10 and/or reproductive TER $\geq$ 5 High Risk cannot be excluded, see Section 9.4.2.2.

9.4.2.2. Further steps

When residue data are available measuring residues of both, the parent and the metabolite(s), it is possible to estimate the proportion of the parent and the metabolite over time. Therefore, a worst-case proportion may be identified and used for the exposure estimation. The endpoint used in the risk assessment can be the lowest between the parent and metabolite(s). Alternatively, a 'relevant endpoint mix' can be calculated, as explained in Section 12.3.

Section 6.4.3 and Annex A on refining residues decline give recommendations on how to estimate a TWA factor in the case of a toxic metabolite. If the risk assessment conducted considering 'a relevant endpoint mix' and a refined combined DT_{50} results in low risk, no further action is needed.

If no residues data are available for estimating the proportion of parent and metabolite(s) over time, or after this has been done, still a low risk cannot be concluded, then additional refinements may be considered, as explained in Section 6.5.

[50] DD for acute and DDD for reproductive.

9.4.2.3. Toxic metabolites in germinating seedlings

Information on the level of metabolites in germinating seedlings will not be available in the majority of assessments since the studies performed for the residue section will focus primarily on later growth stages. In the case that metabolite(s) are expected to have higher toxicity than the parent then it is recommended that a study is performed to measure the level of residues of both the active substance and metabolite in germinating seedlings.

9.4.3. Application of (Q)SAR

In very specific cases, the risk assessment for birds and mammals may be assisted by the use of computational models (see Section 9.4.1). In particular, the use of (quantitative) structure–activity relationship ((Q)SAR) models may be considered to predict acute toxicity endpoints for birds and mammals for metabolites when no experimental data are available.

A software tool for the prediction of oral and dietary toxicity to quail was developed as part of the DEMETRA EU funded project. For mammals, the only freely available software programs for acute toxicity is T.E.S.T. (Toxicity Estimation Software Tool), which is made freely available by the US EPA. Other available programs are commercial programs and a list of available QSAR models evaluated by the joint research centre (JRC).[51]

Guidance on the use of QSAR models is provided by the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals (ECHA, 2008). It is recommended that this guidance is followed when QSAR models are used in the risk assessment of pesticide metabolites for birds and mammals.

The two main aspects to consider from the ECHA Guidance are how to assess (Q)SAR applicability and the documentation of the (QSAR) model and prediction (EFSA PPR Panel, 2013, 2016).

The adequacy of a (Q)SAR prediction is evaluated based on the following conditions (EFSA PPR Panel, 2016):

- The prediction should be generated by a scientifically valid (i.e. relevant and reliable) model.
- The model should be applicable to the chemical of interest with the necessary level of reliability.
- The model endpoint should be relevant for the purpose (i.e. acute or reproductive toxicity assessment).
- The information should be well documented.

The first condition for using the (Q)SAR in a regulatory context is the demonstration of the model validity. A set of five validation principles has been established by the OECD (OECD, 2007a) to evaluate the validity of a (Q)SAR model prediction. According to those principles, a (Q)SAR model should be associated with:

- a defined endpoint;
- an unambiguous algorithm;
- a defined domain of applicability;
- appropriate measures of goodness of fit, robustness and predictivity;
- a mechanistic interpretation, where possible.

Information which covers the above-listed five principles should be available to the assessor as a part of the relevant documentation of the prediction.

For each model prediction, it is fundamental that full documentation is provided allowing an independent evaluation of the adequacy and validity of each model prediction.

Detailed reporting information may not be needed when the model is included in the JRC database. In that case, referring to the JRC QSAR model reporting format database (QMRF) may be sufficient.

In order to reduce uncertainty around a prediction (i.e. to maximise its sensitivity and specificity), at least two independent (Q)SAR models, where possible, (e.g. based on different training sets and/or algorithms as knowledge-based and statistical-based models) should be used for the estimation. If both model predictions are adequate and valid, it is recommended to use the lowest estimated endpoint for risk assessment purposes as defined above.

If both models are not valid and/or in both cases the substance under assessment is outside the applicability domain of the models, it is clear that the (Q)SAR prediction cannot be used. If only one of

[51] https://data.jrc.ec.europa.eu/dataset/e4ef8d13-d743-4524-a6eb-80e18b58cba4

the 2 available estimations is adequate and valid, a weight of evidence approach may be used considering all available information provided by the models, e.g. applicability domain, proposed mechanistic information, prediction for similar substances and available endpoints for the active substance under evaluation.

10. Bioaccumulation, biomagnification and secondary poisoning of birds and mammals

10.1. Introduction

Bioaccumulation is the net result of exposure of an organism to a contaminant from various sources over time. This represents the balance between the fluxes into the organism and the losses through protective processes such as biotransformation (ability to metabolise the contaminant) and elimination (Landrum et al., 1996). Note that these losses also may include losses of the contaminant due to reproduction and dilution due to growth. Biomagnification refers to the progressive build-up of persistent substances by successive trophic levels, so it relates to the concentration ratio in a tissue of a predator organism as compared to that in its prey. Bioaccumulation in prey organisms and biomagnification may lead to secondary poisoning, the poisoning that results when one organism ingests another organism that has contaminants in its system.

Bioaccumulation may be quantified in different ways, amongst which the Bio-Concentration Factor (BCF) and the Bio-Accumulation Factor (BAF). Biomagnification is quantified with the Bio-Magnification Factor (BMF) and the Trophic Magnification Factor (TMF).

The bioconcentration factor (BCF) can be expressed as the ratio of the concentration of a chemical in an organism to the concentration of the chemical in the surrounding environment. For example, the BCF for an aquatic organism is the ratio of the steady state chemical concentration in an aquatic organism (C_B, g chemical/kg wet weight (ww)) and the water (C_W, g chemical/L) determined in a controlled laboratory experiment in which the test organisms are exposed to a chemical in the water but not in the diet (BCF = C_B/C_W). From kinetic studies the steady state BCF (= BCFk) can also be derived from the ratio of the uptake rate constant (k1) and the elimination rate constant (k2) (BCFk = k1/k2) (OECD, 2012).

The BAF for an organism is the ratio of the steady state chemical concentration in the organism (CB, g chemical/kg ww) and the surrounding medium such as water, sediment or soil determined from laboratory or field data in which sampled organisms are exposed to a chemical in the surrounding medium and in their diet (in case of water organisms the BAF = C_B/C_W (see OECD, 2012); in case of soil/sediment-inhabiting organisms the BAF may be $C_B/C_{pore\ water}$ or $C_B/C_{total\ soil}$ (see OECD, 2008b; OECD, 2010)).

The BMF is the ratio of the steady state chemical concentration in a water- or air-respiring organism (C_B, g chemical/kg ww) and in the diet of the organism (C_D, g chemical/kg ww) determined from laboratory or field data (BMF = C_B/C_D).

The TMF is the average factor by which the normalised chemical concentration in biota of a food web increases per trophic level.

Bioaccumulation of pesticides from different environmental compartments may lead to the building up of internal exposure concentrations in organisms, causing toxic effects, if critical internal toxic levels are exceeded. Bioaccumulation may proceed over long periods even when external concentrations are low. In addition, feeding and predation on contaminated prey may lead to food-web transfer and, in the case of highly bio-accumulative compounds, to biomagnification and toxic effects at higher trophic levels. Therefore, the potential bioaccumulation and food-web transfer of pesticides from terrestrial and aquatic organisms to birds and mammals needs to be addressed.

A risk assessment through secondary poisoning is triggered for lipophilic substances that may bioaccumulate. This applies to both the active substances and potential metabolites in soil and surface water. The potential for bioaccumulation of organic substances can be estimated from the value of the n-octanol/water partition coefficient (log P_{OW} or log K_{OW}). It is accepted in European legislation that values of log K_{OW} greater than or equal to 3 indicate that the substance may bioaccumulate.

For assessing the risk through secondary poisoning of organic substances, three different routes are considered, viz.:

- For the terrestrial compartment
  - ✓ Earthworm-eating birds and mammals
- For the aquatic compartment:
  - ✓ Fish-eating birds and mammals
  - ✓ Benthic invertebrate-eating birds and mammals.

The exposure routes described above do not cover all possible exposure routes for secondary poisoning that may occur in agricultural fields treated with organic pesticides or surface waters contaminated with these chemicals. Nevertheless, we consider them as a pragmatic and realistic worst-case proxy to assess their risks of secondary poisoning to birds and mammals. If, however, information is available that other types of chemicals (e.g. metals used as pesticide) and/or other routes of bioaccumulation may play an important role in secondary poisoning of birds and mammals, this should not be ignored in the risk assessment.

10.1.1. Current data requirements for PPPs

The data requirements (Commission Regulation (EU) 283/2013[4] and 284/2013[5]) prescribe consideration of the risk of bioconcentration and results of a fish bioconcentration test (OECD guideline 305; OECD, 2012), for compounds that are not rapidly degraded in water (< 90% loss in 24 h) and with a log K_{OW} > 3 or when other indications for bioconcentration potential are present (see Table 22).

Table 22: Regulations and data requirements for PPPs regarding bioconcentration, bioaccumulation and secondary poisoning

Regulation	Data requirements
Commission regulation (EU) No 546/2011[6] implementing Regulation (EC) No 1107/2009[3] of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of **PPPs**	Section C 2.5.2.2 ' Where there is a possibility of aquatic organisms being exposed, no authorisation shall be granted if …. the maximum bioconcentration factor (BCF) is **greater than 1000** for plant protection products containing active substances which are readily biodegradable or **greater than 100** for those which are not readily biodegradable … unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on the viability of exposed species (predators) occurs — directly or indirectly — ….'
Commission Regulation (EU) No 283/2013[4] of 1 March setting out the data requirements for **active substances**	Section 8.1.3. *Active substance bioconcentration in prey of birds and mammals* 'For active substances with a log P_{ow}[(1)] > 3, an assessment of the risk posed by bioconcentration of the substance in the prey of birds and mammals shall be provided.' 8.2.2.3. *Bioconcentration in fish* 'The bioconcentration of the substance, shall be assessed where: — the log P_{ow} is greater than 3 (see point 2.7) or there are other indications of bioconcentration, and — the substance is considered stable, that is to say there is less than 90% loss of the original substance over 24 h via hydrolysis[(2)] (see point 7.2.1.1).'
Commission Regulation (EU) No 284/2013[5] of 1 March setting out the data requirements for **PPPs**	Refers to Part A of the Annex to Regulation (EU) No 283/2013[4]

(1): P_{ow} old symbol for n-octanol water partitioning coefficient K_{ow}.
(2): Inconsistencies: degradation is only evaluated through hydrolysis; biodegradation is not included whereas it was included in the Regulation (EU) No 546/2011[6] (readily biodegradable).

10.2. Tier 1 secondary poisoning assessment for earthworm-eating birds and mammals

For earthworm-eating birds and mammals, two different approaches are presented: the pore water approach and the dry soil approach.

In the Scientific Opinion on in-soil organisms (EFSA PPR Panel, 2017), the PPR Panel confirmed that for soft-bodied soil organisms such as earthworms, the major uptake route is from soil pore water. However, based on the properties of the assessed substance, e.g. increasing hydrophobicity, other routes may need to be considered. The WG considers the pore water approach as the most scientifically appropriate option. Although the concentration in total soil may be consistently higher than the concentration in pore water, the exposure through pore water is considered more relevant for organisms like earthworms, as stated above. However, the tool currently used for the estimation of the concentration in soil does not include the option of calculating the concentration in pore water (FOCUS, 1997).

Software tools have been developed/updated (PERSAM for Tier 1 and 2, PEARL and PELMO for higher tiers) implementing the exposure assessment recommended in the EFSA Guidance for predicting environmental concentrations in soil (EFSA, 2017). However, until this Guidance and the new model are implemented, it is recommended to use the dry soil approach (as described in Section 10.2.2). When EFSA (2017) is implemented the pore water approach should be followed, unless there is information that the concentration in total soil may be more relevant when considering the properties of the substance under assessment. To this respect, it would be more appropriate that when the soil risk assessment is revised, a consistent approach is taken, and the same ERC (environmental relevant concentration) is considered.

A risk assessment through secondary poisoning may be triggered by the active substance under assessment and/or its pertinent metabolites in soil. The approaches described below are fully applicable to metabolites. When no toxicity endpoints are available for metabolites, it is recommended to assume the metabolite as 10 times more toxic than the parent (relevant reproductive endpoint/10). However, if it is clearly demonstrated (see Section 9.4.1 for further guidance) that the metabolite is not expected to be more toxic than the parent, the metabolite can be assumed to be equally toxic as the parent.

10.2.1. Pore water approach

As highlighted above, the pore water approach may be used when the EFSA Guidance for predicting environmental concentrations in soil (EFSA, 2017) and the related tools are implemented.

The following decision scheme for a Tier 1 risk assessment for secondary poisoning through the pore water approach of earthworm-eating birds and mammals is proposed:

1) The substance (i.e. a.s. and/or major soil metabolite) has a log $K_{OW} \geq 3$

   No: risk assessment for secondary poisoning of earthworm-eating birds and mammals not required
   Yes: perform the risk assessment by following steps 2–5

2) Select the worst-case[52] 7-d TWA $PEC_{soil;pw}$ for the upper soil layer as used in the risk assessment for soil invertebrates (currently a PEC_{soil} for the upper layer of 5cm is always used).

The use of the 7-d TWA PEC is suggested for Tier 1 as a conservative and pragmatic approach accounting for the lack of information on the time required for reaching steady state of the substance between the organism and the environment. Furthermore, note that a moving time-window approach should be used to select the highest 7d- TWA PEC_{soil} in which accumulation of the substance due to multi-year use is taken into account.

1) Calculate the $PEC_{earthworm}$ by means of the Equation 31 (also see Table 23 below):

$$PEC_{earthworm} = \left(eBCF_{earthworm} \times PEC_{soil;\,pw} + PEC_{soil;\,tot} \times F_{gut} \times CONV_{soil}\right) / \left(1 + F_{gut} \times CONV_{soil}\right) \quad (31)$$

The estimated Bioconcentration factor (eBCF) can be described as a hydrophobic partitioning between the pore water and the phases inside the organism and can be modelled according to Equations 32 and 33 as described by Jager (1998):

[52] PERSAM calculates different PEC values for different locations, i.e. North, Central and South EU. If the representative uses of the a.s. are for EU, the worst-case value from the 3 different locations should be selected.

$$eBCF_{earthworm} = (F_{water} + F_{lipids} \times K_{ow})/(RHO_{earthworm}) \quad (32)$$

Since $RHO_{earthworms}$ is 1, the formula is reduced to:

$$eBCF_{earthworm} = (F_{water} + F_{lipids} \times K_{ow}) \quad (33)$$

4) Estimate daily dose residues in earthworms (mg/kg bw per day) with the following equations:

$$\text{Daily dose } PE_{Cearthworm} \text{for bird/mammal GMS} = FIR/bw \times PEC_{earthworm} \quad (34)$$

where:
FIR/bw= Food Intake Rate divided by the body weight for earthworm-eating bird and mammal generic model species. For FIR/bw, see Annex B and Appendix G.

5) Calculate the TER for birds and mammals as follows:

$$TER = (\text{reproductive endpoint})/(\text{daily dose } PEC_{earthworm}) \quad (35)$$

For the selection of the relevant reproductive endpoint, see Sections 5.2.6.5 and 5.2.7

TER ≥ 5 Low risks due to secondary poisoning for earthworm-eating birds and/or mammals
TER < 5 High Risk due to secondary poisoning for earthworm-eating birds and/or mammals; consider a higher tier refinement

Table 23: Explanation of symbols and value for the pore water approach in the risk assessment for secondary poisoning of earthworm-eating birds and mammals

Symbol	Definition	Unit	Value (when available)	Reference
$CONV_{soil}$	Conversion factor for wet to dry soil $CONV_{soil} = RHO_{soil}/(RHO_{soild} \times F_{solid})$	$[kg_{wwt}/kg_{dwt}]$	1.07	ECHA, 2017b
$eBCF_{earthworm}$	Estimated Bioconcentration factor related to soil pore water	$[L/kg_{wwt}]$	Equation 33	
F_{solid}	Volume fraction of solids in soil	$[m^3/m^3]$	0.6	ECHA, 2017b; EFSA, 2009
F_{lipids}	Volume fraction lipids in earthworms	$[m^3/m^3]$	0.012	Jager, 1998
F_{gut}	Fraction of gut loading in worm	$[Kg_{dwt}/kg_{wwt}]$	0.1	Jager et al., 2003
F_{water}	Volume fraction water in earthworms	$[m^3/m^3]$	0.84	Jager, 1998
$PEC_{earthworm}$	Pesticide concentration in earthworm	$[mg/kg_{wwt}]$	Equation 31	
$PEC_{soil;pw}$	Pesticide concentration in soil pore water	[mg/L]		
$PEC_{soil;tot}$	Pesticide concentration in dry soil	[mg/kg]		
$RHO_{earthworm}$	Bulk density of worm	$[kg_{wwt}/L]$	1	Jager, 1998
RHO_{soil}	bulk density of wet soil	$[kg_{wwt}/m^3]$	1700	ECHA, 2017b
RHO_{solid}	bulk density of solid phase	$[kg_{dwt}/m^3]$	2650	Blake, 2008

10.2.2. Dry soil approach

The following decision scheme for a Tier 1 risk assessment for secondary poisoning of earthworm-eating birds and mammals by means of the dry soil approach is proposed (also see Table 24 below):

1) The substance (i.e. active substance and/or major soil metabolite), has a log $K_{ow} \geq 3$

No: risk assessment for secondary poisoning of earthworm-eating birds and mammals not required
Yes: perform the risk assessment by following steps 2–6

2) Select the 7-d TWA $PEC_{soil;tot}$ at a depth as used for the risk assessment of soil invertebrates (currently a PEC_{soil} at 5 cm is always used).

3) Calculate the $PEC_{earthworm}$ by means of Equation 36:

$$PEC_{earthworm} = PEC_{soil} \times eBCF_{earthworms} \quad (36)$$

Estimate the bioconcentration factor for the earthworm ($eBCF_{earthworm}$) by using the following equation (also see Table 24 below):

$$eBCF_{earthworm} = \left(F_{water} + F_{lipid} \times K_{ow}\right)/\left(f_{oc} \times K_{oc}\right) \quad (37)$$

4) Estimate daily dose residues in earthworms (mg/kg bw per day) by applying Equation 34 for both birds and mammal GMS.
5) Calculate the TER for birds and mammals by applying equation 34 above.

TER ≥ 5 Low risks due to secondary poisoning for earthworm-eating birds and/or mammals
TER < 5 High Risk due to secondary poisoning for earthworm-eating birds and/or mammals; consider a higher tier refinement.

Table 24: Explanation of symbols and value for the dry soil approach

Symbol	Definition	Unit	Value (when available)	Reference (if any)
$PEC_{earthworm}$	Pesticide concentration in earthworm	[mg/kg_{wwt}]	Equation 36	
$BCF_{earthworm}$	Bioconcentration factor	[L/kg_{wwt}]	Equation 37	
$PEC_{soil;tot}$	Pesticide concentration in dry soil	[mg/kg]		
F_{water}	Volume fraction water in earthworms	[m^3/m^3]	0.84	Jager (1998)
F_{lipids}	Volume fraction lipids in earthworms	[m^3/m^3]	0.012	Jager (1998)
K_{OW}	Octanol-water partition coefficient of the substance under assessment	[–]		
K_{OC}	Organic carbon adsorption coefficient	[L/kg]	It is recommended to use the same K_{oc} as used in fate as input parameter for modelling. This means that if the Koc geomean is available, then this can be used	
f_{OC}	Organic carbon content of soil	[kg/kg]	0.02	

10.2.3. Possible higher tier approaches to address secondary poisoning in the risk assessment procedure for earthworm-eating birds and mammals

Tier 2 approaches:

- Replacing the default $eBCF_{earthworm}$ by an experimentally derived BAF for terrestrial oligochaetes according to OECD guideline 317 (OECD, 2010);
- Refinement of the $PEC_{soil;pw}$ or $PEC_{soil;tot}$ by using a better-defined time frame for TWA PEC. Based on the experimentally derived BAF, as indicated above, information may be gathered whether a longer time frame than that recommended for Tier 1 (7-day) may be considered;
- TKTD modelling could also be a suitable approach but methods for soil organisms and guidance on how to evaluate those are not available, yet.

Tier 3 approaches:

- Identification of region/crop-specific focal species for earthworm-eating birds and mammals and refining the 'daily dose PEC_{worm}' (see Section 6.5.2);
- Refinement of PT and PD following the identification of region/crop-specific focal species (see Sections 6.5.3 and 6.5.4).

Tier 4 approaches:

- Food-chain modelling approach (see Appendix R);
- Harmonised technical guidance not yet developed;
- Case-by-case evaluation based on expert judgement;
- Recommendations on Good Modelling Practice should be followed (EFSA PPR Panel, 2014).

It has to be noted that earthworm field studies where residues in earthworms are measured in general are not considered a suitable refinement for the risk assessment though secondary poisoning, unless this is done for a representative large number of studies realistically covering the variability in environmental conditions (e.g. soil types) together with sufficiently frequent measurement of earthworm residues (to capture the peak value). Measurements of residues in earthworms in a limited number of experiments, covering a limited number of environmental conditions (e.g. soil types), may not provide a good estimate of the amount of a substance that may bioaccumulate in earthworms.

Following any type of refinement, it is expected that an uncertainty analysis is performed according to the recommendations in Section 13.3.

10.3. Bioaccumulation, biomagnification and secondary poisoning of fish-eating birds and mammals

10.3.1. Introduction

In this update of the Birds and Mammals GD, the Tier 1 assessment for secondary poisoning of pesticides from pelagic fish to fish-eating birds and mammals is considered covering the bioaccumulation and food-web transfer via the overlying water compartment. It is acknowledged, however, that in aquatic food webs secondary poisoning may involve more than two trophic levels (e.g. residues in water – phytoplankton – pelagic invertebrates – fish primary consumer – fish secondary consumer – fish-eating bird/mammal) so that in principle in the risk assessment for secondary poisoning both the BCF and BMF should be considered.

The recommendations with respect to bioconcentration in fish, and secondary poisoning from fish to fish-eating vertebrates, as mention in Section 7.6 of the Aquatic Guidance Document (EFSA PPR Panel, 2013) were considered in the proposal presented below, but note that in the new decision scheme the body weight of the Generic Model Species for a fish-eating bird and mammal is lowered and consequently the multiplication factors are different (see decision scheme in Section 10.3.2 below). Furthermore, the default BMFs from European Commission (2002b) were updated by adopting the default BMFs mentioned in ECHA (2017b) (see Table 25).

10.3.2. Proposed Tier 1 secondary poisoning assessment for fish-eating birds and mammals

The following decision scheme for a Tier 1 risk assessment for secondary poisoning to active substances and major metabolites of fish-eating birds and mammals is proposed:

1) The log K_{ow} (= log P_{ow}) ≥ 3 or there are other indications of bioconcentration, and the substance is considered stable, that is to say there is less than 90% loss of the original substance over 24 h via hydrolysis[53]

   No: risk assessment for secondary poisoning of fish-eating birds and mammals not required
   Yes: perform the risk assessment and go to 2

2) Based on expert judgement, is the default time window of 21 days a realistic worst-case TWA PEC_{sw} (moving time-window approach; taken from the aquatic environmental fate section) in the long-term (reproductive) ERA for (standard) bird and mammal species?[54] In

[53] There may, however, be other cases where it could be justified that dissipation is so fast that a fish BCF study is not needed.
[54] The time-window of the TWA PEC_{sw} should be aligned to the time required for the substance reaching equilibrium in the fish BCF study. When equilibrium is not reached during the BCF test, then a time window of 21 days can be used. For pragmatic reasons either a time window of 7 and 14 days can be selected if the time for reaching equilibrium is shorter than, respectively, 14 and 21 days (it is not anticipated that an averaging interval shorter than 7 days would be needed). Since in edge-of-field surface waters (particularly in streams) short-term pulse exposures are more often the rule than the exception, the WG decided not to select the $PEC_{max;sw}$.

the case of estimated BCF values, a 7-day TWA PEC_{sw} value should be used unless there is a clear justification as to why a different averaging period is more appropriate.

No: Select a time frame of either 7 or 14 days for the TWA PEC_{sw}, motivated by scientific reasoning, and perform the risk assessment by following steps 3–5
Yes: Select the default 21-day TWA PEC_{sw} and perform the risk assessment by following steps 3 to 5

3) Select the whole-body BCF_{fish} from the aquatic section in the dossier (BCF_k values should be reported as lipid-normalised values; default 5% lipid content) as well as the default BMF from Table 25. In case BCF_{fish} data are not available (e.g. for metabolites) QSAR should be used to predict a BCF_{fish}. If the QSAR prediction cannot be used (e.g. in the case it is not reliable) then the log K_{OW} can be used to estimate the BCF according to Table 25 (taking the highest value in the range).

Table 25: Default BMF (= estimated BMF = eBMF) values for organic substances (based on ECHA, 2017b). In principle, experimental BCF_{fish} values should be used to derive default BMF values. Only in case BCF_{fish} values are not available (e.g. for metabolites) the log K_{OW} should be used

log kow of substance	BCF(fish)	BMF
< 4.5	< 2,000	1
< 4.5–< 5	2,000–5,000	2
5–8	> 5000	10
> 8–9	2,000–5,000	3
> 9	< 2,000	1

4) Estimate daily dose residues in fish (mg/kg bw per day) with Equation 38 for both bird and mammals GMS:

$$\text{Daily dose } PEC_{fish} = FIR/bw \times \text{TWA } PEC_{sw} \times BCF_{fish} \times eBMF \quad (38)$$

Where:

FIR= Food Intake Rate divided by the body weight for fish-eating bird and mammal generic model species. For FIR/bw, see Annex B and Appendix G.
TWA PEC_{sw} = the time weighted average PEC (see step 2) in mg/L
BCF_{fish} = Bioconcentration Factor in fish (see step 3)
eBMF = Estimated Biomagnification Factor (see step 3)

5) Calculate the TER for birds and mammals as follows:

$$TER = (\text{reproductive endpoint})/(\text{daily dose } PEC_{fish}) \quad (39)$$

TER ≥ 5 Low risks due to secondary poisoning for fish-eating birds and/or mammals
TER < 5 High risk due to secondary poisoning for fish-eating birds and/or mammals cannot be excluded; consider a higher tier refinement

10.3.3. Possible higher tier approaches to address secondary poisoning in the risk assessment procedure for fish-eating birds and mammals

Tier 2 approaches:

- Refinement of PEC_{sw} and/or consideration of exposure risk mitigation;
- Replacing the default BMF for fish with an experimentally derived BMF according to OECD guideline 305 (OECD, 2012);
- Refinement of the internal body burden of fish (PEC_{fish}) due to time-variable exposure in water by means of TKTD modelling approaches;
- Refinement of the time-window for the TWA PEC_{sw} for PEC_{fish} calculation by means of TKTD modelling approaches.

Tier 3 approaches:

- Identification of region-specific focal species for fish-eating birds and mammals and refining the 'daily dose PEC_{fish}' due to deviating FIR/bw factors (see Section 6.5.2);
- Refinement of PT and PD following the identification of region/crop-specific focal species (see Sections 6.5.3 and 6.5.4).

Tier 4 approaches:

- Food-chain modelling approach (see Appendix R):
- Harmonised technical guidance not yet developed;
- Case-by-case evaluation based on expert judgement;
- Recommendations on Good Modelling Practice should be followed (EFSA PPR Panel, 2014).

Following any type of refinement, it is expected that an uncertainty analysis is performed according to the recommendations in Section 13.3.

10.4. Bioaccumulation and secondary poisoning of benthic invertebrate-eating birds and mammals

10.4.1. Introduction

In the Commission Regulation (EU) no 283/2013[4], there is no trigger for bioaccumulation testing with benthic invertebrates and currently only limited data are available on benthic invertebrate bioaccumulation studies with pesticides in existing dossiers and literature. Bioaccumulation, however, is of high relevance for benthic organisms since they may take up contaminants via different uptake routes, including the sediment compartment. The sediment compartment is a sink for substances being persistent and/or with a high bioaccumulation factor, and bioaccumulation processes are often slow. Consequently, benthic invertebrates have a great potential of accumulating toxic substances and transferring them to higher trophic levels. For this reason, OECD guideline 315 (OECD, 2008b) is developed, to test bioaccumulation in sediment-dwelling benthic oligochaetes.

The test described in OECD guideline 315 consists of two phases, usually a 28-days uptake phase, and an elimination phase of a maximum of 10 days. The uptake rate constant (ks), the elimination rate constant (ke) and the kinetic bioaccumulation factor (BAFK = ks/ke) are calculated. Besides, the worm lipid content, the sediment total organic carbon content and the residue level in worms at the end of the elimination phase are useful for the interpretation of the results.

Tuikka et al. (2016) evaluated bioaccumulation studies with the benthic oligochaete worm *Lumbriculus variegatus* and organic substances like polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons. They conclude that the freely dissolved pore water concentration can be used as a useful predictor of organic contaminant accumulation in benthic oligochaetes. They also conclude that when accumulation is predicted from the concentrations in dry sediment, the reliability may suffer.

EFSA PPR Panel (2015) recommends further developing an ERA scheme for secondary poisoning of pesticides (and major metabolites in sediment) also considering the uptake route via sediment and benthic organisms when certain triggers are met. These triggers are based on occurrence and persistence in sediment and lipophilicity of the pesticide and/or major metabolite under evaluation. A decision scheme to assess the risks of secondary poisoning of benthic invertebrate eating birds and mammals, however, is not given in EFSA PPR Panel (2015).

The International Centre for Pesticides and Health Risk Prevention (ICPS) in Italy recently published the (draft) report 'Update and harmonization of rice pesticide risk assessment and revision of European guidelines Ecotoxicology: birds and mammals' (ICPS, 2019). This report also addresses bioaccumulation of pesticides in benthic organisms. Rice cultivated areas are considered of high importance for the protection of several bird species typical of wet environments that use this environment for feeding and reproduction. Several of these bird species feed on aquatic organisms, including benthic invertebrates. In rice fields, the most relevant exposure for potential bioaccumulation and secondary poisoning is through the food chain from benthic invertebrates to benthophagous birds.

A risk assessment through secondary poisoning may be triggered by the active substance under assessment and/or its pertinent metabolites in sediment. The approaches described below are fully applicable to metabolites. When no reproductive toxicity endpoint is available for it, it is recommended to assume the metabolite as 10 times more toxic than the parent (relevant reproductive endpoint/10). However, if it is clearly demonstrated (see Section 9.4.1 for further guidance) that the metabolite is

not expected to be more toxic than the parent, the metabolite can be assumed to be equally toxic as the parent.

10.4.2. Proposed Tier 1 secondary poisoning assessment for benthic invertebrate-eating birds and mammals

In this update of the Birds and Mammals GD the Tier 1 assessment for secondary poisoning of pesticides and its major metabolites from benthic invertebrates to benthos-eating birds and mammals is considered covering the bioaccumulation and food-web transfer via the sediment compartment. Furthermore, it is assumed that the sediment-dwelling benthic oligochaete *L. variegatus* can be used as model species for benthic invertebrates to assess risks of secondary poisoning from benthic invertebrates to benthos-eating birds and mammals. In addition, it is assumed that the 'pore water approach' to assess secondary poisoning as developed for earthworm-eating birds and mammals (Jager, 1998; Section 10.2.1 above) can be used for the aquatic oligochaete *L. variegatus* as well. Earthworms are terrestrial soil-inhabiting oligochaetes and resemble benthic oligochaetes in their potential role to bioaccumulate organic chemicals partitioned to the soil/sediment compartment. Adopting the pore water approach also implies that the secondary poisoning assessment proposed can only be done when the FOCUS repair document (EFSA, 2020) is officially implemented.

The concentration of a pesticide in the aquatic oligochaete *L. variegatus* can be assessed by Equation 40:

$$PEC_{aq-worm} = \left(eBCF_{aq-worm} \times PEC_{sed;\ pw} + PEC_{sed;\ tot} \times F_{gut} \times CONV_{sed}\right)/\left(1 + F_{gut} \times CONV_{sed}\right) \quad (40)$$

where

$$eBCF_{aq-worm} = \left(F_{water} + F_{lipid} \times K_{OW}\right)/RHO_{aq-worm} \quad (41)$$

and

$$CONV_{sed} = RHO_{sed}/\left(F_{solid} \times RHO_{solid}\right) \quad (42)$$

The bulk density of the *L. variegatus* (= $RHO_{aq\text{-}worm}$) is assumed to be equal to water, so 1 kg/L (see Jager, 1998). Consequently, the fraction of water on the wet weight basis of *L. variegatus* is assumed to be the same as on volume basis (= F_{water}). According to the reported water content of 80% for *L. variegatus* by Mount et al. (1999) the F_{water} becomes then 0.80. According to Jager (1998) the bulk density of lipids can be assumed to be equal to octanol (= 0.83 kg/L). Consequently, when dividing the fraction of lipid on wet weight basis reported for *L. variegatus* by Mount et al. (1999) by the bulk density of lipids, the fraction of lipids on volume basis (= F_{lipid}) becomes 0.01/0.83 = 0.012. The $eBCF_{aq\text{-}worm}$ in Equation 41 then becomes as in Equation 43:

$$eBCF_{aq-worm} = (0.80 + 0.012 \times K_{OW})/1 \quad (43)$$

The concentration of a pesticide (or major metabolite) in *L. variegatus* can then be assessed by Equation 44:

$$PEC_{aq-worm} = \left((0.80 + 0.012 \times K_{OW}) \times PEC_{sed;\ pw} + PEC_{sed;\ tot} \times F_{gut} \times CONV_{sed}\right)/ \left(1 + F_{gut} \times CONV_{sed}\right) \quad (44)$$

Since the gut content of *L. variegatus* reported by Mount et al. (1999) is on average 15% on dry weight basis of the worm, its F_{gut} is calculated to be 0.03 [kg_{dwt}/kg_{wwt}] when considering a water content of 80% of the worm. Equation 44 becomes as in Equation 45:

$$PEC_{aq-worm} = \left((0.80 + 0.012 \times K_{OW}) \times PEC_{sed;\ pw} + PEC_{sed;\ tot} \times 0.03 \times CONV_{sed}\right)/ \left(1 + 0.03 \times CONV_{sed}\right) \quad (45)$$

The values for $PEC_{sed;pw}$ and $PEC_{sed;tot}$ for edge-of-field surface waters can be assessed by means of the approach described in FOCUS repair (EFSA, 2020). Since the vast majority of sediment-dwelling

invertebrates occur in the upper sediment layer, it is proposed to use the $PEC_{sed;pw}$ and $PEC_{sed;tot}$ values as calculated for the upper 1 cm of the sediment layer in the risk assessment as suggested by EFSA PPR Panel (2015).

The following decision scheme for a Tier 1 risk assessment for secondary poisoning of benthos-eating birds and mammals is proposed:

1) The substance has a log $K_{ow} \geq 3$ and a degradation half-life ($DegDT_{90}$) > 100 days in the OECD guideline 308 water-sediment fate study for the total system (OECD, 2002),[55] or, when the $DegDT_{50} < 120$ days, (i) 10% or more of the substance (applied radioactivity) can be found in the sediment at or after 14 days in the experimental water-sediment study, or (ii) 10% or more of the annual dose applied occurs in sediment at the time of maximum PEC_{sed} on basis of appropriate model calculations (e.g. FOCUS repair, EFSA, 2020).[56]

   No: risk assessment for secondary poisoning of benthos-eating birds and mammals not required
   Yes: perform the risk assessment by following steps 2–5

It should be noted that the criteria for considering persistence in sediment in point 1, above, is taken from the EFSA Aquatic Guidance document (EFSA PPR Panel, 2013). Should a different trigger be included in a future guidance document for sediment dwelling organisms, then the above trigger should be reconsidered and aligned.

2) Select the 7-d TWA $PEC_{sed;pw}$ and 7-d $PEC_{sed;tot}$ for the sediment layer as used for the risk assessment of benthic invertebrates (currently a PEC_{sed} for the upper 1cm is used).

The use of the 7-d TWA PEC is suggested for Tier 1 as a conservative and pragmatic approach in line with the approach selected for earthworm-eating birds and mammals (see Section 10.2). Note that a moving time-window approach should be used to select the highest 7-d TWA PEC_{sed} in which accumulation of the substance due to multi-year use is taken into account.

3) Calculate the $PEC_{aq\text{-}worm}$ by means of the Equation 45 (also see Table 26 below)
4) Estimate daily dose residues in benthos (mg/kg bw per day) with the Equation 46 for both bird and mammals GMS:

$$\text{Daily dose } PEC_{aq-worm} = FIR/bw \times PEC_{aq-worm} \quad (46)$$

where:

FIR= Food Intake Rate divided by the body weight for benthic invertebrate -eating bird and mammal generic model species. For FIR/bw, see Annex B and Appendix G.

5) Calculate the TER for birds and mammals by applying Equation 47:

$$TER = (\text{reproductive endpoint})/(\text{daily dose } PEC_{aq\text{-}worm}) \quad (47)$$

TER ≥ 5 Low risk due to secondary poisoning for benthic worm-eating birds and/or mammals
TER < 5 High Risk due to secondary poisoning for benthic worm-eating birds and/or mammals could not be excluded.

Table 26: Explanation of symbols and values in the Tier 1 risk assessment of secondary poisoning for benthos-eating birds and mammals

Symbol	Definition	Unit	Value (when available)	Reference
$PEC_{aq\text{-}worm}$	Pesticide concentration in benthic oligochaete worm	[mg/kg_{wwt}]	Equation 45	
$eBCF_{aq\text{-}worm}$	Estimated Bioconcentration factor related to sediment pore water	[L/kg_{wwt}]	Equation 43	

[55] If several valid $DegDT_{90}$ are available, the geometric mean value can be used.
[56] Selected triggers inspired by recommendations in EFSA PPR Panel, 2013 and EFSA PPR Panel (2015).

Symbol	Definition	Unit	Value (when available)	Reference
$PEC_{sed;pw}$	Pesticide concentration in sediment pore water	[mg/L]		
$PEC_{sed;tot}$	Pesticide concentration in dry sediment	[mg/kg]		
F_{water}	Volume fraction water in benthic oligochaete worms	[m^3/m^3]	0.80	Mount et al. (1999) Jager, 1998
F_{lipids}	Volume fraction lipids in benthic oligochaete worms	[m^3/m^3]	0.012	Mount et al. (1999)[57] Jager, 1998
RHO_{worm}	Bulk density of worm	[kg_{wwt}/L]	1	Jager, 1998
F_{gut}	fraction of gut loading in worm	[Kg_{dwt}/kg_{wwt}]	0.03	Mount et al. (1999)[57]
$CONV_{sed}$	conversion factor for wet to dry sediment $CONV_{sed} = RHO_{sed}/(RHO_{soild} \times F_{solid})$	[kg_{wwt}/kg_{dwt}]	2.6	
RHO_{sed}	bulk density of wet sediment	[kg_{wwt}/m^3]	1300	ECHA, 2017b
RHO_{solid}	Bulk density of solid phase in sediment	[kg_{dwt}/m^3]	2500	ECHA, 2017b
F_{solid}	Volume fraction of solids in sediment	[m^3/m^3]	0.2	ECHA, 2017b

10.4.3. Possible higher tier approaches to address secondary poisoning in the risk assessment procedure for benthic invertebrate-eating birds and mammals

Tier 2 approaches:

- Refinement of PEC_{sed} and/or consideration of exposure risk mitigation;
- Replacing the eBCF for *L. variegatus* by an experimentally derived BAF according to OECD guideline 315 (OECD, 2008b);
- Refinement of the $PEC_{sed;pw}$ or $PEC_{sed;tot}$ by using a better-defined time frame for TWA PEC. Based on the experimentally derived BAF, as indicated above, information may be gathered whether a longer time frame than that recommended for Tier 1 (7-day) may be considered;
- Refinement of the internal body burden of *L. variegatus* ($PEC_{aq\text{-}worm}$) due to time-variable exposure in sediment by means of TKTD modelling approaches;
- Refinement of the time-window for the TWA PEC_{sed} for $PEC_{aq\text{-}worm}$ calculation by means of TKTD modelling approaches.

Tier 3 approaches:

- Identification of region/crop-specific focal species for benthic invertebrate-eating birds and mammals and refining the 'daily dose $PEC_{aq\text{-}worm}$' (see Section 6.5.2);
- Refinement of PT and PD following the identification of region/crop-specific focal species (see Sections 6.5.3 and 6.5.4).

Tier 4 approaches:

- Food-chain modelling approach (see Appendix R);
- Harmonised technical guidance not yet developed;
- Case-by-case evaluation based on expert judgement;
- Recommendations on Good Modelling Practice should be followed (EFSA PPR Panel, 2014).

Following any type of refinement, it is expected that an uncertainty analysis is performed according to the recommendations in Section 13.3.

[57] Mount et al. (1999) report for *L. variegatus* a lipid content of 1% on wet weight basis, a water content of 80% and a gut content of 10-20% on dry weight basis. Several papers (e.g. Leppänen and Kukkonen, 1998; Nybom et al., 2012; Zhang et al., 2013; Abel et al., 2017) report lipid contents of *L. variegatus* within a range of 0.52–1.32 % on wet weight basis. Of these papers, Leppänen and Kukkonen (1998) also report a water content of 90% for *L. variegatus*, but none of these papers provide information on the fraction of sediment/detritus in the gut of this species. For this reason, we selected the information provided by Mount et al. (1999) to predict the PEC in *L. variegatus*.

11. Risk assessment via contaminated water

11.1. Leaf scenario and calculation of PEC_{pool}

Birds may be acutely exposed to pesticides when drinking contaminated water from leaf whorls. This exposure route, hereby defined as the 'leaf scenario', was developed by EFSA (2009), in response to concerns that this oral uptake may be linked to poisoning incidents. Hommes et al. (1990) and Schietinger and Hofmann (1984) in Germany reported an episode of outstanding bird mortality and attributed it to this exposure route. Specifically, authors reported that pesticide spraying of Brassica crops followed by overhead irrigation resulted in the transient collection of pesticide-contaminated water in leaf whorls. Therefore, consequent to the dry weather and the local scarcity of water resources, birds were attracted to, and drank this contaminated water, therefore, getting poisoned.

The leaf scenario is a worst-case exposure relevant for any treatment method that may lead to the leaf deposition of pesticide residues (e.g. spray applications, drenching, dusting, broadcast granules above crops). However, given that the prolonged collection of sufficient volumes of water in leaf structures requires specific morphological features, the leaf scenario should only be addressed in a sub-set of the crops reported in Table 27. Specifically, the morphological features that may potentially favour the formation of sufficiently large, contaminated water reservoirs for a sufficient time frame allowing acute exposure of birds are the following:

i) leaves should point upwards and should be closely pressed against each other and/or;
ii) leaves and/or the stem at their basis should form cavities and;
iii) these structures must be accessible to birds.

Based on these criteria, and the update of Appendix E (i.e. crop group selection for sprays), the crop categories, as reported in Table 27, are proposed to be relevant for an assessment according to the leaf scenario.

Table 27: Crop groups for which the leaf scenario should be addressed

Crop group[2]	Crop stage (BBCH[1])	EPPO Name and link
Leafy vegetable crops (excluding brassica)	From principal growth stage 4, till harvest	leafy vegetable crops (excluding brassica) (3LEAC)
ornamental cactuses and succulents	All[3]	ornamental cactuses and succulents (3ORSC)
ornamental herbaceous plants	All[3]	ornamental herbaceous plants(3ORHC)
Non-bulb ornamental herbaceous plants	All[3]	–
Bulb-like ornamental herbaceous plants	All[3]	–
Vegetable brassica crops (3VBRC)	From principal growth stage 4, till harvest	vegetable brassica crops (3VBRC)

(1): Meier, U. (Meier, 2001). *Growth stages of mono-and dicotyledonous plants*. Blackwell Wissenschafts-Verlag.
(2): More information on crop groups can be found in Appendix E.
(3): BBCH growth stage cannot be specified for ornamentals. However, the criteria for morphological features described above should be considered when deciding whether there is a need for such assessment.

The leaf scenario should be addressed for any of crop groups listed in Table 27, except when the assessment is only relevant to a sub-set of these crops, whose morphology is such that exposure via this route can be excluded.

The leaf scenario is not deemed relevant for mammals, since no record of poisoning incidents of mammals has ever been linked to the consumption of contaminated water from leaf whorls.

11.1.1. Decision scheme for products and active substances applied as a liquid

For PPP applied as a liquid, the assessment should be performed according to the following decision scheme:

1) Does the GAP include a crop/plant listed in Table 27?
   a) If **no,** further assessment is not triggered.
   b) If **yes,** proceed to step 2.
2) Calculate the concentration of the active substance in the pools collected in the leaf axils, PEC_{pool} (mg/L), by the following:

Based on measurements conducted in Hommes et al. (1990) and Schietinger and Hofmann (1984), it was concluded that, for a worst-case exposure characterisation, it should be assumed that the pesticide concentration in leaf whorls is 5 times lower than the spray concentration (i.e. the concentration of active substance when the plant protection product is diluted in the required amount of water).

$$C_{application\ solution} = AR \times AV \quad (48)$$

AR = application rate (mg a.s./ha) – where a range of application rates are specified in the GAP, the highest rate can be used in the first instance in a risk envelope approach.
AV = application volume (L/ha) – where a range of volumes is proposed in GAP, the lowest value should be used

$$PEC_{pool} = \frac{C_{application\ solution}}{5} \quad (49)$$

$C_{application\ solution}$ = worst-case concentration of active substance in the treatment solution (mg/L)
PEC_{pool} = Predicted environmental concentration in the pool of the leaf whorl (mg/L)

3) Perform an acute risk assessment for birds from consumption of contaminated water from leaves:
   a) Calculate exposure to small birds by multiplying the PEC_{pool} by the water intake rate (0.46 L/kg bw per day) of small birds.
   b) Calculate an acute TER value.
      i) If the acute TER $\geq$ 10, low risk is indicated. If $<$ 10 high risk is not excluded. Consider refinement options.

For mixtures of multiple active substances, an additive assessment should be done (see chapter 12).

11.1.2. Consideration for products and active substances applied as a solid formulation

As discussed above, concentrations of active substances in leaf whorls are also expected after the application of solid formulations above crops followed by subsequent irrigation. A theoretical calculation of the concentration in the pool would be complex and need information of the collection of the formulation/active substance on the plant leaves which will differ pending on the formulation type. It was noted that solid formulations applied above the crop (e.g. dusting) are rarely used in the EU. However, in the case that a solid formulation is applied to the crops/plants in Table 27 then the applicant should address the acute risk to birds through the consumption of contaminated water in leaf whorls. A study to measure the concentration of the active substance in the leaf whorls (i.e. PEC_{pool} (mg/L)) may be the simplest way to address such assessment (90th percentile residue value should be used). The risk assessment can then be performed calculating the residue intake to small birds by multiplying PEC_{pool} (mg/L) by the water intake rate (0.46 L/kg bw per day). Acute TER values can then be calculated and compared to the trigger value of 10.

11.2. Puddle scenario and calculation of PEC_{puddle}

A risk assessment is needed for birds and mammals taking contaminated water from puddles, containing residues of pesticides and/or metabolites, formed in the field after a heavy rainfall event. This is needed in all cases where there is contamination of the soil and for soil metabolites requiring further assessment.

EFSA (2009) recommended that a risk assessment was performed for birds and mammals taking contaminated water from puddles, containing residues of pesticides and/or metabolites, formed in the

field after a heavy rainfall event. A quantitative risk assessment was only needed for those substances which did not 'pass' as screening step. It was questioned whether a risk assessment was warranted as experience had shown that few substances failed the assessment. However, the methodology for calculating the PEC in puddle water has also been questioned (EFSA PPR Panel, 2012). Two approaches for estimating PEC_{puddle} have been suggested over the past years by EFSA (EFSA, 2017). Based on those a scoping assessment was performed (Appendix S) and on this basis the WG decided to propose the use the pore water PEC value in the top 1 cm of soil to estimate PEC_{puddle}. The assessment should be performed according to the following decision scheme:

1) Is the ratio of the total dose application rate (g/ha) (i.e. accounting for multiple applications) to the lowest relevant, acute or reproduction toxicity endpoint for birds or mammal (mg/kg bw (per day)) > 50 (for substances with a K_{oc} < 500 L/Kg) or > 3,000 (for substances with a $K_{oc} \geq 500$ L/Kg)?

   If **yes,** screening assessment is not sufficient to indicate a low risk. Proceed to step 2.
   If **no,** the screening assessment is sufficient to indicate a **low** acute and reproductive **risk** to birds and mammals.

2) Perform an acute and reproductive assessment for birds and mammals from consumption of contaminated water from puddles:

   Calculate PEC in puddles (mg/L) by most appropriate methodology (see following section).
   Calculate exposure to small birds by multiplying the PEC in puddles by the water intake rate (0.46 L/kg bw per day) for small birds.
   Calculate exposure to small mammals by multiplying the PEC in puddles by the water intake rate (0.24 L/kg bw per day) for small mammals
   Calculate acute and reproductive TER values.
      If the acute TER ≥ 10, low risk is indicated. If < 10 high risk is not excluded. Proceed to step 3.
      If the reproductive TER ≥ 5, low risk indicated. If < 5 high risk is not excluded. Proceed to step 3.
      Consider possible options for refinement (see note 1 below).

3) Soil metabolites should follow the same decision scheme. If toxicity data are not available for the relevant soil metabolites, a surrogate endpoint may be selected (see Sections 9.4.1 and 9.4.3).

Note 1: When the assessment indicates a high risk to birds and mammals from the consumption of water from puddles then a refined assessment should be performed. In first instance, refinement of the exposure, i.e. PEC_{puddle}, should be evaluated. Generation of further vertebrate data should be only considered as a last resort.

PEC in puddle water

The concentration in maximum PEC_{pw} (1 cm) calculated according to EFSA (2017), should be used to estimate the PEC in puddle water when this guidance document becomes officially implemented.

Until EFSA (2017), is implemented then the PEC puddle water using the methodology as described in EFSA (2009), and as given below, should be used. If there are multiple K_{oc} values available, then the geometric mean of the available values should be taken. Please note that if an implementation date for EFSA (2017), is agreed before the current guidance document is finalised then the following section will be deleted.

$$\mathbf{PEC_{puddle}} = \frac{\mathbf{AReff/10}}{\mathbf{1000} \times (\mathbf{0.02} + \mathbf{K_{oc}} \times \mathbf{0.0015})} \quad (50)$$

Where:
PEC_{puddle} = Predicted Environmental Concentration in puddles [mg/L]
AR_{eff} = application rate [g/ha]; divisor of 10 to achieve rate in mg/m^2
w = 0.02 (pore water term: volume)
s = 0.0015 (soil term: volume, density, organic carbon content)

When multiple spray applications are considered, in the first instance it is simpler to use the total application rate for all applications. If needed a MAF_{repro} based on the DT_{50} in soil (the one selected for the soil exposure assessment) may be applied to achieve the effective application rate AR_{eff}. The MAF_{repro} equations are given in Section 6.2.5.2.

$$\mathbf{AReff} = \mathbf{AR} \times \left(\frac{\mathbf{1} - \mathbf{e}^{-\mathbf{nki}}}{\mathbf{1} - \mathbf{e}^{-\mathbf{ki}}} \right) \quad (51)$$

Where:
AR = application rate [g/ha]
k = ln(2)/DT_{50} (rate constant)
n = number of applications
i = application interval (days)

12. Risk assessment for formulated Plant Protection Products

12.1. Approach to the risk assessment for formulated PPP

According to Regulation 284/2013[5], toxicity data with the PPP on birds and mammals should be provided when exposure to the PPP is possible and the toxicity of the PPP cannot be predicted on the basis of the data for the active substance. Generally, while acute toxicity data with PPPs are commonly available for mammals (since those are also needed for classification and labelling purposes), acute toxicity data with PPPs are rarely available for birds.

To this regard and considering the regulatory requirement to minimise vertebrate testing according to EU Directive 2010/63[31], it is recommended to avoid requesting and submitting PPP toxicity data with birds. An approach is provided to check if the active substance is more toxic when formulated and how to conduct the risk assessment for birds. In general, reproductive toxicity data with PPP are not warranted as long-term exposure to the intact PPP is not expected. It is acknowledged that it is feasible that long-term exposure to a combination of the active substance and the co-formulants is possible. However, when considering the need for vertebrate studies there is a careful balance between requesting data and acknowledging uncertainty.

12.2. Approach for PPP with one active substance

Step 1

Is it possible to predict the toxicity of the plant protection product on the basis of the data for the active substance?

To answer the question, available acute toxicity data with mammals for both the active substance and the PPP should be checked and the respective LD_{50}s compared.

In the case that the toxicological assessment has concluded that experimental data are not needed for estimating the acute oral toxicity to mammals (7.1.1 of Regulation 284/2013[4]), then a judgement will be needed to decide whether the same reasoning can be applied to exclude the need for an acute oral toxicity study with birds. It is expected that, only in rare cases, an acute oral toxicity study with birds would be warranted.

Yes: no additional assessment needed. The risk assessment for the active substance can be considered to address the risk from the PPP.
No: go to step 2

Step 2

Do the active substance and the PPP show a comparable acute toxicity to mammals?

Yes: conduct the risk assessment with the data with the active substance for both birds and mammals. Please note that when the difference between the LD_{50}s is within a factor of 3, the active substance and the PPP are considered comparable (European Commission, 2012; EFSA, 2019b).
No: go to step 3

Step 3

If the PPP exhibits a higher toxicity than the active substance (more than a factor of 3), the acute risk assessment for mammals should be done using the toxicity endpoint of the PPP, expressed in terms of active substance content. For birds, since as described above, formulation data are not commonly available, the risk assessment should be performed by using the relevant LD_{50} of the active substance adjusted by a correction factor.

$$\text{Correction factor}_{\text{birds}} : LD_{50PPP}/LD_{50a.s.} \quad (52)$$

where the LD_{50}s are the ones related to mammals and are expressed in terms of mg a.s./kg bw.

If the low risk cannot be demonstrated, see Sections 6.4, 6.5 and chapter 7, for possible refinements.

It has to be noted that the recommendations of using a correction factor for birds for formulations with one active is in line with the recommendations for the mixture for the use of an adjustment factor, as explained in Section 12.3.3.

12.3. Approach for formulation with more than one active substance

The Regulation (EC) No 1107/2009[3] requires in Article 29 that 'interaction between the active substance, safeners, synergists and co-formulants shall be taken into account' in the evaluation and authorisation. This explicitly refers to marketed PPPs, which are, by origin, technical mixtures containing one to several a.s., plus, typically, several co-formulants. Furthermore, the standard data requirements for PPP (Commission Regulation (EU) No 284/2013[5]) do request 'any information on potentially unacceptable effects of the plant protection product on the environment, on plants and plant products shall be included as well as known and expected cumulative and synergistic effects'. First steps into guidance for an adequate consideration of mixture toxicity in the RA of PPPs had already been undertaken in the former EFSA GD for birds and mammals (EFSA, 2009). Since then, a recent EFSA 'Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals' (EFSA Scientific Committee, 2019) has been published. The approach of this guidance builds on existing methods and international experience in assessing potential concerns about chemical mixtures. The default option consists in adding up the doses for common effects to estimate the overall risk, i.e. it adopts the so-called 'dose addition' (DA) paradigm. However, sometimes, the chemicals 'interact', meaning their toxicity increases or decreases, i.e. synergistic or antagonistic effects. Interactions need checking particularly if toxicity increases.

The previous EFSA GD for birds and mammals (EFSA, 2009) already recommended dose addition as the concept of choice for combined toxicity and risk assessment.

The basic concept of the risk assessment for birds and mammals is that animals are exposed to residues of active substances in the environment, e.g. via their food. Thus, the following steps do not refer to an assessment of formulation toxicity as such, but of the expected effects from simultaneous exposure to a mixture of active substances (and possibly also toxic co-formulants or metabolites, see Section 9.4.2) in the environment resulting from use of that formulation. Further below, two approaches are proposed for addressing the risk posed by PPPs with multiple active substances, depending on the availability of experimental data with the formulation and potential for synergism. For both approaches, the full toxicological profile to birds and wild mammals should be known for each active substance included in the formulation.

Theoretically, the same approaches could also be used for co-formulants if toxicity data were available. For combined risk assessments for active substances and toxic metabolites – please also see Section 9.4.2.

12.3.1. Default approach

This approach should be followed as a default whenever experimental studies with the formulated mixture are not available for either birds or mammals. In addition, the same approach can be followed when experiments with the formulated mixture are available, but they do not indicate any synergic effects (see next Section 12.3.2).

In this case, a consideration should be made of whether information from other sources (e.g. literature, regulatory assessments of comparable PPP) raises a concern that the assumption of DA may

not be met for the substances in the mixture. If a concern is raised, then it should be considered whether toxicity data shall be requested

In the absence of an explicit concern, it is considered appropriate to rely on the DA assumption, in line with the recommendations from EFSA Scientific Committee (2019).

One way of looking at the DA paradigm is to express the toxicity of a mixture as the sum of the toxic units (hereafter TU) of its components. A TU is defined as the ratio between the dose of a mixture component and its toxicological endpoint.[58] In the context of the risk assessment presented in this guidance document, this is equivalent to the reciprocal of the TER, or, which is the same, TU = 1/TER.

Thus, the combined risk for a mixture of *n* components, under a pure DA assumption, can be expressed as:

$$\mathbf{TER_{combi}} = \sum_{i=1}^{n} \mathbf{TU_i} = \left(\sum_{i=1}^{n} \frac{\mathbf{1}}{\mathbf{TER_i}} \right)^{-1} \quad (53)$$

where TU_i and TER_i indicate the TU and the TER for the i-th substance. Trigger values are the same used in the single substance assessment (10 for acute, 5 for reproductive).

Note that this approach does not pose any limitation in terms of refinement for the TER calculation of single substances, as the integration at the mixture level only occurs after the individual TERs have been calculated.

12.3.2. Data-informed approach

Whenever experimental data are available with the formulated mixture, these can be used to check the appropriateness of the DA assumption.

In some cases, it may be that data are available for PPP/mixture which is not the one under assessment. It is considered reasonable to perform a check of the DA assumption for comparable PPPs or mixture, but care must be taken to decide if the PPP/mixture is comparable as the formulation type and co-formulants can influence the toxicity. For the assessment of DA for terrestrial vertebrates, if the PPP is the same formulation type and the amount of co-formulants are within ±10%, or co-formulants are not expected to influence the toxicity, then PPPs can be considered comparable.

STEP 1: To check whether the DA assumption holds true, the first step is the calculation of the mixture toxicity in accordance with the dose addition model (often referred to as Finney's equation). This can be done for either acute or reproductive endpoints.

$$\mathbf{Endpoint_{mix,DA}} = \left(\sum_{i=1}^{n} \frac{\mathbf{p_i}}{\mathbf{Endpoint_i}} \right)^{-1} \quad (54)$$

Where:

- p_i indicates the relative proportion of the i-th substance in the mixture (formulation)
- $Endpoint_i$ indicates the endpoint for the i-th substance in the mixture (formulation) containing n components

STEP 2: the DA assumption can be checked by using the concept of 'Model Deviation Ratio' (MDR), proposed by Belden et al. (2007), which is simply the ratio between the estimated mixture endpoint obtained with the DA approach ($Endpoint_{mix,DA}$) and the experimental mixture endpoint ($Endpoint_{mix,exp}$)

$$\mathbf{MDR} = \frac{\mathbf{Endpoint_{mix,DA}}}{\mathbf{Endpoint_{mix,exp}}} \quad (55)$$

If MDR is < 3, DA is either confirmed (0.33 < MDR < 3) or antagonistic effects cannot be excluded (MDR < 0.33). In both cases it is considered sufficiently conservative to base the risk assessment on additive toxicity and the default approach illustrated above (see 12.3.1) should be followed.

[58] While TU are most often associated with acute data, the same principle can be applied to reproductive/chronic data without any conceptual restriction. The lowest reproductive/chronic endpoint value used for the risk assessment from each substance has to be used in a first Step.

If MDR > 3, the mixture is proven more toxic than what is predicted by the DA model. Synergistic effects cannot be excluded, thus the calculation of the TER_{combi} should be amended by correcting the TU =(1/TER) of each component by the appropriate MDR.

$$\mathbf{TER_{combi}} = \left(\sum_{i=1}^{n} \frac{\mathbf{1}}{\mathbf{TER_i}} \times \mathbf{MDR}\right)^{-1} \quad (56)$$

Trigger values are the same used in the single substance assessment (10 for acute, 5 for reproductive).

Note that, in this case, the TER_{combi} is numerically equivalent to a standard TER obtained as a ratio between the experimental endpoint ($Endpoint_{mix,exp}$ expressed either as sum of the active substances doses or as product dose) and an exposure expressed in the same currency.

Note also that the calculated MDR reflects the difference between a modelled and an experimental endpoint for a mixture whose component proportions are fixed. It is not possible to predict whether the MDR would change (and in which direction) in case such proportions were different.

This has no consequences for screening and tier-1 risk assessment. However, it may have practical implications for higher tiers, if the exposure is refined. Care should be taken that any of the refinements do not result in a change in the relative proportion of the mixture components. For example, a refinement of the RUDs or of the DT_{50} will most likely trigger such change, unless the refined RUDs and/or the DT_{50} are the same for all components of the mixture.

To address this issue, this kind of refinement is acceptable only if refined figures are available for all components of the mixture. In such a case, the worst-case (i.e. highest RUD and longer DT_{50}) among the components should be applied transversally to all the components of the mixture.

In the case that one of the substances in the mixture does not meet the fTWA criteria described in Section 6.1.4, then the mixture cannot be assumed to meet the fTWA criteria and dissipation for any component cannot be accounted for.

12.3.3. MDR extrapolation

Data, if any, will most likely allow calculation of MDR only for the acute endpoint for mammals. Formulation/mixture data are unlikely available for birds or for reproductive toxicity.

Nevertheless, whenever a formulation study is available (for example for acute toxicity to mammals) and synergistic effects are found, the derived MDR > 3 should be used in the TER_{combi} calculation for all assessments, for both birds and mammals, and for both acute and reproductive assessments.

The only exception to this is the unlikely situation when multiple MDR can be calculated (for example for acute toxicity to both birds and mammals). In such a case, the most representative MDR should be used for each assessment, in agreement with the following prioritisation:

1) MDR is available for the specific endpoint. That is, when doing an acute bird risk assessment, if an MDR is available for acute birds, this should be applied.
2) MDR is not available for the specific endpoint, but available for another endpoint of the same taxonomic group (e.g. birds). That is, when doing a reproductive bird risk assessment, if an acute MDR is available for birds, but not a reproductive one, the acute MDR should be used for reproductive bird risk assessment as well.
3) MDR is not available for the specific taxonomic group, but at least one is available for the other. That is, when doing either an acute or a reproductive risk assessment for birds, if no MDR for birds is available, but at least one for mammals is available, the highest MDR for mammals can be used for birds (acute and reproductive) as well.

12.3.4. Special considerations for secondary poisoning and consumption of contaminated water

Considerations of mixture for exposure via secondary poisoning and consumption of contaminated water are only needed when multiple active substances in the product meet the relevant thresholds described in Chapters 10 and 11. When this is the case, it is expected that the relative exposure levels will not mirror the relative proportion of the substances in the formulated product. In consideration of this, the MDR approach is unprofitable. Thus, while acknowledging that potential effects due to

synergy are underestimated, the only feasible solution is to use the default approach (see Section 12.3.1) based on a pure DA model.

13. Uncertainty analysis and weight-of-evidence

13.1. Introduction to uncertainty analysis

Uncertainty surrounding decision-making is an ever-present (though often not explicitly discussed) reality. The lower the uncertainty in the risk assessment, the higher the level of confidence can be in the decision making. That is why, besides acknowledging and transparently communicating uncertainty, it is extremely important to provide the appropriate context for an understanding (weighing) of the uncertainty involved Reckhow (1994).

In the context of environmental risk assessment and management decisions, uncertainty plays a role in both the outcome of the risk assessment and the eventual risk management decision(s). In the former, an understanding and weighing of the inherent uncertainties in the risk assessment will help with eventual weight-of-evidence considerations and conclusions as to the risk level predicted from the risk assessment. These will in turn inform the latter, allowing more detailed and useful risk management decisions (EFSA Scientific Committee, 2018).

Uncertainty, either qualitative or quantitative, refers to the lack of data or an incomplete understanding of the context of the risk assessment. With more, or better data, the uncertainty can be reduced or, in the best case, even eliminated. Uncertainty should not be confused with variability, the quantitative description of the range or spread of a set of values, often expressed in terms of statistical metrics such as variance and standard deviation. Variability is an inherent property of data that cannot be reduced, but it can be better characterised. Sources of uncertainty in environmental risk assessment comprise:

- Errors of measurement.
- Absence of information (including known unknowns, unknown unknowns, poor characterisation of the variability of an endpoint).
- Poor or partial understanding of the driving forces and mechanisms (including flaws in expert judgement, model inadequacy, a poorly developed weight of evidence approach to assess the strengths and weaknesses of the pieces of evidence).
- The use of a fixed parameter, from a variable data set, results in uncertainty related to the representativeness of the selected value (e.g. the assumed body weight of a GMS).

For more information on addressing uncertainties in risk assessments that fall under the remit of EFSA refer to EFSA Scientific Committee (2018).

13.1.1. Responsibilities of the risk assessor

The lower tiers of any risk assessment will contain inherent uncertainties. Many of these are well-known, such as laboratory-to-field extrapolation or inter/intra-species variability. Others are related to particular decisions made regarding default values or the methodology of the risk assessment (see Section 13.2). In order to cover both the larger and smaller uncertainties inherent to the generic tiers, a level of conservativeness generally is 'built-in' to the lower tiers so that the protectiveness as defined in the operational protection goal is believed to be secured. At higher tiers, more specific information may be provided for the particular risk assessment question, which will lower some areas of uncertainty (e.g. field effect studies will lower the laboratory-to-field uncertainty) but may also introduce new levels of uncertainty (e.g. other variables present in the field may have a positive or negative influence on the effect in question, and the scope of these may be largely unknown depending upon the amount and scope of data available). For that reason, it is necessary for the risk assessor to have a clear understanding of the inherent uncertainties for each tier and what they mean for the outcome (in the sense of meeting the protection goal) of the risk assessment. In the case of the lower tiers those are assumed to be covered since they are largely based on regulatory data requirements and trigger values/assessment factors (see Section 3.2), but ideally the protectiveness of the lower tier assessment should be calibrated with results of trustworthy higher tier studies (assigned as reference tier). However, at higher tiers, it is the responsibility of the risk assessor to understand the effect of the new data on the required level of protection and the relative weight of any new uncertainties inherent to the new data when used in the risk assessment. In this context, it is noted that available technical guidance for scientifically underpinned higher tier approaches supported by all

relevant stakeholders is limited for birds and mammals, but ideally would reduce some higher tier uncertainties. Furthermore, knowledge/published data is lacking for many of the variables considered in the risk assessment scheme, making concrete proposals to address these uncertainties purely quantitatively difficult.

The specificity and detail in an answer is directly related to the specificity and detail of the question asked. Therefore, the more specific a risk assessment question, the more specific an answer can be provided. In risk management, it can be difficult to adequately define the risk assessment question without input from the scientists/assessors as to what type of parameterisation is feasible and appropriate. As a result, the process is more of a give and take than a direct one-time answer/question. In the case of providing Guidance for recurring decisions such as pesticide (re)registrations, a certain amount of parameterisation is provided (via Protection Goals) which is applicable to each risk assessment, however, a key responsibility of the risk assessor is to inform the risk manager not only about whether the protection goal was met, but also as to the parameters of the present risk assessment, and important factors/situations which are thus not covered. This is in line with point 3.8.1. of Regulation 1107/2009[3] where it is stated that the ecotoxicology assessment should take into account the uncertainty of the data.

13.1.2. General uncertainties

In addition to individual uncertainties, there are general uncertainties which are not listed in the Table 28 but should nevertheless be borne in mind. A number of these 'general' uncertainties relate to the remit of this Guidance Document and the state-of-the-science and thus do not need to be specifically addressed or mentioned in an uncertainty analysis but must be acknowledged if considering the overall conservatism of the risk assessment. The following list reflects a number of such 'general' uncertainties.

- The present risk assessment methodology does not, and cannot, account for the potential effects of multiple stressors, which are both variable and unknown, but can include anything from climatic circumstances to anthropomorphic pressures on suitable habitats.
- The risk assessment is limited to a representative field, thus not taking into account the potential for multiple exposures to one or more different pesticides within the whole home range of the non-target species and additional treatment of that field with other pesticides within the same time frame.
- It must be noted that the risk assessment is limited to oral exposures to pesticides, via food and water, but not via grooming, nor does it directly account for dermal or inhalation exposures. Although it is assumed that oral exposures constitute the largest and most direct exposures in most cases, it is noted that for some species (e.g. bats) or life-stages (e.g. *in ovo*) 'contact' exposures may play a larger role.
- As this Guidance Document focusses upon direct toxic effects on birds and mammals only, it is not possible to specifically consider 'indirect' effects of pesticides on, for example, the impact on the presence of suitable food items in a given area, or the availability of appropriate habitats. Ideally, other areas of the risk assessment, for example those dealing with invertebrates or plants, would consider the ecosystem service as a basis of many food webs as a part of the protection goal for those trophic levels.
- Owing to the lack of a proper reference tier, the tier 1 risk assessment methodology in this guidance could not be fully calibrated. Consideration of the calibration exercise performed, for the acute risk assessment methodology for spray applications for birds, done in EFSA PPR Panel (2008) is still useful. Nevertheless, it should be acknowledged that the substances used in the studies used for the calibration may not be completely relevant to those in the EU now and in the future.

It should be noted that many of these 'general' uncertainties relate to risk management decisions, since the scope of the risk assessment (i.e. the risk assessment question) is defined by the regulatory framework and underlying regulations. Although these aspects are not directly within the sphere of influence of risk assessors, the assessor must nevertheless be aware of the scope of the assessment in question in order to adequately communicate the results. Furthermore, other general uncertainties are because of the state-of-the-science and could be addressed more readily in the risk assessment once the data and knowledge are available to allow this.

13.2. Uncertainty in Tier 1 assessments

Both lower and higher tier risk assessments as outlined in this guidance document are intended to meet the operational Protection Goals as stated in chapter 3. As shown in chapters 5 and 6, uncertainties cannot be avoided, even in the lower tiers. In the decision-making criteria of 546/2011[6], assessment factor or trigger values of 10 for acute and 5 for the reproductive assessment are used to account for, amongst other issues, uncertainty. These values are considered to account for known factors such as laboratory to field extrapolation, inter/intra-species variability, and likely other areas of uncertainty in the risk assessment.[59]

As reflected in Section 13.3, where a refined risk assessment is performed, it is expected that consideration of the uncertainty is given. To do this, the risk assessor needs to have a good understanding of the uncertainties that already exist in the Tier 1 exposure and/or effect assessment (i.e. to understand the impact of the uncertainty with the refinement of a parameter, a risk assessor needs to first understand the uncertainty for the parameter which is being refined). Therefore, the following section aims to direct the risk assessor to the uncertainties with the Tier 1 parameters and assumptions.

[59] Whilst the trigger or assessment factors of 5 and 10 are fixed in the legislation, it is the responsibility of the risk assessor to communicate with the risk manager the level of protectiveness which is achieved by the complete risk assessment, especially in higher tiers where the trigger/assessment factor may in effect be lowered to 1 (i.e. in integrated exposure and effect field and model studies).

Table 28: Summary of relevant information for consideration of the uncertainty for exposure and effect Tier 1 parameters and assumptions

Parameter/assumption	General description of Data set	Sources of uncertainty
Body weight of Tier 1 GMS	See Annex B and Appendix F. The selected body weights for the GMS are for adults of the lightest species expected to be present in that crop. Where a range of BW was given the mid-range value was taken.	– The use of a single value to represent all individuals within the wild species. – The body weight of juveniles (mammals) and chicks (birds) are not specifically considered.
Dietary proportions (PD) for Tier 1 GMS	See Annex B and Appendix F. The PD values were selected to be relatively conservative in order to cover species represented by the GMS. In reality, dietary proportions are extremely variable.	– The assumption of a fixed diet (for all animals in variable geospatial conditions).
Proportion of treated food in the diet (PT)	PT is assumed to be 1 for all Tier 1 GMS.	– The assumption of a fixed value (for all animals in variable geospatial conditions).
RUD (for foliar sprays)	See summary statistics in Appendix J. The RUD values are derived from data sets with variable sample sizes. Large sample sizes give a better characterisation of the variability and therefore reduces uncertainty. For several matrices, no, or few data were available and therefore surrogate values were taken.	– The assumption of a fixed value (for variable geospatial conditions). – Small data sets and surrogate values for certain matrices. – Data were grouped to several, rather broad, categories e.g. all RUD values for monocotyledon plants were merged. It is known that some avian/mammalian species are known to have preference for certain plant species.
Concentration on treated seed (seed treatments)	Nominal concentration from GAP. In reality, there will be some variability in the measured concentrations on treated seeds.	– The assumption of a fixed value.
Dilution factor and fTWA for seedlings germinating from treated seeds	See Appendix M. Dilution value and fTWA were derived from a relatively small data set for a limited number of plant species grown under laboratory conditions.	– Small data sets and limitations to the representativeness of the data – The assumption of a fixed value
DT_{50} values used for MAF and fTWA	See Appendix K. The DT_{50} used in the calculation of MAF and fTWA, for spray applications, is assumed to be 10 for all matrices. Data was only available for plants and invertebrates. All other values are surrogates. No default DT_{50} (for calculation of fTWA) was available for treated seed.	– The assumption of a fixed value (for variable geospatial conditions). – Surrogate values are used.
Spray drift values (small mammals only)	See Appendix I. The spray drift values used for the exposure assessment for non-target organisms are those derived in Rautmann et al. (2001).	– The representativeness of such values to multiple crops and to all sprayers across the EU. – The assumption of a fixed value. – The use of surrogate values.

Parameter/assumption	General description of Data set	Sources of uncertainty
Deposition values (DV)	See Appendix L.	– Representativeness of such values to multiple crops and to all sprayers across the EU. – The assumption of a fixed value. – The use of surrogate values.
Daily energy expenditure (DEE) (used for calculation of FIR)	See Appendix G.	The values do not consider animals in life stages where a higher than-normal energy is required (lactating mammals, pre-hibernation, during migration, during growth, etc.).
Energy content of food items (used for calculation of FIR)	See Appendix G. For the majority of food items, data come from a reasonably large data set with a coefficient of variation (CV) of less than 15%. Some exceptions do exist, where the data set is smaller, or extrapolation was necessary.	– Grouping of food item types. – The use of surrogate values.
Moisture content of food items (used for calculation of FIR)	See Appendix G. The size of the data set and variability in the moisture content of food items varies amongst the different food items.	– Grouping of food item types. – The use of surrogate values.
Assimilation efficiency for the food items (used for calculation of FIR)	See Appendix G. For birds, the assimilation efficiency assumptions come from reasonably sized data sets. For mammals, few data were used for most food items and no data was available for fruits and flower buds.	– Grouping of food item types. – The use of surrogate values.
TK (TD) (part of FIR)	Not accounted for in Tier 1.	– Formalised TK and TKTD modelling approaches to address metabolism and excretion of active substances, and the reversibility of the damage due to internal exposure, are not included in the Tier 1 assessment. – Ignoring TK and TD processes may result in more worst-case assessments, particularly under time-variable exposure conditions. – The test conditions (climatic control; health of test individuals) of standard toxicity tests may be more optimal for metabolism and excretion than realistic field conditions, resulting in an overall lower internal exposure under laboratory conditions and thus higher LD_{50} and ED_{10} values.
Selection of toxicity endpoints	See Sections 5.2.2 and 5.2.4. Tier 1 toxicity endpoints are defined by Regulation (EU) No 283/2013[4] and Regulation (EU) 284/2013[5].	– In the case an NOAEL is used rather than a BMD_{10} please refer to Section 5.2.7 where the uncertainties are described.

Parameter/assumption	General description of Data set	Sources of uncertainty
Sensitivity of test species relative to wild species	Tier 1 toxicity endpoints are derived for a limited number of test species. The species of birds and mammals tested in toxicology studies are mainly chosen for ease of handling and husbandry, rather than because they represent sensitive members of the *Aves* or *Mammalia* classes.	– No species sensitivity distribution (SSD) or other method was available to the WG to investigate the relative sensitivity of the tested species in relation to a variety of wild species.[60]
Extrapolation of effect endpoint from laboratory studies to the field	Tier 1 toxicity endpoints are derived from studies performed under laboratory conditions.	– Effects observed in the laboratory can differ greatly from observations in the field. Food and environmental pressures have a large influence on normal parameters relating to survival, reproduction and development. The laboratory generally represents a 'best case' situation as far as food and environmental pressures are concerned and therefore likely minimises the magnitude of the effect were it to occur in the field.
Timing of application (for reproductive assessment)	It is assumed that the breeding season coincides with the exposure. This assumption is based on the fact that is improbable that there is no potentially exposed species is breeding in Europe at a given time, and on the assumption that even if exposure occurs outside the breeding season, this exposure may cause delayed effects on reproduction.	– It is possible that: (i) exposure does not overlap with the breeding season of any exposed species; or (ii) exposure causes no delayed effects on reproduction. The latter is difficult to demonstrate under lab (considering no additional vertebrate testing) and field conditions.
Agronomic practices	The Tier 1 exposure assessment accounts only for a few agronomic practices.	– Agronomic practices will influence the exposure to birds and mammals to a variable extent.

[60] Extrapolation of toxicity data between species is a common uncertainty for most risk assessments, as testing of all the species is neither feasible nor ethical.

13.3. Uncertainty and weight-of-evidence in higher tier assessments

As mentioned above, since each higher tier assessment will be different, the risk assessor should contextualise the risk assessment in order to provide the risk manager with the best information on the outcome. Generally, refining the risk assessment may result in a reduction of the overall uncertainty in the risk assessment, however, the refined parameters are specific to the conditions of the study where/when it was performed (e.g. landscape, climate, season, species present, agronomic practices, etc.). As a result, the uncertainty is reduced for those specific conditions, but the conclusions cannot be extrapolated with equal certainty outside those circumstances (see Figure 9). For example, a higher risk assessment using a robust field study performed in five cereal fields in Germany in a certain year: For these five fields and under the conditions of the study the uncertainty is reduced relative to the Tier 1. However, when the results of the field study are used as part of a risk assessment with a wider scope then the certainty that the protection goal is met under other spatial–temporal conditions may be less certain unless additional data to support a broader scope is available. Therefore, the risk assessor must consider numerous factors and understand the impact they have on the level of uncertainty in the outcome of the risk assessment achieving the protection goal. The concept of the relationship between (un)certainty and spatial–temporal scope in lower and higher tier assessments is shown in Figure 9. To facilitate appropriate decision-making, this must be clearly communicated to risk managers. Hence, the WG recommends a transparent way to communicate the 'safety' parameters and also areas where due to the more specific nature of the higher tier risk assessment, a conclusion of low risk may be uncertain or impossible. For those circumstances which are outside the exact parameters of the refined assessment, weight of evidence may be used to identify additional circumstances in which a low risk can be concluded (extrapolated). Both parameter reporting and weight-of-evidence tables can be found in Section 13.4, along with instructions for their most appropriate use.

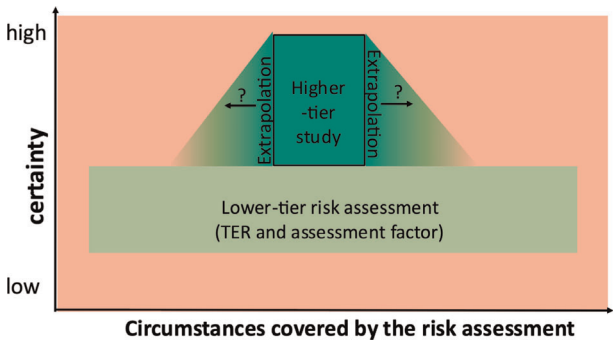


Figure 9: Illustration of the relationship between (un)certainty and spatial–temporal scope of lower and higher tier risk assessments. The red colour illustrates situations when and where risks cannot be excluded and the green colours when and where risks are low

13.4. Uncertainty and weight of evidence risk communication tables for the higher tier risk assessment

In order transparently consider the higher tier data and communicate the decision-making process (and any areas where risk management decisions may be necessary), the WG has created two weight-of-evidence communication (WOEC) tables to assist the risk assessor (EFSA Scientific Committee, 2017b). The two WOEC tables are presented separately in order to allow a detailed consideration and clear communication of the decision-making process for the use of the data from each refinement individually (Table 28) without unduly complicating the ultimate weighing of the complete data set and communication of the results to the risk manager(s) (Table 29). The two WOEC tables together should provide a complete picture to the risk assessor themselves and to the risk manager(s) as to the use of any refined data, the scope of the risk assessment as presented, and any remaining risk management decisions which are inherent to the assessment as presented. As described in Section 6.5.8, where Tier 3 data (ecological refinement) have been used to refine the exposure, it is recommended that the Tier 3 database is consulted, and any relevant data is reflected as part of the uncertainty analysis for higher tier assessments.

13.4.1. How to use the WOEC Tables

The **point-by-point uncertainty consideration** table (Table 28) should be filled in ONLY by the risk assessor (applicants should not complete this table). The line for each study should be filled in at the time of consideration of that study. This means that in practice the assessor should place a blank table at the bottom of the (higher tier) risk assessment and fill in a line for each type of higher tier study as they go through the risk assessment and get to each study. This table provides a succinct and harmonised way of communicating information which is otherwise only present in various areas of text throughout the risk assessment. It provides the assessor with a quick way of visualising and weighing the higher tier risk assessment, as well as informing the full weight-of-evidence consideration and communications to the risk manager(s). It is recommended that the levels of certainty and uncertainty be communicated as 'high', 'medium' and 'low', to avoid confusion regarding (+) or (–) signs. To avoid ambiguity, the focus should always be to consider if the outcome of the assessment is under-protective relative to the protection goal e.g. 'this parameter is highly uncertain and may result in the risk assessment being under-protective' or 'this parameter is of low uncertainty and the assessment will reach the desired protection level'.

Once each higher tier refinement has been considered fully, the assessor can complete the 'conclusion' row, which provides the vital conclusion on the overall uncertainty on the higher tier risk assessment for the risk assessment question and whether this overall uncertainty will result in a more conservative or more liberal risk assessment.

Table 29 is filled in with an example (a mammalian risk assessment in maize) to provide the risk assessor with extra guidance on how they support and improve the risk assessment conclusions and communication.

Table 29: Weight-of-evidence communication (WOEC) Table: Point-by-point higher tier uncertainty considerations

Point of higher tier refinement	Parameters of certainty	Level of certainty inherent to the study (reliability)	Parameters of uncertainty	Use of the data in the risk assessment (relevance)	Resulting potential for under-protection for the risk assessment area under consideration
DT_{50} studies	DT_{50} studies were carried out in maize in the spring in Germany and the Netherlands from BBCH 40–80 showing a mean DT_{50} of 3.2 days.	high	Only 5 studies in Germany and 5 studies in the Netherlands are presented. Variation may not be fully captured, extrapolation to other CZ countries may be uncertain.	The DT_{50} values can be used in the Central Zone. The highest DT_{50} of 4 days was used rather than the mean DT_{50} of 3.2 in order to address uncertainties in the decline calculations (see study evaluations for specific points).	low
Focal species study	Wood mice, common shrews and field voles were found in late growth stage maize fields in Germany in the spring of 2021	medium	Other species may be present in maize fields in other areas of the EU or Germany and during other time frames/other years	It may be concluded that wood mice common shrews and voles are acceptable mammalian focal species in late growth stage maize in Germany. Other MS should determine the applicability of these data and whether other (endangered) species may be present.	low
PT study	A PT study in wood mice was carried out in maize fields in Germany in the spring of 2021. Studies in wood mice were also carried out in the North of France, and Southern Denmark. A total of 250 wood mice were tracked showing a 90th %-ile PT of 0.8.	high	A PT of < 1 is less certain than the assumed PT of 1 in the lower tier risk assessment particularly for mammals, due to the size of the typical home ranges for small mammals. Nevertheless, significant data was provided to support a lower PT for wood mice in this situation. Although much data was provided, extrapolation outside of the study areas may be nevertheless uncertain, particularly considering other agricultural practices and landscape structures.	A 90th %-ile PT value was used in the risk assessment in order to further decrease the potential uncertainty of using a PT < 1. This can also be considered to cover some uncertainty inherent to extrapolation outside the study area. The PT of 0.8 can therefore be considered appropriate for wood mice in the Central Zone.	medium

Point of higher tier refinement	Parameters of certainty	Level of certainty inherent to the study (reliability)	Parameters of uncertainty	Use of the data in the risk assessment (relevance)	Resulting potential for under-protection for the risk assessment area under consideration
PD study	A PD study in Austria in 20 wood mice in spring was available. The wood mice were in 'agricultural areas' but these were not further defined.	low	The low number of animals sampled means that there is high variability, decreasing the overall certainty in the result and its potential for extrapolation. Furthermore, the study area is not clearly defined, and it therefore cannot be assumed that the area is comparable/worst-case for the requested uses.	The study cannot be used in the risk assessment. The default diet from Tier 1 will be used for wood mice, instead. It is noted that should no adequate PD study be available for any focal species, the worst-case diet should be calculated and used in the risk assessment.	high
Field effect study	Field effect studies on field voles were carried out in grassland in Germany in 2021. A total of 20 studies year-long following 300 voles showed a power to detect a 10% effect on population (measured via MNA and population structure)	high	The study was well performed, appropriate endpoints and methodologies were used. The analysis is considered adequate; however, the study took place in a 'boom year' for vole populations. It is not certain whether the results would be similar in a year when vole populations overall were less robust.	Since the study was performed in a 'boom year', it may not adequately represent the potential for effects in years when a less robust population is present. This should be considered in the final decision-making, particularly considering the length of the registration and the yearly use projections.	medium
Population modelling	Population modelling for common voles showed that grasslands in Germany were a worst-case landscape compared to maize fields with comparable off-field areas.	medium	Some of the input parameters were from older or potentially less-applicable literature data.	Since the modelling is used to support the extrapolation of the results of the field study, rather than to show no effect, the uncertainties identified are considered appropriate to the use of the study in the risk assessment.	low

Once the point-by-point higher tier uncertainty consideration table (Table 29) is complete, the assessor should also complete the **weight of evidence/conclusions** table (Table 30). Again, this table should not be completed by the applicant and should only be the providence of the risk assessor. The weight of evidence/conclusions table is key for organising and communicating the specific conclusions made for each study (evidence) and for the full risk assessment in order to assist in risk management decisions and should be presented at the end of the full risk assessment. It may also be useful to present it in a summary communication to risk manager(s), should this be applicable. In this table, it may also be noted if there are areas of the risk assessment which were not refined and/or areas which show a tendency towards over-protection (as well as the relative (un)certainty in these). In this table, a clear conclusion on the impact of each refinement on the overall conclusions of the risk assessment should be presented, including a clear communication of the scope of the risk assessment (i.e. for which circumstances is there high certainty of achieving the protection goal, and for which circumstances is a specific risk management decision required).

The example table below (Table 30) has been completed considering the fictitious risk assessment presented in Table 29, above, for clarity.

Table 30: Weight of evidence/conclusions table

Point of higher tier refinement	Impact on the risk assessment	Weight of evidence for the risk assessment including potential for over-protectiveness
DT_{50} studies	Key study	The residue decline of the active substance in question in maize in the spring was based on highly reliable and relevant data. A worst-case value was used in order to support extrapolation to AREA X^1, which covers the uncertainty introduced by the study.
Focal species study	Key study	Focal species of mammals in late growth stage maize in DE in the spring were identified in a highly reliable study. **Since similar mammalian species and agricultural practices are present in AREA X, the risk assessment also covers use in late growth stage maize in AREA X, except for the red-bellied shrew, which is only present in the northwest of AREA X and is completely absent from DE**.
PT study	Key study	The PT has an immediate and dramatic influence on quantitative risk assessment. In this case, high-quality studies were used to refine the PT and the main uncertainty is as regards extrapolation to other areas/time frames/situations. A 90th %-ile PT is used in the risk assessment to address these and the introduced uncertainties are therefore covered.
PD study	Not used	The available PD study was too unreliable to be used in the risk assessment due to low numbers of mice and unclarity regarding the landscapes/study location(s). This is an area where more/better data could be provided. However, as the default values from Tier 1 were used in the risk assessment, the overall effect on the certainty in the outcome is minimal in this case.
Field effect study	Key study	The potential risk to voles from the proposed use was concluded as negligible based upon a field effect study. To somewhat account for spatial uncertainties, an assessment factor of 2 was applied to the application rate used in the effect field study. As noted, possibly the most important uncertainty is that **the study was performed in a 'boom' year for vole populations and a risk cannot be excluded for vole populations in less prosperous years**.
Population modelling	Supporting study	There were several points where the model was considered to be less certain; however, as it was used to support the landscape chosen for the field effect study and this was agreed upon as an appropriate/worst-case landscape, the study supports a higher certainty in the conclusion of the field effect study.
Additional literature information	Information only	The available literature review indicated a single associating substance X with an impact on Y species. This study was performed in farmland in Bavaria, Germany, where winter cereals and maize were reported as the dominant crops. The study monitored Y species over a 3-year period. The authors correlated an impact with the use of pesticides including

Point of higher tier refinement	Impact on the risk assessment	Weight of evidence for the risk assessment including potential for over-protectiveness
		substance X. Residues of 15 pesticides were measured in soil and substance X was found at concentrations ranging from 0.05 to 1 mg a.s./kg soil. The impact on species Y cannot be attributed to a single substance nor PPP.
Conclusion of risk assessment based on higher tier refinements		A low risk to mammals for the proposed use in late growth stage spring maize in AREA X. However, the following should be considered in the final registration/management decision: The conclusion does not apply to red-bellied shrews in AREA X. A risk cannot be excluded for red-bellied shrews, which are an endangered species in the northwest of AREA X. The conclusion does not apply to field voles in non-boom years. A risk cannot be excluded for field voles in maize in non-boom years. Voles serve as a basis of several food webs, including for kestrels and barn owls in AREA X. If populations are impacted during non-boom years, it cannot be concluded whether the negligible risk to voles during boom years will remain. The proposed use would result in yearly exposures in various areas of AREA X throughout April, May and June.

(1): For the purposes of this example, 'AREA X' represents the regulatory area (i.e. country, zone, EU) for which the risk assessment has been performed.

14. Risk Mitigation

Risk mitigation can be considered to fall into several broad categories:

- Generic mitigation (unquantifiable) are those actions which are undertaken to reduce the risk to birds and mammals (e.g. areas of set-aside). Commonly these are better suited to reducing the indirect effects of pesticides and farming (e.g. provision of suitable foraging areas, suitable roosts, hedgerows, etc.).
- Specific risk mitigation (quantifiable) is targeted action which is needed to mitigate an identified risk due to pesticide exposure via risk assessment. It is important to note that specific mitigation should sufficiently reduce the risk to a level which is considered to be acceptable by risk managers (i.e. a low risk using a refined exposure estimate considering the mitigation must be demonstrated). As such any suggested mitigation must be accompanied by an appropriate risk assessment for which additional data may be needed.
- Changes to the GAP can also be used to mitigate an identified risk e.g. a restriction on growth stage or growing structure, reduced application rate, etc. It should be noted that changes to the GAP during EU level assessments are not allowed, and therefore, applicants should carefully consider their range of selected GAPs when developing a dossier. When a restriction on the growth stage is used to mitigate the risk, then it must be checked that it is not in contradiction to the proposed use of the PPP (i.e. the PPP must still be efficacious for the intended pest).
- Changes to the plant protection product. Although these will be done prior to an approval or authorisation assessment, it is worth noting that the applicant may consider changes to the product to mitigate the risks to birds and mammals when developing a formulation (e.g. size of granules, change of formulation type, change of the base of the granule, inclusion of repellent substances, etc.).

It is also worth noting that for EU level assessments, mitigation measures should be proposed by the applicant in the dossier and considered/evaluated by the RMS in assessment report (EFSA, 2019a).

Poor farming practices (e.g. spillages of treated seeds, leaking chemical irrigation pipes, damaged application machinery, etc.) and other misuses of pesticides should not be considered in the prospective risk assessments under Regulation 1107/2009[3]. However, Member States authorities may

consider mitigating the risk to birds and mammals through label phrases or stewardship programmes. Commission regulation (EU) 547/2011[61] provides information on the labelling requirements for plant protection products. The regulation also indicates when the label phrases are necessary. Three of these phrases are specific for birds and mammals. These are listed below and two of them are also discussed in Table 31.

'SPe 5 *To protect birds/wild mammals the product must be entirely incorporated in the soil; ensure that the product is also fully incorporated at the end of rows*.

The phrase shall be assigned to plant-protection products, such as granules or pellets, which must be incorporated to protect birds or wild mammals.

SPe 6 *To protect birds/wild mammals remove spillages*.

The phrase shall be assigned to plant-protection products, such as granules or pellets, to avoid uptake by birds or wild mammals. It is recommended for all solid formulations, which are used undiluted.

SPe 7 *Do not apply during bird breeding period*.

The phrase shall be assigned when an evaluation according to the uniform principles shows that for one or more of the labelled uses such a mitigation measure is necessary'.

An SETAC publication titled 'Mitigating the Risks of Plant Protection Products in the Environment, MAgPIE' (Alix et al., 2017) was published following two workshops held in 2013. The aim of the workshops was to produce a toolbox of risk mitigation techniques. Below is a summary of the possible specific mitigations measures which can be used in a quantitative risk assessment for birds and mammals. This list is a mixture of those in Alix et al. (2017) and others collected from the WG based on experience for EU level assessments. The list is not exhaustive and there are more possible types of mitigation available in the Member States, especially in the case of rodenticides. It would be beneficial if the following list was periodically updated.

[61] Commission Regulation (EU) no 547/2011 of 8 June 2011 Implementing regulation (EC) no 1107/2009 of the European Parliament and of the Council as regards labelling requirements for plant protection products.

Table 31: Possible specific mitigations measures which can be used in a quantitative risk assessment for birds and mammals

Type of mitigation	Area of the assessment for which the mitigation may be considered	Points to consider for implementing to the risk assessment
Spray drift reduction	Exposure to small mammals in the Terrestrial Area of Interest (TAI) Exposure via secondary poisoning in surface waters	Spray drift mitigation may be achieved by low-drift nozzles or non-spray buffer zones, or a combination of both. To mitigate the risk to mammals using no-spray buffer zones, the no-spray zone must be between the TAI and the sprayer. The FOCUS Landscape and Mitigation Factors in Aquatic Ecological Risk Assessment (FOCUS, 2007) indicates that the maximum spray drift mitigation is 95% as higher levels become uncertain. It would seem logical that such a limit is also applied to the risk assessment for vertebrate wildlife.
Closing protected structures at the time of application would reduce (possibly eliminate) spray drift	Exposure to small mammals in the Terrestrial Area of Interest (TAI) Exposure via secondary poisoning in surface waters	For many protected cropping systems, the closing of the structures during application is not possible. It is important to note that for many walk-in tunnel designs, shade nets and low structures (that are actually removed during spraying, then replaced following an application), this mitigation of spray drift is not practical. For a subset of walk-in tunnel designs, if closure during spraying, is possible, direct spray drift reaching TAI will be prevented. For drift to TAI, such mitigation will effectively reduce the exposure to a low level such that a risk assessment for small mammals is not required. For exposure to surface water, mitigating the spray drift will reduce the exposure, but routes of exposure other than spray drift still need to be assessed (EFSA, 2014a).
Closing semi-protected structures for a period of time after application to allow for residues to dissipate to a level which no longer pose a risk to birds or mammals	In-field exposure to birds and mammals	Such mitigation is potentially feasible for a short period but is not considered realistic for extended periods as ventilation is needed to maintain a suitable environment for the crop. Consequently, if such mitigation was proposed, a risk assessment using measured residue data (or residue decline) should be provided as well as a consideration of whether the suggested period is an accepted mitigation in the Member State(s) where the product will be used.
Soil incorporation (e.g. **'SPe 5** *To protect birds/wild mammals the product must be entirely incorporated in the soil; ensure that the product is also fully incorporated at the end of rows*).	In-crop exposure to birds and mammals taking treated seeds/granules. In-crop exposure to birds and mammals taking weed seeds, invertebrates contaminated with residues following a spray.	Irrespective of the formulation type, data would be needed to support the refined exposure parameters in the risk assessment (e.g. revised seed availability values or revised residue values).

Type of mitigation	Area of the assessment for which the mitigation may be considered	Points to consider for implementing to the risk assessment
Restriction to an application machinery which results in sufficiently low number of available seeds/granules on the soil surface.	In-crop risk assessment to birds and mammals taking treated seeds/granules	Currently specific application machinery is not specified in the GAP (other than sometimes precision drilling) and it would need to be checked with risk managers if they considered such mitigation to be feasible to implement. However, such restrictions could have the potential to allow for more precise exposure parameters to be considered in the risk assessment. Data would be needed to support the refined exposure parameters in the risk assessment (e.g. revised seed availability values or revised residue values).
Restrictions to only apply outside of breeding periods (e.g. **SPe 7** *Do not apply during bird breeding period)*.	Reproductive risk assessment to birds and mammals	Commission regulation (EU) 547/2011[43] includes a label phrase to restrict applications to outside of the breeding season. However, considering that, it needs to be demonstrated that exposure will not occur during the breeding season for, not only the focal species, but for those birds potentially exposed, it is a difficult assessment. At the time of writing, for EU level assessment, sufficient data to demonstrate this has not been provided (see Section 5.2.1).
Restricted treatment areas (i.e. 'spot' application, precision farming or band application, etc.).	Reproductive risk assessment to birds and mammals	Restricted treatment areas are a clear way to reduce the risks to birds and mammals. However, to be able to account for it in a risk assessment there must be a clear indication of the size of area to be treated (i.e. specified in the GAP). Additional data (e.g. residue values, PT data) may also be required to support the assumptions in the risk assessment. Owing to the fact that birds and mammals make take a lethal dose in a single feeding session it is unlikely that such a restriction can be used to mitigate the acute risks to birds and mammals.
Restricted application timing	Acute risk assessment	The acute risk may be mitigated by preventing applications during the active foraging period (e.g. no evening/night-time applications). It is expected that residue data would be needed to demonstrate that the residue levels had decreased to a level which no longer pose a risk to birds and/or mammals.

15. Use of the EFSA Birds and Mammals Calculator Tool

In the calculator tool, a list of common focal species is provided, including body weights. These are listed in Annex B. Please note that the list is not exhaustive and is provided in order to increase ease and speed of use of the calculator tool. Focal species studies must be performed in order to determine the appropriate Tier 3 species for the risk assessment question. The fields (species and body weight) in the calculator tool may also be filled in with other species determined in specific focal species studies.

At the time of submission of the risk assessment to the (R)MS, it is highly recommended to submit the zip files created during the input and calculation by the applicant. This will facilitate the speed with which the (R)MS can evaluate the submission, as the assessor can use the zip file in the calculator tool and, e.g. adjust only refinements which are not accepted in order to calculate a new risk assessment conclusion.

In addition, it may be useful to submit the Excel files created. Although the tables will be copied into the risk assessment document, the Excel files can serve as a reference for the (R)MS. Additional adjustments and investigations of the data are possible using the Excel files (e.g. checking the worst-case FIR/bw for various GMS).

Conclusions

An updated risk assessment for birds and mammals is presented in the current document. The risk assessment methodology was specifically revised to address the needs identified in the context of the approval and authorisation process under Regulation 1107/2009[3]. Therefore, all those aspects which, although scientifically valid, go beyond the scope of that regulation were not taken into consideration in this updated guidance. The risk assessment methodology covers dietary exposure via direct residues on food items, dietary exposure via residues on food items following bioaccumulation (secondary poisoning) and exposure from consumption of contaminated water. Several activities were undertaken towards the harmonisation of higher tier risk assessments within EU Regulatory zones.

It is noted that the lower tier acute ERA for birds was calibrated against direct toxic effects of individual PPPs (EFSA, 2009; Appendix C). However, more uncertainty surrounds whether an appropriate level of protection is given for seed treatments and granules. Also, the protectiveness of the current acute ERA scheme for mammals, as well as for the current reproductive ERA procedure for birds and mammals, is not yet validated due to the lack of appropriate reference tier information.

Recommendations

The WG identified many research needs and recommends that those gaps are addressed before an in-depth revision of the risk assessment for birds and mammals is initiated. The main recommendations are the following:

Risk Assessment methodology

- Specific protection goals in accordance with EFSA methodology should be discussed and agreed with risk managers.
- Appropriate field effect studies and/or population models to calibrate the tiered approach via a reference tier are of vital importance and should be developed and agreed upon.
- The assumption that dietary exposure is the most important exposure route for the majority of birds and mammals needs to be verified.
- The adequate coverage of all life stages (e.g. juveniles) and physiological stages (e.g. lactation, pre-migration) in the risk assessment should be verified.
- The methodology included in this guidance document covers the main application techniques for PPPs used in Europe. The WG recommends that a methodology is developed for in-field and TAI exposure characterisation for additional application techniques. This should include innovative techniques and targeted applications (e.g. spot application, precision farming, etc.).
- A unified approach under several EU Regulations would be advisable in order to develop a meaningful methodology for the assessment of indirect effects. In addition, it is considered essential to address indirect effects from reduced food availability to birds and mammals when developing the risk assessment guidance documents for non-target terrestrial plants, non-target arthropods and non-target soil organisms.

- A holistic approach should be considered to develop harmonised and robust exposure models to cover additional exposure routes (e.g. dermal and inhalation) covering all terrestrial non-target organisms, including bats.
- A unified approach under several EU Regulations would be advisable to link Regulations and directives (e.g. Regulation (EC) 1107/2009[3], Endangered species, CAP,[62] etc.) in support of the EU Green Deal.
- Regulation (EU) No 546/2011[6] ((iii) of point 2.5.2.1) suggests that, where relevant, a cumulative ERA should be conducted in the area of envisaged use in case of authorised uses of PPPs that contain the same active substance or that give rise to the same metabolites. The legislative phrase 'area of envisaged use' was not operationally defined. Therefore, it should be verified whether cumulative exposures (e.g. over time, in multiple fields) are covered by the current risk assessment conclusions or may require further development.
- It is further recommended that a similar exercise (as described in the preceding bullet point) is performed for exposures to different pesticide active substances.
- It should be investigated to what extent birds consume nectar in agricultural, horticultural and forestry environments in the EU.
- A dedicated guidance on the risk assessment for rice should be developed.
- Assessment methodology for microbial pesticides should be addressed in a specific guidance document.
- It is recommended to further investigate the maximum search area for birds and mammals. This should be done for a range of focal species and conditions and may be considered via expert elicitation.
- Appropriate and harmonised methodology needs to be developed for calculation of concentration of active substances and metabolites in puddles which are formed in treated areas after heavy rainfall events.

Risk assessment parameters

- Additional residue data for deriving default parameters used in the screening and Tier 1 risk assessment for sprays should be developed and collected. Specifically, the WG noted that data were lacking for flower buds, weeds seeds and flying insects.
- For plant DT_{50} values specifically, the WG recommends a comprehensive data collection and analysis in a harmonised manner.
- The crop groups used in this guidance document for the risk assessment of spray applications uses the EPPO harmonised classification, as far as possible. The WG considers that it would be beneficial if the exposure parameters (such as crop interception) are aligned to these crop groups.
- Information on bioaccumulation factors (BAFs) for pesticides in soil- and sediment-dwelling prey organisms of birds and mammals should be developed and collected.
- Further development of alternative methods to vertebrate testing as well as validation of additional endpoints (e.g. behavioural) for currently available test methods and implementation of these into the risk assessment is recommended.
- Additional data should be developed and analysed regarding species sensitivity for terrestrial vertebrates for various effect endpoints and substances.
- It is recommended that more extensive guidance on the use of HCD (for both birds and mammals) is produced in concert with the mammalian toxicology experts.
- Development of regulatory technical guidance on how to conduct and interpret (statistically – including MDD; ecologically; spatially/temporally) higher tier exposure, effects and integrated exposure effects field studies for birds and mammals should be developed.
- Further development of environmental scenarios, food web bioaccumulation models, TKTD models and population models (including their documentation) for birds and mammals, following the comprehensive suggestions of EFSA PPR Panel (2014, 2018).
- Ecological data for birds and mammals to underpin risk assessment methodology, and for in-silico modelling, should be updated and expanded to include information on normal operating

[62] European Commission, 2010. The CAP towards 2020: meeting the food, natural resources and territorial challenges of the future. http://ec.europe.eu/agriculture/cap-post-2013/communication/index.en.htm; European Commission, 2013c. CAP Reform – an explanation of the main elements. EC/MEMO/13/621.

range (NOR), various ecological niches and habitats in the EU and appropriate landscapes (e.g. agricultural).
- Field studies on animal behaviour not captured by standard exposure assessment scenarios (e.g. birds using drip irrigation systems for drinking water) are scarce and more data are needed in order to consider this and other possible new/developing routes of exposure.

Monitoring

- Further development of post-registration monitoring of birds and mammals to assess unforeseen side effects of pesticides, verify the risk assessment approach and to evaluate the effectiveness of risk mitigation measures, is recommended.

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Abbreviations

AChE	Acetilcolinesterase
ADME	Absorption, Distribution, Metabolism and Excretion
AF	Assessment Factor
ALT	Alanine Aminotransferase
AFSSA	Agence française de sécurité sanitaire des aliments
AR	Application rate
a.s.	Active substance
AUC	Area under the curve
AV	Avoidance factor
BAF	Bioaccumulation factor
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical industry
BC	Broadcasting of seed or granule
BCF	Bioconcentration factor
BMD	Benchmark dose
BMD_{10}	The benchmark dose responsible for a 10% response, adjusted to take account of the usual background response by organisms.
$BMDL_{10}$	The lower confidence limit of the benchmark dose responsible for a 10% response, adjusted to take account of the usual background response by organisms.
bw	body weight
C	Concentration
CAT	Critical Appraisal Tool
CLP	Classification, Labelling, Packaging
CRO	Contract Research Organisation
CSL	Central Science Laboratory (now: The Food and Environment Research Agency)
d	day
DA	Dose Addition
DAR	Draft Assessment Report

DD	Dietary dose
DDD	Daily dietary dose
DEE	Daily energy expenditure
$DP_{lethal-50}$	Dose profile causing 50% mortality
DT_{50}	Time for 50% degradation
DT_{90}	Time for 90% degradation
DV_i	Deposition Value and is the proportion of substance reaching the food item *i* accounting for interception by the crop.
EC	European Commission
ECPA	European Crop Protection Association
EEC	European Economic Community
EfAGs	Effect Assessment Goals
ExAGs	Exposure Assessment Goals
EPPO	European and Mediterranean Plant Protection Organization
ERA	Environmental Risk Assessment
ERC	Environmental Relevant Concentration
ETR	Exposure-Toxicity Ratio
F1	First filial generation
F2	Second filial generation
FIR	Food intake rate
FO	Frequency of occurrence
FOB	Functional Observation Battery
FOCUS	Forum for the Co-ordination of pesticide fate models and their Use
FS	Focal species
fTWA	Time-weighted average factor
GAP	Good Agricultural Practice. Often used to refer to the representative/proposed use of an a.a. or PPP (e.g. applications rate, timing, growth stage, etc.)
GD	Guidance Document
GFS	Generic Focal Species
GLP	Good Laboratory Practice
GMS	Generic Model Species
HCD	Historical Control Data
HD_5	hazardous dose to 5% of the species
HR	Highest residue level. Often used in regulatory residue assessments under Regulation 1107/2009
ICPS	International Centre for Pesticides and Health Risk Prevention
IMS	Indicator Model Species
JRC	Joint Research Centre
k	rate constant
K_{OC}	Organic carbon absorption coefficient
K_{OW}	Octanol-water partition coefficient
LC_{50}	Lethal concentration; the concentration at which 50% of the test organisms die.
LD_{50}	Lethal dose; the dose at which 50% of the test organisms die.
LOD	Limit of detection
LOQ	Limit of quantification
LP_{50}	Lethal profile: exposure profile causing 50% mortality
MAF	Multiple application factor
MDD	Minimum detectible difference
MDR	model deviation ratio
mg/kg	Milligram per kilogram
mg/L	Milligram per litre
MNA	Minimum-number-alive
MoA	Mode of Action
MRL	Maximum residue levels
MS	Member State
n	Sample size
nAChR	nicotinic acetylcholine receptor

NEU	Northern Member States of the European Union also referred to as NZ (Northern Zone)
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOEL	No observed effect level
NOR	Normal Operating Range
OECD	Organisation for Economic Co-operation and Development
OPPTS	US EPA's Office of Pesticide Programs and Toxic Substances
P	Parental
PBTK	physiologically based TK models
PD	Composition of the diet obtained from the area containing a sufficient coverage of the crop of interest
PEC	Predicted environmental concentration
PHI	pre-harvest interval
ppm	Parts per million
PPP	Plant Protection Product
PPR Panel	EFSA Scientific Panel on Plant Protection Products and their Residues
PrD	Precision drilling
PT	Proportion of an animal's daily diet obtained in the in-field area for the crop under assessment
Q_{met}	Ratio of concentration of metabolite to concentration of parent.
QSAR	Quantitative structure-activity relationship
RA	Risk assessment
RAD	Regulatory Acceptable Dose
RAR	Renewal Assessment Report
RIVM	Netherlands National Institute of Public Health and the Environment
RMS	Rapporteur Member State
RUD	Residue unit dose
SANCO	European Commission Health and Consumer Protection Directorate General
SAR	Structure-activity relationship
SD	Standard drilling
SETAC	Society of Environmental Toxicology and Chemistry
SEU	Southern Member States of the European Union also referred to as SZ (Southern Zone)
SSD	Species Sensitivity Distribution
STMR	supervised trial median residue. Often used in regulatory residue assessments under Regulation 1107/2009
TAI	Terrestrial Area of Interest
TD	Toxicodynamics
TER	Toxicity-exposure-ratio
TK	Toxicokinetics
TKTD	ToxicoKineticToxicoDynamic
TMF	Trophic Magnification Factor
ToR	Terms of Reference
TRR	Total Radioactive Residue
TU	Toxic Unit
TWA	Time weighted average
UA	Uncertainty Analysis
US EPA	United States Environmental Protection Agency
WF	Water flux
WG	Working Group
WoE	Weight of evidence
WOEC	Weight-of-evidence communication
ww	Wet weight

Glossary

Absorption	Process(es) of uptake of substances into or across tissues. Absorption refers to parent compound and all its metabolites
Abundance	The total number of individuals of a taxon in an area
Accuracy	The degree to which a measured quantity approaches the true value of what is being measured
Acute toxicity	Responses, often lethal, caused by short-term exposure to a toxicant
Acute DD	Acute Dietary Dose (mg a.s./kg bw per day)
Ad libitum feeding	The diet is available at all times
Adsorption	The adhesion of a substance (e.g. active ingredient of a plant protection product) on the surface of solids or fluids
Antagonism	Opposition of force or principle; of chemicals, producing opposing effects
Arithmetic mean	Average of *n* values, calculated as the sum (Σn) divided by the number (n)
Assessment endpoint	An explicit expression of environmental value that is to be protected. Operationally, it is defined by an identified environmental entity of value that is susceptible to harm and an attribute that provides evidence of harm.
Assessment factor	Numerical adjustment used to extrapolate from experimentally determined (dose-response) relationships to estimate the exposure to an agent below which an adverse effect is not likely to occur.
Attribute	One of a set of descriptive terms
Avifauna	The bird fauna of an area or period
Bait	A ready-to-use bait (RB), a formulation designed to attract and be eaten by the target pests, can be assessed in the same manner as a seed treatment.
Benchmark Dose (BMD)	A benchmark dose (BMD) is a dose or concentration that produces a predetermined change in the response rate of an adverse effect.
Benchmark Response (BMR)	The predetermined change in response of a BMD is called the benchmark response (BMR).
Benchmark dose, lower confidence limit (BMDL)	The BMD calculated from a statistical model is a range rather than a fixed number. The lower limit of the range is denoted as the BMDL and is typically used in risk assessment.
BMDL10	The BMDL10 denotes the lower limit of the BMD calculated for a BMR equal to a 10% change in the response rate of an adverse effect relative to the response of control group. The smaller number after the BMDL will always denote the level of change in the response rate, though for the purposes of this Guidance the BMDL10 is the most important.
BMDU10	The BMDU10 denotes the upper limit of the BMD calculated for a BMR equal to a 10% change in the response rate of an adverse effect relative to the response of control group.
Bias	A systematic error in sampling inherent in the sampling technique
Bimodal	Used of a population or frequency distribution having two modes or peaks
Biomagnification	Is the process whereby the tissue concentrations of a contaminant increase as it passes up the food chain through two or more trophic levels. It is a typical issue for persistent chemicals with a high affinity for fat tissue and/or that are poorly metabolised or excreted.
Biodiversity	The variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.
Biological community	An association of interacting populations that consists of different species of plants, animals and microbes occupying the same area at the same time

Birth rate	The number of offspring produced per head of the population per unit time
Bush fruit	A small fruit growing on a woody bush (e.g. currant, gooseberry)
Cane fruit	Applies to any *Rubus* species or hybrid which is commonly grown with supports such as wires or canes, including raspberries, blackberries, and hybrids such as loganberry, boysenberry, marionberry and tayberry.
Carnivorous	Flesh eating
Carrying capacity	The maximum number of organisms that can be supported in a given area or habitat
CD-1 IGS mice	CD-1 IGS mice are outbred mice derived from a group of outbred Swiss mice developed at the Anti-Cancer Center in Lausanne, Switzerland. The CD-1 IGS mice are generally used for genetics, toxicology, pharmacology, and aging research.
Conceptual model	A hypothesis regarding the structures and important factors that govern the behaviour of an object or process of interest.
Confidence interval (band)	The range of parameter values that would all be accepted under the assumed statistical error distribution and the chosen significance level
Confidence limits	The interval that contains the given parameter value with a certain probability (e.g. 95%).
Control	A parallel experiment or test carried out to provide a standard against which an experimental result can be evaluated
Continuous data	Data that can be measured on an infinite scale.
Correlation quotient	A measure of the linear relationship between two quantitative variables, indicating the degree to which they vary together
Crepuscular	Animals which are active at twilight
Daily Dose	Estimated dose an animal will obtain in a single feeding bout
Daily Dietary Dose	Estimated dose through the diet that an animal will obtain over a 24 h period
Dam	In animal breeding, the female parent
Death (Mortality) rate	The number of deaths in a population per unit time
Degradation	Loss process by which a substance is physically transformed from one chemical species to another. This can ultimately result in the formation of unextracted residues and CO_2, but not necessarily in all cases
Dehusking	The removal of the husk (dry outer integument) from a grain, seed or fruit
Deshelling	The removal of the shell (hard outside covering) from a flower bud
Delayed effect	An effect which occurs after the lapse of some time after the onset of exposure.
Demography	The study of populations, especially of growth rates and age structure
Deterministic model	A mathematical model in which all the relationships are fixed and the concept of probability is not involved
Direct effect	An effect that is mediated solely by the interaction between a specified ecological receptor and an environmental stressor.
Dissipation	Result of one or more loss processes leading to the disappearance of a substance from an environmental matrix, e.g. soil. Loss processes contributing to dissipation include degradation within the soil matrix by biotic and/or abiotic processes, soil surface photolysis, volatilisation, plant uptake and leaching
Ecosystem	A dynamic complex of plant, animal and microorganism communities and their non-living environment interacting as a functional unit.
Ecosystem function	See Ecosystem process.
Ecosystem process	Action or event that results in the flow of energy and the cycling of matter. Examples of ecosystem processes include decomposition, production, water and nutrient cycling.

Ecosystem service (ES)	The benefit people obtain from ecosystems. Ecosystem services include provisioning services such as food and water; regulating services such as flood and disease control; cultural services such as spiritual, recreational, and cultural benefits; and supporting services such as nutrient cycling that maintain the conditions for life on Earth.
Ecosystem structure	Attributes related to the instantaneous physical state of an ecosystem. There are several characteristics to describe ecosystem structure. For example, species population density, species richness or evenness, and standing crop biomass.
Effect	In general, an effect is something that inevitably follows an antecedent (cause or agent). A biological effect is the biological result of exposure to a causal agent.
Empirical	Based upon direct observation and experience rather than theory or preconception
Endangered species	A species threatened with (local) extinction
Environment	Natural environment, encompassing all living and non-living entities occurring naturally on earth or some region thereof.
Environmental risk assessment (ERA)	The evaluation of the probability and seriousness of harmful (or adverse) effects to human health and the environment, whether direct or indirect, immediate or delayed, following exposure to a potential stressor.
Expert judgement	The judgement of a person with relevant knowledge or skills for making that judgement
Expert Knowledge Elicitation (EKE)	A systematic, documented and reviewable process to retrieve expert judgements from a group of experts, often in the form of a probability distribution
Exposure	Contact or co-occurrence of a stressor with a receptor organism, population, community; usually expressed in terms of concentration, duration and frequency.
Exposure scenario	A set of conditions or assumptions about sources, exposure pathways, amount or concentrations of agents involved and exposed organisms, systems or (sub)populations (i.e. numbers, characteristics, habitats) used to aid in the evaluation and quantification of exposures in a given situation.
Extrapolation	The process of estimating a value beyond the range of actual values, on the basis of an equation obtained from the actual values
Focal species	Focal species are usually selected based on their ecological relevance, their likely exposure to the potential stressor under field conditions, their susceptibility to the potential stressor, and their testability. Ideally, focal species should have equal or greater sensitivity to the potential stressor than do the species they represent in the ERA and thus knowledge of the effects on these species provides reliable predictions about effects on many other species. In the B&M guidance document: A representative subset of real species that actually occur in the crop when the plant protection product is being used.
Foliar invertebrates	Invertebrates dwelling on plant surfaces.
Food web	A representation of the various paths of energy and matter flow through populations in the community.
Frugivorous	Feeding on fruit
Functional group	A collection of organisms with similar functional trait attributes and that are likely to be similar in their response to environmental changes and effects on ecosystem functioning.
Functional Observational Battery	The (FOB) is a neurobehavioral valuation tool describing numerous behaviour and neurological activity related parameters of a rat strain.
Functional redundancy	A system resilience principle that states that there should be two or more different ways to perform a critical task.
Functional trait	A measurable property (e.g. mobility, feeding behaviour, trophic level, and place in the food web) of an organism, which has demonstrable links to the organism's function.

Gavage	Force-feeding
Generic model species	Not a real species, but a composed 'realistic worst-case' species representing a specific feeding guild and stratum. It is considered to be representative of all those species potentially at risk, i.e. it is based on ecological knowledge of a range of species that could be at risk. It has a high food intake rate and may consume a mixed diet. The 'generic model species' is also considered to be a representative of the types of birds or mammals that occur across Member States.
Generic focal species	Old terminology for generic model species
Generalist	A species having a broad habitat range or food preference
Generation time	The average duration of a life cycle between birth and reproduction
Geometric mean	Is a mean or average, which indicates the central tendency or typical value of a set of numbers by using the product of their values. It is calculated by multiplying the numbers in the data set, and taking the nth root of the result, where 'n' is the total number of data points in the set.
Graminivorous	Feeding on grass
Granivorous	Feeding on seeds
Grooming	The act of cleaning and tidying the body surface, by licking, nibbling or other directed behaviour
Ground invertebrates	Invertebrates dwelling on the soil surface.
Guild	A group of species having similar ecological resource requirements and foraging strategies
Habitat (Ecological habitat)	of a species is the place where an organism normally lives, often characterised by a dominant plant form (e.g. forest habitat) or physical characteristic (stream habitat).
Hawking	Feeding in flight
Hazard (harmful characteristics)	The characteristics of a potential stressor that can cause harm to or adverse effects on human health and/or the environment.
Herbivorous	Feeding on plants
Home range	The area, usually around the domicile, over which an animal normally travels in search of food
Incidence	The probability of an occurrence within a certain time.
In-crop area	Surface covered by the crop plants including the space between the crop rows.
Indicator model species	A species, the presence or absence of which is indicative of a particular habitat, community or set of environmental conditions.
Indirect effect	An indirect effect involves effects of a stressor being transmitted to a specified receptor through an indirect route involving one or more other, intermediary, receptors. For example, a predatory non-target organism could be affected indirectly by a stressor in several ways, including effects of the stressor reducing the abundance of its prey species, its intra-specific or inter-specific competitors, its pathogens or its parasites.
In-field area	The crop area and its boundaries that are managed by the farmer in the context of crop management.
Insectivorous	Feeding on insects
Lactation	Production of milk
Landscape	Any geographical area of interest at a relatively large scale resulting in heterogeneity in space such as fields or habitat patches (e.g. in the context of this scientific opinion, it usually refers to an area that encompasses a mixture of agricultural and non-agricultural land-use types (e.g. field and off-field), at spatial scales which are defined according to the ecological entities of concern).
Large seeds	Crop seeds with a diameter > 0.5 cm.
Lethal	Pertaining to, or causing, death by direct action
Life-history trait	Also referred to as a demographic trait. A trait that influences the population growth rate and ultimately drives population densities and age distributions.

Life span	Longevity; the maximum or mean duration of life of an individual or group
Line of evidence	A set of relevant information of similar type grouped to assess a hypothesis. There is no fixed rule on how much similarity of the information is required within the same line of evidence. This is for the assessor(s) to decide, and depends on what they find useful for the purpose of the scientific assessment.
Litter	Those animals produced at a multiple birth
Mammal	Any member of the Mammalia, a class of tetrapod vertebrates
Maternal	Pertaining to, or derived from, the female parent
Maximum searching area	The maximum area that can be feasibly exploited by an individual of a certain bird or mammal species with the assumption it takes all of the sown seeds/granules available in the surface of that area.
Measurement endpoint	A measurable quality related to the valued characteristics chosen for the assessment (Suter et al., 1993). Within the context of ERAs that fall under the remit of EFSA this concerns a quantifiable response to a potential stressor that is related to the specific protection goal.
Median	That value of a variable in an ordered array that has an equal number of observations or items above and below it
Metapopulation	An overall population comprising populations of the same species connected through immigration and emigration.
Minimum Number Alive (MNA)	A widely used index of abundance and trappability (% of population trapped during a capture session) in mark-recapture programmes. MNA is a negatively biased abundance index, and is sensitive to capture probability and the capture session number.
Moving time window approach	A calculation of the worst-case residue after multiple applications accounting for residue decline and accumulation of residues.
Native	Indigenous; living naturally within a given area
Niche	The ecological role of a species in a community
NOAEL	Concentration or dose of a substance that causes no detectable adverse alterations in an organism in the context of a given (safety) experiment. Although data requirements as laid down in Regulation 283/2013 and 284/2013 refer to NOEC (no observed effect concentration), the use of NOAEL is recommended for birds and mammals as the endpoint is expressed as a dose level per kg body weight.
Nocturnal	Active during the hours of darkness
Non-target organism (NTO)	An organism that is not intended to be affected by the potential stressor under consideration.
Normal operating range (NOR)	The range of values of a given ecological measurement endpoint that is normally observed during a predefined period for a reference population, community, ecosystem or process
Off-crop area	Any uncropped area. It includes also uncropped areas in-field, and such areas can be, e.g. the minimal required zone for agricultural management, buffer strips or beetle banks.
Off-field area	Area surrounding a field; either (semi)natural habitats with high ecological value (such as hedgerow or grass strip) or simple structures (fence or a bare strip of land); normally no short-term changes in cultivation, in most cases not to be influenced by the farmer. Another off-field category comprises man-made structures, e.g. an adjacent field, roads, etc.
Omnivorous	Eating a diet of both plants and animals
Ornithology	The study of birds
Parameter	A characteristic of a distribution of a variable or population. Term in the model that is fixed when conducting a model run or simulation.
Parameterisation	Parameter definition, the process of defining parameters that are used to represent the (biological) processes in a model

Passerines	Any bird of the order Passeriformes (songbirds)
Paternal	Pertaining to, or derived from, the male parent
Pelleted seed	Pelleted seeds are seeds coated with inert materials to change their size and shape usually to help with handling/planting. The seed may be treated with pesticide products prior to the coating or the coating itself may include pesticide products.
Pest	The concept of pest organisms is anthropocentric and thus a pest is defined as any organism that is perceived by humans to interfere with their activities. Ecologically there are no such organisms as pests. Organisms in several phyla are considered to be pests: e.g. arthropods, nematodes, molluscs, vertebrates. In particular, any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products are called plant pests.
Pesticide	Substance/agent that prevents, destroys, or controls a harmful organism ('pest') or disease, or protects plants or plant products during production, storage and transport.
Plant Protection Product (PPP)	A substance (or device) used to protect (crop) plants from damage by killing or reducing pest organisms or by mitigating their effects.
Population	A group of individuals of the same species.
Precision Drilling	Drills whose metering mechanism distributes seeds singly by means of a burying device at predetermined intervals to form a sowing line.
Prevalence	The proportion of a population estimated to have a certain effect/ disease/etc. at a certain time.
Primary toxicity	Primary toxicity is the specific toxicity caused by the substance in question. See also Secondary toxicity.
Probability	Defined depending on philosophical perspective: (1) the frequency with which samples arise within a specified range or for a specified category; (2) quantification of uncertainty as degree of belief regarding the likelihood of a particular range or category
Problem formulation	Phase of environmental risk assessment which includes a preliminary description of exposure and environmental effects, scientific data and data needs, key factors to be considered, and the scope and objectives of the assessment. This phase produces the risk hypotheses, conceptual model and analysis plan, around which the rest of the assessment develops.
Protection goals	The objectives of environmental policies, typically defined in law or regulations.
Pulses	Edible seeds of plants in the legume family
Quantal data	When the response is a binary value (e.g. alive/dead)
Quotient	The value obtained by dividing one quantity by another
Random distribution	A distribution in which the outcome is the result of pure chance
Reciprocity	The observation that effects of exposure are similar when exposed for a short time to a greater concentration or for a longer time to a smaller concentration. Reciprocity relates to Haber's law, which assumes that toxicity depends on the product of concentration and time. In case of linear reciprocity, different exposure patterns with the same are under the curve concentration result in the same effect, the basis of the time-weighted average (TWA) approach.
Recovery	Ecological recovery is the return of the perturbed ecological endpoint (e.g. species composition, population density) to its normal operating range.
Recruitment	The influx of new members into a population by reproduction or immigration
Reproductive	The reproductive risk assessment is based on a selected relevant endpoint which may be derived from a long-term study. The term 'reproductive' is used as a general term referring to an effect on development/growth and/or reproduction, that is likely to be relevant for population maintenance.
Refuge	An area in which an ecological entity can survive through a period of unfavourable conditions.

Regulated products	Claims, materials, organisms, products, substances and processes submitted to EFSA for evaluation in the context of market approvals/ authorisation procedures for which an ERA is required.
Relevance	The contribution a piece or line of evidence would make to answer a specified question from a regulatory, biological and/or exposure point of view
Reliability	The inherent quality of a piece of information, particularly considering the experimental procedure and the resulting plausibility of the findings.
Risk	The likelihood of consequences (of specified type, magnitude and duration) arising if an ecological entity is exposed to a specified stressor.
Risk assessment	A scientifically based process consisting of four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation
Risk management	Decision-making process involving considerations of political, social, economic and technical factors with relevant risk assessment information relating to the hazard.
Secondary toxicity	Secondary toxicity is toxicity resulting from other substances or effects produced as a result of the presence of the original toxin in the body. An example would be effects on reproduction as a result of significant body weight decreases caused by the original toxin.
Sex ratio	The relative numbers of males and females in a population, expressed as the number of males per 100 females
Short cut	A method of achieving something more quickly or more easily than if you use the usual methods.
Ruminant	An even-toed ungulate mammal that chews the cud regurgitated from its rumen. The ruminants comprise the cattle, sheep, antelopes, deer, giraffes, and their relatives
Scavenger	Any organism that feeds on carrion or organic refuse
Scenario	'plausible and often simplified descriptions of how the future may develop, based on a coherent and internally consistent set of assumptions about key driving forces and relationships'
Sensitivity analysis	The quantification of the effect of changes in input values or assumptions (including boundaries and model functional form) on the outputs. By investigating the relative sensitivity of model parameters, a user can become knowledgeable about the relative importance of parameters in the model
Service providing unit (SPU)	The systematic and functional components of biodiversity necessary to deliver a given ecosystem service at the level required by service beneficiaries.
Small seeds	Seeds with a diameter of $\leq$ 0.5 cm.
Species diversity	The number of different species/taxa within a given community
Species sensitivity distribution (SSD)	Models of the variation in sensitivity of species to a particular stressor. They are generated by fitting a statistical or empirical distribution function to the proportion of species affected as a function of stressor concentration or dose. Traditionally, SSDs are created using data from single-stressor laboratory toxicity tests, such as median lethal concentrations (LC_{50}s).
Specific Protection Goal (SPG)	An explicit expression of the environmental value to be protected, operationally defined as an ecological entity and its attributes.
Standard Drilling	Drilling of seeds by means other than broadcast and precision.
Stratum	A horizontal layer of vegetation within a stratified plant community.
Synergism	Activity of two or more chemical agents given together that is greater than the sum of activity had the agents been given separately.
Systemic pesticides	Pesticides which move within the plant.
Systemic toxicity	It refers to toxic effects caused as a result of absorption and distribution of a substance that affects the whole body rather than a specific (local) area, i.e. to an area distant from its entry point.

Taxon	A taxonomic group of any rank sufficiently distinct from other such groups to be treated as a separate unit.
Terrestrial Area of Interest (TAI)	The area where the risk assessment is performed e.g. for small mammals outside of treated area.
Thousand grain weight	The weight of 1000 seeds.
Threshold	The minimum level of a value of a stimulus to elicit a response
Tillage	The agricultural preparation of soil by mechanical agitation of various types (e.g. digging, stirring, overturning).
Toxicodynamics	All processes that lead to the damage and/or mortality of the organism exposed to toxic compounds. Biological effects are caused by the toxic compounds on the molecular level, where the molecules of the toxic compound interfere with one or more biochemical pathways. Toxicodynamic part of TKTD models integrate all those processes into only a few equations that capture the dynamics of responses or effects over time.
Toxicokinetics	All processes that influence the dynamics in internal exposure of an individual to the toxic compound, and include absorption, distribution, metabolism and elimination. Toxicokinetic models are used to estimate internal exposure concentrations.
Trait	A well-defined, measurable, phenotypic or ecological character of an organism, generally measured at the individual level, but often applied as the mean state of a species.
Translaminar	Pesticides which move into the leaf where a reservoir of active substance remains for a period of time providing longer control against the target pest.
Uncertainty	Uncertainty is the inability to determine the true state of affairs of a system and it may arise in different stages of risk assessment due to lack of knowledge and to natural variability.
Uncertainty analysis	Analysis of all factors that could reduce the appropriateness and precision of a model to describe a certain phenomenon
Validation	The process of establishing that the procedure/model is a sufficiently accurate representation of the real world to be used as the basis for regulatory decisions.
Variability	The inherent heterogeneity or diversity of data in an assessment.
Variable	A measured or estimated quantity that describes an object that can be observed in a system and that is subject to change. Two kinds of variables can be distinguished. The state variables (e.g. body mass) are the dependent variables calculated within a model, which are also often the performance indicators of the models that change over the simulation. The forcing variable corresponds to input data to the model (e.g. toxicity of the substance). This input data may be defined in the exposure/ecological scenario.
Vermivorous	Feeding on worms
Vulnerable species	A species is a with a relatively high sensitivity to a specific stressor, a high chance of exposure and/or high risk of indirect effects, plus a poor potential for population recovery.
Weight of evidence assessment	A process in which evidence is integrated to determine the relative support for possible answers to a question

Appendix A – Generalised description of the tiered approach in prospective ERA for pesticides

An appreciation of the tiered risk assessment scheme is needed in order to help contextualise the outcome of any assessment. Figure A.1 and Table A.1 help to describe and explain the tiered approach. Furthermore, it provides clarity as to why a risk assessment indicating a high risk can change to low risk if additional data and risk assessments are made available.

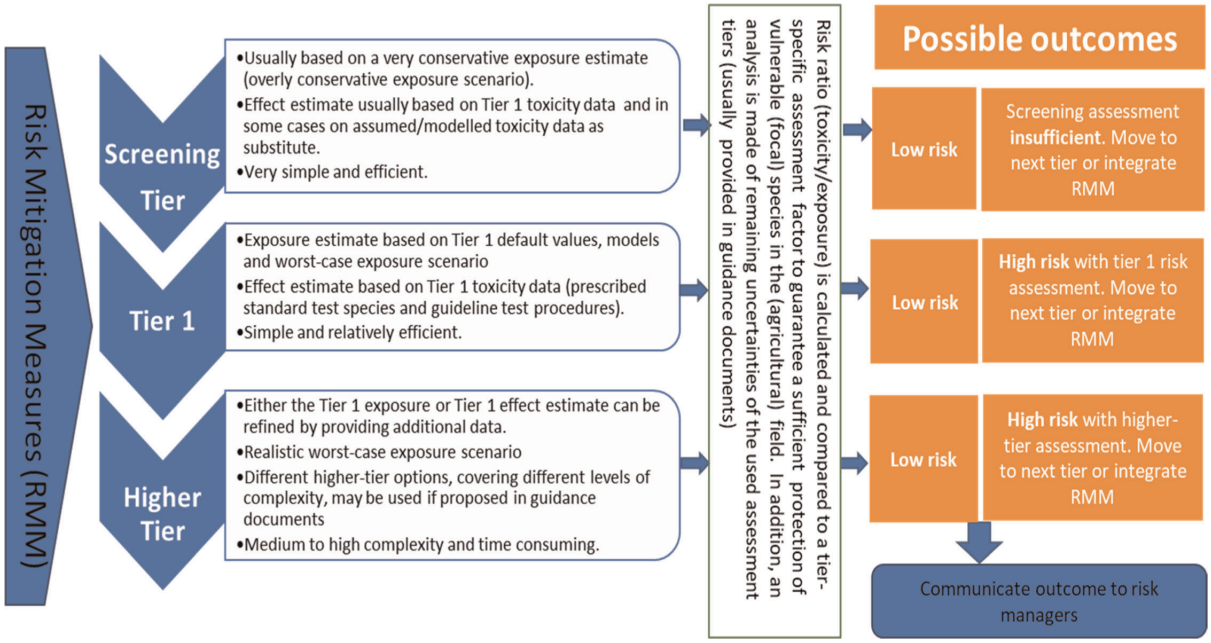


Figure A.1: Generalised description of the tiered approach in prospective ERA for pesticides and non-target organisms

Table A.1: Generalised explanation of the different effect assessment and exposure assessment tiers and their use in risk assessment

	Effect assessment	Exposure assessment	Interpretation and use in risk assessment
Screening Tier	The screening level hazard assessment can be based on standard Tier 1 toxicity data when available. In some cases, where data are lacking, conservative estimates can be made. For example, it is common to assume that a metabolite is 10 times more toxic than the parent substance. Should the risk assessment pass with such assumption it negates the need for testing with the metabolite. Another example of screening Tier is the Tier 0 effect assessment for sediment organisms by using the experimentally derived Regulatory Acceptable Concentration for pelagic water organisms and the Equilibrium Partitioning approach (EFSA PPR Panel, 2015)	The exposure assessment used at the screening tier may be an overestimation because of the use of overly conservative exposure scenarios. It may be assumed that in case of repeated pesticide applications, all pesticide loadings in the environmental compartment of concern occur simultaneously (see e.g. Step 1 exposure scenario of FOCUS, 2001). It may be the case that in the exposure assessment many crop groups are merged together and therefore, when a screening level assessment 'passes' it can be useful for extrapolation of risk assessments between different uses (i.e. application of the risk envelope).	The purpose of a screening step is to provide an efficient and cost-effective method to identify uses of substances that pose low risk Usually, the risk assessment is performed as much as possible in line with the Tier 1 risk assessment but could be with conservative estimates for the hazard and/or exposure. Should a screening assessment fail then this should simply inform the risk assessor that a more detailed risk assessment is needed.
Tier 1	Tier 1 laboratory toxicity data should be used. These data are defined by the data requirements in the EU (Regulation 283/2013[63] and 284/2013[64]) and occasionally supplemented by additional data (e.g. generated to meet data requirements outside the EU) pending on the relevant guidance document. Guidance on the selection of the endpoint should be given in the respective guidance documents, as well as how to deal with multiple Tier 1 toxicity data for the same standard test species.	Tier 1 exposure assessment may be conservative relative to the actual exposure because of the use of worst-case exposure scenarios. Guidance on the (default) parameters, models and scenarios to be used in the exposure assessment should be given in the respective guidance documents. The exposure scenarios account for crop-specific applications of the same active substance/regulated product including its repeated application in the crop of concern.	The risk assessment based on Tier 1 data (in both the exposure or hazard assessment) should be a relatively conservative prediction of the level of risk. Ideally, this needs verification by appropriate higher tier assessments, e.g. comparison of the Tier 1 regulatory acceptable exposure concentration with that of the (surrogate) reference tier (see EFSA PPR Panel, 2010). This reference tier may include (semi-)field studies and modelling approaches. However, owing to the scarcity of suitable data in some cases this may not have been possible and other approaches needed to be pursued.

[63] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

[64] Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the EuropeanParliament and of the Council concerning the placing of plant protection products on the market.

	Effect assessment	Exposure assessment	Interpretation and use in risk assessment
			The risk assessment based on Tier 1 data (in both the exposure and hazard assessment) is usually a ratio of the toxicity to exposure (TER) or vice versa (ETR). The resulting number is compared to the 'standard' assessment factor. This assessment factor is mentioned in legislation and is assumed to be compliant with the protection goal.
Higher Tiers	Different higher tier options to refine the Tier 1 effect assessment, covering different levels of complexity, may be used if proposed in the guidance document for the non-target species of concern. The approaches should not be in conflict with the effect assessment goals (EfAGs) that underlie the SPG. Tier 2 approaches usually are based on toxicity data for standard and additional test species (e.g. allowing the SSD approach), but may also include the application of validated TKTD models for standard and additional test species (see e.g. EFSA PPR Panel, 2013, 2018). Tier 3 approaches usually are based on 'no observed effect levels' or treatment-related responses (effect classes) due to realistic worst-case exposure conditions in (semi-)field studies (e.g. mesocosms or field effect studies). In addition, population-level modelling studies (focus on potential vulnerable species at risk) may be an appropriate Tier 4 approach, although official EFSA guidance on population-level modelling is limited for most groups of non-target organisms.	Different higher tier options to refine the Tier 1 exposure assessment, covering different levels of complexity, may be used if proposed in the guidance document for the assessment of concern. The higher tier exposure values should be a more realistic estimate and not in conflict with the exposure assessment goals (ExAGs) that underlie the SPG (realistic worst-case exposure scenarios). Relatively simple Tier 2 refinements may concern a better estimation of specific parameters sensitive for the exposure assessment (e.g. the use of an environmentally more realistic DT50 value for food items instead of a fixed default value). More complex refinements may concern the definition of sophisticated exposure scenarios (e.g. covering more realistic combinations of soil, weather, topography and land-use conditions) and modelling approaches (see e.g. EFSA PPR Panel, 2012b). Integrated effect and exposure modelling on basis of environmental scenarios (combination of ecological and exposure scenarios) and models that allow spatial-explicit assessments of exposures to vulnerable focal species may be a future option (official EFSA guidance not yet available).	The risk assessment - based on higher tier data in either the exposure or effect assessment, or both - should be a more realistic prediction of the level of risk. Again, this needs verification by comparing the results with the (surrogate) reference tier (see EFSA PPR Panel, 2010). However, owing to scarcity of suitable experimental, monitoring and/or modelling data this may not have been possible and other approaches may need to be pursued (e.g. Expert Knowledge Elicitation; EFSA Scientific Committee, 2014). The risk assessment based on higher tier data is usually a ratio of the toxicity to exposure (TER) or vice versa (ETR) but in some higher tiers the exposure and effect assessment may be integrated. The resulting number usually is compared to the assessment factor (which may differ from Tier 1). Such assessment factor should address the remaining uncertainties and be compliant with the protection goal. In some cases (e.g. integrated exposure and effect modelling), the resulting number may also be directly used, although a small assessment may be required for spatial–temporal extrapolation. Should multiple higher tier data be available then it is strongly recommended that this is considered in an appropriate weight of evidence assessment.

Appendix B – Description of available protocols for an avian reproductive study

The Appendix describes the 2 available standard protocols for conducting an avian reproductive study (OECD, 1984b; USEPA, 2012a). Both protocols require birds are acclimated to laboratory conditions. The substance to be tested is mixed into the diet. The birds are fed *ad libitum* for a recommended period of 10 weeks before they begin laying in response to a change in photoperiod. The egg-laying period should last 8–10 weeks. Eggs are removed from the adults the day they are laid, stored and then artificially incubated.

Biological variables recorded during the study are presented in Table B.1.

Table B.1: Key biological variables recorded in the OECD 206 and US OCSPP 850.2300 studies(1)

OECD 206	USEPA OCSPP 850.2300
Frequency, duration, and description of signs of toxicity, along with severity, numbers affected and any remissions	Description of any signs of intoxication or any other abnormal behaviour in adult and young birds including time of onset, duration, severity (including death), and numbers affected (including accidental deaths or injuries), and any remissions.
Food consumption and body weights for adults and young birds	Body weights and food consumption of adult male and female birds and the body weight gain for each sex by pen between test initiation and termination. Tabulation of body weights of adult male and female birds by pen and observation time (provide raw data) and the body weight gain for each sex by pen between test initiation and termination.
Details of gross pathological examinations(2)	Details of the necropsies (see section on recommendations below)
Egg production: • eggs set, • viability, • hatchability (including normal hatchlings), • survival of young, and • eggshell thickness	Egg production: • number of eggs laid, • number of irregular or abnormal eggs, number of cracked eggs, • number of eggs set, • number of viable embryos, • number of live embryos, • number of normal hatchlings, and number of 14-day old survivors • percentage of eggs set of eggs laid, • viable embryos of eggs set, • live 18-day old embryos, • 14-day old survivors of eggs laid, and • 14-day old survivors of hatchlings • eggshell thickness
Anything unusual about the test and other relevant information which might have influenced the results	

(1): Please note that full details of the key parameters can be found in the respective guideline.

(2): It is noted that OECD TG 206 recommends gross pathology examinations, although further guidance on this assessment is not given in this test guideline. Normally, weights of some organs and results of visual pathology are reported. Further recommendations on how to report gross pathology findings may be found in the report of the SETAC/OECD workshop on avian toxicity testing, OECD (1996) and Martin et al. (2020). It is recommended that gross pathology findings are reported when available with particular reference to potential endocrine target organs (thyroid and gonads/reproductive organs) as well as liver. Information on gross pathology is normally reported but effects on gross pathology are not used to derive an endpoint.

As regards statistical analysis of key parameters, the **OECD 206** (OECD, 1984b) states that 'test groups' should be individually compared to the control group by a statistical procedure designated in the study plan. Any generally acceptable statistical method, such as analysis of variance or other applicable methods may be used. As regards which parameters should be assessed, the guideline highlights that the following should be assessed:

- Egg production – number of eggs laid per hen (ten weeks)
- Percentage of cracked eggs
- Viability (per cent viable embryos of eggs set)
- Hatchability (per cent hatching of eggs set)
- Percentage of hatchlings that survive to 14 days
- Number of 14-day old survivors per hen
- Eggshell thickness

It further highlights that 'if possible', the following should also be subject to statistical analysis:

- the percentage of hens laying eggs,
- the body weight of adult birds and
- the body weight of 14-day-old survivors'.

As for the **USEPA OCSPP 850.2300** (USEPA, 2012a), it is stated that for the parameters presented below, 'escriptive statistics (mean, standard deviation, standard error, 95% confidence interval, median, first and third quartiles, minimum, maximum)' along with plots of the effects (mean, median, first and third quartiles, minimum, maximum) observed by treatment level should be provided. It also indicates that 'percent inhibition calculations as compared to control' should be presented.

- eggs laid,
- number of irregular or abnormal eggs,
- number of cracked eggs,
- number of eggs set,
- number of viable embryos,
- number of live embryos,
- number of normal hatchlings,
- number of 14-day old survivors by treatment,
- percentage of eggs set of eggs laid,
- viable embryos of eggs set,
- live 18-day old embryos,
- 14-day old survivors of eggs laid,
- 14-day old survivors of hatchlings,
- eggshell thickness,
- body weights of adult male and female birds,
- body weight gain for each sex,
- body weights of surviving young at 14 days of age by pen and observation period.

Since the **US EPA OCSPP 850.2300** has been updated recently and clarified the statistical analysis and the parameters which should be statistically analysed, it is recommended to always follow that guideline for this particular aspect. In case of replicates not performing as expected, see Section 5.2.6.1 on biological relevance.

Appendix C – Long-term and reproductive studies with mammals

This Appendix gives an overview and a basic description of the available long-term and reproductive test protocols for mammals. These studies are used for the selection of the reproductive endpoint for wild mammals, see Section 5.2.6.5.

a) OECD 416 – Two-generation reproduction toxicity study

In this test (OECD, 2001c), two or sometimes more generations can be assessed. It is specifically designed to address male and female reproductive performance including gonadal function, oestrous cycling, mating behaviour, conception, parturition, lactation and weaning. The results of such tests are the ones most often used for assessing toxicity in wild mammals. The test uses rats or (less frequently) mice. Males are dosed during growth and, at least, during a complete spermatogenic cycle (56 days in mice, 70 days in rats). Females are dosed for two complete oestrous cycles. The animals are then mated.

The test substance is given throughout the study, typically in the diet. Sufficient pregnancies and offspring must be produced to enable assessment of maternal behaviour as well as of suckling, growth and development of the initial offspring generation (F1) right up to weaning. As the name implies, the two-generation test means that the F1 pups are kept on-dose and bred to produce a second generation, the F2 generation. The highest dose level should induce toxicity, but not mortality, in the parent animals.

If necessitated by a decrease in food consumption, a pair-fed group could be added. Other than the functional endpoints such as fertility, litter size and survival, test endpoints include gross necropsy and pathology of the reproductive tract as well as histopathology where indicated (especially if reproductive organ histopathology was not performed on the shorter-term studies). The latest revisions to the test emphasised more detailed examinations of sperm parameters, sexual maturation and functional measurements of the reproductive output. The two-generation study allows an examination of the full growth, development and sexual maturation of the F1.

b) OECD 443[65] Extended one-generation reproduction toxicity study

This test (OECD, 2018c) was developed to reduce animal use in the two-generation study, while still maintaining, and in several cases adding more, relevant endpoints for reproduction and development.

The test may be conducted using three cohorts. Cohort 1 (A and B), which is intended to determine reproductive/developmental endpoints, should always be present, and may be extended to two generations, under certain circumstances. Cohorts 2 (A and B) (developmental neurotoxicity cohort) and 3 (developmental immunotoxicity cohort) may be used depending on prior knowledge of the substance in question.

A 2-week premating treatment is advised, which covers three to four oestrous cycles in females. The length of the premating treatment is intended to assure steady-state exposure levels at the time of mating and to be able to detect any potential adverse effects on cyclicity and on post-testicular effects on sperm at the time of mating. It also ensures that at termination males will have been exposed for one full spermatogenic process.

The test then follows similarly to OECD 416 (OECD, 2001c), until postnatal day 21, at which time all of the F1 pups are randomly allocated into the various cohorts mentioned above. Cohorts 1A and 1B include 20 pups/sex/group. Cohort 1A investigates primary effects upon reproductive systems and of general toxicity. Cohort 1B provides a follow-up assessment of reproductive performance by mating F1 animals (when considered necessary), and for obtaining additional histopathology data (e.g. in cases of suspected reproductive or endocrine toxicants, or when results from cohort 1A are equivocal). Cohort 2A and 2B include 10 pups/sex/group and investigate (A) (one male or one female per litter) assigned for neurobehavioral testing followed by neurohistopathology assessment as adults and (B) (one male or one female per litter) assigned for neurohistopathology assessment at weaning (Post Natal Day 21 or PND 22). Cohort 3 includes a total of 20 pups per group (10 males and 10 females per group; one per litter, where possible, plus more control animals, if necessary, as positives in the assay) which are tested in a T-cell dependent antibody response assay (TDAR) at PND 56 $\pm$ 3.

[65] Note that OECD 415, 1983, (adopted 26 May 1983) is a one-generation reproduction toxicity study that follows the same dosing schedule as an extended one generation study except that there is no further dosing of pups or dam after weaning, and there are no separate cohorts used for various investigations. Due to the limited dosing and the (relatively) limited endpoints measured, this test is rarely seen, but, if available, may be investigated for relevant endpoints.

Throughout the study, clinical observations, body weights and food consumption are measured at regular intervals in all animals. Further, in the P generation, oestrous cycles, mating and pregnancy parameters are measured. A wide variety of offspring parameters are measured, including general toxicity and development endpoints, but also sexual development endpoints such as anogenital distance for all pups and balano-preputial separation or vaginal patency in males or females, respectively, for select pups.

Cohort 2 animals are used to investigate neurodevelopmental effects, similarly to OECD 424 and 426, discussed below. These include both behavioural investigations, performed at appropriate time points in development, and histopathology performed both immediately (Cohort 2B) or upon reaching adulthood (Cohort 2A).

Cohort 3 animals are used in a T-cell dependent antibody response assay at around PND 56.

c) OECD 414 – Prenatal developmental toxicity study

The mammalian data package will typically contain two prenatal developmental toxicity tests according to OECD 414 (OECD, 2018d), one in rats and one in rabbits. The reason for using both rats and rabbits is the differential sensitivity of rats and rabbits to different types of teratogens, though in some cases only rat will have been used. This test doses pregnant female animals from the approximate day of implantation (ca. day 5 or 6 of gestation in rats and rabbits) to the day before delivery (ca. day 21 of gestation in rats). An earlier protocol used a shorter dosing period, restricted to the time of major organ and system differentiation.

Doses are normally given by oral gavage. The dose spacing is typically quite large in these studies, as it must be conducted so that the lowest dose shows no toxicity, the middle dose some minor toxicity and the top dose significant toxicity. A fourth dose group may be added, usually using factor of up to 10 between dose groups. The highest dose tested should produce some degree of maternal toxicity or be the limit dose of 1,000 mg/kg bw per day.

The study is designed to determine adverse effects on the dam such as reduced body weight, clinical signs and ability to maintain pregnancy. The study also identifies structural abnormalities in the fetus (e.g. teratogenic effects). The fetuses are examined for viability, size, weight, sex ratio and specifically, for abnormalities of the skeleton and soft tissues/organs. The possible developmental abnormalities are too numerous to list exhaustively. They consist, generally, of external, visceral, skeletal, and materno-fetal abnormalities. More information on the types of abnormalities and a definition of each can be found in (Makris et al., 2009). Fetal abnormalities are normally divided into severe cases (malformations), i.e. those that would compromise the ability to survive or function normally, and minor cases (variations/anomalies) that would have a minimal impact on the animal. Malformations would generally be considered ecologically relevant, whereas variations/anomalies are unlikely to be ecologically relevant. Please note that the same effect (absence of vertebrae, for example) could be classified as a malformation or as a variation/anomaly depending upon severity and location, therefore the evaluation of the mammalian toxicologist(s) will be of key importance in determining ecological relevance.

For some endpoints, the effects of maternal toxicity may play a significant role, and this is carefully weighed in endpoint determination. Finally, as described above, if the incidence of even a severe effect is low, it may be considered to be not ecologically relevant.

d) OECD 421 – Reproduction/Developmental Toxicity Screening Test

This guideline (OECD, 2016c) is intended to provide initial, limited information on possible effects on male and female reproductive performance, such as gonadal function, mating behaviour, conception, development of the conceptus and parturition. It is intended to be used as a preliminary screening test and is therefore not always present in the data set and is also not relevant for ecologically relevant endpoints.

Males are dosed for a minimum of 4 weeks, and females are dosed for approximately 63 days (2 weeks prior to mating, the variable time to conception, the duration of the pregnancy and at least thirteen days after delivery). Doses are typically administered via gavage but may also be dosed in the diet or drinking water. At least 10 males and 12–13 females per group are used (the extra females are intended to ensure that there are 10 females with the typical oestrous cycle available per group). The minimal number of pregnant females per group is 8. Clinical observations, body weight and food/water consumption are recorded regularly throughout the live part of the test. Oestrous cycles are also measured and recorded before pregnancy. Duration of gestation, number and sex of pups, stillbirths, live births, runts and the presence of gross abnormalities are recorded, along with pup weight, which

is also recorded at regular intervals post-partum. The ano-genital distance of each pup is also measured between PND0 and PND4. Blood samples are taken regularly from all dams and from at least two pups per litter (and from males at termination). Thyroid hormones (T4 and Thyroid Stimulating Hormone) are then measured at each time point. Pathology and histopathology are performed on the parental generation and all pups, with special attention to reproductive organs and thyroid.

e) OECD 422 – Combined Repeated dose Toxicity Study with Reproduction/ Developmental Toxicity Screening Test

This test (OECD, 2016d) provides information on the possible hazards likely to arise from a repeated exposure over a relatively limited period of time. It is intended to be used on substances where no 90-day study is available or warranted, or as a preliminary study. It also includes a reproduction/developmental toxicity screening, as outlined above. There is also some emphasis placed on clinical observations and related endpoints for neurotoxicity and immunotoxicity.

The test is conducted as above in OECD 421, with additional observations of sensory reactivity to different modalities, assessment of grip strength, and motor activity assessments in five adults of each sex, per group. Additional haematology is also performed in five males and five females per group, including haematocrit, haemoglobin concentrations, erythrocyte count, reticulocytes, total and differential leucocyte count, platelet count and a measure of blood clotting time/potential. Clinical biochemistry is performed to determine effects on liver and kidney on all dams and at least two pups, and on all adult males at termination. Furthermore, additional pathological examinations and histopathology are covered (as in a repeated dose study) for all major organs.

OECD 421 and 422 are screening tests and may be used indicatively for the elucidation of potential toxicities and toxic doses. Consultation with the toxicology section is recommended should it seem that the relevant lowest endpoint was determined in one of these tests, as presumably the same/a similar endpoint was further investigated in a definitive test (i.e. OECD 414, 416, 443, 426).

f) OECD 426 – Developmental Neurotoxicity Study

In some cases, where available data or known mechanism of action indicate neurotoxicity, a developmental neurotoxicity study (OECD, 2007d) may be available. The test is mainly performed in the rat, and test substance is administered, typically orally through the food, during gestation (minimally starting at implantation, GD6) and lactation (PND 21). Usually, enough animals are used to produce 20 litters per dose group. Pups are selected from each dose group and assigned for endpoint assessments on or after PND 4. Selection of pups should be performed so that to the extent possible both sexes from each litter in each dose group are equally represented in all tests.

Dams are observed for clinical signs, and body weight and food and water consumption are measured at regular intervals. The same observations are made in pups, with additional tests for physical and developmental landmarks and the battery of tests associated with developmental neurotoxicity, including behavioural ontogeny (such as righting reflex, negative geotaxis and motor activity). Motor activity should be observed from weaning to adulthood and it is recommended that it is used to assess behavioural ontogeny, in which case the same individuals should be used in order to assess the ontogeny of intersession habituation. Additional motor-sensory observations should also be made including auditory startle habituation, for example. Learning and memory tests should be conducted post-weaning and in young adults. Neuropathological examination is performed after sacrifice including all CNS organs and PNS organs as well for animals sacrificed at termination (adults).

Table C.1: Behavioural endpoints from developmental neurotoxicity studies

Endpoint	Description	Potential ecological relevance
Righting reflex	Righting reflex, also known as the labyrinthine righting reflex, is a reflex that corrects the orientation of the body when it is taken out of its normal upright position. It is tested mainly by flipping the rat onto its back and recording the time to return to upright. It may also be tested in the air (righting whilst falling).	As righting reflex is key to survival in all animals, except in cases of low severity this endpoint would be considered ecologically relevant.
Negative geotaxis	For the negative geotaxis test, a rat is placed on a platform with a slightly roughened surface. The platform is then swung through 90° in 3–4 seconds and the angle at which the animal loses its balance is measured. This tilting plane test examines the sensory system, and above all, the intactness of the placing reflexes.	Similar to righting reflex, the functioning of the sensory system and particularly placing reflexes is key to survival. This endpoint would be considered ecologically relevant except in cases of very low severity.
Other sensory system tests	Crossing of rods of different width, cliff avoidance, the ability to stay on a rotating rod, and pivoting and forelimb grip strength are all additional ways in which the sensory system may be tested.	Similar to the other sensory system tests (righting reflex, negative geotaxis) these tests are indicative of functions basic to survival and effects would be considered ecologically relevant should they show appropriate severity.
Motor activity, exploration and habituation	Mainly an open field test where intensity of motor activity (ambulation) and rearing is recorded. Time spent in the central and/or peripheral parts of the open field arena and defecation rate are considered the measure of emotional reactivity to stressful stimuli in a new environment. A separate but related endpoint is 'habituation', which refers to whether the animal will 'get used to' the environment after multiple exposures or will remain alert and active.	These endpoints can be highly variable between individuals and are difficult to measure rigorously. The link to population-level effects also may be more tenuous. The severity and prevalence of the effect should be considered and only in cases of relatively high severity and prevalence would these endpoints be considered to be relevant. In those cases of high severity, a lack of motor activity and/or exploratory behaviour could result in an inability to adequately gather food. A lack of ability to habituate would result in high energy expenditure and unusually fast habituation could result in a lack of vigilance against predation.
Olfactory orientation	Various methods may be used to generally measure a rat's tendency to react to/explore new scents and/or habituate to repeated exposure to the same scent.	In cases of clear effects, the endpoint could be considered ecologically relevant. Reaction to new scents increases the likelihood of appropriate reaction to the environment relevant for both reproduction (mate finding/pup care) and survival (food finding/predator awareness).
Auditory startle reflex/habituation	Similar to olfactory orientation, this can be tested using various methodologies, with the general theme being introduction of a startling sound and measuring reaction time and magnitude. Generally, whole-body ballistic movements are measured using a startle response system that consists of a piezoelectric accelerometer mounted under a platform that detects the corresponding startle responses upon introduction of high decibel (±80 db) sound.	Both the rapidity of the response and habituation to the sound can be used as endpoints and both may be considered ecologically relevant. Alterations to either could be fatal for wild animals.

Endpoint	Description	Potential ecological relevance
Memory and learning	There are various methodologies to measure learning and memory. Common variants include the Morris water maze, radial arm maze, and various other task learning and memory options.	Defects in memory and learning would be considered ecologically relevant as they could directly affect both survival and reproduction. As with all endpoints, the severity of the effect should be considered, as natural variation will be high.
Neuropathology CNS	At the end of the test the brain should be investigated for any (histo) pathologies.	Changes in brain structures should be considered potentially ecologically relevant.
Neuropathology PNS	At the end of the test the peripheral nervous system will be investigated for any (histo) pathologies.	Changes in the PNS should be considered on a case-by-case basis as to ecological relevance. Factors to consider would be the severity and type of effect.
Other general endpoints including body weight of dams and pups, developmental milestones, clinical signs/chemistry, etc. are also measured and should be considered as discussed above.		

Further information on DNT tests, endpoints, and interpretation of those endpoints can also be found in the recent Guidance of the NAFTA TWG on Pesticides (Moser et al., 2016).

Subchronic and repeated dose tests

a) **OECD Test 407 – Repeated dose 28-day oral toxicity in rodents (OECD, 2008c)**
b) **OECD Test 408 – repeated dose 90-day study oral toxicity study in rodents (OECD, 2018e)**
c) **OECD Test 409 – Subchronic oral toxicity – non-rodent 90-day study (OECD, 1998b)**

The above three tests are essentially the same except for the duration of the dosing period and among others the number (and type) of animals per group. They consist of repeated oral dosing of the test substance either by gavage or in the diet

In the 28-day test, at least ten animals (five male and five female) are used per dose level. A satellite group of ten animals in the control and top dose may be used to look for reversibility, persistence or delayed occurrence of toxic effects, for at least 14 days post-treatment.

In the 90-day tests, 20 animals are used per dose level, with an additional optional satellite group of at least ten animals, as in the 28-day test.

In non-rodent 90-day tests (frequently dogs), eight animals are used per dose level, with an additional optional satellite group of eight.

Clinical observations are made regularly throughout the test. In the final week of exposure in the 28-day test, reactivity to stimuli is tested, but only in cases where the test is not conducted as a preliminary test to a subsequent 90-day study (where functional observations are performed not earlier than week 11). Body weight and food/water consumption are measured regularly (at least weekly). Haematology and clinical biochemistry (specifically for liver and kidney toxicities) are performed. In some cases (tests performed according to the most up-to-date version of OECD 408, for example) thyroid hormone measurements should be available, along with High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and serum total cholesterol measurements (all potentially indicative of liver/thyroid effects). Gross pathology and histopathology are performed on all major organs.

These studies are generally of lower relevance (see Section 5.2.6.5). Endpoints from studies performed in dogs may be less relevant for physiological reasons.

Chronic/carcinogenicity tests

a) OECD Test 451 (OECD, 2018f) – Carcinogenicity Studies

Carcinogenicity studies (OECD, 2018f) provide information on the possible hazards arising from repeated exposure for a period lasting up to the entire lifespan of the species used. This information will include potential carcinogenicity, including indicate target organ(s), and possible accumulation. As such, the dosing in the study is daily, for the majority of the lifespan of the animal (between 12 and 18 months in mice and 24 in rats; when the number of survivors in the lower dose groups or controls falls below 25%), beginning preferably before the age of 8 weeks. Because of the relatively high

incidence of spontaneous tumours in older animals, and the desire to link tumour formation to exposure as opposed to natural processes, the number of animals used in these tests is relatively high (at least 50 animals of each sex, with more in low dose groups if an aim of the study is to estimate carcinogenic potential at low doses).

The test substance is usually administered orally via diet or drinking water, or by gavage (dermal or inhalation exposure may be used under circumstances where this is warranted by the properties of the test substance and expected exposure regime). Body weight, food/water consumption and food efficiency are measured regularly (weekly for ~ 13 weeks and monthly thereafter). Clinical observations are made daily. Haematology and urinalysis may be performed. Gross pathology is performed at study termination, with histopathology of the organs in the table below, taken from OECD 451 (2018f).

all gross lesions	heart	pancreas	stomach (forestomach, glandular stomach)
adrenal gland	ileum	parathyroid gland	[teeth]
aorta	jejunum	peripheral nerve	testis
brain (including sections of cerebrum, cerebellum, and medulla/pons)	kidney	pituitary	thymus
caecum	lacrimal gland (exorbital)	prostate	thyroid
cervix	liver	rectum	[tongue]
coagulating gland	lung	salivary gland	trachea
colon	lymph nodes (both superficial and deep)	seminal vesicle	urinary bladder
duodenum	mammary gland (obligatory for females and, if visibly dissectable, from males)	skeletal muscle	uterus (including cervix)
epididymis	[upper respiratory tract, including nose, turbinates, and paranasal sinuses]	skin	[ureter]
eye (including retina)	oesophagus	spinal cord (at three levels: cervical, mid-thoracic, and lumbar)	[urethra]
[femur with joint]	[olfactory bulb]	spleen	vagina
gall bladder (for species other than rat)	ovary	[sternum],	section of bone marrow and/or a fresh bone marrow aspirate
Harderian gland			

b) OECD Test 452 – Chronic Toxicity Studies

Chronic toxicity studies (OECD, 2018g) provide information on the possible hazards arising from repeated exposure for a period lasting up to the entire lifespan of the species used, including chronic toxicity and target organs, as well as possible accumulation. The test substance is administered daily for 12 months (other durations may be chosen with appropriate justification) via the oral route (unless the properties of the substance or the expected exposure suggest another route of exposure to be more appropriate). At least 20 animals per sex per group are used (for rodents, a minimum of 4 per sex per group for non-rodents). Detailed clinical observations are made on all animals at the end of the first week and monthly thereafter. Depending on the results from the 28-day and/or 90-day studies, additional observations of reactivity to sensory stimuli and motor activity may be conducted before and at three-month intervals during the study. Haematological examination, clinical

biochemistry and urinalysis are carried out in at least 10 male and 10 female animals per group every 3 months (with smaller samples for non-rodents), unless no effects were seen in 90-day studies, in which case the investigation at 3 months can be skipped. At termination, all animals are subjected to detailed gross necropsy and histopathological examination of the same organs as in OECD 451 (2018f).

c) OECD Test 453– Combined Chronic Toxicity/Carcinogenicity Studies

The combined study design (OECD, 2018h) consists of two parallel phases, a chronic phase and a carcinogenicity phase. The chronic phase animals are dosed for a minimum of 12 months and the carcinogenicity phase animals are dosed for the major portion of their lives. 50 animals per sex per dose group are used for the carcinogenicity phase and 10 per sex per group are used for the chronic phase. Similar durations to OECD 451 and 452 are proscribed for the relevant phases of this study, with a satellite 'recovery' group possible for the chronic phase. Further, the observations and data recordings are according to OECD 451 and 452 (OECD, 2018f, 2018g).

Appendix D – Determining an extrapolated LD50 for mammals

Background and methodology

Poorly toxic substances may be tested using limit designs. However, risk assessment schemes may still require estimating surrogate LD_{50} values. In these circumstances, extrapolation factors are often applied to results from limit toxicity tests to derive LD_{50s}. EFSA (2009) provided guidance on how to extrapolate a surrogate LD_{50} for birds, but not for mammals.

CropLife Europe (formerly European Crop Protection Association, ECPA) recently collected and analysed a data set of mammalian acute toxicity studies to derive a suitable extrapolation factor.[66]

EFSA reviewed this data set and concluded that it could not be relied upon, because: (i) the choice of active substances and studies was not justified; (ii) the original reports were not always available, and (iii) the information included in the data set was insufficient for a comprehensive assessment. Therefore, EFSA conducted a parallel analysis, which followed the same methodology used for birds (appendix 5 of EFSA, 2008) and the principles of the CropLife Europe approach. This analysis used a 5-step process to derive a suitable extrapolation factor for mammals:

1) selection of candidate pesticide active substances for the analysis;
2) extraction of acute oral toxicity data from renewal assessment reports (RARs) and Draft assessment reports (DARs) available in the EFSA database;
3) validity and quality appraisal of the resulting data set;
4) statistical analysis of the dose-response using probit regression and the extraction of probit slopes;
5) comparison of the resulting distribution with the data set provided by CropLife Europe; the analysis of the distribution of probit slopes and the derivation of a single representative slope for the derivation of the mammalian extrapolation factor.

Step 1 – preliminary step: selection of active substances

A list of all approved pesticide active substances was downloaded from the database of the European Commission (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en - last access: March 2021), across which we retained pesticides classified as Acute Tox (1, 2, 3 and 4). This classification is generally based on evident lethality (e.g. an LD_{50}/LC_{50} value), according to the Classification, Labelling and Packaging (CLP) Regulation ((EC) No 1272/2008).[67] Although it does not specifically focus on the oral exposure route, we selected substances falling into this category to narrow down the list of approved substances to those more likely to cause acute oral lethality.

For each of these substances, we retrieved the mammalian acute LD_{50} (s) reported in the PPDB database (https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm, last access: March 2021). Where this was not possible, data were extracted from EFSA conclusions.

The resulting list of pesticide active substances covered an adequately wide distribution of hazard scenarios and pesticide categories (see Figure D.1). Therefore, the list of candidate pesticides was considered a sufficient basis for the data extraction and analysis.

[66] Data set, analyses and some of the raw data were submitted to EFSA in February 2021.
[67] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

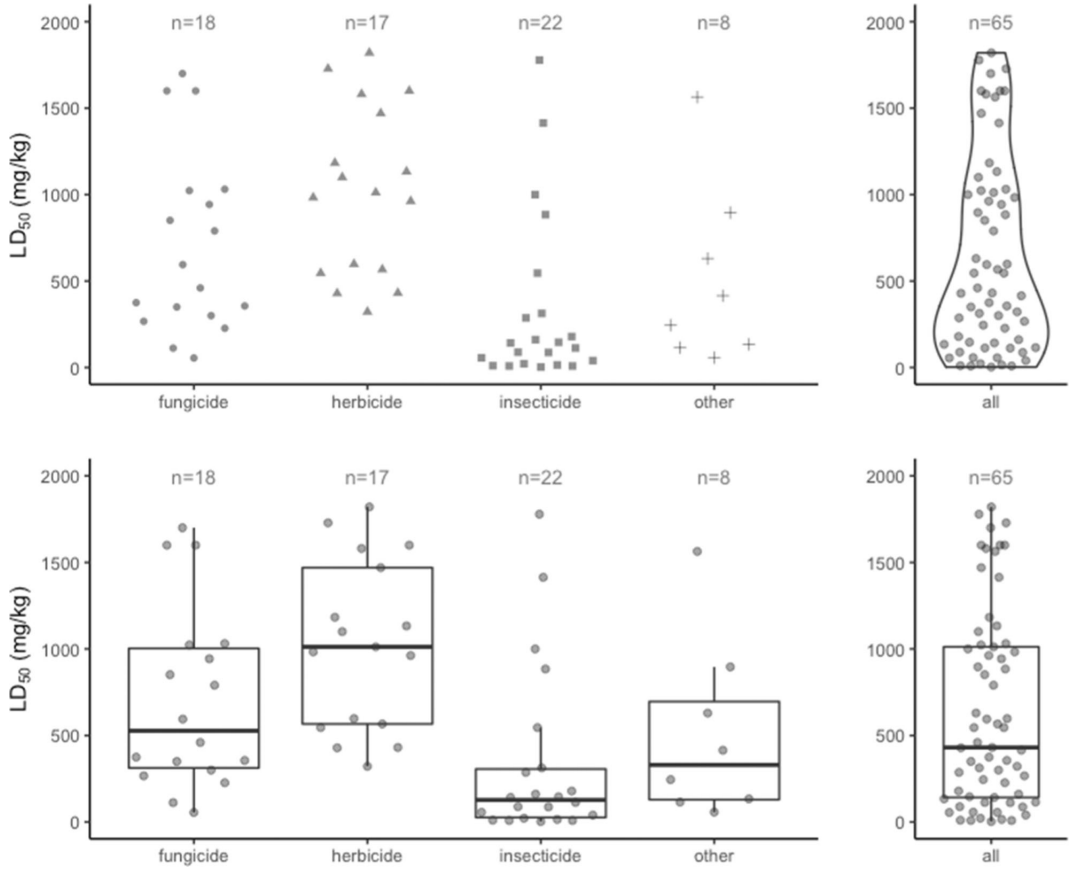


Figure D.1: Distribution of LD_{50} values across substances selected for the data extraction process (i.e. step 2 of this protocol). All non-exact (i.e. 'greater than') values, and all substances not classified as Acute tox 1, 2, 3, and 4 were excluded from this analysis

Steps 2 and 3: data extraction process and validity/quality appraisal

Data extraction:

The list of substances drafted in step 1 was used as basis for a targeted search of acute oral dose-response studies for the derivation of a representative slope for mammals. The PPDB database was not used further, as it did not report raw mortality data. Instead, we searched and re-assessed toxicity studies in the Renewal Assessment Reports and Draft Assessment Reports (RARs and DARs, respectively).

Specifically, their validity was checked against the most recent guidelines. If they were confirmed to be valid, mortality data, along with key information on the experimental design and study identification were extracted.

We retrieved data for a total of 478 dose responses, covering five mammal species (i.e. rat, mouse, rabbit, hamster and dog). However, following the quality appraisal detailed below, a number of studies were considered not sufficiently reliable to be used in the final assessment. Therefore, the final analysis may only be considered representative of mouse and rat (see Figure D.5).

Quality appraisal:

We categorised the dose response relations into the following 3 categories:

- Quality 1 (reliable): the study was considered fully suitable for the derivation of the probit slope. There was a minimum of two increasing intermediate mortality levels between lowest (< 10%) and highest (> 80%) effect.
- Quality 2 (reliable with restrictions): the study was considered potentially suitable for the derivation of a LD_{50}, although model fitting issues may be expected, which may undermine the reliability of the resulting probit slope. There was only one intermediate mortality level between lowest (< 20%) and highest (> 80%) effect.

- Quality 3 (unreliable): the study may be suitable for risk assessment purpose, but it cannot be used to reliably analyse the dose response. This category includes the following:
    - limit experimental designs or dose-response designs where the highest effect level is below 20%;
    - poor monotonicity: where (i) multiple (> 2) increasing concentrations resulted in the same mortality level or where (ii) there was no intermediate effect level between the lowest (< 20%) and highest (> 80%) mortality;
    - incomplete dose responses (i.e. where the highest effect was < 80% and the lowest effect is > 20%).

Following the quality appraisal, 286 dose responses were ranked as quality 3, and discarded. Additionally, 73 and 118 dose–responses were categorised as quality 1 and 2, respectively. One study was discarded because the sample size could not be retrieved.

Additionally, a subset of the data set submitted by CropLife Europe for which raw data was available was re-analysed using the same assessment criteria described above. Since CropLife Europe only provided raw data for a subset of acute oral toxicity studies included in their Excel database, their data could only be re-analysed when the corresponding original study report was provided or was available to EFSA. When this was not the case, the studies were discarded.

We assessed a total of 131 new dose responses from CropLife Europe, 42 of which were classified as quality 1. Additionally, 37 dose responses were classified as quality 2 and the remaining 51 were classified as quality 3.

Step 4 – dose response analysis

Each study categorised as quality 1 or 2 in the previous step was analysed using probit analysis. The resulting probit slopes were collected in a database, which also included the data retrieved during steps 1 and 2.

It should be noted that – due to the nature of data - several quality 2 studies presented goodness-of-fit issues, which often made it impossible to derive a confidence interval around the LD_{50}.

The distributions of the EFSA and CropLife Europe slopes were compared in step 5.

Step 5 – comparative analysis of EFSA and CropLife Europe data sets + derivation of a unique representative probit slope

The original aim was to analyse the data set using generalised linear models (GLMs). However, the assumption of heteroscedasticity and normality of residuals were not met, even after logarithmic transformation. Therefore, the non-parametric Kruskal–Wallis test was used for statistical comparison. No post hoc analysis was necessary.

Results

When quality 1 and 2 slopes were pooled together, the EFSA representative slope was higher than the one presented by CropLife Europe (Kruskal-Wallis, $p < 0.001$, Figure D.2). However, several dose responses classified as quality 2 presented fitting issues. Therefore, only quality 1 data were finally relied upon.

It should be noted that, when the EFSA data set and CropLife Europe data were pooled together, the slopes from quality 2 studies were significantly higher than the slope of quality 1 studies (Kruskal-Wallis, $p < 0.001$, Figure D.4).

When only quality 1 slopes were considered, the EFSA and CropLife Europe distributions did not differ from each other (Kruskal-Wallis, $p = 0.88$, Figure D.3).

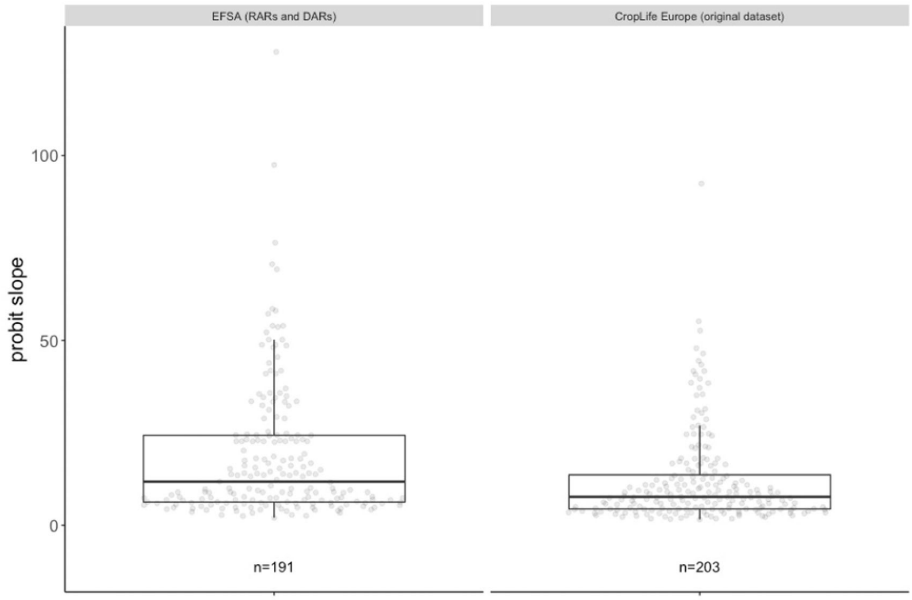


Figure D.2: Distribution of probit slopes of the data set (i) extracted by EFSA from RARs/DARs (left) and (ii) originally submitted by CropLife Europe (right). Quality 1 and 2 slopes were pooled

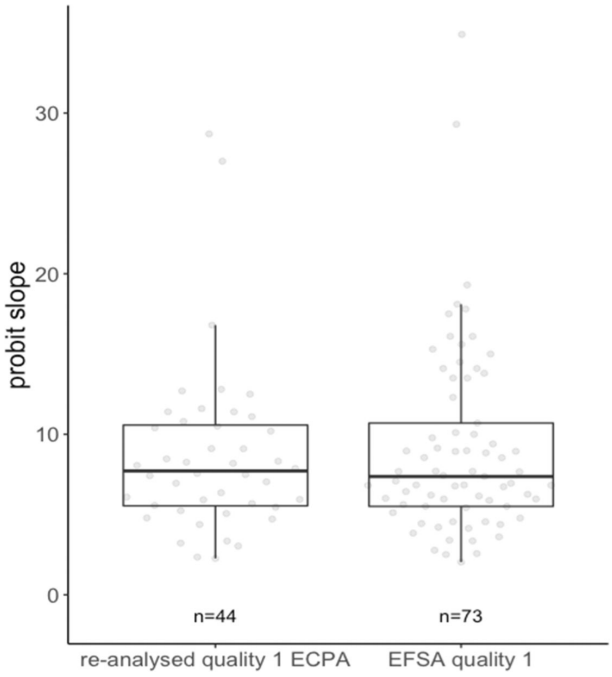


Figure D.3: Distribution of probit slopes of the data set (i) extracted by EFSA from RARs/DARs (left) and (ii) originally submitted by CropLife Europe (former ECPA, right). Quality 1 and 2 slopes were plotted separately

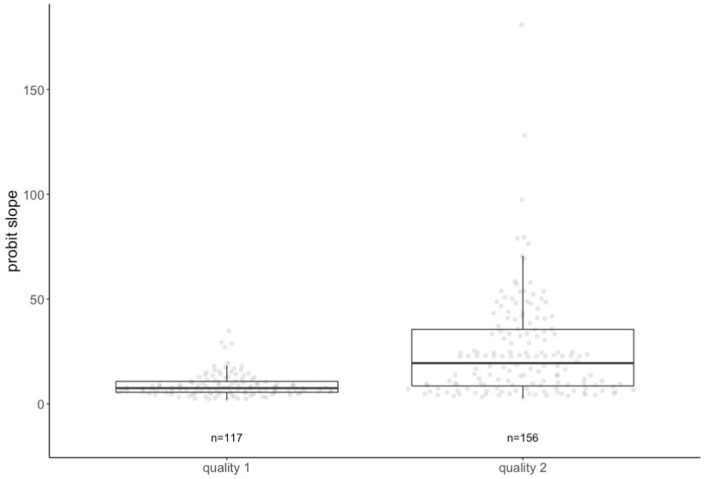


Figure D.4: Distribution of probit slopes of the pooled data set consisting of (i) extracted by EFSA from DARs/RARs + (ii) the subset of Croplife Europe data which could be re-analysed and validated by EFSA (same data as Figures D5 and D6). Quality 1 and 2 slopes were plotted separately.

Consistent with the analysis provided by CropLife Europe, we found no statistical support that the differences across types of test items (i.e. active substance vs. formulation), mammal species, modes of action (i.e. fungicide, herbicide, etc.) or sex were significant (Kruskal-Wallis, p (active substance vs. formulation) = 0.96, p (mammal species) = 0.82, p (mode of action) = 0.46, p (sex) = 0.9; Figure D.5).

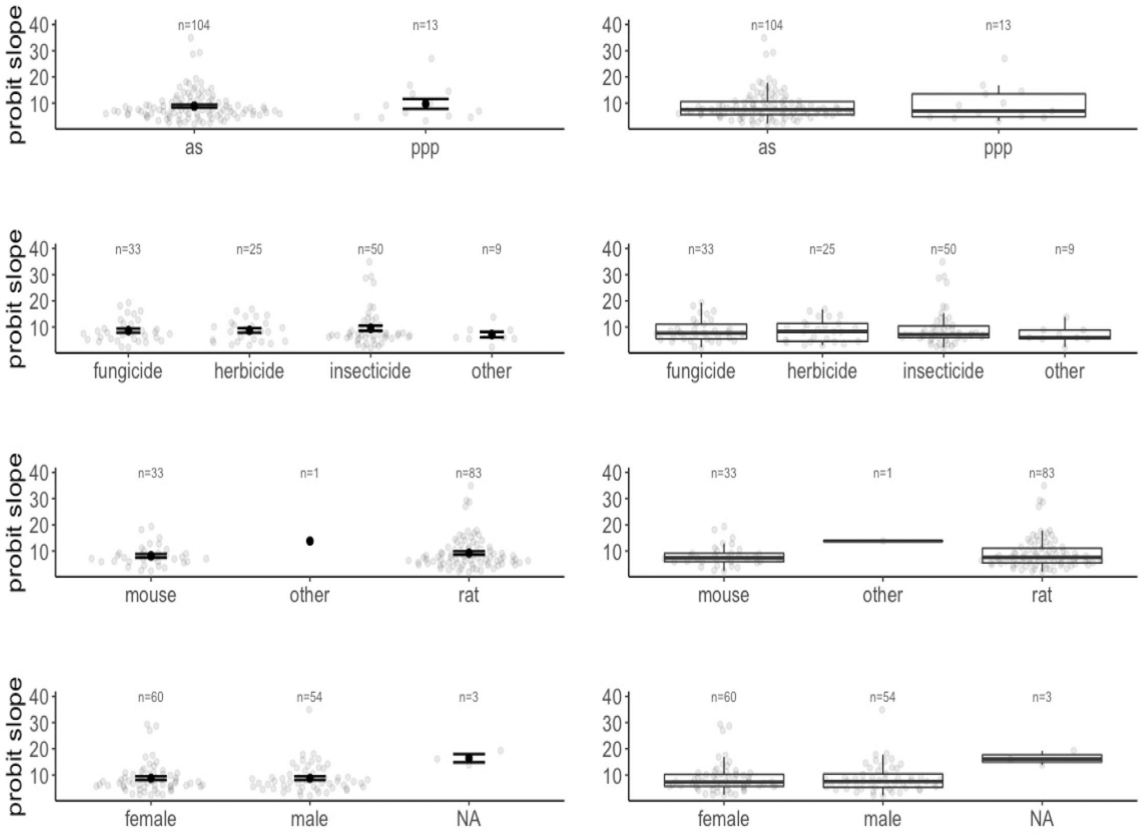


Figure D.5: Distribution of probit slopes of the pooled data set consisting of (i) extracted by EFSA from DARs/RARs + (ii) the subset of Croplife Europe data which could be re-analysed and validated by EFSA (same data as Figures D.4 and D.6). Data pooled by pesticide type; mode of action; mammal species and sex (top to bottom)

Conclusions

This re-analysis addresses some of the uncertainties previously identified by the WG in the CropLife Europe proposal for mammalian acute endpoint extrapolation:

- It uses a repeatable methodology to justify the selection of toxicity data.
- It implements a transparent validity/reliability assessment.

It was considered inappropriate to pool the EFSA data set with the originally submitted data set by CropLife Europe for the following reasons:

i) The selection criteria behind the choice of active substances and studies were not specified in the CropLife Europe data set;
ii) It is unclear if and how the studies were evaluated for their reliability and validity in the CropLife Europe data set;
iii) The original study reports were only provided for a subset of the analysed data from CropLife Europe. Where raw data were not submitted, their validity and reliability could not be confirmed. However, the subset of data submitted by CropLife Europe and re-analysed by EFSA could be validated by EFSA, and, therefore, they were pooled together with the EFSA data for the derivation of a representative slope;
iv) Important information such as the identity of the active substance remains unknown for the subset of CropLife Europe data for which no raw data was provided. Consequently, double counting cannot be excluded if the full CropLife Europe data were to be pooled with the EFSA data set.

The slope derived in quality 2 studies was higher (hence, worst-case) comparatively to quality 1 studies.

However, a high proportion of quality 2 dose responses presented fitting issues. Therefore, it was considered inappropriate to include them in the final analysis. Quality 1 slopes were not different across data sets (i.e. EFSA and validated CropLife Europe).

Overall, the WG:

- Retained only quality 1 slopes.
- Pooled the EFSA data set with the subset of data submitted by CropLife Europe which could be validated.
- Did not exclude any data from this pooled data set (Figure D.6), although, seemingly, about 4 data points may be statistically identified as outliers.

The following distribution (Figure D.6) and descriptive statistics (Table D.1) were thus derived:

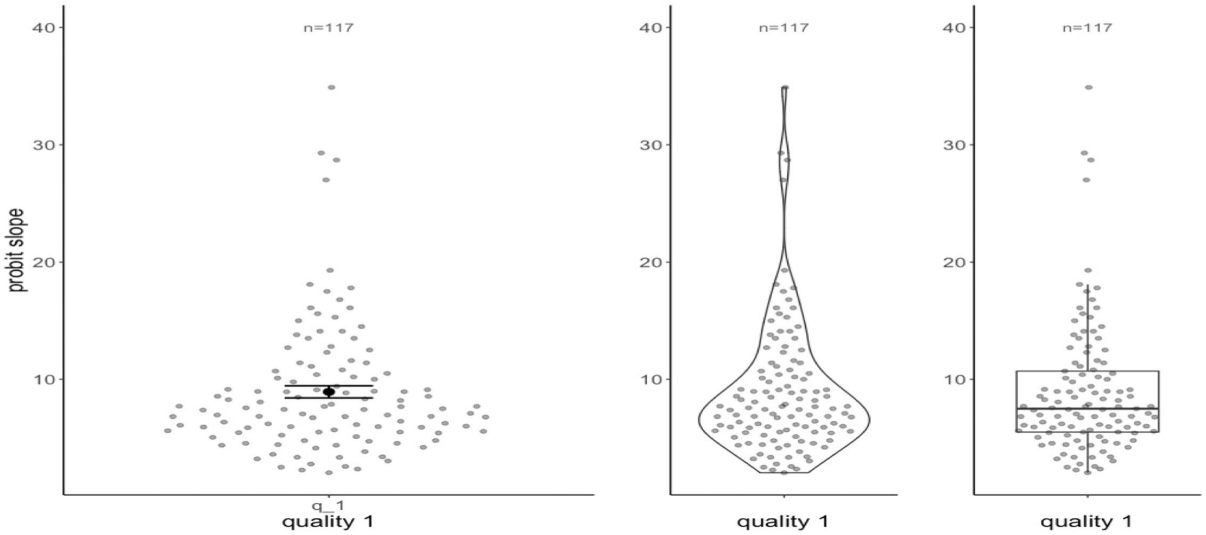


Figure D.6: Distribution of probit slopes of the pooled data set consisting of (i) extracted by EFSA from DARs/RARs + (ii) the subset of Croplife data which could be re-analysed and validated by EFSA (same data as Figures D.4 and D.5). Data were plotted in a single distribution. See also Table 1 for descriptive statistics and Table D.2 for extrapolation factors

Table D.1: The descriptive statistics for the distribution of probit slopes shown in Figure D.6

min	max	mean	percentile						
			5%	10%	25%	50%	75%	90%	95%
2.04	34.9	8.93	2.99	3.75	5.51	7.49	10.7	15.4	17.9

Consistent with the assumption used for birds in EFSA (2009), the WG derived the extrapolation factors assuming a 50% confidence interval. Calculations were repeated for the mean, median and 95% percentile slopes from the distribution shown in Figure D.6. It should be noted that for birds the mean slope has been used to determine the extrapolation factors. Thus, the same is recommended for mammals, as the data does not indicate any reason why a more conservative value should be used that was used for birds.

Table D.2: The Extrapolation factors at increasing sample sizes, assuming 0 or 1 deaths and mean, median and 90th percentile probit slopes, as calculated in Table D.1. Consistent with EFSA (2009), a 50% confidence limit around the mortality level was assumed

Sample size	Mean slope	Median slope	90th perc. slope	Mean slope	Median slope	90th perc. slope
	Mortality = 0 (50% Conf. interval)			Mortality = 1 (50% Conf. interval)		
3	1.24	1.29	1.13	1.00	1.00	1.00
5	1.34	1.42	1.18	1.13	1.16	1.08
6	1.37	1.46	1.20	1.18	1.21	1.10
10	1.47	1.59	1.25	1.29	1.35	1.16

Appendix E – Crop group selection for foliar spray crops

The crop groups used in this guidance document are primarily those from the EPPO harmonised classification and coding of the uses of plant protection products (https://gd.eppo.int/PPPUse/). However, some adjustment was needed to account for the specific needs of the risk assessments for birds and mammals (e.g. division of winter sown and spring sown crops and splitting bulb ornamentals from other types). The crop groups used for the guidance are given in Table E.1.

A note on the use to ornamentals:

Ornamental plants are a diverse group of plants, grown in a variety of ways, which can vary from small herbaceous plants to large ornamentals trees. For this reason, the selection of appropriate parameters for environmental risk assessment has often been problematic. For the purposes of this guidance document, risk assessment scenarios have been developed for birds and mammals using the crop (plant) codes in the EPPO harmonised classification and coding of the uses of plant protection products. Furthermore, the EPPO Global Data Base also provides definitions for the treated object which will inform the risk assessor whether the plant itself is treated or whether the ground below the plant will be treated. Nevertheless, it is likely that an applicant may wish to have an authorisation for a single, or a sub-set, of ornamental species. In this case, the risk assessor should consider what is the most appropriate surrogate crop/plant to use for the risk assessment taking into consideration the plant structure, application methodology and other relevant information (e.g. daffodils and other bulb ornamentals may be best considered as equivalent to onions). However, the risk assessor should take particular care with the crop interception for ornamentals, as this can vary depending on the plant type, plant density as well as the growth stage. Please note that the inclusion of the group 'Ornamental plants (unspecified)' is because many proposed GAPs do not indicate the type of ornamental plant that the PPP will be used. In these cases, the risk assessment must encompass all types of ornamental plant and therefore worst-case parameters need to be selected.

Table E.1: Crop groups and their definition

Name for GD	EPPO name	Further definition
All field crops BBCH 0–9	–	This crop group covers all annual and biannual field crops before emergence of the crop. It is assumed that weeds may be present as some farming practices do not remove weeds (e.g. low tillage) or weeds may emerge faster than the crop. This also covers emerging seedlings.
Allium vegetable crops	allium vegetable crops (3ALLC)	Onions, garlic, shallots, leeks, chive, etc.
Amenity grassland	amenity grassland (3AMGC)	Any ornamental, recreation or sport areas composed of mown grass.
Artichokes and cardoons	*Cynara scolymus* (CYUSC) *Cynara cardunculus* (CYUCA)	Artichokes, cardoons
Asparagus	*Asparagus officinalis* (ASPOF)	Asparagus
Banana	*Musa* × *paradisiaca* (MUBPA)	Banana
Bare fallow	bare fallow (treatment of) (3BARFO)	Land, whether worked (e.g. ploughed, tilled) or not, that is not seeded or planted for one or more growing seasons. The inactive period is usually less than 5 years.
Biomass trees	biomass trees (3BMTC)	Populus sp. Salix sp. (SAXSS)
Broadleaf forest tree	broadleaf forest trees (3FOBC)	*Acer* spp., *Alnus* spp., *Betula* spp., *Castanea* spp., *Crataegus* spp., *Cornus* spp., *Fraxinus* spp., *Juglans* spp., *Populus* spp., *Prunus* spp., *Salix* spp., *Sorbus* spp., *Quercus* spp., *Tilia* spp., *Ulmus* spp., *Carpinus betulus, Corylus avellana, Fagus sylvatica, Malus sylvestris* and *Pyrus pyraster*
Buckwheat	*Fagopyrum esculentum* (FAGES)	Buckwheat
Bulb-like ornamental herbaceous plants	–	Bulbous plants (e.g. *Crocus* spp., *Lilium* spp. and *Tulipa* spp.)

Name for GD	EPPO name	Further definition
Citrus fruit crops	citrus fruit crops (3CITC)	Oranges, manderines, clementines, lemons, etc.
Coniferous forest trees	coniferous forest trees (3FOCC)	*Abies* spp., *Cupressus* spp., *Picea* spp., *Pinus* spp., *Cedrus* spp. *and Larix* spp., *Juniperus communis, Pseudotsuga menziesii* and *Taxus baccataf*
Cotton	–	–
Fig	*Ficus carica* (FIUCA)	Fig
Fruiting cucurbitaceous vegetable crops	fruiting cucurbitaceous vegetable crops (3FCVC)	Zucchini, courgette, watermelon, cantaloupe, cucumber, gherkin, pumpkin, marrow, squash
Fruiting solanaceous vegetable crops	fruiting solanaceous vegetable crops (3FSVC)	Bell pepper, chilli, paprika, red pepper, sweet pepper, *Physalis* sp., tomato, aubergine, eggplant
Grass crops	grass crops (3GRAC)	Ryegrasses (3RYGC) and fescue grasses (3FESC)
Grassland	grassland (3GRLC)	Both rotational and permanent grassland
Herb crops	herb crops (3HERC)	Chervil, parsley (and parsley root), etc.
Hops	*Humulus lupulus* (HUMLU)	Hops
Jerusalem artichoke	*Helianthus tuberosus* (HELTU)	Jerusalem artichoke
Kiwifruit	Actinidia deliciosa (ATIDE)	Kiwifruit
Leafy vegetable crops (excluding brassica)	leafy vegetable crops (excluding brassica) (3LEAC)	Lambs lettuce, lettuce, escarole, spinach, chicory, beetroot, etc.
Legume crops, except soybean	legume crops (3LEGC)	Alfalfa, clover, lupin
Legume vegetable crops	legume vegetable crops (3LEVC)	Field (faba) beans, Peas, lentils, French beans
Linseed (= flax)	*Linum usitatissimum* (LIUUT)	Linseed (= flax)
Maize and millet crop	maize and millet crops (3MAMC)	Millet, maize, sorghum
Nut crops	nut crops (3NUTC)	Chesnut, walnut, pine nut, pistachio
Olives	*Olea europaea* (OLVEU)	Olives
Ornamental broad-leaved trees, shrubs, and climbing plants	ornamental broad-leaved trees, shrubs, and climbing plants(3ORBC)	This group includes ornamental broad-leaved trees of e.g. *Acacia* spp., *Acer* spp., *Betula* spp., *Erica* spp., *Fagus* spp., *Ilex* spp., *Hibiscus* spp., *Hydrangea* spp., *Populus* spp., *Quercus* spp., *Tilia* spp., ornamental shrubs of e.g. *Buxus* spp., *Crataegus* spp., *Ligustrum* spp., *Rosa* spp., *Viburnum* spp., *Rhododendron* spp., and woody climbing plants such as *Bougainvillea spectabilis, Hedera helix, Jasminum nudiflorum*.
Ornamental cactuses and succulents	ornamental cactuses and succulents(3ORSC)	This group includes stem succulents (most of which are cactuses) as well as root, caudiciform and pachycaul succulents e.g. *Aeonium* spp., *Kalanchoe* spp., *Schlumbergera* spp. and *Sempervivum* spp.
Ornamental conifers	ornamental conifers(3ORCC)	This group includes e.g. *Abies* spp., *Cedrus* spp., *Chamaecyparis* spp., *Cupressaceae* spp., *Ephedra* spp., *Juniperus* spp., *Larix* spp., *Picea* spp., *Pinus* spp., *Pseudotsuga* spp., *Thuja* spp. and *Tsuga* spp. Species such as *Araucaria araucana* and *Taxus baccata* are also relevant.
Ornamental herbaceous plants	ornamental herbaceous plants (3ORHC)	Ornamental plants which have no persistent woody stem above ground. This group includes annuals, biennials and perennials herbaceous ornamental plants such as green herbaceous plants (e.g. Dieffenbachia spp. and *Salvia* spp.), flowering plants (e.g. *Begonia* spp., *Chrysanthemum* spp., *Cyclamen* spp., *Pelargonium* spp., *Veronica* spp.), bulbous plants (e.g. *Crocus* spp., *Lilium* spp. and *Tulipa* spp.), ferns and ornamental grasses, cereals and vegetables.

Name for GD	EPPO name	Further definition
Ornamental herbaceous plants excluding bulbs		Ornamental plants which have no persistent woody stem above ground. This group includes annuals, biennials and perennials herbaceous ornamental plants such as green herbaceous plants (e.g. *Dieffenbachia* spp. and *Salvia* spp.), flowering plants (e.g. *Begonia* spp., *Chrysanthemum* spp., *Cyclamen* spp., *Pelargonium* spp., *Veronica* spp.), ferns and ornamental grasses, cereals and vegetables.
Ornamental plants (unspecified)[(a)]	ornamental plants (terrestrial) (3ORTC)	All types of plant structures
ornamental woody monocotyledonous plants	ornamental woody monocotyledonous plants (3ORMC)	This group includes trees and shrubs such as palms and bamboos. *Cycads* spp., *Ginkgo* spp., and *Gnetum* spp. are also included.
Ornamental woody plants	ornamental woody plants (3ORWC)	ornamental broad-leaved trees, shrubs, and climbing plants ornamental conifers ornamental woody monocotyledonous plants
Pineapple	*Ananas comosus* (ANHCO)	Pineapple
Pome fruit crops	pome fruit crops (3PMFC)	Pear, apple
Poppy	*Papaver somniferum* (PAPSO)	Poppy
Potato	*Solanum tuberosum* (SOLTU)	Potato
Quinoa	*Chenopodium quinoa* (CHEQU)	Quinoa
Rhubarb	*Rheum rhabarbarum* (RHERH)	rhubarb
Root and stem vegetables	umbelliferous vegetable crops (3UMBC)	Carrot, celeriac, parsnips, celery, fennel
Salsify	*Scorzonera hispanica* (SCVHI)	Salsify
Sesame	Sesame	Sesame
Small fruit crop	small fruit crops (3SMFC)	Cranberry, blueberry, bilberry (whortleberry), lingonberry (cowberry), huckleberry, Raspberries, blackberries, dewberries, blackcurrant, redcurrant, white currant, gooseberry
Soybean	Glycine max (GLXMA)	Soybean
Spring sown brassica arable crop	brassica arable crops (3BRAC)	Oilseed rape, mustard, turnip rape
Spring sown cereal crop	cereal crops (3CERC)	Wheat, barley, oats, rye, triticale, etc.
Stone fruit crop	stone fruit crops (3STFC)	Peach, Plum, Almond, cherry, apricot
Strawberry	*Fragaria x ananassa* (FRAAN)	Strawberry
Stubbles	stubble (cereal)(YSTEG) stubble (maize)(YSTZE)	All types of crop stubbles including fields before direct sowing with no tillage
Sugar beet	beet crops (3BEEC)	Sugar beet
Sunflower	*Helianthus annuus* (HELAN)	Sunflower
Sweet potatoes	*Ipomoea batatas* (IPOBA)	Sweet potatoes
Tobacco	*Nicotiana tabacum* (NIOTA)	Tobacco
Vegetable brassica crops	vegetable brassica crops (3VBRC)	Broccoli, cauliflower, Brussels sprouts, cabbage, Chinese cabbage, kale, cress, horseradish, swedes, turnips, kohlrabi, radish
Vines	Vitis vinifera (VITVI)	Grape
Winter sown brassica arable crop	brassica arable crops (3BRAC)	Oilseed rape, mustard, turnip rape
Winter sown cereal crop	cereal crops (3CERC)	Wheat, barley, oats, rye, triticale, etc.

(a): Please note that the inclusion of the group 'Ornamental plants (unspecified)' is because many proposed GAPs do not indicate the type of ornamental plant that the PPP will be used. In these cases, the risk assessment must encompass all types of ornamental plant and therefore worst-case parameters need to be selected.

Appendix F – Generic model species parameter definition

The proper definition of Tier 1 generic model species (GMS) and their parameters was considered essential to ensure that the risk assessment methodology recommended in this guidance document is sufficiently protective and encompasses the wide range of environmental conditions in EU Member States. The WG used the generic focal species and their parameters as defined in EFSA (2009) as the basis for the analysis and refinement performed for this guidance document.

As discussed previously, Tier 1 GMS are not real species but model species with assumed characteristics meaning that the risk assessment for this model is protective of all other species exposed. GMS are representative of different feeding guilds (herbivore, omnivore, insectivore, granivore or frugivore) but it is important to note that they are not identical (e.g. the animal referred to as a frugivore is actually omnivorous but at certain times of the year takes a high proportion of fruit). The defining characteristics – or parameters – of a GMS are:

- Presence in the field and field margins. This is based on the crop (type, structure and growth stage), food availability and the ecology of the species represented by the GMS.
- Body weight and relative food intake rate. A smaller body weight results in relatively higher energy requirements hence higher food intake.
- The different proportions of dietary components taken within the field/terrestrial area of interest. In the context of this guidance document, for direct dietary exposure, this is split to the following categories: ground-dwelling arthropods (those on the soil surface), foliar-dwelling arthropods (those on plant surfaces), seeds on the soil surface (both weeds and crops), seed on plants (crops and weeds), monocotyledon foliage (both weeds and crop if relevant), dicotyledon (both weeds and crop if relevant), treated seeds and seedlings germinating from treated seed. For dietary exposure via secondary poisoning these are fish, earthworms and sediment-dwelling invertebrates.

The WG refined the appropriate GMS parameters according to a number of considerations:

i) In the first instance it was considered whether any feeding guild (herbivore, omnivore, insectivore, granivore or frugivore) was clearly not relevant for the various scenarios. If there was no clear evidence to indicate that a feeding guild was not relevant (e.g. frugivorous species are only considered relevant when fruit is available), the feeding guild was considered to be potentially relevant.

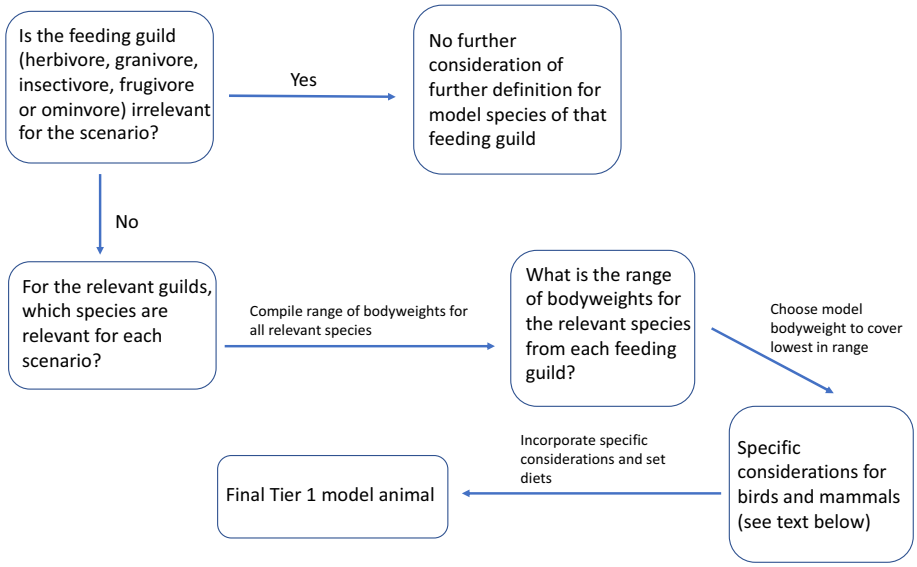
ii) Next, data was gathered considering the different species for each feeding guild that could be expected to be present in the specific scenarios. Data included the information from the procurement (Lahr et al., 2018), from well-known ecological surveys and summaries (e.g. Buxton et al., 1998, Gurney et al., 1998, IUCN, online, red list species summaries), and from literature beyond the scope of the data in Lahr et al. (2018) (for example, for flower bud-feeding species). The WG considered that the data was of acceptable quality for consideration of the species when it was from the procurement, (including both specific focal species studies submitted in the context of dossiers and high-quality literature data), and from the well-known ecological surveys/summaries. The few data not included under these was evaluated for quality by the WG members. Pending on the specificities of the GMS, these data were used to consider the presence of the species represented by the GMS. These are described in the following sections of this appendix. Such assessment encompassed the potential species (with low body weight) covered by the GMS as far as practicable.

iii) For the next step, the WG considered the range of body weights of the species identified as potentially relevant as model species for each feeding guild. A body weight was then chosen for the model species from each relevant feeding guild which covered the body weights of the species identified as relevant. A representative body weight covering the lower range for all the identified relevant species was chosen. Avian body weights were taken from Dunning (2007). The lowest value of the sexes and subspecies were taken. Where a range was indicated the mid-value was taken. For mammals a single source for body weights was not available and various sources were used. The same approach for birds with regard to the range of values was followed.

iv) The assumption regarding the difference proportions of food items in the diet of the GMS in the field/field margin was then considered. This was done in different ways as described below for each of the relevant GMS in the following sections. It should be noted that the assumption for the proportions of the mixed diet is based on the values which the WG considered to be a reasonable worst case. Nevertheless, it must be acknowledged that, obviously, not all birds and mammals feeding in the fields with a certain crop will take the same proportion of food items as assumed in the Tier 1. In some cases, an animal will take proportion of food items which result in a higher level of exposure whilst in other cases lower level of exposure.

v) It was noted that in most cases the in-field assessment for birds and mammals is considered sufficiently protective of the population outside of the treated area (Section 6.2.1), but some exceptions exist, particularly for small mammals, considering the species that inhabit in the immediate area. This will be considered further in the proceeding sections, and also when discussing the performing and evaluation of Tier 3 focal species studies (Section 6.5.2).

The following flow-chart shows the process the WG used to consider the GMS parameters for each scenario.



Specific considerations for birds

Besides the general considerations above, concerning feeding guild, general habitat utilisation, and body weight, special consideration was given to further differentiation within feeding guilds.

Omnivorous birds

The WG considered whether the assumptions of the omnivorous diet for birds could be considered to be a realistic worst-case. It was acknowledged that the proportions of the diet resulting in the worst-case exposure was highly dependent upon the availability of food items and the level of residue in each matrix. Both of these factors will vary throughout the year. As a generic consideration, a higher proportion of herbivorous diet was considered worst-case since a higher level of consumption would be required due to the relatively lower energy available in herbivorous food items. It was determined that, while the default diet as proposed for model omnivorous birds in EFSA (2009) was a reasonable worst-case for spring and summer, this was not the case for those scenarios which represent the exposure of the GMS in autumn and winter (or when the application timing is not specific; dietary studies in Lahr et al. (2018)). In those cases, a higher proportion (i.e. 50%) of crop foliage or weeds (depending on the crop and the growth stage) was considered.

Therefore, in summary the WG agreed the following diet assumptions for a 27 g GMS.

Table F.1: Summary of Tier 1 GMS diet assumptions for small omnivorous birds

Crop group	BBCH	Diet
All field crops	0–10	50% seeds, 25% ground arthropods, 25% foliage
Allium vegetable crops	All	25% foliage, 25% seeds, 50% arthropods
Spring sown cereal crop	All	
Fruiting cucurbitaceous vegetable crops	All	
Fruiting solanaceous vegetable crops	All	
Leafy vegetable crops (excluding brassica)	All	
Maize and millet crop	All	
Spring sown brassica arable crop	All	
Potato	All	
Legume vegetable crops	All	
Root vegetables	All	
Strawberry	All	
Sugar beet	All	
Sunflower	All	
Winter sown cereal crop	> 30	
Winter sown brassica arable crop	> 20	
Hops	All	
Cotton	All	25% foliage, 50% seeds 25% arthropods
Small fruit crop	All	50% foliage, 25% seeds, 25% arthropods
Legume crops	All	
Grassland	All	
Amenity grassland	All	
Ryegrasses	All	
Citrus fruit crops	All	
Pome fruit crops	All	
Stone fruit crop	All	
Nut crops	All	
Olives	All	
Vines	All	
Bare fallow	NA	
Stubbles	NA	50% foliage, 50% seeds
Winter sown cereal crop	09–29	
Winter sown brassica arable crop	09–19	

Regarding the type of the foliage consumed in each case, the WG agreed the following practical solution based on reasonable worst-case assumptions:

- For monocot crops up to BBCH 30–100% monocot foliage should be considered.
- For monocot crops above BBCH 30–50/50% monocot/dicot foliage should be considered.
- For dicot crops – all growth stages, 50/50% monocot/dicot foliage should be considered.
- For legume crops, leafy vegetables and grassland – all growth stages, 100% crop foliage should be considered.
- For bare fallow, orchard, bush and cane fruit, vines, hops and crop stubbles – 80/20% monocot/dicot weeds should be considered.
- For all crops (BBCH 00–10) – 50/50% monocot/dicot foliage should be considered.

Regarding the location of the arthropods consumed in each case, the WG agreed the following practical solution based on reasonable worst-case assumptions:

- For all crops (BBCH 0–10) and all ground directed applications of pesticides – 100% ground arthropods should be considered.
- For all other crops/growth stages-75/25% ground/foliar arthropods should be considered.

A special consideration was given to omnivorous birds likely to forage in forestry plantations (biomass trees, broadleaf forest tree and coniferous forest trees). The WG decided that an omnivorous bird GMS is needed; an 11 g bird with a diet of 50% seeds, 25% dicotyledon plants and 25% monocotyledon plants. It is assumed that the GMS will mostly feed from the ground.

Insectivorous birds

In the analysis, insectivorous birds were divided in ground, foliar or aerial feeders, or some combination of the three, were considered in detail.

In the first instance, the growth stage of the crop was considered in order to make a reasonable assumption about the likelihood of birds taking a high proportion of foliar insects. To further delineate this information, the presence of arthropod pests in the crop resulting in the need for insecticide treatment at particular growth stages was used as a surrogate where necessary. Data from the UK (Fera Science Ltd; Pesticide Usage Surveys) was considered in this case, as no other such data was immediately available. In addition, since as the data was (and the scenarios are) linked to the growth stage of the crop, it was assumed that although the time of year and the type of arthropod may vary throughout Europe, the mere presence of any foliar/flying arthropods would be relatively stable. This data was used together with data from Lahr et al. (2018) regarding the presence of focal species with a foliar foraging stratum in that crop/growth stage. Data for focal species on some less common crops (i.e. cotton, hop, sunflower) were lacking. For those cases, an assumption regarding the presence of birds with foliar foraging strata was done based on the crop structure. When both sources of information indicated that foliar arthropod consumption is highly probable (because of a high density of foliar arthropods and the presence of a bird species with a foliar foraging strata), the WG considered that a 100% foliar insectivore species is relevant (Table F.2); for all the other scenarios (except for all crops BBCH 00–09 and ground directed applications) a mixture of 50% ground and 50% foliar arthropods was considered. Whenever a ground directed application of pesticides is used, and in all crops BBCH 00–09, 100% ground arthropods should be considered.

Table F.2: Summary of crops and growth stages triggering an insectivorous bird GMS and the respective diet assumptions

Crop group	Spray direction	Growth stage BBCH	Body weight (g)	Diet
All field crops	Crop	0–9	17	100% ground arthropods
Spring sown cereal crop Winter sown cereal crop	Crop	< 40	10	50% ground and 50% foliar arthropods
	Crop	≥ 40	6	100% foliar arthropods
Spring sown brassica arable crop Winter sown brassica arable crop	Crop	< 40	15	50% ground and 50% foliar arthropods
	Crop	≥ 40	10	100% foliar arthropods
Hops	Crop	All	17	100% foliar arthropods
	Ground	All	17	100% ground arthropods
Vines Small fruit crop Citrus fruit crops Pome fruit crops Stone fruit crop Nut crops Olives	Crop	All	8	100% foliar arthropods
	Ground*	All	17	100% ground arthropods
Biomass trees Coniferous forest trees Broadleaf forest tree	Crop	All	5.5	100% foliar arthropods
	Ground*	All	8	100% ground arthropods
Sunflower	Crop	< 30	15	50% ground and 50% foliar arthropods
	Crop	≥ 30	9	100% foliar arthropods
Cotton	Crop	< 40	15	50% ground and 50% foliar arthropods
	Crop	≥ 50[£]	10	100% foliar

Crop group	Spray direction	Growth stage BBCH	Body weight (g)	Diet
Maize and millet crop	Crop	< 30	15	50% ground and 50% foliar arthropods
	Crop	≥ 30	9	100% foliar
Allium vegetable crops Amenity grassland Bare fallow Buckwheat Fruiting cucurbitaceous vegetable crops Fruiting solanaceous vegetable crops Grassland Herb crops Leafy vegetable crops (excluding brassica) Legume crops Legume vegetable crops Linseed (= flax) Poppy Potato Root vegetables Ryegrasses Strawberry Stubbles Sugar beet Vegetable brassica crops	Crop	All	15	50% ground and 50% foliar arthropods
Ornamental plants (unspecified)	Crop	All	5.5	100% foliar arthropods
	Ground	All	8	100% ground arthropods
Ornamental herbaceous plants Non-bulb ornamental herbaceous plants	Crop	< 40	10	50% ground and 50% foliar arthropods
	Crop	≥ 40	6	100% foliar arthropods
Ornamental woody plants Ornamental woody monocotyledonous plants Ornamental conifers	Crop	All	5.5	100% foliar arthropods
Ornamental broad-leaved trees, shrubs, and climbing plants	Crop	All	8	100% foliar arthropods
Ornamental cactuses and succulents	Crop	All	15	50% ground and 50% foliar arthropods
Ornamental woody plants Ornamental woody monocotyledonous plants Ornamental conifers Ornamental broad-leaved trees, shrubs, and climbing plants Ornamental cactuses and succulents	Ground	All	17	100% ground arthropods

*: In the case of applications to below crops such as in orchards, ornamentals trees, etc.
£: There is no principal growth stage 4 (BBCH 40–49) for cotton.

It was further noted by the WG that many species which are normally omnivorous may show a fully insectivorous diet during nesting/chick feeding. This should be considered for any focal species studies performed in order to refine the risk assessment and is discussed further in Section 6.5.2.

A special consideration was given to insectivorous birds likely to forage in forestry plantations (biomass trees, broadleaf forest tree and coniferous forest trees). The WG decided that two insectivorous birds GMS are needed. The first is a 5.5 g bird with a diet of 100% foliar arthropods. It is assumed that the GMS will feed from the tree. The second is an 8 g bird with a diet of 100% ground arthropods. It is assumed that the GMS will feed from the ground.

Herbivorous birds

In addition to truly herbivorous birds, the WG noted that there are several omnivorous birds which are heavily reliant on plant material in some circumstances or at certain times of the year. Therefore, within the herbivorous bird feeding guild it was decided that there is the need to have multiple Tier 1 GMS. These are a large herbivorous bird, a medium omnivorous bird and a bud-eating bird. The analysis and selection of the Tier 1 parameters and the crop groups for which these GMS are needed are described in the following paragraphs.

Large herbivorous birds

First, it was recognised that the herbivorous bird species in Europe are also mostly wetland species, and therefore the presence of water was likely to have an impact on the presence of these species. Furthermore, it was noted that many of the species covered by this GMS are migratory and their presence can be localised. Nevertheless, species are present in all regulatory zones and all year. However, it was recognised that it may be possible to eliminate the need for consideration of the large herbivorous bird in Member State specific risk assessments, but it would be unlikely at a zonal or EU level assessment. However, to do so would need an in-depth analysis of the presence of large herbivorous birds in the Member State for which the authorisation is sought. It was further acknowledged, that owing to the characteristics described above, care is needed when interpreting their presence based on the standard focal species studies performed to data (Lahr et al., 2018). To check the crops for which a large herbivorous bird GMS should be included firstly information from Lahr et al. (2018) was extracted. Secondly a search of the literature was performed looking for reports of agricultural conflict and grazing birds in EU. To check the body weight assumption for the Tier 1 GMS, a consideration was given to the occurrence of grazing birds using distribution maps (e.g. European Bird Portal (online) and the distribution maps from data zone on the Bird Life international factsheets (2004)). The lowest body weight of the species covered by the GMS, including those who migrate, was selected. It was noted that the food intake rate calculation uses parameters which are not specific for the period before or during hibernation where more intense feeding is needed. However, taking the lowest body weight for the GMS was considered sufficiently protective for the Tier 1 exposure assessment. Therefore, on the basis of the analysis, the WG agreed what reported in Table F.3.

Table F.3: Summary of Tier 1 GMS for large herbivorous birds

Crop group	BBCH range	Body weight (kg)	Diet assumptions
Amenity grassland	10–30	0.7	100% crop leaves (monocotyledon)
Grassland	10–30	0.7	100% crop leaves (monocotyledon)
Ryegrasses	10–30	0.7	100% crop leaves (monocotyledon)
Winter sown cereals	10–30	1	100% crop leaves (monocotyledon)
Spring sown cereals	10–30	1	100% crop leaves (monocotyledon)
Winter sown brassica arable crop	10–30	1	100% crop leaves (dicotyledon)
Spring sown brassica arable crop	10–30	1	100% crop leaves (dicotyledon)
Legume vegetable crops	10–30	1	100% crop leaves (dicotyledon)
Stubble fields	N/A	1	50% dicotyledon 50% monocotyledon weeds or left-over crop foliage

Bud-eating birds

In addition, it was noted that some birds are specifically herbivorous at particular times of the year. Several species of birds are known to consume flower buds of fruiting/flowering crops almost exclusively when they are available, though their diet may vary at other times of year. This type of GMS was not considered in the Tier 1 assessment in EFSA (2009). To derive the appropriate body weight a literature search was performed investigating the species of birds which forage on flower buds. With regard to the crops, the WG decided that the scenario was relevant for orchards, small fruit and flowering ornamentals. The diet assumption in the Tier 1 should be 100% flower buds as the

information gathered suggested that when present, birds are heavily reliant on the flower buds as a food source. The WG noted that this emphasises the need for relevant data when considering Tier 3 refinement of the dietary proportions. The relevant BBCH growth stage groups were selected to be between BBCH 50 and 69. The reason for the inclusion of BBCH 60–69 (flowering) was because buds are still present during principal growth stage 6 (e.g. only 50% of flowers are open by BBCH 65). Therefore, in summary the WG agreed what reported in Table F.4.

Table F.4: Summary of Tier 1 GMS for bud-eating birds

Crop group	BBCH range	Body weight (g)	Diet assumptions
Pome fruit crops	50–69	22	100% flower buds
Small fruit crop	50–69	22	100% flower buds
Stone fruit crop	50–69	22	100% flower buds
Citrus fruit crops	50–69	22	100% flower buds
Nut crops	50–69	22	100% flower buds
Olives	50–69	22	100% flower buds
Non-bulb ornamental herbaceous plants	50–69	22	100% flower buds
ornamental broad-leaved trees, shrubs and climbing plants	50–69	22	100% flower buds
ornamental herbaceous plants	50–69	22	100% flower buds
ornamental plants	50–69	22	100% flower buds
ornamental woody monocotyledonous plants	50–69	22	100% flower buds
Bulb-like ornamental herbaceous plants	50–69	22	100% flower buds
Broadleaf forest tree	50–69	22	100% flower buds

Medium omnivorous birds consuming a high proportion of foliage

EFSA (2009) already included the need to consider a medium omnivorous bird, consuming 100% crop or weed leaves, for several crops at certain growth stages. Only for oilseed rape (BBCH 20–40) was a mixed diet assumed. The body weight of the Tier 1 generic model species was 490 g. The medium omnivorous bird generic model species is considered to represent birds which are attracted to feeding in the field by the presence of the crop itself.

When considering the bird species covered by this GMS, the WG agreed further analysis was needed to understand whether the assumed body weight in EFSA (2009) was protective of all species on this feeding guild and if there are other crop scenarios for which this GMS is relevant. For this the database compiled by Lahr et al. (2018) was consulted. Secondly a complimentary search of the literature for small/medium birds foraging on crop seedlings was performed. On this basis the WG agreed that the assumed body weight should be reduced to 390 g in order to be protective of all species. The WG also agreed that a medium bird may forage on all types of crop seedlings and young weeds. Therefore, it was agreed that a medium omnivorous bird taking 100% crop seedlings or weeds would be needed for early growth stages of field crops (i.e. BBCH 00–20). It was noted that EFSA (2009) also included the need for a medium omnivorous bird in legume forage (BBCH 21–49) and oilseed rape (BBCH > 20). The WG agreed that these should be maintained in the Tier 1 risk assessment for the revised guidance document.

Therefore, in summary the WG agreed what reported in Table F.5.

Table F.5: Summary of Tier 1 GMS for Medium omnivorous birds consuming a high proportion of foliage

Crop group	BBCH range	Body weight (g)	Diet assumptions
All non-permanent crops	00–09	390	100% weed leaves (50 monocot, 50% dicot)
All non-permanent crops	10–19	390	100% monocot leaves in a monocot crop 100% dicot leaves in dicot crops
Legume crops	21–49	390	100% crop
Winter sown brassica arable crop	20–99	390	50% crop, 50% weed leaves (25% monocot, 25% dicot)

Crop group	BBCH range	Body weight (g)	Diet assumptions
Spring sown brassica arable crop	20–99	390	50% crop, 50% weed leaves (25% monocot, 25% dicot)

A special consideration was given to herbivorous birds likely to forage in forestry plantations (biomass trees, broadleaf forest tree and coniferous forest trees). The WG decided:

- A large herbivorous GMS was not needed.
- A 280 g medium omnivorous GMS was needed. The dietary assumptions are 20% seeds, 40% dicotyledon plants and 40% monocotyledon plants. It is assumed that the GMS will feed from the ground.
- A 22 g bud-eating GMS was needed. It is assumed to eat 100% flower buds from the tree.

Granivorous birds

Granivorous birds, which may be ground or foliar feeders, or some combination of the two, were considered by the WG.

The WG considered that weed seeds are always available in agricultural soils either from recently flowering weeds or from the seedbank. Furthermore, some birds will also forage on weed seeds directly from the plant if flowering weeds are present. For these reasons, the WG concluded that a scenario with granivorous birds should always be part of the risk assessment. This is in contrast to EFSA (2009), where a granivorous scenario was not triggered for several crops.

Also, seed-bearing crops, with seeds for both for feed/food and seed production, are relevant scenarios at BBCH growth stages > 70, that are eaten by granivorous birds (Buxton et al., 1998). Both the ground and foliar strata representing weed seeds and crop seeds, respectively, is therefore relevant for granivorous birds. This was also the case in EFSA (2009), where species covering the same feeding strategy as mentioned above were selected as GMS. The WG was concerned, however, that GMS should sufficiently cover all European granivorous bird species potentially at risk. In the table below the body weight of granivorous birds foraging on seeds from the ground or in the crop is presented. The body weights were selected to cover all small European granivorous species.

Table F.6: Summary of Tier 1 Granivorous GMS for all crops other than forestry plantations

Crop	Feeding strata	BBCH	BW (g)	Diet assumptions
All crops other than forestry plantations	Ground	All	11	100% weed seeds
All seed-bearing crops	Crop	> 69	15	100% crop seeds

A ground feeding GMS may encounter both weed seeds and crop seeds when the crop is in the seed-bearing growth stages. The WG considered that this scenario will be covered by the foliar granivorous GMS due to the higher residue levels found in seeds attached to the plant.

A special consideration was given to granivorous birds likely to forage in forestry plantations (biomass trees, broadleaf forest tree and coniferous forest trees). The WG decided that two granivorous bird GMS are needed. The first is a 13 g bird with a diet of 100% tree seed. It is assumed that the GMS will feed from the tree. The second is a 12 g bird with a diet of 100% weed seeds. It is assumed that the GMS will feed from the ground.

Frugivorous birds

As with other GMS, the WG recognised that there are not frugivorous birds (relying on 100% fruit) in Europe. However, several species are known to consume high proportions of fruit when available. Data on frugivorous birds in Europe were sparse. The majority of the available data were generated in North America. Where data were severely lacking for a specific crop in Europe, the WG considered data for other related crops and the possibility of read across to determine the adequate frugivorous GMS body weight. In addition to the diet and presence in crops, the crop structures and preferred habitats of various species were considered in order to determine the most appropriate body weight GMS for each crop.

The diet assumption in the Tier1 should be 100% fruit as the information gathered suggested that when present, some bird species are heavily reliant on fruits as a food source. The WG noted that this

emphasises the need for relevant data when considering Tier 3 refinement of the dietary proportions. Therefore, in summary the WG agreed what reported in table F.7.

Table F.7: Summary of Tier 1 Frugivorous GMS for all crops other than forestry plantations

Crop group	BBCH range	Body weight (g)	Diet assumptions
Pome fruit crops	80–89	12	100% fruit
Stone fruit crop	80–89	12	100% fruit
Citrus fruit crops	80–89	12	100% fruit
Nut crops	80–89	70	100% fruit
Olives	80–89	12	100% fruit
Small fruit crop	70–89	12	100% fruit
Strawberries	70–89	83	100% fruit
Vines	80–89	67	100% fruit
Fruiting cucurbitaceous vegetable crops	80–89	83	100% fruit
Fruiting solanaceous vegetable crops	80–89	83	100% fruit
Legume vegetable crops	70–89	219	100% vegetable

A special consideration was given to fruit-eating birds likely to forage in forestry plantations (biomass trees, broadleaf forest tree and coniferous forest trees). The WG decided a fruit-eating GMS is needed. An 11 g bird with a diet of 50% berries and 50% foliar arthropods. It is assumed that the GMS will feed from the tree.

Avian GMS for treated seeds and germinating seedlings

The avian GMS were defined for the following scenarios:

i) Small granivorous bird consuming 100% small (≤ 0.5 cm) treated seeds.
ii) Medium granivorous bird consuming 100% large (> 0.5 cm) treated seeds.
iii) Large omnivorous bird consuming 100% potato tubers.
iv) Medium omnivorous bird consuming 50% potato tubers.
v) Small omnivorous bird consuming seedlings (BBCH 09–20; including the seed case).
vi) Medium omnivorous bird consuming 100% seedlings (BBCH 09–20; including the seed case).

First of all, data from Lahr et al. (2018) was used to identify which species are relevant in each scenario. This was not possible for all concerning crops as only some of the entries in the database allowed to finely select the growth stage of interest. Then, literature searches were done; in the case of treated seeds (small and large), reports and papers about the selection of focal species in recently drilled fields or about the ingestion of treated seeds by birds were reviewed and used. In the case of seedlings and potato tubers, reports and papers about agricultural conflicts with birds were used. These reviews were complemented with diet studies of specific species which the WG agreed needed special consideration. For each scenario, the body weight and the diet assumptions were considered. In all cases, with the exception of small omnivorous bird consuming seedlings, 100% of the treated food item in the diet was considered a reasonable worst-case assumption. From those species where it is reasonable to assume 100% diet, the lowest body weight from the range was selected.

Therefore, in summary the WG agreed what reported in Table F.8.

Table F.8: Summary of Tier 1 GMS for seed treatments

Treated food item	GMS	Body weight (g)	Diet assumption
Small treated seeds (≤ 0.5 cm)	Small granivorous	16	100% treated seeds
Large treated seeds (> 0.5 cm)	Medium granivorous	130	100% treated seeds
Treated potato tubers	Large omnivorous	5,500	100% potato tubers
	Medium omnivorous	400	50% potato tubers
Seedlings	Small omnivorous	27	*Depending on the crop
	Medium omnivorous	390	100% seedlings

*: Diet composition, and thus the % of seedlings, will be the same as that for the spray application in the specific crop from BBCH 10 to 19.

Specific considerations for mammals

Small mammal species

Most small mammals, with the notable exception of the wood mouse (*Apodemus sylvaticus*), are quite sensitive to the presence or absence of ground cover. However, it is noted that this sensitivity does not preclude these species from ever foraging in relatively low-coverage areas under specific circumstances e.g. presence of food or feeding pressure. In addition, even if these species are indeed less likely to forage throughout the field, their presence at the edge of field is likely, and foraging in the field close to the field edge is equally likely, depending upon the other environmental circumstances. Furthermore, larger mammals within the feeding guild may have less sensitivity to the presence or absence of coverage. As a result, only in very clear cases did the WG consider the presence or absence of coverage in the field to preclude the presence of particular species in a crop field, for example when the plant structures necessary for the preferred methods of ambulation would not be expected to be present. In cases where cover/plant structure was influential to the presence of small mammals, the literature indicated that a plant height of approximately > 20 cm for arable crops and grasses corresponded to sufficient coverage. This corresponds to a BBCH growth stage of between 30 and 40 for most crops. For simplicity's sake, and in agreement with the approach taken in EFSA (2009), it was assumed that plants at ≥ BBCH 40 have sufficient coverage and aerial structure for small mammals to be present. Similar to birds, if a focal species study is performed at higher tiers in order to refine the risk assessment, the species present in the immediate off-field area should also be considered. This is discussed further in Section 6.5.2.

Another critical point was that many small mammal focal species studies performed historically (e.g. those included in Lahr et al., 2018) were considered not likely to adequately capture some of the smallest species of mammals due to the trapping methodologies utilised, including trap location, and the very small size of these species. Nevertheless, the literature and common ecological knowledge (e.g. Gurney et al., 1998, Yigit et al., 2016, Hutterer et al., 2016, Kryštufek et al., 2019, etc.), indicate the presence of some of these very small species in areas relevant to the risk assessment of pesticides. In addition, to support the choice of mammalian GMS, a narrative review of the literature was performed covering small mammal ecological studies (limited to Europe and covering the period 1925–2020). The WG therefore carefully considered the very small species including the ecological data available on habitat use and diet, when considering the appropriate body weights for the Tier 1 GMS. Should focal species studies be performed in order to refine the risk assessment, attention should be paid to utilising methods appropriate to detect all species in the field and in the immediate off-field areas. This is discussed further in Section 6.2.1.

Omnivorous mammals

The WG considered that omnivorous species might be present in almost all crops and growth stages, owing to food availability, and given that several species are less sensitive to the perils of open ground. The WG then considered whether the appropriateness of the currently assumed diet for the model omnivorous mammal in EFSA (2009). It was acknowledged that the worst-case-ness of the diet was highly dependent upon the level of exposure of the various food items, which might vary, and the availability of the various food items at the time of application. As a generic consideration, a higher proportion of herbivorous diet was considered worst-case as a higher level of consumption would be required due to the relatively lower energy of these food items. It was determined that although the diet of omnivorous mammals is highly variable, the default diet as proposed for model omnivorous mammals in EFSA (2009) was a reasonable worst-case.

In summary: A 23-g small omnivorous species is needed for all crops and growth stages. The proportions of the different food items in the diet are – 50% weed seeds, 25% ground arthropods and 25% dicotyledon weeds. Only an infield assessment is needed for this GMS.

Insectivorous mammals

Whilst insectivorous mammals were generally assumed to consume ground arthropods, the amount of the diet which is taken from the surface versus from the upper layers of the soil beneath the surface, may vary. The WG considered this information particularly when determining appropriate GMS body weight for the different application scenarios and secondary poisoning assessment. In general, the evidence collected indicated that smallest insectivores have a very high feeding pressure, which may overwhelm concerns of the need for cover under many circumstances and they therefore may feed infield. In addition, several small insectivore species may be found in a very wide variety of

habitats, including various agricultural and other anthropogenic landscapes. Consequently, the WG agreed that there is a need to consider small insectivorous mammals for the majority of crops and growth stages.

In summary: A 4 g small insectivorous species is needed for almost all crops and growth stages. For forestry uses (broadleaf forest trees and biomass trees) a 9 g insectivorous specie. For both GMS the diet is 100% ground arthropods. Only an infield assessment is needed for this GMS.

Herbivorous mammals

Small herbivorous mammals can be found in a wide variety of landscapes where herbaceous food is plentiful. They tend to be quite sensitive to the presence of sufficient cover to avoid predation and are therefore not likely to be present in early growth stages of many crops, regardless of the presence of potential food items. However, it is noted that under no-till, or low-till, practices these species may nevertheless persist in the field, also during earlier growth stages. Since the assessment cannot be directly linked to agricultural practices at the moment, it is suggested that the evaluators in the MS should ascertain whether the crop in question is commonly grown under no-tillage and decide whether the small herbivore might be relevant at early growth stages, dependent upon the case in question. It may also then be considered whether the plant parts consumed in those circumstances would be subject to interception (i.e. they are below or above the leaf mat).

A review of the literature on diets of small herbivorous mammals determined some preferences for vegetative diet, but these could not be specifically linked to monocotlyous or dicotylous species only as both were represented. It was also reported that the diet of younger or older animals varied, based upon the distance of the preferred vegetative food source from the burrow. On this basis, and considering the foraging range of small herbivorous mammals, it decided that in monocotyledon crops or in monocotyledon-dominated landscapes (e.g. grassland, orchards), the diet should be 100% monocotyledon, and in all other scenarios, 50% monocotyledons, 50% dicotyledons should be applied.

In summary:

At BBCH > 39 in arable crops, small herbivorous mammals of 25g should be considered in-field. In perennial crops, where for example, the cover in-field may be persistently available, small herbivores may be present at all growth stages. A TAI assessment is needed for this GMS, with a diet of 50% monocotyledons, 50% dicotyledons, for all crops at all growth stages.

Medium herbivorous mammals may be present in many crops, and certainly in agricultural areas, either consuming crop or consuming other vegetative matter in or around the field. The WG considered it difficult to eliminate medium herbivores from most crops unless it could be determined that there would be no food source available. In addition, medium herbivores are copiously present in immediate off-field areas.

EFSA (2009) quantified the diet composition of large herbivorous mammals based on conservative assumptions. However, the WG considered that further evidence on the ecology of these mammals was needed to substantiate the characterisation of their diet compositions. Therefore, a narrative review was conducted with the aim of determining the proportion of monocotyledons and dicotyledons in the diet of large herbivorous mammals.

Based on a quantitative and qualitative analysis of these data, the WG agreed that the diet of medium herbivorous mammals should be quantified as:

- 80% monocotyledons, 20% dicotyledons, when the treated crop is a palatable monocotyledon or in monocotyledon-food item dominated landscapes (e.g. grasslands, orchards);
- 50% monocotyledons, 50% dicotyledons in scenarios when the GMS is consuming only weeds. For palatable dicot crops, it is assumed the diet is 100% dicot crop leaves.

Summary: A medium herbivore GMS of 1500g should be considered in all crops at all growth stages.

It must be noted that the leaves of plants of the *Solanaceae* family (e.g. potato, tomato, eggplant, etc.) contain alkaloids including tropanes which may be toxic. As a result, these are mostly avoided by foraging mammals (and birds), however, under certain circumstances some individuals or groups of individuals may become habituated and consume e.g. potato leaves. This has been occasionally reported amongst lagomorphs. The WG carefully considered this point and determined that since the consumption of these leaves is an exception rather than a rule an herbivore consuming the leaves of these plants could not be included in the GMS as a default. Furthermore, it must also be noted that a

large herbivore GMS is critical when potato plants are not present, or still very small (i.e. no small herbivore is present), at which time the consumption of weeds is as worst-case as consumption of crop leaves (i.e. the deposition is the same and the energy/moisture content are similar). At higher growth stages, the small herbivorous GMS is present (also consuming weeds), which represents a worst-case situation due to the much lower bw. Should an applicant wish to suggest that weeds are not present in any crop, they are required to provide data on this, and to suggest new/appropriate focal species. These species may, indeed, then be, for example, large herbivores eating crop leaves.

Frugivorous mammals

Similar to birds, data was relatively scarce for frugivorous mammals in Europe. The WG considered the data from the sources previously indicated, as well as the potential for read across from similar/related crops, as was also done for the frugivorous birds. These data were used to determine the appropriate body weight of the generic model species of frugivorous mammal for various fruit and nut crops. In addition to the diet and presence in crops, the crop structures and preferred habitats of various species were considered in order to determine the most appropriate species for each crop.

Different frugivores were determined to have clear preferences for different types of fruits and nuts and different crop structures. Some leave the canopy mainly only to cross open areas between trees and bushes, whereas others spend significant time on the ground. It was noted that the species which consume fruit are also known to take buds at earlier growth stages. Therefore, for orchards, trees and fruits, a bud-eating GMS should also be considered.

In summary, for spray uses:

In small fruit and nut crops the GMS should be a frugivore of 25g, eating 100% fruits/nuts or 100% buds at growth stages where these are present. In orchards, citrus and ground-growing fruits and vegetables, a frugivore of 82g should be present, eating 100% fruits or 100% buds at growth stages where these are present.

Granivorous mammals

The WG considered whether granivorous mammals were likely to take grains/seeds directly from the (top of) crop itself at late growth stages, similar to granivorous birds. Additionally, it was considered whether this might also be true for weeds at the seed production stage. Some species of granivores are indeed known to do this and are present in various agricultural crops. These species are not likely to be present until the crop structure is high enough to support them. Other species, which may be majority granivorous when seeds and nuts are available, may only take seeds from the ground. It was therefore decided to consider the crop and crop structure in order to determine which species were likely to be present and whether the species in question would consume seeds from the crop heads or from the ground.

In summary, for spray uses:

In crops at > BBCH 39, a small granivore of 6g should be considered in-field, consuming weed seeds, or at appropriate growth stages (i.e. BBCH > 69), crop seeds. In all crops and growth stages a small granivore of 23g should be present, consuming weed seeds (from the ground). Both an infield and a TAI assessment is needed for this GMS. The body weight of the GMS in the TAI is 6 g.

Mammalian GMS for treated seeds and germinating seedlings

An additional consideration was which granivorous species would be appropriate for seed treatments (i.e. consumption of treated seeds). Considering the crop structure at the time of seeding and the food source present it was decided that a granivore of 23g was appropriate taking seeds from the ground was appropriate. Some larger mammals are known to consume potatoes tubers and bulbs and consequently a (10 kg) GMS covering this scenario is needed.

For germinating seedling, it is logical that the same GMS species are used as were selected for early growth stages for spray applications. On this basis the GMS are a 1,500 g medium herbivorous mammal (consuming 100% crops seedlings) and a 23 g small omnivorous mammal (consuming 50% weed seeds, 25% crop seedlings and 25% ground arthropods).

Bird and mammal GMS for secondary poisoning assessment

The WG considered the species might be most representative for the Tier 1 assessment of secondary poisoning via fish, earthworms and aquatic invertebrates. There are a number of fish-eating species in Europe, and of these the lowest body weight was chosen as representative for the Tier 1

GMS. Similarly, small insectivores (both terrestrial and aquatic) may be found throughout Europe. Consideration was given to the likelihood of exposure mainly via earthworms, or via aquatic invertebrates and the GMS were chosen based on the species most likely to have a worst-case diet with high percentages of earthworms/aquatic invertebrates.

Piscivorous birds

There are many types of fish-eating birds (piscivorous). For some piscivorous bird species the fish part of the diet varies, depending on habitat and season (Peris et al., 1995). For other piscivorous bird species, fish always comprise a major part of diet, but also non-fish food items comprise a significant part (Hampl et al., 2005; Gagliardi et al., 2007). Some species, however, are always fully piscivorous and as such, they are relevant to consider in a risk assessment for fish-eating birds exposed to bioaccumulating substances in the food chain. A small, fully piscivorous, GMS with a body weight of 27 g is considered to be realistic worst case by the WG.

Vermivorous birds

Earthworms are important prey for many ground-feeding birds, and they have strong bills that enable them to catch earthworms. A small earthworm-eating bird GMS, consuming 100% earthworms, with a body weight of 66 g is considered to be realistic worst case by the WG.

Insectivorous bird (aquatic)

The WG considered that birds foraging on aquatic invertebrates, e.g. waders, also needs to be considered in a food chain risk assessment. The appropriate weight of a small aquatic invertebrate-eating to be assumed for the GMS was considered to be 21 g.

Piscivorous mammal

There are relatively fewer mainly piscivorous mammals in Europe, so an appropriate GMS was considered to have the lowest body weight and highest percentage of fish in the diet. As a result, a 500g mammal consuming 100% fish is the appropriate GMS for secondary poisoning to mammals through fish.

Vermivorous mammal

Small insectivorous mammals were considered. In order to consume large percentages of earthworms in the diet, the mammal must be able, and inclined, to dig for worms under the surface of the ground. The most appropriate worst-case GMS consuming 100% earthworms is a 9 g insectivore.

Insectivorous mammal (aquatic)

Amongst the insectivorous mammals, some have specialised in taking aquatic (sediment) invertebrates, the smallest of which were considered to be appropriate to use as GMS for assessing the risk to mammals from secondary poisoning through benthic organisms. A 15 g mammal consuming 100% aquatic invertebrates was considered the appropriate GMS for secondary poisoning through sediment organisms.

GMS parameters for new crops/situations or for those crops outside of Europe

Although the WG made a substantive effort to include all crops grown in the EU, there are some crops for which recommendations for appropriate GMS parameters could not be proposed (e.g. pineapples and bananas) which are grown in some territories of MS but are outside of Europe. For these cases it is suggested that the risk assessor considers an appropriate surrogate crop scenario, considering the crop structure, for the screening and Tier 1 risk assessment. For example, Member State risk assessors already agreed that orchard crops were a reasonable surrogate for uses to banana trees (EFSA, 2019b). It should be acknowledged, as with the use of any surrogate information there is uncertainty in the risk assessment.

In addition, there were a number of additional crops which could be grown in the EU which were not covered by EFSA (2009). For many of these crops, data were lacking for detailed consideration of the appropriate GMS parameters. Therefore, the WG has made suggestions for surrogate crops based on the plant structure and way the crops are grown. These are given in Table F.9 below.

In cases where a higher tier risk assessment is triggered then, in order to perform a scientifically robust risk assessment it is essential that relevant data from the region where the crop is grown is

provided by the applicant. This is of particular importance to ecological data but also to exposure parameters such as DT_{50}, RUDs and crop interception.

Table F.9: Proposed surrogate crops for identification of GMS parameters

New crop	Surrogate crop for GMS parameter selection
Artichokes and cardoons	Root and stem vegetables
Asparagus	Root and stem vegetables (but noting the crop is a monocot)
Banana	citrus
Buckwheat	Spring sown brassica arable crop
Fig	Olives
Jerusalem artichoke	Sunflower
Kiwifruit	Vines
Linseed (= flax)	Spring/winter sown brassica arable crop
Pineapple	Fruiting solanaceous vegetable crops
Quinoa	Maize and millet crop
Rhubarb	Root and stem vegetables
Ryegrasses	Grassland
Salsify	Herb crops
Sesame	Spring/winter sown brassica arable crop
Sweet potatoes	Potato
Tobacco	Maize

Appendix G – Background information for Food Intake Rate Calculations

As described in the Section 6.1.3 of the guidance document a key parameter in the estimation of the dietary exposure of birds and mammals to residues of pesticides substances is the food intake rate (FIR) by the model species. The equation for estimating the FIR is given in Section 6.1.3 of the guidance document (equation 6). Clarifications include indicating which values are relevant for the screening indicator model species and tier 1 generic model species. Furthermore, assimilation efficiency data for additional avian orders have been added to Table G.3 as have the energy and moisture content of additional food items (Table G.2).

Daily energy expenditure (DEE)

Data for the DEE are derived from a research project carried out for Defra (2007).

Relationship between body weight (bw in g) and daily energy expenditure (DEE in kJ) can be described by the equation:

$$\log DEE = \log a + b \times \log bw$$

To obtain the specific equation for the relevant species group, the respective log a and b from Table G.1 have to be inserted.

Table G.1: Species groups, log a and b, the standard errors for a and b (SE), the number of species in each group (N), and the proportion of variation explained by each equation (r2)

Species group	GMS	log a	SE log a	b	SE b	N	r2
Non passerines	Large herbivorous medium omnivorous Benthic invertebrate-eating Fish-eating Large omnivorous	0.839	0.161	0.669	0.063	18	0.87
Passerines (*)	Small insectivorous, Small omnivorous Granivorous Frugivorous bud-eating earthworm-eating	1.032	0.058	0.676	0.045	44	0.84
Mammals (*)	All	0.814	0.046	0.715	0.019	46	0.97

(*): Excluding desert passerines or desert and marine eutherians.

It should be noted that the allometric equations to predict its daily energy expenditure identified significant differences between taxonomic groups and between species occupying different habitats hence the differentiation between passerines and non-passerines in the above table. As discussed in Defra (2007) there are circumstances where a bird or mammal will have a higher energy requirement than represented by the data e.g. when feeding young birds, lactating mammals, during migration, pre-hibernation hyperphagia, etc. This is acknowledged by the working group as an uncertainty, nevertheless, it was decided to maintain the current assumptions for the risk assessment to avoid making the exposure assessment overly conservative for the majority of situations. It should be acknowledged that there are circumstances where the energy requirement, hence food intake rate, will be higher.

Energy and moisture content of food items

In EFSA (2008, appendix 13), an analysis was done for the energy and moisture content of food items taken by birds and mammals. This analysis combined data from Crocker et al. (2002), and Bairlein (1998). These combined data are used for calculating the FIR for the scenarios defined for the indicator model species and generic model species. For higher tier assessments, tier 3, it is sometimes necessary to include other food categories. Data energy and moisture content for these food items can be found in EFSA (2008, appendix 13), Buxton et al. (1998) and Crocker et al. (2002).

Table G.2: Different food items, their energy content [kJ/g dry] and moisture content [%]

Food items (as defined in EFSA, 2008)	Corresponding diet components used for IMS and GMS	kJ/g dry	Moisture [%]
Grasses and cereal shoots	Monocot. Foliage (including maize)	17.6	76.4
Non-grass herbs	Dicot. foliage	17.8	88.1
Cereal seeds	Crop seeds	18.4	14.7
Weed seeds	Weed seeds	21.7	9.9
Tree seeds	Tree seeds and nuts	22.9	15.0
–	Flower buds	19.4[(a)]	38.2[(a)]
Fruit	All fruits/vegetables/tubers/bulbs[(b)]	14.8	83.9
Arthropods (including caterpillars)	Ground-dwelling and foliar-dwelling arthropods	22.7	68.8
Soil invertebrates (slugs and earthworms)	Soil invertebrates	19.4	84.3
Fish	Fish	21.0	73.7
Aquatic invertebrates	Benthic invertebrates	20.9	76.3
Aquatic vegetation	–	15.0	81.4

(a): These values are taken from Reynolds (2003) and are related to cherry flower buds. In Tischler and Karschon (1983) the energy content of *Eucalyptus camaldulensis* is reported as 19.8 kJ/g. Guglielmo et al. (1996) reported an energy content for quaking aspen flower buds around 20 kJ/g.

(b): All fruits/vegetables = citrus fruit, fruit from cucurbitaceous vegetable crops, fruiting from solanaceous vegetable crops, pome fruits, stone fruits, grapes, small fruits, strawberries, bananas, figs, vegetables from legume vegetables, kiwifruit, olives, pineapples.

Assimilation efficiency

Some food passes through the gut unabsorbed to emerge as faeces. The true energy value of a food is given by the energy content of the food minus the energy value of the faeces. Therefore, in calculating the likely food intake we need to take account of different species' assimilation efficiencies for different foods. EFSA (2008), took the assimilation efficiencies for birds are from Bairlein (1998) the assimilation efficiencies for mammals are from Crocker et al. (2002), and Smit (2005). For higher tier assessments, it is sometimes necessary to include other food categories. Data for some food items can also be found in these three references (see also EFSA, 2008).

Table G.3: Assimilation efficiency of different food items for mammals and different bird species[a]

Assimilation efficiency of different food items (as defined in EFSA (2008))	Corresponding diet components used for IMS and GMS	Mammal	Passeriformes (e.g. song-birds)	Anseriformes (e.g. ducks/ geese)	Columbiformes (e.g. pigeon)	Galliformes (e.g. fowl)	Charadriiformes (e.g. gulls/ waders)	Coraciiforms	Gruiformes
IMS and GMS[d]		All	Small insectivorous, Small omnivorous, Granivorous, frugivorous, bud-eating, earthworm-eating birds	Large herbivorous birds		Medium omnivorous birds	Benthic invertebrate-eating birds	Fish-eating birds	Large omnivorous bird
Grasses and cereal shoots	Monocot. Foliage (including maize)	0.47	0.76	0.41	0.53[c]	0.42	–	–	
Non-grass herbs	Dicot. Foliage	0.76	0.76	0.41	0.53[c]	0.42	–	–	0.59
Cereal seeds	Crop seeds	0.84	0.80	0.83	–	0.65	–	–	
Weed seeds	Weed seeds	0.84	0.80	0.83	0.76[b]	0.65	–	–	
	Tree seeds and nuts	0.84	0.80	0.83	0.76[b]	0.65	–	–	
–	Flower buds	0.74[f]	0.67[d]						
Fruit	Large fruit, small fruit, grapes, vegetable-fruits, potato tubers, plant bulbs	0.74[f]	0.67	–	–	0.57	–	–	0.45
Arthropods (including caterpillars)	Ground-dwelling and foliar-dwelling arthropods	0.87	0.76	0.87	–	0.70	–	–	
Soil invertebrates	Earthworms	0.87	0.76	0.87	–	0.70	–	–	
Fish	Fish	0.87	0.76	0.87	–	0.70	0.69	0.75[e]	
Aquatic invertebrates	Benthic invertebrates	0.87	0.76	0.87	–	0.70	0.69	0.75[e]	
Aquatic vegetation		0.76	0.76	0.41	–	0.42	–	–	

Assimilation efficiency of different food items (as defined in EFSA (2008))	Corresponding diet components used for IMS and GMS	Mammal	Passeriformes (e.g. song-birds)	Anseriformes (e.g. ducks/ geese)	Columbiformes (e.g. pigeon)	Galliformes (e.g. fowl)	Charadriiformes (e.g. gulls/ waders)	Coraciiforms	Gruiformes
Summary statistics		Dicot foliage N = 26, SD = 0.11 Monocot foliage N = 35, SD = 0.12 Seeds N = 23, SD = 0.76 Insects (as surrogate for invertebrates) N = 8, SD = 6.3	N = 184, 18 species	N = 441, 67 species	Surrogate	N = 184, 18 species	N = 19, 7 species	Surrogate	

(a): Note that these assimilation efficiencies are for the screening IMS and tier 1 GMS. For higher tier assessment using focal species data for the correct order should be used.
(b): No data available for Columbiformes, the value for seeds is the average of 3 data (83% for Anseriformes + 65% for Galliformes + 80% for Passerriformes).
(c): No data available for Columbiformes, the value for the assimilation efficiency of herbage is the average of 6 data (36% for Struthioniformes, 59% for Gruiformes, 41% for Anseriformes, 42% for Galliformes, 61% for Piciformes and 76% for Passerriformes).
(d): no data available. Values for fruits are used as surrogate.
(e): No data available for Coraciiforms, the value for the assimilation efficiency of fish is the average of 15 data (34% for Gruiformes, 69% for Charadriiformes, 79% for Lariformes, 76% for Alciformes, 75% for Sphenisciformes, 87% for Procellariiformes, 80% for Pelecaniformes, 77% for Strigiformes, 84% for Falconiformes, 82% for Accipitriformes, 80% for Ciconiiformes, 87% for Anseriformes, 70% for Galliformes, 64% for Piciformes and 76% for Passerriformes).
(f): No value available, surrogate for general vegetation used.

It should be noted that all of the above data on moisture content and calorific content have been used to determine food intake rates for indicator model species as well as generic model species.

FIR in the case of PD refinement

If PD values are refined (see Section 6.5.4) then it may be necessary to recalculate the FIR for the refined diet. PD values may be defined in terms of wet weight or dry weight. If the values are given in wet weight, then equation 6 (Section 6.1.3) can be used. However, if PD values are given in dry weight, then there is no need to correct for the moisture content of the food. The following equation should be used:

$$FIR = \left(DEE / \sum_i \left(PD_i \times FE_i \times AE_i\right)\right),$$

Where:
FIR: Food Intake Rate [g dry weight/day]
DEE: Daily Energy Expenditure [kJ/day] of the focal species
PD_i: Proportion of food item i in the diet
FE_i: Food energy of food item i [kJ/dry g]
AE_i: Assimilation Energy for food item i by the focal species (obtained by assimilation energy in % divided by 100).

Appendix H – Background information on the fTWA and the selected averaging period to be used

According to EFSA (2009), the conceptual model for estimating the exposure for reproduction assessments accounts for dissipation (in the form of a DT_{50}) of the active substance on the food item consumed by birds and mammals. The DT_{50} is used to calculate a time weighted average factor (fTWA) which is then applied to the exposure estimation. The reproductive risk assessment for birds and mammals according to European Commission (2002a), included a default fTWA value of 0.53 which assumed a 21 day averaging period in the exposure assessment, saying simply, '*It is obvious that a constant exposure level (if above the response threshold) will have more serious long-term effects than an exposure pattern which starts with the same level and then rapidly declines, either due to accumulation of the substance (increase of body burden) or due to accumulation of effects. This has to be considered when relating toxicity (constant exposure level) to field exposure. Also, when assessing a persistent and a non-persistent substance the degradation rate in some way should be reflected in the exposure estimate and the risk indicator. An appropriate means to reduce such kind of bias is to average the exposure over a certain time interval. Unfortunately, there is no sound scientific basis and no generally accepted rule on how long this interval should be; to simply take the study duration is disapproved by most experts. For the time being a period of 3 weeks is proposed as a convention, unless there are good reasons to take shorter or longer times. For example, cases where the effects data used are derived from a study with a shorter exposure period, or where a short delay between the onset of exposure and the onset of effects is observed, or where effects are to be ascribed to the exposure during a brief sensitive period would call for a shorter averaging time*.'

EFSA (2008) contains an information box for risk managers, which outlined the key issues and how they were proposed to be addressed:

'A detailed critique of the approach outlined in EC *(*2002a*) is provided in Appendix 16, however the key points are*:

- *Lack of clarity regarding whether the assessment in EC (*2002a*) is aimed at assessing*
- *Non-specific effects resulting from long-term or chronic exposure to pesticides*
- *Reproductive effects due to long-term or prolonged exposure*
- *Reproductive effects possibly after short-term exposure*

This lack of clarity leads to confusion regarding the aim of assessment, and hence how to refine the risk should that be necessary.

- *The use of an arbitrary time-weighted average approach*.
- *Lack of clarity as regards the scenario and hence level of protection being assessed, i.e. is it an average field or average bird*.
- *No consideration of the breeding behaviour or time of breeding and its potential overlap with applications*.
- *Current approach only uses one endpoint from the toxicity study*.

The above points are addressed by the following:

- *The risk assessment now focuses on the potential effects on reproduction*.
- *Time-weighted averages are still used in the current risk assessment. However, time windows are generally justified rather than being arbitrary. There is still use of an arbitrary time window in the avian assessment; however, this is used to highlight whether the effects could have been due to long-term exposure*.
- *The proposed approach considers the potential overlap of applications with breeding*.
- *The level of protection is evaluated qualitatively in Section 3.5*.

See Section 3.5 for evaluation of the level of protection provided by the proposed scheme and also for a comparison of the outcome for 9 representative substances, compared to that with the existing procedure'.

One of the main points mentioned is the 'use of an arbitrary time-weighted average approach'. The statement suggests that this arbitrary fTWA approach is 'generally justified' in the opinion, particularly considering 'the potential overlap of applications with breeding'. This was hitherto referred to as the 'phase-specific approach'.

Instead of a default fTWA averaging period of 21 days, the opinion foresaw 'the use of a period according to the demands of the reproductive risk assessment'. However, this proposal was for the most part dropped from the final Guidance document (EFSA, 2008, 2009), as the application of the phase-specific approach, whilst theoretically attractive, was technically difficult. It was possible potentially to use it for specific species, however, it was not clear that by using the appropriate phase-specific assessment for one representative species, other species also intended to be protected/addressed via the risk assessment for that species would also be protected. Furthermore, effects on breeding can be as a result of parental exposure outside the breeding season. As a result, the Guidance eventually maintained the default fTWA averaging period of 21 days, but mentioned that this was not considered entirely appropriate, though 'it should be assumed as a default that the effects are caused by long-term exposure, unless there is specific evidence for the pesticide under assessment that the effect could be caused by short-term exposure' (EFSA, 2009, p 34). The phase-specific approach was allowed as a refinement-option which was henceforth rarely used due to the aforementioned technical difficulties.

As mentioned in European Commission (2002a) the default averaging period of 21 days is arbitrary. It was not then, nor is it now, supported by any specific reference. However, the Working Group considers that no other value may be considered to be supported by data, making it impossible to 'update' this value according to any specific references. Furthermore, the best attempt to update it to be more appropriate to the endpoint used in the risk assessment (the phase-specific approach), is not feasible, at least at the level of the EU, due to lack of information on the exact species which are appropriate and necessary for every crop in all zones at every possible time frame of exposure, and lack of ability (in most cases) to differentiate effects as a result of exposure outside the breeding season. The WG therefore determined that the most appropriate way forward is to maintain the fTWA averaging period of 21 days, except in those cases where, based upon the available data, it is not justified to account for dissipation by the use of a time-weighted average. If evidence is provided regarding the exposure time frame required to achieve the onset of an effect, the assessment might be refined according to that time frame, however, this would presumably only be possible to ascertain via relatively complex modelling (e.g. DEB modelling) (Muller et al., 2019; Kooijman et al., 2020). Section 6.1.4 of the GD provides guidance regarding which endpoints might be considered incompatible with the use of a fTWA.

Appendix I – Spray drift values to be used for the exposure assessment for small mammals

Spray drift values used for the exposure assessment of mammals in the off-crop environment should be aligned to those for other non-target organisms. Currently, the spray drift values used for the exposure assessment for non-target organisms are those derived in Rautmann et al. (2001) and are currently used for several groups of non-target organisms e.g. non-target arthropods and non-target terrestrial plants (European Commission, 2002a).

Rautmann et al. (2001) derived spray drift values (% of applied application rate per hectare) for a number of crops with some differentiation of the growth stage (only in terms of early and late). Table I.1 provides a summary of the spray drift values from Rautmann et al. (2001) with an added clarification for which sprayer technique and conditions the values are being used to represent. For single applications 90th percentile values should be used. For multiple applications, a lower percentile value may be taken. For ease of reference, the drift values for each spray technique for single and multiple applications and considering different distances from the crop are given in Tables I.3–I.7.

In Table I.2, the crop groups used for the Tier 1 exposure assessment for spray are summarised together with a proposal for a spray drift value which can be used in the Tier 1 exposure assessment. With the exception of strawberries, the spray drift values have been selected to cover the conditions which will lead to the highest drift. However, the applicant may wish to propose a different value together with a justification considering the specific GAP and sprayer conditions. An indication is given in the last column of the table. The selected value should be aligned to that used for other exposure assessments (e.g. surface water). An exception for taking the worst-case values was made for strawberries as, although they can be grown vertically, it was considered that the majority assessments will be for standard (non-vertical) fields. Applicants and risk assessors should consider a higher spray drift value if vertical farming of strawberries is common in the Member State where the PPP will be used or it is specified in the GAP. Furthermore, applicants may wish to provide studies to refine the default spray drift values. These should be evaluated and utilised in harmonised manner (logically presented in the context of the surface water exposure assessment).

The WG recommends that default spray drift values are updated including a consideration of additional application machinery. If agreed and harmonised values become available, then the ones below should be replaced.

Table I.1: Spray drift technique and spray drift value from Rautmann et al. (2001)

Spray drift category from Rautmann et al., 2001	Spray technique and conditions for which the Rautman category is being used to represent
Field crops	Standard horizontal boom sprayer
Fruit crops, early (= orchards with lower crop intercept(1))	Air-blast equipment to plants with lower crop intercept(1)
Fruit crops, late (= orchards with higher crop intercept(2))	Air-blast equipment to plants with a higher crop intercept(2)
Vine, late(3)	Sideward sprayer
Hops	Hop sideward sprayer

(1): Deciduous trees at BBCH 0–69 and 97–99.
(2): Evergreen trees and deciduous trees at BBCH 71–95.
(3): EFSA (2020) excluded drift values for 'vines, early' in the exposure assessment as these drift values were based on non-standard application techniques for early vines.

Table I.2: Crop groups used for the Tier 1 exposure assessment for sprays together with the spray drift value to be used for Tier 1 exposure assessment

Name for GD	EPPO name	Spray drift category to be used for Tier 1 (in the first instance)	Considerations for more specific spray drift category (and proposed by applicant)
All field crops BBCH 0–9	–	Standard horizonal boom sprayer	–

Name for GD	EPPO name	Spray drift category to be used for Tier 1 (in the first instance)	Considerations for more specific spray drift category (and proposed by applicant)
Allium vegetable crops	allium vegetable crops (3ALLC)	Standard horizonal boom sprayer	–
Amenity grassland	amenity grassland (3AMGC)	Standard horizonal boom sprayer	–
Artichokes and cardoons	*Cynara scolymus* (CYUSC) *Cynara cardunculus* (CYUCA)	Standard horizonal boom sprayer	–
Asparagus	*Asparagus officinalis* (ASPOF)	Standard horizonal boom sprayer	–
Banana	*Musa* × *paradisiaca* (MUBPA)	Air-blast equipment to plants with a higher crop intercept	–
Bare fallow	bare fallow (treatment of) (3BARFO)	Standard horizonal boom sprayer	–
Biomass trees	biomass trees (3BMTC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95
Broadleaf forest tree	broadleaf forest trees (3FOBC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95
Buckwheat	*Fagopyrum esculentum* (FAGES)	Standard horizonal boom sprayer	–
Bulb-like ornamental herbaceous plants	–	Standard horizonal boom sprayer	–
Citrus fruit crops	citrus fruit crops (3CITC)	Air-blast equipment to plants with a higher crop intercept[2]	–
Coniferous forest trees	coniferous forest trees (3FOCC)	Air-blast equipment to plants with a higher crop intercept[2]	–
Cotton	–	Standard horizonal boom sprayer	–
Fig	*Ficus carica* (FIUCA)	BBCH Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95
Fruiting cucurbitaceous vegetable crops	fruiting cucurbitaceous vegetable crops (3FCVC)	Standard horizonal boom sprayer	–
Fruiting solanaceous vegetable crops	fruiting solanaceous vegetable crops (3FSVC)	Sideward sprayer	Standard horizonal boom sprayer could be used if the GAP indicates that the plants will not be grown vertically
Grass crops	grass crops (3GRAC)	Standard horizonal boom sprayer	–

Name for GD	EPPO name	Spray drift category to be used for Tier 1 (in the first instance)	Considerations for more specific spray drift category (and proposed by applicant)
Grassland	grassland (3GRLC)	Standard horizonal boom sprayer	–
Herb crops	herb crops (3HERC)	Standard horizonal boom sprayer	–
Hops	*Humulus lupulus* (HUMLU)	Hop sideward sprayer	–
Jerusalem artichoke	*Helianthus tuberosus* (HELTU)	Standard horizonal boom sprayer	–
Kiwifruit	*Actinidia deliciosa* (ATIDE)	Sideward sprayer	–
Leafy vegetable crops (excluding brassica)	leafy vegetable crops (excluding brassica) (3LEAC)	Standard horizonal boom sprayer	–
Legume crops, except soybean	legume crops (3LEGC)	Standard horizonal boom sprayer	–
Legume vegetable crops	legume vegetable crops (3LEVC)	Standard horizonal boom sprayer	–
Linseed (= flax)	*Linum usitatissimum* (LIUUT)	Standard horizonal boom sprayer	–
Maize and millet crop	maize and millet crops (3MAMC)	Standard horizonal boom sprayer	–
Nut crops	nut crops (3NUTC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95 Sideward sprayer may be used pending on the application technique.
Olives	*Olea europaea* (OLVEU)	Air-blast equipment to plants with a higher crop intercept[2]	
Ornamental broad-leaved trees, shrubs, and climbing plants	ornamental broad-leaved trees, shrubs, and climbing plants(3ORBC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95 Sideward sprayer may be used pending on the application technique.
Ornamental cactuses and succulents	ornamental cactuses and succulents(3ORSC)	To be proposed by applicant pending on the specific plants in the GAP and the spray technique.	
Ornamental conifers	ornamental conifers (3ORCC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95 Sideward sprayer may be used pending on the application technique.

Name for GD	EPPO name	Spray drift category to be used for Tier 1 (in the first instance)	Considerations for more specific spray drift category (and proposed by applicant)
Ornamental herbaceous plants	ornamental herbaceous plants(3ORHC)	Standard horizonal boom sprayer	–
Ornamental herbaceous plants excluding bulbs		Standard horizonal boom sprayer	–
Ornamental plants (unspecified)[a]	ornamental plants (terrestrial)(3ORTC)	Air-blast equipment to plants with lower crop intercept[1]	This category is for when the plants to be treated is not specified in the GAP and therefore it needs to cover all situations.
Ornamental woody monocotyledonous plants	ornamental woody monocotyledonous plants (3ORMC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95 Sideward sprayer may be used pending on the application technique.
Ornamental woody plants	ornamental woody plants (3ORWC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95 Sideward sprayer may be used pending on the application technique.
Pineapple	*Ananas comosus* (ANHCO)	Standard horizonal boom sprayer	–
Pome fruit crops	pome fruit crops (3PMFC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95 Sideward sprayer may be used pending on the application technique.
Poppy	*Papaver somniferum* (PAPSO)	Standard horizonal boom sprayer	–
Potato	*Solanum tuberosum* (SOLTU)	Standard horizonal boom sprayer	–
Quinoa	*Chenopodium quinoa* (CHEQU)	Standard horizonal boom sprayer	–
Rhubarb	*Rheum rhabarbarum* (RHERH)	Standard horizonal boom sprayer	–
Root and stem vegetables	umbelliferous vegetable crops (3UMBC)	Standard horizonal boom sprayer	–
Salsify	*Scorzonera hispanica* (SCVHI)	Standard horizonal boom sprayer	–

Name for GD	EPPO name	Spray drift category to be used for Tier 1 (in the first instance)	Considerations for more specific spray drift category (and proposed by applicant)
Sesame	Sesame	Standard horizonal boom sprayer	–
Small fruit crop	small fruit crops (3SMFC)	Sideward sprayer	–
Soybean	Glycine max (GLXMA)	Standard horizonal boom sprayer	–
Spring sown brassica arable crop	brassica arable crops (3BRAC)	Standard horizonal boom sprayer	–
Spring sown cereal crop	cereal crops (3CERC)	Standard horizonal boom sprayer	–
Stone fruit crop	stone fruit crops (3STFC)	Air-blast equipment to plants with lower crop intercept[1] or Air-blast equipment to plants with a higher crop intercept[2]	Lower crop intercept = Deciduous trees at BBCH 0–69 and 97–99 Higher crop intercept = Evergreen trees and deciduous trees at BBCH 71–95 Sideward sprayer may be used pending on the application technique.
Strawberry	*Fragaria × ananassa* (FRAAN)	Standard horizonal boom sprayer	Sideward sprayer should be used if the GAP indicates that the plants will be grown vertically
Stubbles	stubble (cereal)(YSTEG) stubble (maize)(YSTZE)	Standard horizonal boom sprayer	–
Sugar beet	beet crops (3BEEC)	Standard horizonal boom sprayer	–
Sunflower	*Helianthus annuus* (HELAN)	Standard horizonal boom sprayer	–
Sweet potatoes	*Ipomoea batatas* (IPOBA)	Standard horizonal boom sprayer	–
Tobacco	*Nicotiana tabacum* (NIOTA)	Standard horizonal boom sprayer	–
Vegetable brassica crops	vegetable brassica crops (3VBRC)	Standard horizonal boom sprayer	–
Vines	Vitis vinifera (VITVI)	Sideward sprayer	–
Winter sown brassica arable crop	brassica arable crops (3BRAC)	Standard horizonal boom sprayer	–
Winter sown cereal crop	cereal crops (3CERC)	Standard horizonal boom sprayer	–

Table I.3: Drift values for standard horizonal boom sprayers for single and multiple applications

Standard horizonal boom sprayer								
Distance [m]	No of applications							
	1	2	3	4	5	6	7	8 and more
	90th centile	82nd centile	77th centile	74th centile	72nd centile	70th centile	69th centile	67th centile
1	2.77	2.38	2.01	1.85	1.75	1.64	1.61	1.52
3	0.95	0.79	0.68	0.62	0.59	0.56	0.55	0.52
5	0.57	0.47	0.41	0.38	0.36	0.34	0.33	0.31
10	0.29	0.24	0.20	0.19	0.18	0.17	0.17	0.16
15	0.20	0.16	0.14	0.13	0.12	0.11	0.11	0.11
20	0.15	0.12	0.10	0.10	0.09	0.09	0.08	0.08

Table I.4: Drift values for sideward sprayers for single and multiple applications

Sideward sprayers								
Distance [m]	No of applications							
	1	2	3	4	5	6	7	8 and more
	90th centile	82nd centile	77th centile	74th centile	72nd centile	70th centile	69th centile	67th centile
3	8.02	7.23	6.90	6.71	6.59	6.41	6.33	6.26
5	3.62	3.22	3.07	2.99	2.93	2.85	2.81	2.78
10	1.23	1.07	1.02	0.99	0.9	0.95	0.94	0.93
15	0.65	0.56	0.54	0.52	0.51	0.50	0.49	0.49
20	0.42	0.36	0.34	0.33	0.33	0.32	0.31	0.31
30	0.22	0.19	0.18	0.17	0.17	0.17	0.16	0.16
40	0.14	0.12	0.11	0.11	0.11	0.11	0.10	0.10
50	0.10	0.08	0.08	0.08	0.08	0.07	0.07	0.07

Table I.5: Drift values for air-blast equipment to plants with lower crop intercept for single and multiple applications

Air-blast equipment to plants with lower crop intercept								
Distance [m]	No of applications							
	1	2	3	4	5	6	7	8 and more
	90th centile	82nd centile	77th centile	74th centile	72nd centile	70th centile	69th centile	67th centile
3	29.20	25.53	23.96	23.61	23.12	22.76	22.69	22.24
5	19.89	16.87	15.79	15.42	15.06	14.64	14.45	14.09
10	11.81	9.61	8.96	8.66	8.42	8.04	7.83	7.58
15	5.55	5.61	5.23	4.91	4.61	4.51	4.40	4.21
20	2.77	2.59	2.36	2.21	2.09	2.04	1.99	1.91
30	1.04	0.87	0.77	0.72	0.69	0.66	0.65	0.62
40	0.52	0.40	0.35	0.32	00.31	0.30	0.29	0.28
50	0.30	0.22	0.19	0.17	0.17	0.16	0.16	0.15

Table I.6: Drift values for air-blast equipment to plants with higher crop intercept for single and multiple applications

Air-blast equipment to plants with higher crop intercept								
Distance [m]	No of applications							
	1	2	3	4	5	6	7	8 and more
	90th centile	82nd centile	77th centile	74th centile	72nd centile	70th centile	69th centile	67th centile
3	15.73	12.13	11.01	10.12	9.74	9.21	9.10	8.66
5	8.41	6.81	6.04	5.60	5.41	5.18	5.11	4.92
10	3.60	3.11	2.67	2.50	2.43	2.38	2.33	2.29
15	1.81	1.58	1.39	1.28	1.24	1.20	1.20	1.14
20	1.09	0.90	0.80	0.75	0.72	0.68	0.67	0.65
30	0.54	0.40	0.36	0.35	0.34	0.31	0.30	0.29
40	0.32	0.23	0.21	0.20	0.20	0.17	0.17	0.16
50	0.22	0.15	0.13	0.13	0.13	0.11	0.11	0.11

Table I.7: Drift values hop sideward sprayer for single and multiple applications

Hop sideward sprayer								
Distance [m]	No of applications							
	1	2	3	4	5	6	7	8 and more
	90th centile	82nd centile	77th centile	74th centile	72nd centile	70th centile	69th centile	67th centile
3	19.33	17.73	15.93	15.38	15.12	14.90	14.63	13.53
5	11.57	9.60	8.57	8.26	7.99	7.79	7.60	7.15
10	5.77	4.18	3.70	3.55	3.36	3.23	3.13	3.01
15	3.84	2.57	2.26	2.17	2.03	1.93	1.86	1.82
20	1.79	1.21	1.05	0.93	0.88	0.83	0.81	0.78
30	0.56	0.38	0.34	0.31	0.29	0.28	0.26	0.25
40	0.25	0.17	0.15	0.14	0.14	0.13	0.12	0.12
50	0.13	0.09	0.08	0.08	0.07	0.07	0.06	0.06

Appendix J – RUDs for Tier 1 spray applications

Introduction

In EFSA (2009), residues of plant protection products on food items were collected for different food items categories and from different sources. In 2018, EFSA published an external scientific report (Lahr et al., 2018) where a unified database of ecological and residue data was developed to be used for the risk assessment of plant protection products for birds and mammals. The main sources of data were the information submitted in the context of approval of active substances and authorisation of products and additional information retrieved through a systematic literature review. Therefore, in the present guidance document residue per unit dose (RUD) values were updated using the residue database published in EFSA scientific report, Lahr et al. (2018).

In the database the information gathered on residues focused on (initial) residue levels after treatment and the quality of the data were scored using criteria based on the study methods recommended by the EFSA guidance document for risk assessment for birds and mammals (EFSA, 2009). The reliability of each study was scored as code 1 (high quality), code 2 (medium quality), code 3 (low quality) or code 4 (quality not assignable, when insufficient information was provided in the description of the study to assign a quality code 1, 2 or 3). For the residue data summarised from the DAR/RAR it was only marked if the summary mentioned if the data were acceptable or not.

Therefore, in the present guidance document it was decided to use RUD values only from studies with codes 1, 2 and data summarised from the DAR/RAR. Non-GLP studies were included in this analysis as in Lahr et al. (2018), it was assumed that non-guideline data from e.g. academic laboratories, based on good principles in design, conduct, reporting and employing appropriate statistics, were of equal quality as studies performed by GLP compliant laboratories according to up-to-date test guidelines.

In general, the highest number of records in the databases came from residue studies on cereals, vegetables, maize, orchards, and vineyards. The database contained few data for food items such as weed/crop seeds, rice, bulbs and onion like crops, root and stem vegetables, sunflowers, and sugar beets. No RUD values were available for tubers, flower buds, tree nuts in orchards and tree in forestry, vegetables from legume vegetable crops, fruit from forests (biomass trees, broadleaf forest tree and coniferous forest trees), bananas, figs, kiwi, olives and pineapple. Therefore, for these crops surrogate RUD values were assigned based on the plant structure and the type of spray equipment used. For weed/crop seeds, it was decided to still use the RUD values reported in EFSA (2009) (surrogate values of mean: 40.2 mg/kg; 90th percentile: 87.0 mg/kg).

The resulting RUD values to be used for the screening step and/or for the first-tier risk assessment are presented for the different food items in the following tables. A descriptive statistical analysis is reported as well, showing the number of data available, the geometric mean, the mean, the confidence interval, the standard deviation, the median, the variance, the minimum and maximum values, and the 10th, 75th and 90th percentiles. Furthermore, the interquartile range (IQR), which is difference between the first (25th percentile) and the third (75th percentile) quartiles, was calculated to represent the middle spread of the data. The skewness and the kurtosis were reported to get information on the symmetry.

The geometric mean and 90th percentile of the available RUD values were derived to be used in the reproductive and acute assessments, respectively. Instead of using the arithmetic mean, as in EFSA (2009) it was decided to use the geometric mean, which is less influenced than the arithmetic mean by the extreme values in the distribution.

For the RUD values for fruits, besides the data collected by Lahr et al. (2018), a residue data set was submitted by CropLife Europe,[68] consisting of a series of trials investigating residue levels in fruits after spray application. The raw data produced by CropLife Europe were analysed and the Working Group agreed to combine with the data in Lahr et al. (2018) to derive RUD values in fruits.

Considering that it was not possible to access all the raw data used in EFSA (2009), it was decided to not use those data in the present Guidance Document to avoid double counting of the data. However, it should be highlighted that the new RUD values are based on a larger data set, relative to the RUD values reported in EFSA (2009). Furthermore, all RUD values contained in this version of the guidance have been assessed for reliability in a harmoised manner.

[68] Submitted to EFSA, March 2020.

Arthropods

RUD values were extracted from the database developed by Lahr et al. (2018), for ground-dwelling arthropods, foliar-dwelling arthropods and flying insects considering the:

- matrix analysed (column R of the database);
- diet category (column S of the database: arthropods soil, arthropods plant, not specified);
- application method (column L of the database: air blast sprayer, sprayed, tractor pulled air blast sprayer, boom sprayer);
- BBCH growth stage at day of application (column T of the database):
    - for ground-dwelling arthropods data were considered only for applications done on bare soil/ground, directed applications on top of crops with BBCH growth stage up to 20, and ground directed applications in orchards/vines (e.g. herbicide);
    - for foliar-dwelling arthropods and flying insects, the whole season was considered.

The results of the statistical analysis are reported in Table J.1, and the graphical representation of the data is reported in Figures J.1–J.3.

Laboratory studies and studies in which matrix and diet category were not specified were excluded. Furthermore, for ground-dwelling arthropods, studies in which both BBCH and month of application were not specified were excluded. The analysis of the data for foliar-dwelling arthropods showed one outlier (max RUD = 242 mg/kg, see Figure J.2); however, considering the overall distribution of the data and the fact that the outlier did not affect the geomean (with outlier: 8.9 mg/kg; without outlier: 8.4 mg/kg) and the 90th percentile (with outlier: 25.3 mg/kg; without outlier: 24.8 mg/kg), it was decided to exclude the outlier when deriving the RUD values for foliar-dwelling arthropods.

The category 'ground-dwelling arthropods with crop interception' (EFSA, 2009) was not considered in the present guidance document, because the level of interception occurred in the trials was considered uncertain.

Table J.1: RUD values for food items 'ground-dwelling arthropods', 'foliage-dwelling arthropods' and 'flying insects' (in the note RUD values from EFSA (2009), are reported for comparison)

Food items	Sample size	Geometric mean (mg/kg)	Arithmetic mean (mg/kg)	Confidence Level (95.0%)	Standard deviation	Median (mg/kg)	Variance	IQR	Skew-ness	Kurtosis	Min (mg/kg)	10th Percentile (mg/kg)	75th Percentile (mg/kg)	90th Percentile (mg/kg)	Max (mg/kg)	Range (mg/kg)
Ground-dwelling arthropods	30	**2.8**	8.4	4.6	12.4	1.9	154	11.9	2.2	5.5	0.11	0.52	13.0	**20.2**	53.8	53.7
Foliar-dwelling arthropods	53	**8.4**	13.6	3.5	12.6	11.4	158	10.0	2.1	5.0	0.24	1.4	15.8	**24.8**	58.9	58.6
Flying insects	14	**2.6**	4.8	4.3	7.4	1.7	55	3.1	2.9	9.1	0.78	1.0	4.5	**9.7**	28.5	27.7

In EFSA (2009) *RUD values for ground-dwelling arthropods without interception (n° data: 21; mean: 7.5 mg/kg; standard deviation: 12.0; 90th percentile: 13.8 mg/kg) and for foliar dwelling arthropods (n° data: 35; mean: 21.0 mg/kg; standard deviation: 21.6; 90th percentile: 54.1 mg/kg)*.

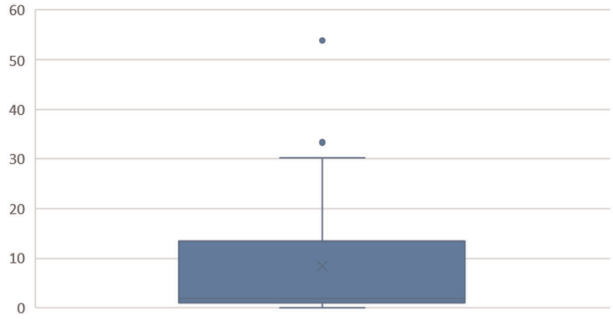


Figure J.1: Distribution of RUD values (mg/kg) for ground-dwelling arthropods

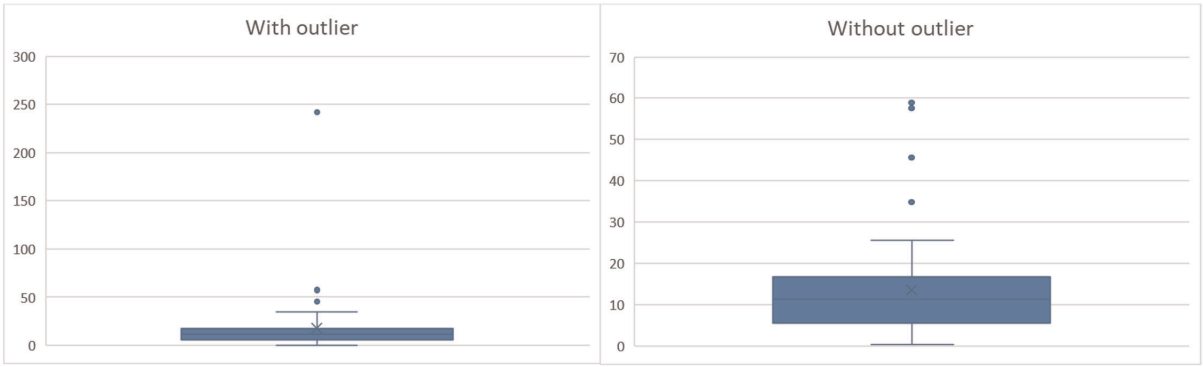


Figure J.2: Distribution of RUD values (mg/kg) for foliar-dwelling arthropods (with and without outlier)

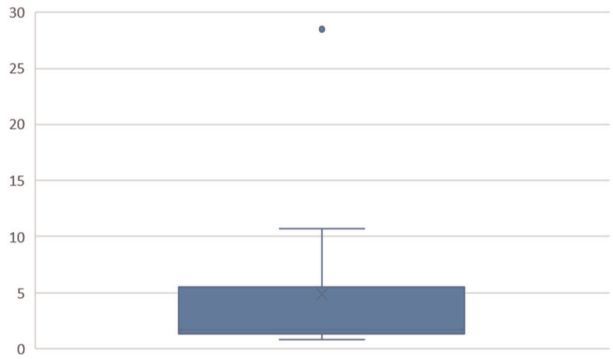


Figure J.3: Distribution of RUD values (mg/kg) for flying insects

Plants

RUD values were extracted from the database developed by Lahr et al. (2018), for monocotyledons (including wheat, grass, and barley), maize and dicotyledons considering the:

- crop group (column G of the database: for monocotyledons: cereals, grassland, maize; for dicotyledons: leafy vegetables, oilseed rape, sugar beet, pulses, etc…);
- matrix analysed (column R of the database: whole plants, immature plant, green material, leaves, foliage, etc…);
- diet category (column S of the database: monocotyledons, dicotyledons, not specified);
- application method (column L of the database: spray);
- BBCH growth stage at day of application (column T of the database):
  - for monocotyledons and maize data were considered only for BBCH growth stage up to 30 as only the first growth stages of the grasses and cereals are eaten; when the BBCH was not specified the month of application was taken into account
  - for dicotyledons, the whole season was taken into account.

The results of the statistical analysis are reported in Table J.2, and the graphical representation of the data is reported in Figures J.4–J.6.

Considering the large number of data available for maize and the difference in the RUD values for this crop compared to the other monocotyledons, it was decided to keep maize separated.

Only few data were available for rice and then considering the peculiarity of this crop, it was decided to not include data in rice in monocotyledons. If additional data will be available for rice, then specific RUD values will be derived. Dicotyledons were defined as non-grass 'weeds' in EFSA (2009).

Data coming from studies in which the application type was not specified, and the matrix was a varying mixture were excluded.

Table J.2: RUD values for plant food items (in the note RUD values from EFSA (2009), are reported for comparison)

Food items	Sample size	Geometric mean (mg/kg)	Arithmetic mean (mg/kg)	Confidence Level (95.0%)	Standard deviation	Median (mg/kg)	Variance	IQR	Skew-ness	Kurtosis	Min (mg/kg)	10th Percentile (mg/kg)	75th Percentile (mg/kg)	90th Percentile (mg/kg)	Max (mg/kg)	Range (mg/kg)
Monocotyledons (63% wheat, 24% grass, 13% barley)	218	**47.2**	61.6	5.5	40.9	50.9	1675	51.2	1.0	0.75	0.71	18.3	85.6	**117.8**	194.3	193.6
Maize	120	**29.7**	38.9	4.4	24.2	33.7	587	32.9	0.90	0.85	1.0	13.2	54.6	**71.3**	116.7	115.7
Dicotyledons	355	**21.9**	36.3	3.4	32.5	24.8	1058	37.2	1.4	1.6	0.1	6.3	49.5	**84.8**	153.4	153.4

In EFSA (2009) *RUD values for grass+cereals BBCH 10–30 (n° data: 132; mean: 54.2 mg/kg; standard deviation: 55; 90th percentile: 102.3 mg/kg) and for non-grass weeds (n° data: 230; mean: 28.7 mg/kg; standard deviation: 27.5; 90th percentile: 70.3 mg/kg).*

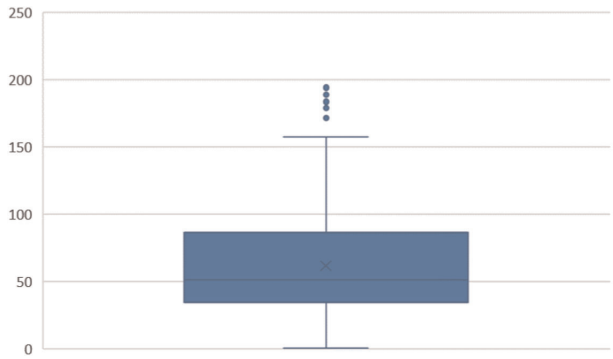


Figure J.4: Distribution of RUD values (mg/kg) for monocotyledons (wheat, barley and grass)

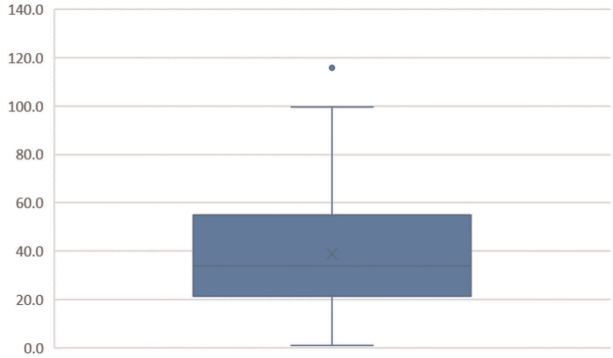


Figure J.5: Distribution of RUD values (mg/kg) for maize

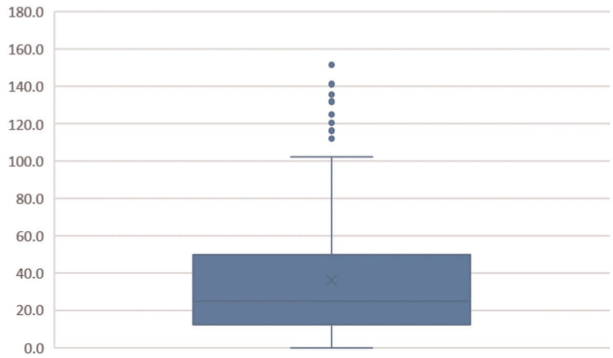


Figure J.6: Distribution of RUD values (mg/kg) for dicotyledons

Fruits

RUD values were extracted from the database developed by Lahr et al. (2018), considering the:

- crop group (column G of the database: vineyards, orchards, fruiting vegetables, strawberries);
- matrix analysed (column R of the database: fruit, bunch of grapes, grapes, berry);
- diet category (column S of the database: fruit);
- application method (column L of the database: spray);
- BBCH growth stage at day of application (column T of the database: BBCH growth stages of development of fruits (71–79) and maturity of fruits (BBCH 81–89)).

It was decided to align the crop groups used for fruits in EFSA (2009) to the EPPO classification. Therefore, crop groups 'large fruits from orchards' and 'berries' were not considered anymore and the category 'small fruits from orchards' was renamed as 'fruit from small fruit crops' and it includes residue data for berries (e.g.: black currants, blueberries, raspberries, etc…). Grapes were included in the crop group 'other berries' in EFSA (2009), but considering the number of data available and

following the EPPO classification it was decided to have a separate group for grapes. The crop groups 'stone fruits' (including apricots, peaches, plums, cherries), 'pome fruits' (including apples, pears) and 'citrus fruits' (including oranges, mandarins, lemons) were considered. Furthermore, the crop group 'gourds' was renamed as 'fruiting cucurbitaceous vegetable crops' (see Appendix E). Tomatoes were included in the crop group 'fruiting solanaceous vegetable crops'; for this crop group data were available only for tomatoes and peppers in the database by Lahr et al. (2018). No data on 'fruit from small fruit crops' (such as black currant, blueberry, raspberry, etc.) were available in the database by Lahr et al. (2018).

The results of the statistical analysis are reported in Table J.3, and the graphical representation of the data is reported in Figure J.7.

Table J.3: RUD values for fruits food items from database by Lahr et al. (2018) (in the note RUD values from EFSA (2009), are reported for comparison)

Food items	Sample size	Geometric mean (mg/kg)	Arithmetic mean (mg/kg)	Confidence Level (95.0%)	Standard deviation	Median (mg/kg)	IQR	Skew-ness	Kurtosis	Min (mg/kg)	10th Percentile (mg/kg)	75th Percentile (mg/kg)	90th Percentile (mg/kg)	Max (mg/kg)	Range (mg/kg)
Citrus fruits (36% mandarins, 64% oranges)	22	**4.4**	10.9	6.1	13.8	3.6	15.2	1.6	1.5	0.5	0.6	16.6	**31.8**	47.8	47.3
Fruiting cucurbitaceous vegetable crops (cucumbers 84%, courgettes 16%)	37	**1.2**	3.7	1.5	4.6	1.0	9.4	0.9	−1.2	0.1	0.2	9.7	**11.0**	11.3	11.3
Fruiting solanaceous vegetable crops (88% tomatoes, 12%peppers)	97	**0.73**	1.1	0.25	1.2	0.71	0.76	2.90	10.3	0.04	0.27	1.25	**2.5**	7.5	7.5
Grapes	177	**3.1**	3.7	0.39	2.6	3.3	2.5	3.5	23.3	0.27	1.4	4.7	**6.6**	24.7	24.4
Pome fruits (81% apples, 19% pears)	42	**1.8**	2.2	0.4	1.3	2.0	1.4	1.0	0.8	0.4	0.7	2.8	**4.2**	6.0	5.6
Stone fruits (23% peaches, 15% apricots, 31% cherries, 31% plums)	26	**1.4**	1.9	0.6	1.4	1.3	1.4	1.4	1.5	0.2	0.6	2.4	**3.8**	5.6	5.4
Fruit from small fruit crops (berries)	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Strawberries	42	**1.0**	1.2	0.22	0.70	1.1	0.83	1.6	3.3	0.25	0.52	1.5	**1.9**	3.7	3.5

In EFSA (2009), RUD values for small fruits from orchards (n° data: 33; mean: 3.3; standard deviation: 2.6; 90th percentile: 6.5), large fruits from orchards (n° data: 33; mean: 19.5 mg/kg; standard deviation: 16.8; 90th percentile: 41.1 mg/kg), tomatoes (n° data: 86; mean: 12.8 mg/kg; standard deviation: 14.6; 90th percentile: 30.6 mg/kg), fruiting cucurbitaceous vegetable crops (named as gourds in EFSA (2009); n° data: 19; mean: 34.3 mg/kg; standard deviation: 54.7; 90th percentile: 61.5 mg/kg).

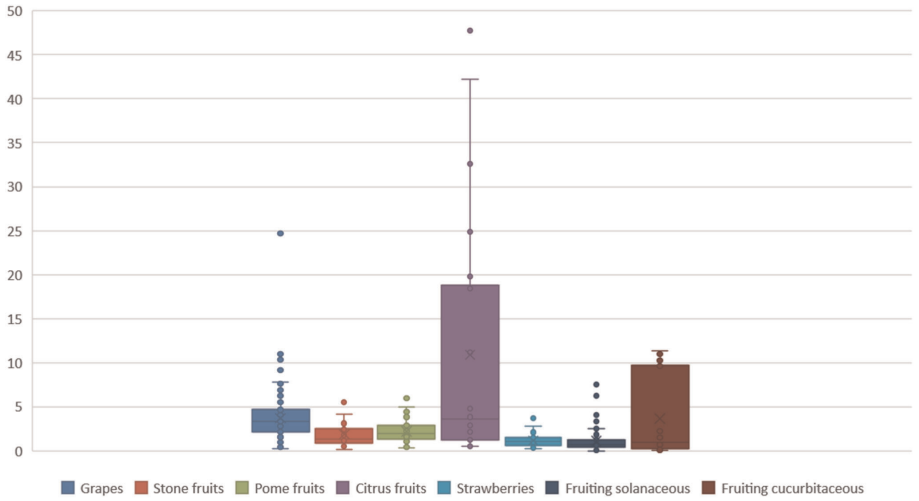


Figure J.7: Distribution of RUD values (mg/kg) for fruits from database by Lahr et al. (2018)

A large residue data set was submitted by Croplife Europe, consisting of a series of trials investigating residue levels in fruits at multiple time points after spray application. The raw data produced by CropLife Europe were analysed, each study was evaluated against a set of criteria, which were aligned to Lahr et al. (2018).

However, first a set of screening (exclusion) criteria was used according to the flowchart shown in Figure J.8. Furthermore, data were excluded whenever there was evidence of control contamination and studies which were already included in Lahr et al. (2018) were excluded from the analysis (i.e. duplicates were identified by searching for study author names, year of publication and study ID).

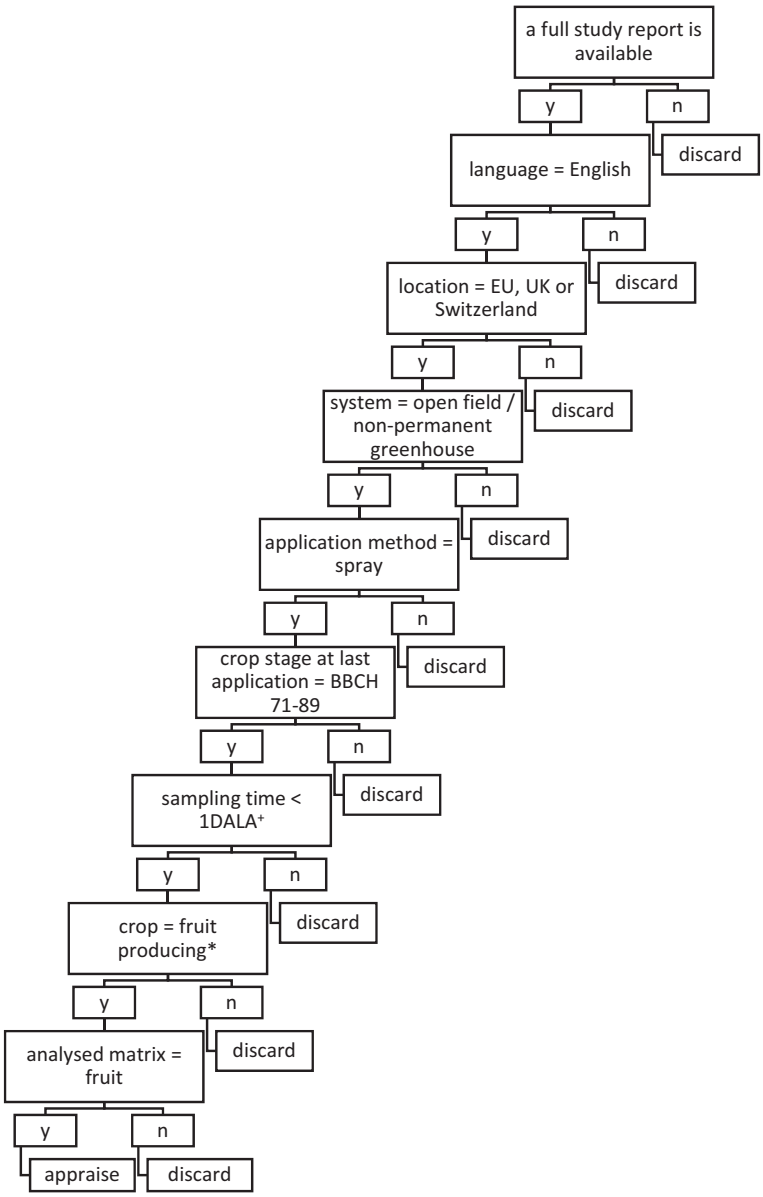


Figure J.8: Screening assessment. +DALA: days after the last application. *Fruit crops (European Commission, 2019): Fruiting vegetables (i.e., solanaceous and cucurbitaceous); sweet corn; citrus fruits; tree nuts; pome fruits; stone fruits; fruit **from small fruit crops; grapes; strawberries**

In addition to the screening outlined above, the following appraisal criteria were used to assess the study quality:

1) the sampling/storage methodology clearly described:
   yes --> 1
   more or less (i.e. some critical information missing) --> 2
   no --> 3
2) damaged samples were excluded from the analysis:
   yes --> 1
   unclear/not reported --> 2
   no --> 3
3) the sampling was randomly performed and/or the samples at the extreme edge of the field were excluded:
   yes --> 1
   not reported --> 2
   no --> 3
4) the report contains the following information: plot dimensions, plant density, trial site history (PPP use), application equipment details; weather data at application (e.g. average temp and rainfall); method of sampling; sampling collection; sampling storage stability; analytical method; crop health; growing conditions; cultivation; maintenance chemicals applied during trial period; irrigation; weather conditions for the duration of the trial:
   yes --> 1
   most of these information --> 2
   no --> 3
5) the distance between replicates was large enough to prevent contamination:
   yes -> 1
   unclear --> 2
   no --> 3

An overall quality score was assigned to each RUD value, which was calculated as the cumulative rating from appraisal criteria 1 to 5, as described above.

The data extraction resulted in 1065 valid RUD values from 15 countries (i.e. Austria, Belgium, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, Poland, Portugal, Spain, Switzerland, The Netherlands, United Kingdom). The representative crop species are listed in Table J.4 and the representative active ingredients are listed in Table J.5.

Table J.4: Number of RUD values for crop groups in CropLife data set

Crop	Crop group	n. of RUD values
Grapes	grapes	148
Black currant	Fruit from small fruit crops	64
Gooseberry	Fruit from small fruit crops	5
Red currant	Fruit from small fruit crops	15
Raspberry	Fruit from small fruit crops	80
Courgette	fruiting cucurbitaceous vegetable crops	57
Cucumber	fruiting cucurbitaceous vegetable crops	87
Melon	fruiting cucurbitaceous vegetable crops	153
Watermelon	fruiting cucurbitaceous vegetable crops	13
Apricot	stone fruits	76
Peach	stone fruits	9
Plum	stone fruits	58
Sweet cherry	stone fruits	54
Sour cherry	stone fruits	15
Strawberry	strawberry	140
Lemon	citrus fruits	7
Mandarin	citrus fruits	9
Orange	citrus fruits	15
Apple	pome fruits	54
Pear	pome fruits	6

Table J.5: Number of RUD values for active substances in CropLife data set

Active substance	Category	n. of RUD values
abamectin	insecticide	2
acetamiprid	insecticide	8
ametoctradin	fungicide	20
azoxystrobin	fungicide	17
boscalid	fungicide	33
bupirimate	fungicide	19
chlorantraniliprole	fungicide	22
copper	fungicide	9
cyflufenamid	fungicide	12
cyproconazole	fungicide	4
cyprodinil	fungicide	19
deltamethrin	insecticide	47
difenoconazole	fungicide	38
dimethomorph	fungicide	36
dithianon	fungicide	20
ethephon	growth regulator	9
fenhexamid	fungicide	16
fenoxycarb	fungicide	2
fludioxonil	fungicide	11
fluopicolide	fungicide	13
fluopyram	fungicide	105
imidacloprid	insecticide	46
iprovalicarb	fungicide	15
lambda cyhalothrin	fungicide	23
mandipropamid	fungicide	4
mandipropamid	fungicide	4
metiram	fungicide	20
penconazole	fungicide	18
pirimicarb	insecticide	4
propamocarb hydrochloride	fungicide	25
pymetrozine	insecticide	1
pyraclostrobin	fungicide	32
pyrimethanil	fungicide	8
spirodiclofen	acaricide	81
tebuconazole	fungicide	102
thiacloprid	insecticide	76
thiamethoxam	insecticide	16
tolylfluanid	fungicide	10
triadimenol	fungicide	7
trifloxystrobin	fungicide	115

A graphical representation of the distribution of the RUD values across Europe is shown in Figure J.9.

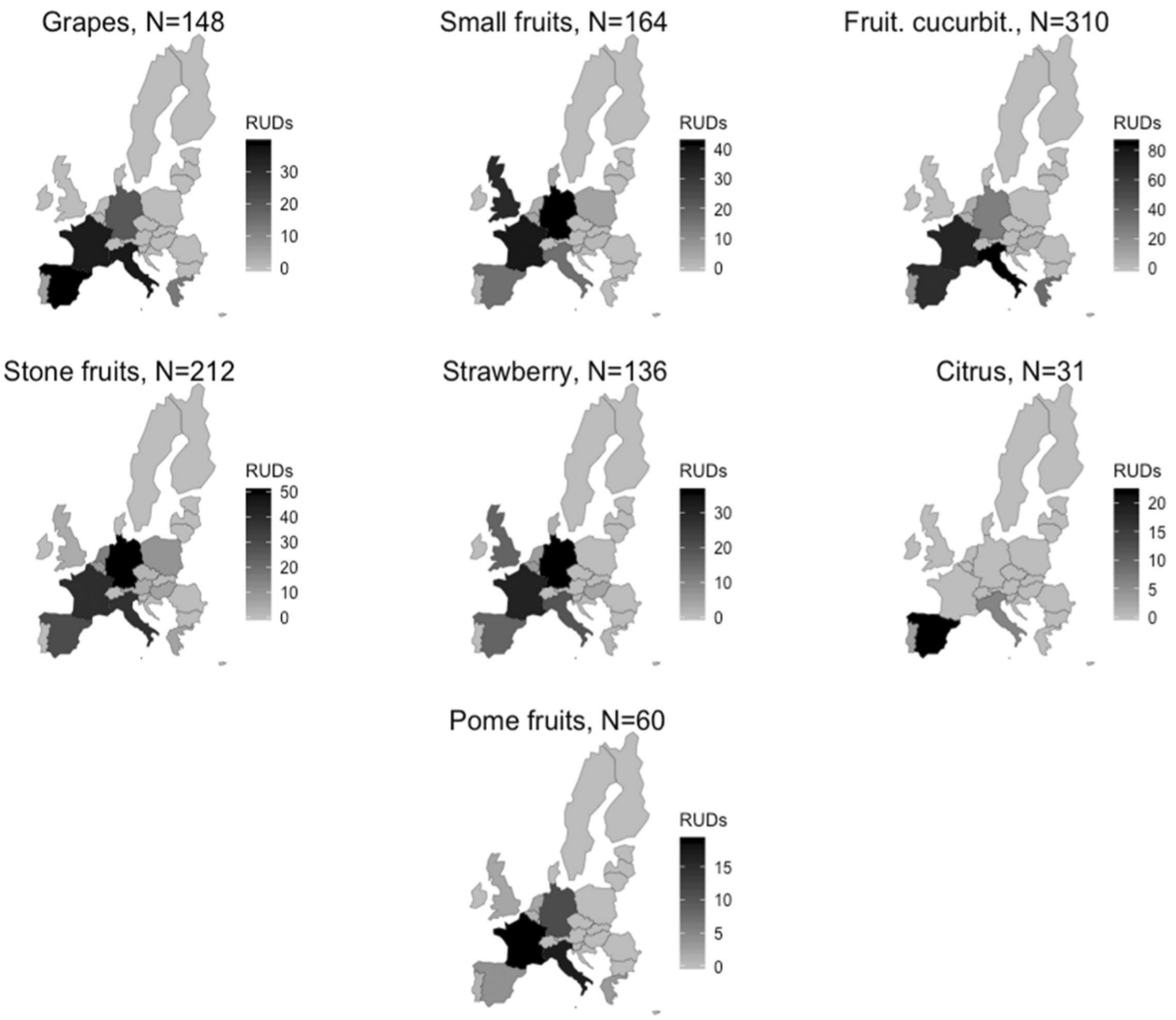


Figure J.9: Geographical distribution of RUD values aggregated by crop groups (EPPO classification)

For consistency with the analysis done by Lahr et al. (2018) in the analysis of the CropLife data set it was considered that:

- trials where rain events occurred were retained, regardless of when they happened or their intensity
- for trials where the product was applied several times at timely intervals, the first residue after the last application was used for the RUD derivation
- when a measurement was below the limit of quantification, the RUD was calculated assuming that the residue level was 1/2 the limit of quantification.

The distribution of RUD values across crops and crop groups is reported for both the CropLife and Lahr et al. (2018) data sets in Figures J.10 and J.11, respectively.

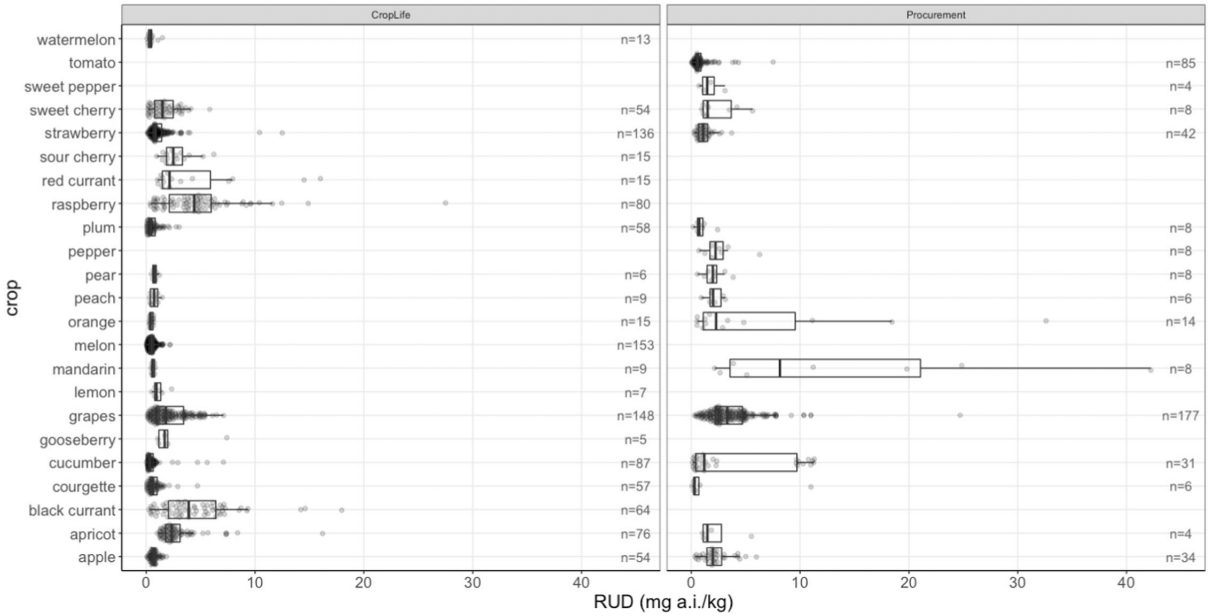


Figure J.10: Distribution of RUD values (mg/kg) across crop species in the CropLife (left) and Lahr et al. (2018) (right) data sets

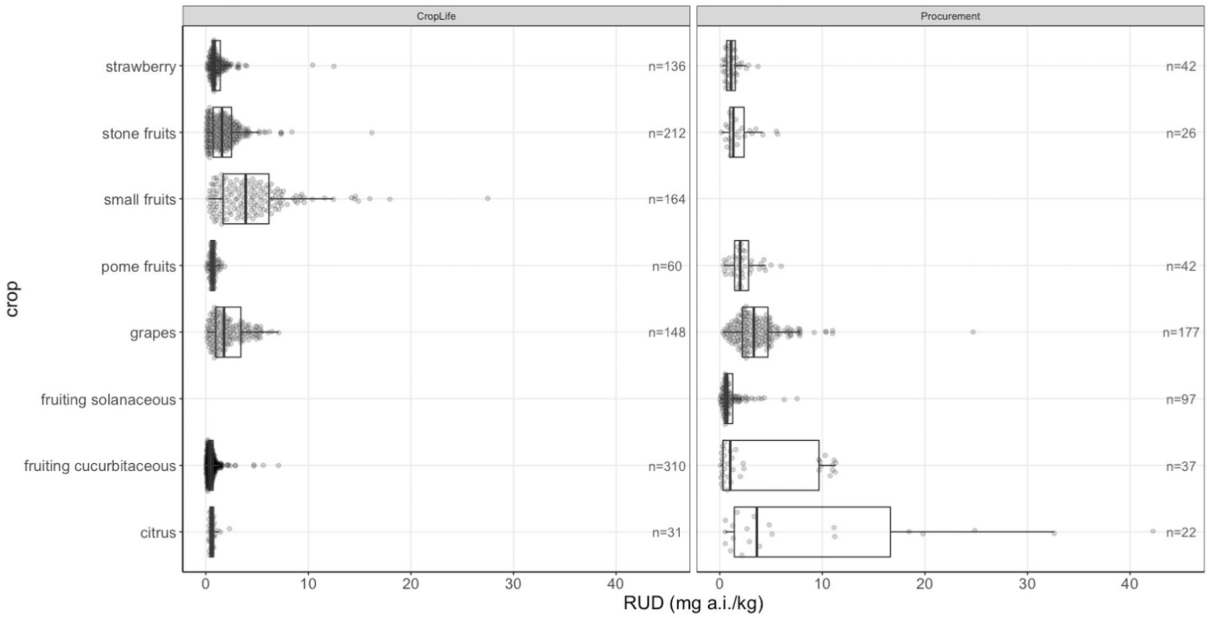


Figure J.11: Distribution of RUD values (mg/kg) across crop groups of CropLife (left) and Lahr et al. (2018) (right) data sets

A descriptive statistical analysis of the separated and of the combination of two data sets is reported in Table J.6.

Table J.6: Statistical analysis of the RUD values derived from the CropLife and the Lahr et al. (2018) data sets aggregated by crop categories

Data set	Crop group	Min (mg/kg)	1st quartile (mg/kg)	Median (mg/kg)	Arithmetic Mean (mg/kg)	3rd quartile (mg/kg)	Max (mg/kg)	Geometric mean (mg/kg)	90th percentile (mg/kg)	95th percentile (mg/kg)	Sample size
CropLife	**Citrus fruits**	0.27	0.4	0.58	0.66	0.76	2.33	0.58	0.93	1.33	31
	Fruiting cucurbitaceous vegetable crops	0.01	0.26	0.48	0.61	0.71	7.1	0.42	1.07	1.07	310
	Fruiting solanaceous vegetable crops	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0
	Grapes	0.1	0.97	1.79	2.22	3.43	7.11	1.61	4.82	5.27	148
	Pome fruits	0.07	0.47	0.67	0.73	0.87	1.86	0.62	1.24	1.43	60
	Stone fruits	0.03	0.71	1.59	1.88	2.53	16.2	1.2	3.59	1.28	212
	Fruit from small fruit crops	0.24	1.71	3.9	4.84	6.16	47.9	3.3	8.88	12.3	164
	Strawberries	0.1	0.64	0.85	1.26	1.41	12.5	0.93	2.12	3.2	136
Lahr et al. (2018)	**Citrus fruits**	0.53	1.41	3.6	10.9	16.63	47.78	4.44	31.8	41.7	22
	Fruiting cucurbitaceous vegetable crops	0.06	0.3	1.02	3.65	9.68	11.31	1.23	11.0	11.2	37
	Fruiting solanaceous vegetable crops	0.04	0.49	0.71	1.11	1.25	7.52	0.73	2.52	3.47	97
	Grapes	0.3	2.2	3.3	3.72	4.7	24.71	3.06	6.64	7.8	177
	Pome fruits	0.35	1.44	1.98	2.21	2.79	5.98	1.83	4.24	4.44	42
	Stone fruits	0.18	0.97	1.32	1.89	2.36	5.63	1.44	3.84	5.19	26
	Fruit from small fruit crops	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0
	Strawberries	0.25	0.67	1.09	1.18	1.51	3.71	1.0	1.91	2.56	42
CropLife + Lahr et al. (2018)	**Citrus fruits**	0.27	0.5	0.76	4.91	2.65	47.78	**1.34**	**17**	28	53
	Fruiting cucurbitaceous vegetable crops	0.01	0.27	0.5	0.94	0.79	11.31	**0.47**	**1.4**	2.38	347

Data set	Crop group	Min (mg/kg)	1st quartile (mg/kg)	Median (mg/kg)	Arithmetic Mean (mg/kg)	3rd quartile (mg/kg)	Max (mg/kg)	Geometric mean (mg/kg)	90th percentile (mg/kg)	95th percentile (mg/kg)	Sample size
	Fruiting solanaceous vegetable crops	0.04	0.49	0.71	1.11	1.25	7.52	**0.73**	**2.52**	3.47	97
	Grapes	0.1	1.46	2.6	3.04	4.19	24.71	**2.28**	**5.33**	6.86	324
	Pome fruits	0.07	0.54	0.87	1.34	1.86	5.98	**0.97**	**2.87**	3.99	102
	Stone fruits	0.03	0.73	1.55	1.88	2.52	16.2	**1.22**	**3.61**	4.5	238
	Fruit from small fruit crops	0.24	1.71	3.9	4.84	6.16	47.95	**3.3**	**8.88**	12.3	164
	Strawberries	0.1	0.64	0.91	1.24	1.46	12.5	**0.95**	**2.1**	3.03	178

The distribution of RUD values from the CropLife data set is different than the one Lahr et al. (2018) (see Figure J.12 and Table J.7, Wilcoxon, W = 167210, p < 2.2e-16). However, considering that two data sets were not done on the same crops is acceptable that they are not fully comparable.

Table J.7: Descriptive statistics of the data sets by CropLife and Lahr et al. (2018) combining all crop groups

Data set	Min (mg/kg)	1st quartile (mg/kg)	Median (mg/kg)	Arithmetic Mean (mg/kg)	3rd quartile (mg/kg)	Max (mg/kg)	Geometric mean (mg/kg)	90th percentile (mg/kg)	95th percentile (mg/kg)	Sample size
CropLife	0.01	0.48	0.91	1.84	2.2	47.95	0.98	4.44	6.13	1061
Lahr et al. (2018)	0.04	0.8	1.91	3.01	3.71	47.78	1.73	5.69	9.68	443
CropLife + Lahr et al. (2018)	0.01	0.53	1.13	2.18	2.67	47.95	1.16	4.9	6.88	1504

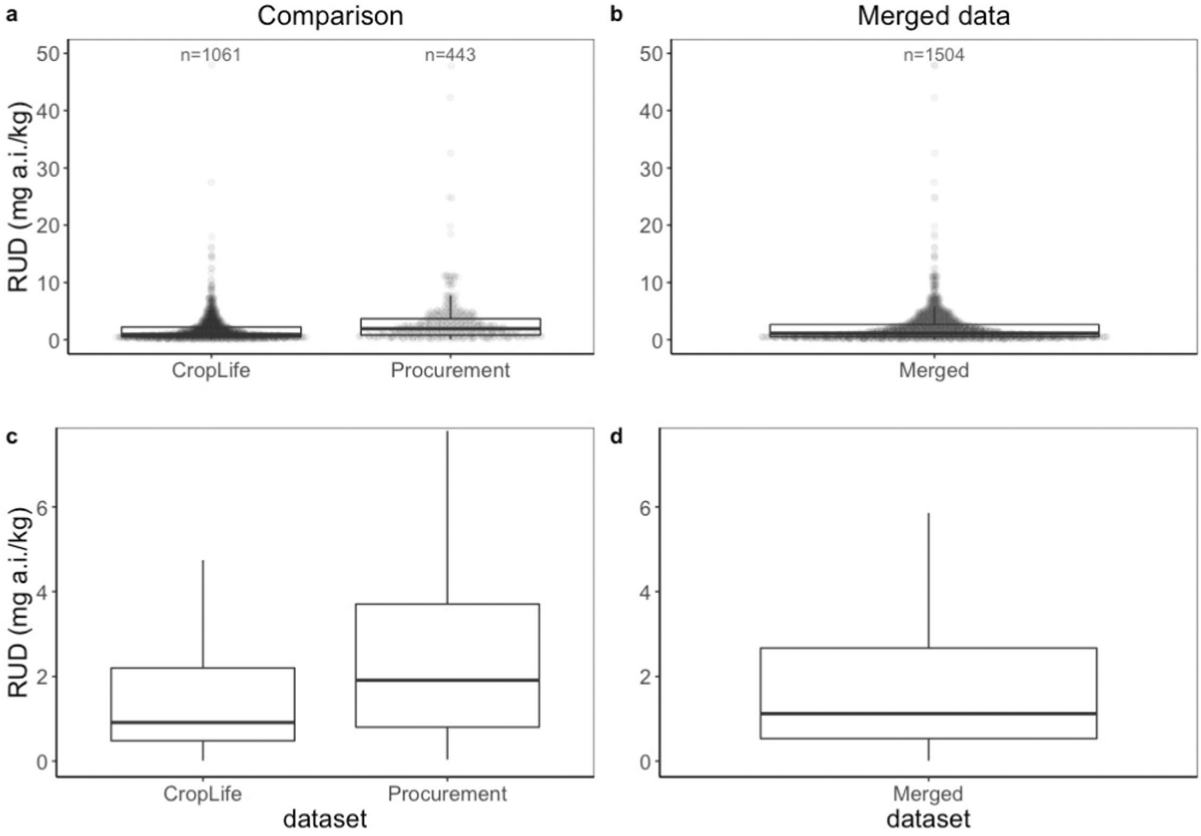


Figure J.12: Distribution of RUD values of the data sets by CropLife and Lahr et al. (2018) separated (a, c) or merged (b, d). Boxplots plotted against raw data (a, b) or alone (c, d)

Overall, it was decided to merge the RUD values derived from the CropLife and Lahr et al. (2018) data sets as this will give more robust values. A summary of the combined RUD values for fruits is shown in Table J.8. It should be noted that no RUD values were available flower buds, tree nuts in orchards and tree seeds in forestry, fruit from forests (biomass trees, broadleaf forest tree and coniferous forest trees), legume vegetables, bananas, figs, kiwifruits, olives and pineapple. Therefore, for these crops surrogate RUD values were assigned based on the plant structure and the type of spray equipment used.

Table J.8: Summary of the geometric mean and 90th percentile of the RUD values for fruits to be used in the reproductive and acute assessments, respectively

Crop group	Sample size	Geometric mean (mg/kg)	90th percentile (mg/kg)	Standard deviation	Variance	Coefficient of variation	Source
Citrus fruits	53	1.34	17	10.4	107	211	CropLife + Lahr et al. (2018)
Fruiting cucurbitaceous vegetable crops	347	0.47	1.4	1.88	3.52	200	CropLife + Lahr et al. (2018)
Fruiting solanaceous vegetable crops	97	0.73	2.52	1.22	1.5	110	Lahr et al. (2018)
Grapes	324	2.28	5.33	2.33	5.42	76.7	CropLife + Lahr et al. (2018)
Pome fruits	102	0.97	2.87	1.14	1.3	85.3	CropLife + Lahr et al. (2018)
Fruit from small fruit crops	164	3.3	8.88	5.10	26	105	CropLife
Stone fruits	238	1.22	3.61	1.75	3.05	92.6	CropLife + Lahr et al. (2018)
Strawberries	178	0.95	2.1	1.32	1.75	107	CropLife + Lahr et al. (2018)
Bananas	0	1.34	17	10.4	107	211	Surrogate from 'citrus fruits'
Figs	0	1.34	17	10.4	107	211	Surrogate from 'citrus fruits'
Flower buds	0	3.3	8.88	5.10	26	105	Surrogate from 'fruit from small fruit crops'
Fruits from forests (biomass trees, broadleaf forest trees, coniferous forest trees)	0	3.3	8.88	5.10	26	105	Surrogate from 'fruit from small fruit crops'
Legume vegetables	0	0.73	2.52	1.22	1.5	110	Surrogate from 'fruiting solanaceous'
Kiwifruits	0	1.34	17	10.4	107	211	Surrogate from 'citrus fruits'
Olives	0	2.28	5.33	2.33	5.42	76.7	Surrogate from 'grapes'
Pineapples	0	0.73	2.52	1.22	1.5	110	Surrogate from 'fruiting solanaceous'
Tree nuts/ seeds	0	1.22	3.61	1.75	3.05	92.6	Surrogate from 'stone fruits'

Appendix K – Residue decline on food items following spray applications

Plant dissipation half-life (DT_{50})

Pesticide dissipation rates in/on various plant matrices are used in regulatory environmental and human risk assessments and different databases have been collated in the past to derive default values for Tier 1 assessments. The EFSA Guidance Document on risk assessment for birds and mammals (EFSA, 2009) recommends a default value of 10 days for residue decline on sprayed plant foliage in order to calculate multiple-application factors (MAF) and the time-weighted average (TWA) factor in the dietary exposure assessment. This value was based on an analysis of the data collected by Willis and McDowell (1987), who reviewed approximately 450 DT_{50} values for a broad spectrum of vegetative plant materials. However, the analysis was conducted by considering the mean values only and the respective standard deviations. The authors of the Guidance already identified some uncertainties regarding this conservative value, taking into consideration that the information available at that time indicated that 'most pesticides have DT_{50}s below 10 days' and that 'dissipation in first few days is often faster than implied by assumption of first order kinetics'. Charles (2004) proposed a default half-life of 5 days for pesticides on plant surfaces based on the same data set from Willis and McDowell (1987).

Although the 10 days value for plant DT_{50} is routinely used in the risk assessment for birds and mammals, it may be argued that this value is not conservative enough to be implemented at Tier 1. In contrast, it could be suggested that the default value was derived from field residue trials conducted in non-EU countries, including also the application of persistent substances that are no longer on the EU market and therefore not representative of the current situation (Ebeling and Wang, 2018).

In the last years several data sets on plant residue decline of pesticides have become available as part of standard residue trials for MRL (maximum residue limit) or from specific residue decline trials conducted to refine the risk assessment to birds and mammals or the exposure assessment for workers. The reported data presents a very large variability due to the different physico-chemical properties of the compound, on the type of the matrix, the physiology and morphology of the plant/crop, environmental conditions and if the residues are measured 'on' or 'in' the plant matrix. It should also be considered that, pending on the purpose of the study, different experimental designs, sampling methods and regime, sample processing and interpretation of the measured data, etc. are considered. For example, half-lives may refer to dissipation during plant growth, thus including growth dilution or to the period after harvest only. In the risk assessment for birds and mammals, the persistence of pesticide on/in the plant treated is relevant considering the period where the plant is consumed by birds and mammals, i.e. small enough to be palatable.

Therefore, a narrative review of the data available in EFSA was carried out to further investigate the adequacy of a default plant DT_{50} value of 10 days for the calculations of MAF and fTWA in the light of the information available nowadays.

1) Lewis and Tzilivakis (2017)

Lewis and Tzilivakis (2017) presents a large database on pesticide dissipation rates in various plant matrices (e.g. leaves, stems, seeds, fruits), based on 1390 published studies and comprising more than 400 compounds and over 200 plants. Records for unique pesticide–plant matrix combination are reported for pesticide residues on the matrix surface as well as total residues (i.e. on the matrix and absorbed within the plant material). In the data set provided as a supplementary MS Excel file, the dissipation rates are reported as arithmetic mean for the pesticide–plant matrix combination as reported in the published literature. Where sufficient experimental data were available within the article, Lewis and Tzilivakis (2017) determined the half-lives via first-order kinetics. Where the article reported more than one experiment on the same pesticide–plant matrix combination, the data range across experiments was reported. As a result, the data set includes 2713 rows, each of which reported minimum, maximum and mean DT_{50} values (where only a single value was available, then minimum, maximum and mean values reported are the same). The single DT_{50} values ranged from 0.02 to 918 days, with the average of the arithmetic means of 7.5 days, the median of 4.0 days and the 90th percentile of 13.8 days. The whole data set is illustrated in Figures K.1 and K.2.

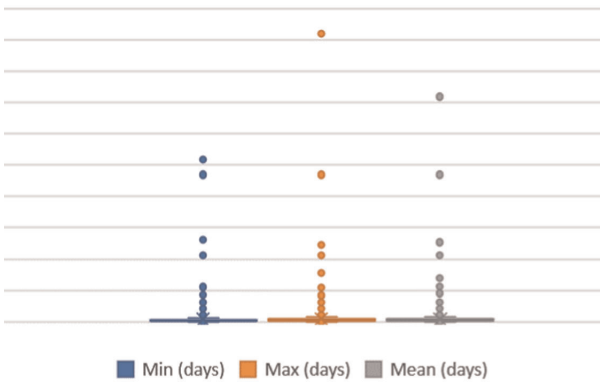


Figure K.1: Plant DT_{50} values (days) based on the whole data set by Lewis and Tzilivakis (2017)

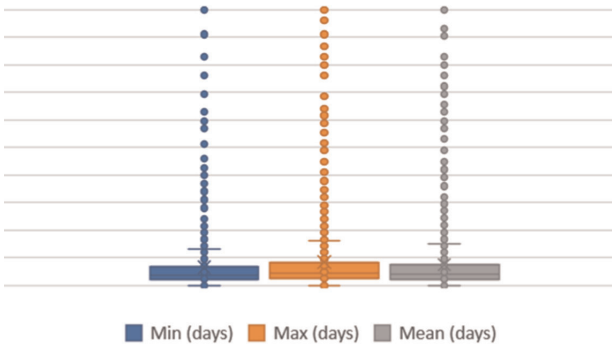


Figure K.2: Plant DT_{50} values (days) up to 100 days based on the whole data set by Lewis and Tzilivakis (2017)

It is worth noting that the residue decline rates higher than 10 days and measured under European open field conditions represent only the 0.6% of the whole data set. It can be considered that this data set confirms that a DT_{50} of 10 days would cover most of the cases.

It should be noted that the article by Lewis and Tzilivakis (2017) does not include many details regarding some important methodological aspects like the method of sampling, method of extraction, number of sampling points, analytics, kinetics used, weather conditions under which the experimental data were measured, etc. To fully understand those details, the original articles should be consulted (the references are provided) and carefully scrutinised. Although the authors included in their review protocol a number of criteria that the published study needed to comply with in order to ensure a sufficient quality of the data, this evaluation check was not performed. Therefore, the uncertainties associated with the quality of this data set remain unresolved.

2) Lahr et al. (2018)

In 2018 EFSA published an external scientific report (Lahr et al., 2018) where a unified database of ecological and residue data was developed to be used for the risk assessment of plant protection products for birds and mammals. Data were collected from regulatory papers submitted for pesticides approval/authorisation and from public literature bibliographies. The External Scientific Report drew by Lahr et al. and the three associated databases (organised in Excel files) were published in 2018. The database collating residue data focussed on (initial) residue levels after treatment in crops, in insects, weeds, seeds, etc., and on residue decline. Almost 3000 records regarding 190 active substances and metabolites are reported. Each record may contain one or more quantitative data. The original Excel file contains significant information about the study, including the methods used, conditions during the trials, parameter values and a global assessment of the data quality. Therefore, in the context of the present Guidance Document, the data set on the residue decline was filtered by considering only records with:

1) reported DT_{50} values (column AG in the original Excel file);
2) a diet category assigned to dicotyledons and monocotyledons (column S in the original Excel file);
3) all the relevant plant matrices except for hay, pods, treated corn seeds (column R in the original Excel file).

The remaining matrices are presented in Table K.1.

Table K.1: Sampled matrices considered to calculate plant DT_{50} (extracted from the original MS Excel file produced by Lahr et al. (2018))

Broad-leaved weeds	Green material	Plants	Whole plant
Cereal shoots	Green over-ground plant material	Seedling	Whole plant (no roots)
English rye grass and others	Leaf with root collar	Seeds and seedlings	Whole plant no roots

Broad-leaved weeds	Green material	Plants	Whole plant
Foliage	Leaves	Shoots	Whole plant without roots
Foliage, stem and leaves	Leaves, foliage	Tops	Whole plant, no roots
Forage	Lettuce	Turf between tree rows in apple orchard	Whole plants
Grass	Non-crop ground foliage	Varying mixture of grass species (monocots) and maize leaves	Whole plants without roots
Grass leaves	Not specified	Varying mixture of grass species (monocots), broadleaf weed species (dicots) and maize leaves	
Grass, above soil	Perennial rye grass and others	Weeds	
Grass, no details given	Plant	Weeds, short vegetation	

Following the data filtering procedure, a total of 230 records were retained. In this selected data set, the DT_{50} values ranged from 0.16 days to 60 days, with an arithmetic mean of 4.2 days (SD: 5.82) and a median value of 2.7 days. The distribution of the whole data set is illustrated in Figure K.3.

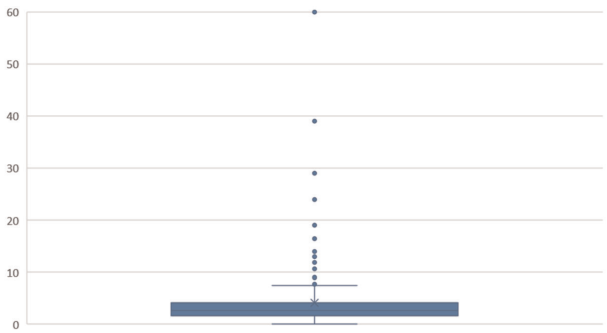


Figure K.3: Distribution of the plant DT_{50} values (days) from Lahr et al. (2018)

Only 16 DT_{50} values were above 10 days and were dominated by peanut leaves/foliage and cereals 'whole plant' samples, confirming that a DT_{50} of 10 days covers most cases.

It should be noted that this data set presents some uncertainties, as well, such as the exclusion of potentially reliable DT_{50} values that were not estimated/reported in the report but which could have been estimated from the time-series of residues provided, etc.

3) Additional information

Ebeling and Wang (2018) conducted an evaluation of regulatory foliage residue dissipation trials covering 30 compounds and 396 field residue trails from various crops (Figure K.4).

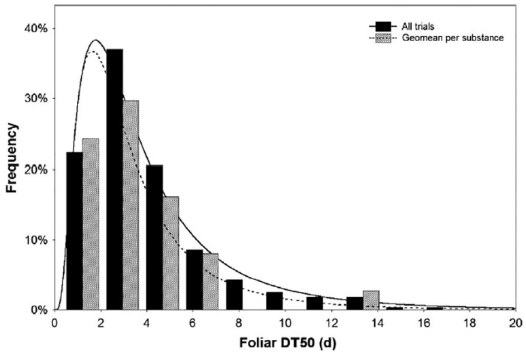


FIGURE 1: Distribution of foliar dissipation half-life values for all trials (approach A) and for all compounds (approach B, geometric means per compound). DT50 = dissipation half-life.

Figure K.4: Distribution of foliar dissipation half-life values evaluated by Ebeling and Wang (2018) (original figure from Ebeling and Wang, 2018)

Besides estimating the foliar residue decline under field conditions, the authors tried to address those factors that potentially determine the dissipation process (i.e. crop group, residue zone and rainfall). In this case, however, in the reliability criteria, trials with measurements for only 3 time points were included, which is not recommended by current regulatory practice (EFSA, 2019b). Nevertheless, over all compounds, trials, zones, and crops the geometric mean foliar DT_{50} was 3.2 days and the 90th percentile was 7.9 days.

Fantke and Juraske (2013) reviewed 811 scientific literature sources published between 1956 and 2012, providing 4513 dissipation half-lives of 346 pesticides measured in experimental studies with 183 plant species, worldwide. The data set, available online, includes dissipation half-lives in plants and on plant surfaces, based on different models to fit residual pesticide concentration curves. As the authors were mainly interested in residues for human exposure, data from sampled plant matrixes such as straw, fruit pulp, tree bark are included. Due to time constraints, no further attempts to further filter the original data set were made by the Working Group. The 95% confidence interval of pesticide dissipation half-lives in plants across all 4513 considered data points for 1485 pesticide–plant species combinations ranges from 0.6 to 28.7 days. Overall, measured half-lives in harvested plant materials ranged from around 1 h for pyrethrins in leaves of tomato and pepper fruit to 918 days for pyriproxyfen in pepper fruits under cold storage conditions, with a mean DT_{50} value of 6.98 days and a median value of 3.89 days.

Conclusions

Overall, the amount of information available is considerable, in terms of number of compounds and plant species, variety of plant components and tissues investigated and diversity of environmental conditions. However, depending upon the final aim of the review in question, different methodologies for collecting, screening, assessing and processing the data were applied. The main quality criteria applied by the different authors and affecting the structure of each data set are:

- Locations of the residue trials (EU studies vs. non-EU studies)
- Regulatory status of the active substance (only currently EU approved substances vs. including substances no longer on the market)
- Relevance of the plant species investigated for the specific exposure assessment where dissipation rates are intended to be used (i.e. workers, consumers, non-target and beneficial organisms)
- Relevance of the plant matrix(ces) for the specific exposure assessment where dissipation rates are intended to be used
- Type of sampling of the plant matrix (measurements 'ON' the matrix vs. measurements 'IN' the matrix)
- Lack and diversity of methodological information (LOD/LOQ, field recovery…) within the retrieved data points
- Availability of raw data to verify the reported DT50 calculations or to perform new kinetic evaluations

- Quality requirements for kinetic evaluation (e.g. minimum number of sampled times after pesticide application and kinetic models used)

It is clear that a proper evaluation of all the available data would require the setting of a fit-for-purpose protocol where quality and assessment criteria are harmonised. In some reviews, the required information is reported in the data set together with the raw data of the measured residues, while for others it would be necessary to check each original study report/publication included in the data set. Additionally, particular attention should be paid to ensuring that the residue database does not contain duplicate entries from the same trial (such as would be the case where data for a substance is taken from a protected study and from a regulatory assessment report). The Working Group was not able complete this challenging and time-consuming task within the available time frame. Future investigations on factors responsible for the pesticide dissipation in/on plants and the variability between substances, plant species, matrices, study-specific environmental conditions, sampling procedure, sampling processing, pesticide application and doses are strongly recommended.

In conclusion, the WG is of the opinion that the overall picture provides sufficient indications that the use of a default DT_{50} value of 10 days will support a sufficiently conservative Tier 1 exposure assessment sufficiently protective for birds and mammals.

Dissipation half-life (DT_{50}) for foliar- and ground-dwelling arthropods

In the database developed by Lahr et al. (2018), the part collating residue data focussed on (initial) residue levels after treatment in crops, insects, weeds, seeds, etc., and on residue decline.

Therefore, in the context of the present Guidance Document, the data set on the residue decline was filtered by considering only records with:

1) reported DT_{50} values (column AG in the original Excel file);
2) with diet category assigned to arthropods plant and arthropods soil (column S in the original Excel file);
3) all the relevant matrices (column R in the original Excel file).

It should be noted that for ground-dwelling arthropods the whole season was taken into account.

Following the data filtering, a total of 44 records were retained. In this selected data set, the DT_{50} values ranged from 0.12 days to 21.1 days, with an arithmetic mean of 3.4 days (SD: 3.9), the median of 2.6 days and the 90th percentile of 8.0 days. The distribution of the whole data set is illustrated in Figure K.5.

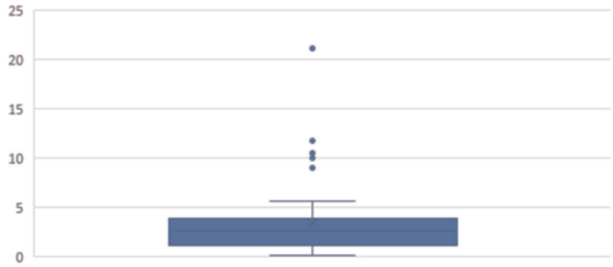


Figure K.5: Distribution of the DT_{50} values (days) in ground and foliar-dwelling arthropods from Lahr et al. (2018)

Only 3 DT_{50} values were above 10 days, confirming that, in general, a DT_{50} of 10 days would cover most of the cases. Furthermore, the size of the data set is considered too small to trigger a change of the default DT_{50} value.

In conclusion, the WG considers that the use of a default DT_{50} value of 10 days in arthropods will enable a Tier 1 exposure assessment sufficiently protective for birds and mammals. It should be noted that also this data set presents some uncertainties such as the exclusion of potential reliable DT_{50} values that were not estimated/reported in the report but that could have been estimated from the time-series of residues provided, etc.

Appendix L – Derivation of deposition values (DV) by considering crop interception

Deposition values (DV) are used in the exposure calculations and account for the amount of active substances deposited on the food items consumed by the bird or mammal, and are derived from the crop interception values (e.g. DV (%) = 100 – crop interception (%)).

When the food item is foliar-dwelling arthropods, crop foliage, crop seeds, fruit, flower buds or any other food item on the crop, the DV is 100%.

For food items located on the soil surface (ground), and for weeds, the DV are determined considering the crop interception values, which are commonly used in soil exposure assessments and account for the impact that the crop canopy has on the amount of active substance reaching the soil surface. In EFSA (2009), the crop interception values reported in the FOCUS surface water report (FOCUS, 2001) for Step 2 calculations were used. In the meantime, crop interception values were updated (EFSA, 2014b), and the exposure assessments in the different environmental compartments (soil, surface water and sediment) were aligned using the same default crop interception values (EFSA, 2017, 2020). Therefore, in this guidance document it was decided to use the same crop interception values reported in EFSA (2014b, 2020) for food items located on the soil surface.

Since weed foliage is not located at the soil surface, care needs to be taken in using the crop interception values developed for soil. Therefore, the crop interception values reported in EFSA (2014b, 2020), are relevant only for BBCH crop stages corresponding to high soil coverage. This reflects the approach followed in EFSA (2009), where reliable predictions using FOCUS interception values were deemed possible for weed foliage food items only when the largest part of the soil surface is covered by the crop and the undergrowth vegetation is smaller than the crop. Therefore, for BBCH stages corresponding to low soil coverage, no crop interception is considered for weed foliage food items (DV = 100%).

A further point for selecting the appropriate DV is that, at early growth stages, bird and mammals eat both crop leaves and weed leaves (DV = 100%). At later growth stages, the crop leaves of most crops become unpalatable to birds and mammals and only weeds are consumed (DV for weed leaves is dependent on the crop interception value). Some crops, such as leafy vegetables, remain palatable to birds and mammals at all growth stages.

In summary, for food items located on soil surface and on weed foliage the crop interception values are the same except for the BBCH stages corresponding to low soil coverage, where no crop interception is considered for weeds foliage.

Crop interception values are not available for all crops covered in this guidance document, and therefore surrogate crops should be used for carrying out the risk assessment. The proposed surrogate crops, derived by experts' judgement and based on common practice when selecting crop interception values for soil exposure assessments, are reported in Table L.2 together with the complete list of crop groups. It should be further noted that in some cases, owing to the use of surrogate values, a DV value was missing for certain BBCH groups. Where this was the case, the DV from the preceding BBCH group was used.

The uses in orchards, vines and tree-like crops/plants needs further consideration. For herbicides which are usually applied below the crop/plant itself, no crop interception is applicable in these situations, and therefore the deposition value to weeds is always 100%. However, in EFSA (2020), it is proposed that for pome/stone fruits and vines there is some soil coverage by the vegetation below the trees (grass, weeds), and the default vegetation interception was set to half of the crop interception in grass; in the present guidance document it was decided to follow the same approach.

Therefore, for herbicides and PPPs which are applied below the crop canopy in pome/stone fruits and vines, deposition to weed foliage food items is always 100%, and deposition to soil surface food items is always 55% irrespective of the crop growth stage; while for herbicides and PPPs which are applied below the crop canopy in other tree-like crops/plants deposition to weed foliage and soil surface food items is always 100%.

For any PPPs applied to the crop canopy, the deposition to weed foliage food items and soil surface food items is dependent on the crop interception and varies depending on the type of crop and the growth stages (see Table L.1).

Furthermore, interception by plants in the Terrestrial Area of Interest (TAI) has not been previously considered. However, the WG considered that it would be a reasonable approach to also apply half of the crop interception in grass to account for plant interception in TAI. This means that deposition to soil surface food items is assumed to always be 55% irrespective of the crop growth stage but

deposition to weeds/plants/foliar-dwelling arthropods, etc. is 100%. It is acknowledged that the interception by plants in the TAI area will vary significantly. However, since plant coverage is key for the small mammal GMS for which the risk assessment is being performed, the WG considered that it was reasonable to assume plant interception in the TAI.

The following table summarises the deposition values (%) for food items located on soil surface and weed foliage and are aligned with the crop interception values reported in EFSA (2014b, 2020).

Table L.1: Deposition values (%) for food items located on soil surface (in alignment with the crop interception values reported in EFSA (2014b, 2020)) and weed foliage

	Food Item location	Germination/ sprouting	Leaf development		Formation of side shoots	Stem elongation/ rosette growth	Vegetative plant parts	Inflorescence emergence	Flowering	Development of fruit	Ripening	Senescence
BBCH stage		**00–09**	**10–13**	**14–19**	**20–29**	**30–39**	**40–49**	**50–59**	**60–69**	**70–79**	**80–89**	**90–99**
Cereals, spring and winter[(d)]	Soil surface	100	100	100	80	20	10	10	10	20	20	(20)
	Weed foliage	100	100	100	100	20	10	10	10	20	20	(20)
Cotton	Soil surface	100	70	70	40	40	n.a.	25	25	25	25	(10)
	Weed foliage	100	100	100	100	100	n.a.	25	25	25	25	(10)
Field beans	Soil surface	100	75	75	60	n.a.	n.a.	30	30	30	30	(20)
	Weed foliage	100	100	100	100	n.a.	n.a.	30	30	30	30	(20)
Grass[(a),(b)]	Soil surface	10	10	10	10	10	10	10	10	10	10	10
	Weed foliage	10	10	10	10	10	10	10	10	10	10	10
Legumes	Soil surface	100	65	65	n.a.	45	n.a.	15	15	15	15	(15)
	Weed foliage	100	100	100	100	100	n.a.	15	15	15	15	(15)
Linseed	Soil surface	100	70	70	40	40	30	30	30	30	30	(10)
	Weed foliage	100	70	70	40	40	30	30	30	30	30	(10)
Maize	Soil surface	100	75	75	n.a.	50	n.a.	25	25	25	25	(10)
	Weed foliage	100	100	100	n.a.	50	n.a.	25	25	25	25	(10)
Oilseed rape, spring and winter	Soil surface	100	60	60	20	20	n.a.	20	20	20	20	(10)
	Weed foliage	100	100	100	100	20	n.a.	20	20	20	20	(10)
Potatoes	Soil surface	100	85	85	40	40	n.a.	15	15	15	15	(50)
	Weed foliage	100	100	100	100	100	n.a.	15	15	15	15	(50)
Soybeans	Soil surface	100	65	65	45	n.a.	15	15	15	15	15	(35)
	Weed foliage	100	100	100	100	n.a.	15	15	15	15	15	(35)
Strawberries	Soil surface	100	70	70	50	50	40	40	40	40	40	(40)
	Weed foliage	100	100	100	100	100	40	40	40	40	40	(40)
Sugar beets	Soil surface	100	80	80	n.a.	30	10	10	10	10	10	(10)
	Weed foliage	100	100	100	n.a.	100	10	10	10	10	10	(10)

	Food Item location	Germination/ sprouting	Leaf development		Formation of side shoots	Stem elongation/ rosette growth	Vegetative plant parts	Inflorescence emergence	Flowering	Development of fruit	Ripening	Senescence
BBCH stage		**00–09**	**10–13**	**14–19**	**20–29**	**30–39**	**40–49**	**50–59**	**60–69**	**70–79**	**80–89**	**90–99**
Sunflower	Soil surface	100	80	80	n.a.	50	n.a.	25	25	25	25	(10)
	Weed foliage	100	100	100	n.a.	50	n.a.	25	25	25	25	(10)
Tobacco	Soil surface	100	50	50	30	n.a.	n.a.	10	10	10	10	(10)
	Weed foliage	100	100	100	30	n.a.	n.a.	10	10	10	10	(10)
Vegetables, bulb	Soil surface	100	90	90	n.a.	n.a.	60	60	60	60	60	(40)
	Weed foliage	100	100	100	n.a.	n.a.	60	60	60	60	60	(40)
Vegetables, leafy	Soil surface	100	75	75	n.a.	n.a.	30	(30)	(30)	(30)	(30)	(10)
	Weed foliage	100	100	100	n.a.	n.a.	30	(30)	(30)	(30)	(30)	(10)
Vegetables, root	Soil surface	100	75	75	n.a.	n.a.	20	(20)	(20)	(20)	(20)	(20)
	Weed foliage	100	100	100	n.a.	n.a.	20	(20)	(20)	(20)	(20)	(20)
Bush berries, application to crop canopy	Soil surface	60	40	40	40	40	40	40	40	40	25	25
	Weed foliage	100	40	40	40	40	40	40	40	40	25	25
Citrus[(a)], application to crop canopy	Soil surface	20	20	20	n.a.	20	n.a.	20	20	20	20	20
	Weed foliage	20	20	20	n.a.	20	n.a.	20	20	20	20	20
Hops, application to crop canopy	Soil surface	100	80	80	50	50	n.a.	40	40	30	30	(30)
	Weed foliage	100	80	80	50	50	n.a.	40	40	30	30	(30)
Olives[(a)], application to crop canopy	Soil surface	30	30	30	n.a.	30	n.a.	30	30	30	30	30
	Weed foliage	30	30	30	n.a.	30	n.a.	30	30	30	30	30
Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy	Soil surface	100	100	100	100	100	100	100	100	100	100	100
	Weed foliage	100	100	100	100	100	100	100	100	100	100	100

	Food Item location	Germination/ sprouting	Leaf development		Formation of side shoots	Stem elongation/ rosette growth	Vegetative plant parts	Inflorescence emergence	Flowering	Development of fruit	Ripening	Senescence
BBCH stage		**00–09**	**10–13**	**14–19**	**20–29**	**30–39**	**40–49**	**50–59**	**60–69**	**70–79**	**80–89**	**90–99**
Pome/stone fruits, application to crop canopy	Soil surface	(50)	40	40	n.a.	40	n.a.	40	40	35	35	50(c)
	Weed foliage	(50)	40	40	n.a.	40	n.a.	40	40	35	35	50(c)
Vines, application to crop canopy	Soil surface	(60)	50	40	n.a.	n.a.	n.a.	40	40	25	25	60(c)
	Weed foliage	(60)	50	40	n.a.	n.a.	n.a.	40	40	25	25	60(c)
Pome/stone fruits and vines, applications below crop canopy	Soil surface	(55)	55	55	55	55	55	55	55	55	55	(55)
	Weed foliage	100	100	100	100	100	100	100	100	100	100	100

n.a.: non-existing crop stages according to the BBCH Compendium.
Deposition values in parentheses indicate when there might be no crop present at this BBCH stage (e.g. after harvest) (EFSA, 2020), and then are only applicable in case the crop is allowed to continue beyond the usual BBCH for harvesting (e.g. seeds production, etc…).
(a): Evergreen, constant crop interception not related to BBCH stage.
(b): Considered to represent established turf all year round in EFSA (2020).
(c): No crop interception in EFSA (2014b) for this BBCH stage, default crop interception was set equal to the interception at BBCH 00–09.
(d): Cereals can be considered as surrogate crop for newly sown grass.

Table L.2: Summary of the crop deposition value to be used for the exposure assessment for spray applicationsn

Crop group (see Appendix E for further definition)	Crop or surrogate crops for deriving crop deposition values	
	For applications to crop/plant canopy	For applications below crop/plant canopy
All crops/plants resulting in exposure to TAI	For weeds and other food items not in the soil in the TAI the DV = 100%, for ground level food items in the TAI the DV = 55%	–
All field crops BBCH 0–9	100% deposition	–
Allium vegetable crops	Vegetables, bulb	–
Amenity grassland	Grass	–
Artichokes and cardoons	Vegetables, leafy	–
Asparagus	Vegetables, leafy	–
Banana	Pome/stone fruits, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Bare fallow	100% deposition	–
Biomass trees	Olives, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Broadleaf forest tree	Olives, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Buckwheat	Oilseed rape	–
Bulb-like ornamental herbaceous plants	Vegetables, bulb	–
Citrus fruit crops	Citrus, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Coniferous forest trees	Olives, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Cotton	Cotton	–
Fig	Pome/stone fruits, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Fruiting cucurbitaceous vegetable crops	Vegetables, leafy(1)	–
Fruiting solanaceous vegetable crops	Vegetables, leafy(1)	–
Grass crops	Cereals(3)	–
Grassland	Cereals(3)	–
Herb crops	Vegetables, root	–
Hops	Hops, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Jerusalem artichoke	Vegetables, leafy	–
Kiwifruit	Vines, application to crop canopy	Pome/stone fruits and vines, applications below crop canopy
Leafy vegetable crops (excluding brassica)	Vegetables, leafy	–
Legume crops, except soybean (alfalfa, clover, lupin)	Legumes	–

Crop group (see Appendix E for further definition)	Crop or surrogate crops for deriving crop deposition values	
	For applications to crop/plant canopy	For applications below crop/plant canopy
Legume vegetable crops (peas, beans, etc.)	Field beans	–
Linseed (= flax)	Linseed	–
Maize and millet crop	Maize	–
Nut crops	Pome/stone fruits, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines),
Olives	Olives, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Ornamental broad-leaved trees, shrubs, and climbing plants	Pome/stone fruits	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Ornamental cactuses and succulents	Field beans[(2)]	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Ornamental conifers	Olives, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Ornamental herbaceous plants	Sunflower[(2)]	–
Ornamental herbaceous plants excluding bulbs	Sunflower	–
Ornamental plants (unspecified)	No interception value to be considered	No interception value to be considered
ornamental woody monocotyledonous plants	Pome/stone fruits, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Ornamental woody plants	Pome/stone fruits, application to crop canopy	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Pineapple	Vegetable, leafy	–
Pome fruit crops	Pome/stone fruits	Pome/stone fruits and vines, applications below crop canopy
Poppy	Vegetable, bulb	–
Potato	Potato	–
Quinoa	Cereals	–
Rhubarb	Vegetable, leafy	–
Root vegetables	Vegetables, root	–
Salsify	Vegetables, root	–
Sesame	Cereals	–
Small fruit crop	Bush berries	Tree/bush like crops (other than pome/stone fruits and vines), applications below crop canopy
Soybean	Soybean	–
Spring sown brassica arable crop	Oilseed rape	–
Spring sown cereal crop	Cereals	–
Stone fruit crop	Pome/stone fruits	Pome/stone fruits and vines, applications below crop canopy
Strawberry	Strawberries	–
Stubbles	100% deposition	–

Crop group (see Appendix E for further definition)	Crop or surrogate crops for deriving crop deposition values	
	For applications to crop/plant canopy	For applications below crop/plant canopy
Sugar beet	Sugar beet	–
Sunflower	Sunflower	–
Sweet potatoes	Potatoes	–
Tobacco	Tobacco	–
Vegetable brassica crops	Vegetable, leafy	–
Vines	Vines, application to crop canopy	Pome/stone fruits and vines, applications below crop canopy
Winter sown brassica arable crop	Oilseed rape	–
Winter sown cereal crop	Cereals	–

(1): Given the diverse crop situations for 'fruiting cucurbitaceous vegetable crops' and 'fruiting solanaceous vegetable crops' the most appropriate surrogate crop interception was considered to be 'leafy vegetables' rather than 'fruiting vegetables' because the lower crop interception values of 'leafy vegetables' represent a realistic worst case of the amount of active substance deposited on the food items consumed by birds and mammals.

(2): Owing to the diverse plants within the 'Ornamental cactuses and succulents' and 'Ornamental herbaceous plants' groups, the crop interception for 'field beans' and 'sunflower' was considered as the most appropriate surrogate for crop interception, respectively. For GAPs which are more limited, then the selection of the surrogate may be reconsidered.

(3): For established or permanent grassland the DV value to ground level food items for BBCH 60–70 may be used. This value is 10% which considers that the crop interception value for grass is 90%.

Appendix M – Dilution factor Germinating seedlings

According to EFSA (2009) the estimation of the concentration of active substances in seedlings germinating from treated seed, the seed loading on the treated seed is divided by five to account for growth dilution. No default value for dissipation was available for the Tier 1 long-term exposure estimate and therefore fTWA is assumed to equal one. The residue database collated by Lahr et al. (2018), did not contain sufficient data on residue decline from seedlings from treated seed to derive a suitable default value for dissipation.

To check the appropriateness of the dilution factor, an analysis of the growth of control seedlings in non-target terrestrial plant studies was done. The following steps were followed:

Step 1 - collection of data:

Ten substances were selected from dossiers available to EFSA. The substances were randomly selected from the dossiers of chemical active substances that have been submitted to EFSA as part of the peer review of active substances under Regulation (EC) 1107/2009. A database was compiled with:

- PPP
- Active substance
- Author
- Study code
- Plant species
- Study methodology (OECD Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD, 2006b) or OECD Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test (OECD, 2006c))
- Type of seedling
- 21-day fresh weight of control seedlings (g)

The 1,000 grain weight (range) for the plant species was collected from the literature (primarily from the supplementary information from Lucchesi et al. (2016)).

Data were collected for ten active substances. Two plant species were excluded as they were not considered relevant to field crops. These were *Oryza sativa* (rice) and *Brassica kaber* (wild mustard). This resulted in 20 plant species (Table M.2).

Step 2 - Calculations:

The following calculations were performed:

- The minimum and maximum weight of an individual seed were determined from the 1000-grain weight.
- The minimum and maximum 21-day growth factor was calculated accounting for the weight of a seed and the 21-day seedling weight.
- Assuming exponential growth of the seedling, the rate constant *k* was calculated (ln(21-day growth factor)/21).
- Using the rate constant, a 5-day dilution factor could be determined for each species (5-day dilution factor = $e^{(5k)}$). 5 days is the time assumed before emergence.
- The mean of the 5-day dilution factors was calculated.
- Using the rate constant, a 21-day TWA average factor (i.e. covering the period of between 5 and 26 days with day 5 being the assumed day of emergence) could also be calculated assuming first order dissipation from growth dilution and the following equations:

$$AUC = \frac{e^{(-t_0 * k)}}{k} - \frac{e^{(-t_1 * k)}}{k}$$

$$fTWA = \frac{AUC}{t_1 - t_0}$$

Where:
AUC = Area Under the Curve
K = rate constant
t_0 = 5
t_1 = 26

Results

Table M.1 summarises the data analysis. The mean 5-day dilution factor is 4.45 based on the minimum dilution and 5.34 based on the maximum dilution. Table M.1 also gives the range and mean values of the calculated 21-day fTWA values.

Table M.1: Maximum, Minimum, arithmetic mean and median values for seed weight, 21-day dilution, 5-day dilution factor and 21-day fTWA for 20 plant species

	21-day fresh weight in control (g)	1000 grain weight (g)		Weight of a single seed (g)		Dilution at 21 days		5-day dilution factor	21-day fTWA	5-day dilution factor	21-day fTWA
		Min	Max	Min	Max	Min	Max	Minimum dilution		Maximum dilution	
Max	97.00	300.00	3000.00	0.30	3.00	37808.33	50411.11	12.30	0.28	13.17	0.20
Min	0.07	0.70	1.20	0.00	0.00	7.01	12.96	1.59	0.01	1.84	0.01
Mean	15.19	30.99	117.14	0.03	0.09	1533.41	3581.27	**4.45**	0.06	5.34	0.04
Median	11.68	10.00	31.00	0.00	0.01	369.44	635.56	4.09	0.04	4.65	0.03

Conclusions and recommendations:

The mean 5-day dilution factor is 4.45 based on the minimum dilution and 5.34 based on the maximum dilution. This is considered to support the assumption in EFSA (2009) of a growth dilution factor of 5. Table M1 also gives the range and mean values of the calculated 21-day fTWA values. The WG agreed that since the fTWA were derived for a relatively small data set, it would be appropriate to take a maximum 21-day fTWA of 0.28 for the reproductive risk assessment.

On the basis of the results the following is recommended:

- The 5-day dilution factor to be applied to the nominal seed concentration should be 5. This can be used to estimate the initial concentration in germinating seedlings and should be used for the acute assessment.
- A 21-day fTWA of 0.28 can be used in the exposure calculation for the long-term assessment for substances where an fTWA is considered appropriate (i.e. concentration in seedlings is given by the nominal seed loading/5 $\times$ fTWA).

Table M.2: Control data from OECD Test No. 208 and OECD Test No. 227 studies and calculation of growth dilution factors

Study code	Test Guideline	Plant species	21-day fresh weight in control (g)	Excluded?	Min 1000 grain weight	Max 1000 grain weight	min weight (g)	max weight (g)	MAX 21 day dilution	MIN 21 day dilution	k (min dilution)	dilution at 5 days (min)	21-day TWA (min)	k (max dilution)	dilution at 5 days (max)	21-day TWA (max)
137 392	208	*Brassica napus*	56.62		2	10	0.002	0.010	28,310	5,662.00	0.41	7.83	0.01	0.49	11.48	0.01
137 392	208	*Pisum sativum*	24.4		88	432	0.088	0.432	277.3	56.48	0.19	2.61	0.09	0.27	3.82	0.05
169374/2423	208	*Avena sativa*	5.26		24	48	0.024	0.048	219.2	109.58	0.22	3.06	0.07	0.26	3.61	0.05
169374/2423	208	*Brassica napus*	4.15		2	10	0.002	0.010	2,075.0	415.00	0.29	4.20	0.04	0.36	6.16	0.02
169374/2423	208	*Glycine max*	3.75		127.9	161.99	0.128	0.162	29.3	23.15	0.15	2.11	0.14	0.16	2.24	0.13
TNW14637, R-29125	227	*Avena sativa*	19.03		24	48	0.024	0.048	792.9	396.46	0.28	4.16	0.04	0.32	4.90	0.03
TNW14637, R-29125	227	*Allium cepa*	15.38		2.9	5	0.003	0.005	5,303.5	3,076.00	0.38	6.77	0.02	0.41	7.71	0.02
TNW14637, R-29125	227	*Beta vulgaris*	27.73		10	31	0.010	0.031	2,773.0	894.52	0.32	5.04	0.03	0.38	6.60	0.02
TNW14637, R-29125	227	*Brassica napus*	36.99		2	10	0.002	0.010	18,495.0	3,699.00	0.39	7.07	0.02	0.47	10.37	0.01
TNW14637, R-29125	227	*Daucus carota*	20.87		0.7	2.5	0.001	0.003	29,814.3	8,348.00	0.43	8.58	0.01	0.49	11.62	0.01
TNW14637, R-29125	227	*Glycine max*	27.79		127.9	161.99	0.128	0.162	217.3	171.55	0.24	3.40	0.06	0.26	3.60	0.05
ACE-08-223	227	*Avena sativa*	11.6		24	48	0.024	0.048	483.3	241.67	0.26	3.69	0.05	0.29	4.36	0.04
ACE-08-223	227	*Allium cepa*	2.07		2.9	5	0.003	0.005	713.8	414.00	0.29	4.20	0.04	0.31	4.78	0.03
ACE-08-223	227	*Cucumis sativus*	40.09		27	33	0.027	0.033	1,484.8	1,214.85	0.34	5.43	0.03	0.35	5.69	0.02
ACE-08-223	227	*Brassica napus*	20.87		2	10	0.002	0.010	10,435.0	2,087.00	0.36	6.17	0.02	0.44	9.05	0.01
ACE-08-223	227	*Glycine max*	17.23		127.9	161.99	0.128	0.162	134.7	106.36	0.22	3.04	0.07	0.23	3.21	0.06
ACE-08-223	227	*Beta vulgaris*	20.82		10	31	0.010	0.031	2,082.0	671.61	0.31	4.71	0.03	0.36	6.17	0.02
IIA 8.12/02	208	*Avena sativa*	97		24	48	0.024	0.048	4,041.7	2,020.83	0.36	6.12	0.02	0.40	7.22	0.02
TNW107662	227	*Avena sativa*	6.12		24	48	0.024	0.048	255.00	127.50	0.23	3.17	0.06	0.26	3.74	0.05
TNW107662	227	*Allium cepa*	3.3		2.9	5	0.003	0.005	1,137.9	660.00	0.31	4.69	0.03	0.34	5.34	0.03
TNW107662	227	*Beta vulgaris*	12.66		10	31	0.010	0.031	1,266.0	408.39	0.29	4.18	0.04	0.34	5.48	0.03
TNW107662	227	*Brassica napus*	17.99		2	10	0.002	0.010	8,995.0	1,799.00	0.36	5.96	0.02	0.43	8.74	0.01
TNW107662	227	*Daucus carota*	14.48		0.7	2.5	0.001	0.003	20,685.7	5,792.00	0.41	7.87	0.01	0.47	10.65	0.01
TNW107662	227	*Glycine max*	17.47		127.9	161.99	0.128	0.162	136.6	107.85	0.22	3.05	0.07	0.23	3.22	0.06

Study code	Test Guideline	Plant species	21-day fresh weight in control (g)	Excluded?	Min 1000 grain weight	Max 1000 grain weight	min weight (g)	max weight (g)	MAX 21 day dilution	MIN 21 day dilution	k (min dilution)	dilution at 5 days (min)	21-day TWA (min)	k (max dilution)	dilution at 5 days (max)	21-day TWA (max)
5549-92-0483-BE-001	208	*Fagopyrum esculentum*	4.31		27.5	36.1	0.028	0.036	156.7	119.39	0.23	3.12	0.07	0.24	3.33	0.06
5549-92-0483-BE-001	208	*Zea mays*	7.82		250	420	0.250	0.420	31.3	18.62	0.14	2.01	0.16	0.16	2.27	0.12
5549-92-0483-BE-001	208	*Cucumis sativus*	2.86		27	33	0.027	0.033	105.9	86.67	0.21	2.89	0.08	0.22	3.03	0.07
5549-92-0483-BE-001	208	*Brassica kaber*	1.48	Y												
5549-92-0483-BE-001	208	*Avena sativa*	1.72		24	48	0.024	0.048	71.67	35.83	0.17	2.34	0.12	0.20	2.77	0.08
5549-92-0483-BE-001	208	*Allium cepa*	0.19		2.9	5	0.003	0.005	65.52	38.00	0.17	2.38	0.11	0.20	2.71	0.09
5549-92-0483-BE-001	208	*Raphanus sativus*	3.23		7	7.5	0.007	0.008	461.43	430.67	0.29	4.24	0.04	0.29	4.31	0.04
5549-92-0483-BE-001	208	*Sorghum bicolor*	0.96		28	33	0.028	0.033	34.29	29.09	0.16	2.23	0.13	0.17	2.32	0.12
5549-92-0483-BE-001	208	*Glycine max*	11.79		127.9	161.99	0.128	0.162	92.18	72.78	0.20	2.78	0.08	0.22	2.94	0.07
5549-92-0483-BE-001	208	*Lycopersicum esculentum*	0.29		3	4	0.003	0.004	96.67	72.50	0.20	2.77	0.08	0.22	2.97	0.07
5549-92-0484-BE-001	227	*Fagopyrum esculentum*	13.88		27.5	36.1	0.028	0.036	504.73	384.49	0.28	4.13	0.04	0.30	4.40	0.04
5549-92-0484-BE-001	227	*Zea mays*	16.76		250	420	0.250	0.420	67.04	39.90	0.18	2.41	0.11	0.20	2.72	0.09
5549-92-0484-BE-001	227	*Cucumis sativus*	29.13		27	33	0.027	0.033	1,078.89	882.73	0.32	5.03	0.03	0.33	5.27	0.03
5549-92-0484-BE-001	227	*Brassica kaber*	9.47	Y												
5549-92-0484-BE-001	227	*Avena sativa*	12.73		24	48	0.024	0.048	530.42	265.21	0.27	3.78	0.05	0.30	4.45	0.04
5549-92-0484-BE-001	227	*Allium cepa*	8.8		2.9	5	0.003	0.005	3,034.48	1,760.00	0.36	5.93	0.02	0.38	6.75	0.02
5549-92-0484-BE-001	227	*Raphanus sativus*	11.76		7	7.5	0.007	0.008	1,680.00	1,568.00	0.35	5.76	0.02	0.35	5.86	0.02
5549-92-0484-BE-001	227	*Sorghum bicolor*	8.12		28	33	0.028	0.033	290.00	246.06	0.26	3.71	0.05	0.27	3.86	0.05
5549-92-0484-BE-001	227	*Glycine max*	22.2		127.9	161.99	0.128	0.162	173.57	137.05	0.23	3.23	0.06	0.25	3.41	0.06
5549-92-0484-BE-001	227	*Lycopersicum esculentum*	33.91		3	4	0.003	0.004	11,303.33	8,477.50	0.43	8.62	0.01	0.44	9.23	0.01
S10-03575	227	*Allium cepa*	16.4		2.9	5	0.003	0.005	5,655.17	3,280.00	0.39	6.87	0.02	0.41	7.82	0.01
S10-03575	227	*Avena sativa*	20.6		24	48	0.024	0.048	858.33	429.17	0.29	4.23	0.04	0.32	4.99	0.03

Study code	Test Guideline	Plant species	21-day fresh weight in control (g)	Excluded?	Min 1000 grain weight	Max 1000 grain weight	min weight (g)	max weight (g)	MAX 21 day dilution	MIN 21 day dilution	k (min dilution)	dilution at 5 days (min)	21-day TWA (min)	k (max dilution)	dilution at 5 days (max)	21-day TWA (max)
S10-03575	227	*Beta vulgaris*	16.2		10	31	0.010	0.031	1,620.00	522.58	0.30	4.44	0.04	0.35	5.81	0.02
S10-03575	227	*Brassica napus*	27.3		2	10	0.002	0.010	13,650.00	2,730.00	0.38	6.58	0.02	0.45	9.65	0.01
S10-03575	227	*Cucumis sativus*	29		27	33	0.027	0.033	1,074.07	878.79	0.32	5.02	0.03	0.33	5.27	0.03
S10-03575	227	*Lolium perenne*	14.6		3.25	3.6	0.003	0.004	4,492.31	4,055.56	0.40	7.23	0.02	0.40	7.41	0.02
S10-03575	227	*Lycopersicum esculentum*	29.8		3	4	0.003	0.004	9,933.33	7,450.00	0.42	8.35	0.01	0.44	8.95	0.01
S10-03575	227	*Sorghum bicolor*	10.2		28	33	0.028	0.033	364.29	309.09	0.27	3.92	0.04	0.28	4.07	0.04
S10-03575	227	*Triticum aestivum*	19		29	58	0.029	0.058	655.17	327.59	0.28	3.97	0.04	0.31	4.68	0.03
S10-03575	227	*Vicia faba*	72.1		300	3,000	0.300	3.000	240.33	24.03	0.15	2.13	0.14	0.26	3.69	0.05
S10-03574	208	*Allium cepa*	1.22		2.9	5	0.003	0.005	420.69	244.00	0.26	3.70	0.05	0.29	4.21	0.04
S10-03574	208	*Avena sativa*	9.23		24	48	0.024	0.048	384.58	192.29	0.25	3.50	0.05	0.28	4.13	0.04
S10-03574	208	*Beta vulgaris*	3.96		10	31	0.010	0.031	396.00	127.74	0.23	3.17	0.06	0.28	4.15	0.04
S10-03574	208	*Brassica napus*	17.69		2	10	0.002	0.010	8,845.00	1,769.00	0.36	5.93	0.02	0.43	8.70	0.01
S10-03574	208	*Cucumis sativus*	9.2		27	33	0.027	0.033	340.74	278.79	0.27	3.82	0.05	0.28	4.01	0.04
S10-03574	208	*Lolium perenne*	4.89		3.25	3.6	0.003	0.004	1,504.62	1,358.33	0.34	5.57	0.02	0.35	5.71	0.02
S10-03574	208	*Lycopersicum esculentum*	9.05		3	4	0.003	0.004	3,016.67	2,262.50	0.37	6.29	0.02	0.38	6.74	0.02
S10-03574	208	*Sorghum bicolor*	2.72		28	33	0.028	0.033	97.14	82.42	0.21	2.86	0.08	0.22	2.97	0.07
S10-03574	208	*Triticum aestivum*	9.76		29	58	0.029	0.058	336.55	168.28	0.24	3.39	0.06	0.28	4.00	0.04
S10-03574	208	*Vicia faba*	60.84		300	3,000	0.300	3.000	202.80	20.28	0.14	2.05	0.15	0.25	3.54	0.05
EA05B2A033	208 B	*Avena sativa*	11.56		24	48	0.024	0.048	481.67	240.83	0.26	3.69	0.05	0.29	4.35	0.04
EA05B2A033	208 B	*Lolium perenne*	3.74		3.25	3.6	0.003	0.004	1,150.77	1,038.89	0.33	5.23	0.03	0.34	5.36	0.03
EA05B2A033	208 B	*Triticum aestivum*	11.3		30	61	0.030	0.061	376.67	185.25	0.25	3.47	0.05	0.28	4.11	0.04
EA05B2A033	208 B	*Allium cepa*	12.99		2.9	5	0.003	0.005	4,479.31	2,598.00	0.37	6.50	0.02	0.40	7.40	0.02
EA05B2A033	208 B	*Brassica napus*	26.32		2	10	0.002	0.010	13,160.00	2,632.00	0.38	6.52	0.02	0.45	9.57	0.01
EA05B2A033	208 B	*Glycine max*	22.81		127.9	161.99	0.128	0.162	178.34	140.81	0.24	3.25	0.06	0.25	3.44	0.06

Study code	Test Guideline	Plant species	21-day fresh weight in control (g)	Excluded?	Min 1000 grain weight	Max 1000 grain weight	min weight (g)	max weight (g)	MAX 21 day dilution	MIN 21 day dilution	k (min dilution)	dilution at 5 days (min)	21-day TWA (min)	k (max dilution)	dilution at 5 days (max)	21-day TWA (max)
EA05B2A033	208 B	*Daucus carota*	12.36		0.7	2.5	0.001	0.003	17,657.14	4,944.00	0.41	7.58	0.02	0.47	10.26	0.01
EA05B2A033	208 B	*Cucumis sativus*	96.49		27	33	0.027	0.033	3,573.70	2,923.94	0.38	6.69	0.02	0.39	7.01	0.02
EA05B2A033	208 B	*Beta vulgaris*	27.03		10	31	0.010	0.031	2,703.00	871.94	0.32	5.01	0.03	0.38	6.56	0.02
EA05B2A033	208 B	*Lactuca sativa*	45.37		0.9	1.2	0.001	0.001	50,411.11	37,808.33	0.50	12.30	0.01	0.52	13.17	0.01
EA05B2A020	208 A	*Avena sativa*	5.96		24	48	0.024	0.048	248.33	124.17	0.23	3.15	0.07	0.26	3.72	0.05
EA05B2A020	208 A	*Lolium perenne*	1.33		3.25	3.6	0.003	0.004	409.23	369.44	0.28	4.09	0.04	0.29	4.19	0.04
EA05B2A020	208 A	*Triticum aestivum*	3.81		30	61	0.030	0.061	127.00	62.46	0.20	2.68	0.09	0.23	3.17	0.06
EA05B2A020	208 A	*Allium cepa*	1.39		2.9	5	0.003	0.005	479.31	278.00	0.27	3.82	0.05	0.29	4.35	0.04
EA05B2A020	208 A	*Brassica napus*	7.71		2	10	0.002	0.010	3,855.00	771.00	0.32	4.87	0.03	0.39	7.14	0.02
EA05B2A020	208 A	*Glycine max*	12.42		127.9	161.99	0.128	0.162	97.11	76.67	0.21	2.81	0.08	0.22	2.97	0.07
EA05B2A020	208 A	*Daucus carota*	1.27		0.7	2.5	0.001	0.003	1,814.29	508.00	0.30	4.41	0.04	0.36	5.97	0.02
EA05B2A020	208 A	*Cucumis sativus*	17.16		27	33	0.027	0.033	635.56	520.00	0.30	4.43	0.04	0.31	4.65	0.03
EA05B2A020	208 A	*Beta vulgaris*	7.48		10	31	0.010	0.031	748.00	241.29	0.26	3.69	0.05	0.32	4.83	0.03
EA05B2A020	208 A	*Lactuca sativa*	8.68		0.9	1.2	0.001	0.001	9,644.44	7,233.33	0.42	8.30	0.01	0.44	8.88	0.01
IIIA, 10.8.1/01	208	*Avena sativa*	12.58		24	48	0.024	0.048	524.17	262.08	0.27	3.77	0.05	0.30	4.44	0.04
IIIA, 10.8.1/01	208	*Lolium perenne*	8.71		3	4	0.003	0.004	2,903.33	2,177.50	0.37	6.23	0.02	0.38	6.68	0.02
IIIA, 10.8.1/01	208	*Oryza sativa*	5.19	Y				0.105								
IIIA, 10.8.1/01	208	*Daucus carota*	14.47		0.7	2.5	0.001	0.003	20,671.43	5,788.00	0.41	7.87	0.01	0.47	10.65	0.01
IIIA, 10.8.1/01	208	*Brassica napus*	22.37		2	10	0.002	0.010	11,185.00	2,237.00	0.37	6.27	0.02	0.44	9.20	0.01
IIIA, 10.8.1/01	208	*Brassica oleracea*	22.23		3	5	0.003	0.005	7,410.00	4,446.00	0.40	7.39	0.02	0.42	8.34	0.01
IIIA, 10.8.1/01	208	*Pisum sativum*	18.14		88	432	0.088	0.432	206.14	41.99	0.18	2.43	0.11	0.25	3.56	0.05
IIIA, 10.8.1/01	208	*Allium cepa*	7.96		2.9	5	0.003	0.005	2,744.83	1,592.00	0.35	5.79	0.02	0.38	6.59	0.02
IIIA, 10.8.1/01	208	*Cucumis sativus*	42.51		27	33	0.027	0.033	1,574.44	1,288.18	0.34	5.50	0.03	0.35	5.77	0.02
IIIA, 10.8.1/01	208	*Gossypium hirsutum*	18.54		104.5	104.5	0.105	0.105	177.42	177.42	0.25	3.43	0.06	0.25	3.43	0.06
TNW15458	227	*Avena sativa*	5.15		24	48	0.024	0.048	214.58	107.29	0.22	3.04	0.07	0.26	3.59	0.05

Study code	Test Guideline	Plant species	21-day fresh weight in control (g)	Excluded?	Min 1000 grain weight	Max 1000 grain weight	min weight (g)	max weight (g)	MAX 21 day dilution	MIN 21 day dilution	k (min dilution)	dilution at 5 days (min)	21-day TWA (min)	k (max dilution)	dilution at 5 days (max)	21-day TWA (max)
TNW15458	227	*Zea mays*	14.94		250	420	0.250	0.420	59.76	35.57	0.17	2.34	0.12	0.19	2.65	0.09
TNW15458	227	*Lolium perenne*	2.34		3.25	3.6	0.003	0.004	720.00	650.00	0.31	4.67	0.03	0.31	4.79	0.03
TNW15458	227	*Allium cepa*	0.27		2.9	5	0.003	0.005	93.10	54.00	0.19	2.59	0.10	0.22	2.94	0.07
TNW15458	227	*Beta vulgaris*	19.52		10	31	0.010	0.031	1,952.00	629.68	0.31	4.64	0.03	0.36	6.07	0.02
TNW15458	227	*Brassica napus*	19.8		2	10	0.002	0.010	9,900.00	1,980.00	0.36	6.09	0.02	0.44	8.94	0.01
TNW15458	227	*Helianthus annuus*	13.56		50	200	0.050	0.200	271.20	67.80	0.20	2.73	0.09	0.27	3.80	0.05
TNW15458	227	*Lolium perenne*	13.29		3	4	0.003	0.004	4,430.00	3,322.50	0.39	6.89	0.02	0.40	7.38	0.02
TNW15458	227	*Daucus carota*	2.8		0.7	2.5	0.001	0.003	4,000.00	1,120.00	0.33	5.32	0.03	0.39	7.20	0.02
TNW15458	227	*glycine max*	7.74		127.9	161.99	0.128	0.162	60.52	47.78	0.18	2.51	0.10	0.20	2.66	0.09
TNK15458	208	*Avena sativa*	1.13		24	48	0.024	0.048	47.08	23.54	0.15	2.12	0.14	0.18	2.50	0.10
TNK15458	208	*Zea mays*	3.241		250	420	0.250	0.420	12.96	7.72	0.10	1.63	0.26	0.12	1.84	0.20
TNK15458	208	*Lolium perenne*	0.069		3.25	3.6	0.003	0.004	21.23	19.17	0.14	2.02	0.16	0.15	2.07	0.15
TNK15458	208	*Allium cepa*	0.18		2.9	5	0.003	0.005	62.07	36.00	0.17	2.35	0.12	0.20	2.67	0.09
TNK15458	208	*Beta vulgaris*	1.386		10	31	0.010	0.031	138.60	44.71	0.18	2.47	0.10	0.23	3.24	0.06
TNK15458	208	*Brassica napus*	1.445		2	10	0.002	0.010	722.50	144.50	0.24	3.27	0.06	0.31	4.79	0.03
TNK15458	208	*Helianthus annuus*	1.401		50	200	0.050	0.200	28.02	7.01	0.09	1.59	0.28	0.16	2.21	0.13
TNK15458	208	*Lolium perenne*	2.101		3	4	0.003	0.004	700.33	525.25	0.30	4.44	0.04	0.31	4.76	0.03
TNK15458	208	*Daucus carota*	0.294		0.7	2.5	0.001	0.003	420.00	117.60	0.23	3.11	0.07	0.29	4.21	0.04
TNK15458	208	*glycine max*	2.017		127.9	161.99	0.128	0.162	15.77	12.45	0.12	1.82	0.20	0.13	1.93	0.18
		Max	97.0		300.0	3,000.0	0.300	3.000	50,411.11	37,808.33	0.50	12.30	0.28	0.52	13.17	0.20
		Min	0.1		0.7	1.2	0.001	0.001	12.96	7.01	0.09	1.59	0.01	0.12	1.84	0.01
		Mean	15.2		39.4	113.4	0.039	0.113	3,581.27	1,533.41	0.28	4.45	0.06	0.31	5.34	0.04
		50th %ile	11.7		10.0	31.0	0.010	0.031	635.56	369.44	0.28	4.09	0.04	0.31	4.65	0.03

Appendix N – Use of Critical Appraisal Evaluation Tools

The EFSA Critical Appraisal Tools (CATs) were developed in order to assist the study evaluator/risk assessor to evaluate all data submitted to support the risk assessment in a logical and harmonised manner, and to clearly communicate that evaluation with others. The tool is based upon the concepts of the CRED system (Moermond et al., 2016), which was developed for laboratory aquatic toxicity studies and ring tested amongst evaluators from Asia, Europe, and North America. The concepts are similar to the Klimisch system (Klimisch et al., 1997), however, separate evaluations of reliability (internal validity) and relevance (external validity) are made and distinctly reported in order to take as much data into account as possible and to more transparently report how data were considered and (potentially) used in the risk assessment. Study evaluators/risk assessors may need to add additional questions pending on the study objective and the suggested endpoints for risk assessment.

The evaluator is responsible for checking whether the submitted data are of sufficient reliability (internal validity) to be used in the risk assessment. Studies of high reliability (no issues determined that would affect the outcome of the study) are ranked with a value of R1. Studies which show some issues which increase uncertainty in the outcome but are nevertheless considered sufficient to provide information to potentially support the risk assessment (pending the relevance evaluation) are ranked with a value of R2. Studies which show major deficiencies which call into question the reported outcome cannot be considered reliable enough to be used in the risk assessment and are ranked with a value of R3. Studies which, from the reported information, appear to be potentially reliable, but for which important data needed to determine the full reliability of the study are missing from the report/not reported, receive a value of R4. R4 studies cannot be used in the risk assessment unless additional data can be provided to address the gaps determined in the evaluation within the appropriate time frame for the risk assessment. The difference between R3 and R4 is important for providing transparency about how studies were considered in a risk assessment and improving the quality of reporting for data submitted for regulatory risk assessment.

Similar to the reliability indices, relevance (external validity) can be ranked according to the same principles. A study which is highly relevant to the risk assessment question without any extrapolation or uncertainty can be considered C1. Data which are required according to Regulation (EU) No 283/2013 or 284/2013 might be considered to be C1, as these are data submitted as required for the risk assessment. However, other data may be just as relevant, depending upon the data itself. Data which shows significant relevance, but for which some extrapolation is required, or which really can only be considered supportive of more relevant data, should be considered C2. Data which are clearly outside the scope of the risk assessment question can be considered C3 and should not be used in the risk assessment, even if it is highly reliable. Similar to R4, the C4 designation is reserved for data which seem potentially relevant based upon the data reported, but for which an important piece or pieces of information are missing meaning that the evaluator is unable to determine the relevance of the study. Again, the difference between C3 and C4 is important for communicating how a study was considered and for showing what type of information is required in order to evaluate the relevance of data.

Table N.1: Summary of the reliability (internal validity; R1–R4) and relevance (external validity; C1–C4) categorisation and guidance on their use for risk assessment purposes

Categorisation	Definition	Use in the risk assessment
R1	Studies of high reliability (no issues determined that would affect the outcome of the study)	Yes, if relevant
R2	Studies with some issues which increase uncertainty in the outcome but are nevertheless considered sufficient to provide information to potentially support the risk assessment	Yes, if relevant, with additional uncertainty noted
R3	Studies which show major deficiencies which call into question the reported outcome cannot be considered reliable enough to be used in the risk assessment	No
R4	Studies which, from the reported information, appear to be potentially reliable, but for which important data needed to determine the full reliability of the study are missing from the report/not reported	No(1)

Categorisation	Definition	Use in the risk assessment
C1	A study which is highly relevant to the risk assessment question without any extrapolation	Yes, if reliable
C2	A study which is significantly relevant, but for which some extrapolation is required, or which really can only be considered supportive of more relevant data.	Yes, if reliable, in weight-of-evidence
C3	Data which are clearly outside the scope of the risk assessment question	No
C4	Data which seem potentially relevant based upon the data reported, but for which an important piece or pieces of information are missing meaning that the evaluator is unable to determine the relevance of the study	No(1)

(1): For R4 and C4 studies it is worth considering if the missing information can be obtained e.g. by contacting the study author.

Possible approaches for evaluating multiple endpoints from a single study (e.g. tracking of multiple bird species for PT determination)

In the majority of higher tier studies multiple endpoints are investigated. It is recommended that an evaluator first identifies what endpoints are assessed and decides on the most appropriate approach for the evaluation. Two approaches are possible:

- In the risk assessment question, state the specific endpoint being evaluated and differentiate clearly where necessary e.g. PT in blue tit in apple orchards in Austria or PT values for all species from a single study
    - In the former case, multiple CAT tables will need to be produced (i.e. one table per species).
    - In the latter case, a single CAT table should be used but some questions need to be answered considering all endpoints being evaluated and a comment added to the comment box to indicate where there is a difference in reliability or relevance between species/endpoints (e.g. the number of species tracked was 12 for the blue tit but only 2 for the greenfinch, etc.)

Generally, unless endpoints closely comparable, it is recommended that the assessment is done endpoint by endpoint.

Appendix O – Dehusking/deshelling behaviour in birds and mammals

Dehusking/deshelling behaviour in birds

Some studies have shown that dehusking of seeds can substantially reduce avian exposure to pesticides (e.g. Avery et al., 1994, 1997). Dehusking is mainly observed in smaller species (body weight < 50 g), concretely in the specialised granivores (finches, sparrows and buntings). Other small bird species with a relatively thin bill, such as skylark, wagtails and other non-granivores, do not have the capability of dehusking. Larger granivorous birds (body weight > 50 g) do not dehusk as they are able to destroy even hard-shelled seeds in their gizzard (Prosser, 1999). It is important to note that dehusking in birds is extremely variable; certain species dehusk some but not all seed types, and in the wild the actual proportion of seeds dehusked may depend on stressors such as feeding pressure, predation risk or competition (Prosser, 1999; Prosser and Hart, 2005). Moreover, even when seeds are dehusked, exposure of the bird will vary depending on the handling time of the seed (i.e. exposure increases with the handling time and handling time depends on a combination of the physical characteristics of the seed with the bill structure and force; Avery et al., 1997; Van der Meij and Bout, 2006; Soobramoney and Perrin, 2007). Therefore, assuming a standard reduction (of any value) of the theoretical exposure in species that dehusk is not justified. Moreover, for birds, a risk assessment for a dehusking focal species shall always be accompanied by an assessment for a second species that does not dehusk. This is part of the iterative consideration of specific focal species that is described in Section 6.5.2.

Regarding buds, bud-eating birds generally deshell buds discarding the dry outside and consuming the central portion (Newton, 1964, 1967; Summers and Huson, 1984), however, the extent of the outer layer varies depending on the stage of development of the bud (Summers and Huson, 1984) and this influences the potential exposure. Moreover, just like for seeds, exposure may also occur while manipulating the bud with the tongue to peel off the outer layers. Currently, there is a lack of data regarding the exposure of birds to pesticides through bud-ingestion and thus, a refinement of exposure by deshelling will require case-specific evidence that deshelling actually occurs under field conditions for the focal species, and experimental data must be available for the relevant type of flower bud. Moreover, as explained for the seeds, a consideration of whether there are other focal species that do not deshell buds.

Dehusking behaviour in mammals

For granivorous mammals, dehusking or cracking of seed or fruit shells is often a part of their typical feeding behaviour (Barber et al., 2003). Several studies have demonstrated that the reduction of the exposure due to dehusking of treated seeds is seed-type and species specific (Ludwigs et al., 2007; Defra, 2010a; Brühl et al., 2011). Therefore, if dehusking is to be considered in a higher tier risk assessment, standardised methods with wild (focal) species under realistic worst-case scenarios must be used. Also, the exposure to the pesticide during the dehusking process (i.e. exposure during handling) has to be considered (see examples of how to do this in Defra, 2010a; Morris and Thompson, 2011).

As an example, dehusking efficiency in wood mouse has been quantified in two studies.

Brühl et al. (2011) studied dehusking behaviour in wild wood mouse (*Apodemus silvaticus*) individually caged (12/14 mice per seed type, the sex of the individuals was not mentioned). Two experimental setups were performed; seeds treated with a fungicide and seeds treated with a generic pigment. The amount of fungicide or pigment was measured in the seeds and in the remaining husk to calculate the exposure reduction. Dehusking efficiency was approximately the same, no matter the kind of coating nor whether the mice were deprived of food before the experiment (16 h) or not. Percentages of exposure reduction for the four kinds of seeds tested are presented in Table O.1.

Defra (2010a) studied reduction of exposure through dehusking in wood mouse for seven seed types (wheat, maize, barley, pelleted sugar beet, peas, oilseed rape and beans). Additionally, in order to study possible differences between species, they studied dehusking behaviour in bank voles (*Clethrionomys glareolus*) for wheat and barley. They used 10 individuals per seed type and a 12 h food deprivation period (after verifying that longer deprivation periods produce similar effects). Seeds were treated with a palatable blue dye which, once ingested, is 100% eliminated in the first 48 h. The amount of dye recovered from the faecal pellets was used to calculate the percentage of dye which was consumed. The weight of the husk generated, and the amount of seeds consumed were used to calculate the dehusking efficiency. Hoarding was also measured (by assessing the amount of seeds taken from the dish). Some, seed-type dependent, differences were found between species. For some

seed types, the correlation between the amount of dye (seeds) consumed and the amount recovered in the faeces was improved when the hoarding variable was considered. Percentages of exposure reduction for wood mouse and bank vole are presented in Table O.1.

The use of these values for refined exposure assessments should be assessed on a case-by-case basis and consider their relevance to the substances under assessment (e.g. considering the properties of the PPP and the active substance under assessment).

Table O.1: Percentages of exposure reduction through de-husking measured in two different studies (Defra, 2010a; Brühl et al., 2011) for different seed types. Results for seeds treated with a pigment for wood mouse and bank vole (Defra, 2010a), and for seeds treated with a pigment and a fungicide for wood mouse (Brühl et al., 2011) are presented separately

		% reduction through dehusking							
		Wheat	**Barley**	**Maize**	**Sunflower**	**Pelleted sugar beet**	**Peas**	**Oil seed rape**	**Beans**
Defra, 2010a (mean ± SE)	Wood mouse (n = 10 per seed type)	60.2 ± 6.6	55.4 ± 8.5	62.2 ± 8.3	–	98.6 ± 0.5	89.4 ± 2.9	41.7 ± 8.6	66.7 ± 6.7
	Bank vole (n = 10 per seed type)	27.6 ± 9.5	46.7 ± 8.7	–	–	–	–	–	–
Brühl et al., 2011 (mean ± SD)	Wood mouse/ Pigment	58.0 ± 14.5 (n = 12)	83.9 ± 9.3 (n = 11)	59.0 ± 1 3.1 (n = 12)	98.8 ± 2.0 (n = 11)	–	–	–	–
	Wood mouse/ Fungicide	61.4 ± 15.1 (n = 13)	79.5 ± 7.5 (n = 14)	–	–	–	–	–	–

Appendix P – Estimating seed availability

As described in Section 6.5.7 it is possible to refine the risk to birds and mammals, via consumption of treated seed, by considering the maximum searching area to reach the RAD.

This approach requires information on the density of seeds/granules available on the soil surface after drilling or application. The density of seeds/granules on the surface will depend on the drilling rate of the crop for seeds (Table P.3) or the application rate for granules, the type of drilling machine/application machine and the efficiency of these techniques. Please note that the data presented in Table P.3 are taken from Lucchesi et al. (2016). Should additional information be available in a harmonised guidance document in the future then these values should be used. Table P.2 summarises factors that should be considered when performing seed availability studies together with possible mitigation measures.

There are few studies that estimated the number of seeds on the soil surface after sowing (the WG is not aware of any studies investigating on granules on the soil surface in the literature). Within these studies, most of them focus on cereal seeds (Tamis et al., 1994; Pascual et al., 1999; Barber et al., 2003; de Snoo and Luttik, 2004; Defra, 2010b; Lopez-Antia et al., 2016; Roy et al., 2019; Lennon et al., 2020) and maize (de Snoo and Luttik, 2004; Defra, 2010b; McGee et al., 2018; Roy et al., 2019), while information on other seeds is limited to one or none. Table P.1 summarises available data in literature (some studies are not reported as the used methodologies are considered inappropriate). Unless reliable and relevant data are available in the scientific literature on the seed/granule availability, the applicant is expected to provide such data. Furthermore, to use the data in Table P.1, the applicant needs to demonstrate the relevance of the data to the GAP under assessment.

It should be borne-in-mind, that the risk for birds/mammals cannot be entirely excluded when even when the density of seeds in the surface is very low as some species can still dig up buried seeds. In the case of birds, this behaviour has been described in corvids and pigeons (e.g. Pascual and Hart, 1997; Kennedy and Connery, 2008; Curtis et al., 2019), thus the consideration of a digging species is necessary when exposure assessment is driven by medium granivorous birds consuming seeds (i.e. in the case that it is demonstrated that there are few seeds remaining on the soil surface). The WG did not find detailed information about the sowing depth that would make seeds unavailable to birds, although there are indications that the limit may be close to 5cm (Kennedy and Connery, 2008). Wood mice are known to dig up seeds (as well as bury them), mainly likely detecting the buried seeds via smell (Jennings, 1976). For seeds which are buried within the top 3cm of soil, it is not appropriate to consider reduction in exposure to mammals based on reduced seed availability on the soil surface.

Table P.1: Available data from public literature on the density of seeds found in the soil surface after drilling

Location in field	Crop	Study location	Drilling technique	N fields	Number of seeds per m^2				Reference
					Mean	SD	SE	90th percentile	
In-field	Wheat (spring)	The Netherlands	SD	7	1.7	1.3			De Snoo and Luttik, 2004
	Wheat (autumn)	The Netherlands	SD	31	25.5	28.5			De Snoo and Luttik, 2004
	Wheat (autumn)	UK	SD	31	4.7		1.3		Pascual et al., 1999
	Wheat (autumn)	UK	SD	20	3.6			8.2	Defra, 2010b
	Barley (autumn)	UK	SD	20	4.6			10.1	Defra, 2010b
	Barley (spring)	UK	SD	20	9.7			27.4	Defra, 2010b
	Cereal (autumn)	Spain	SD,BC	28	11.3		1.2	32.4	Lopez-Antia et al., 2016
	Wheat (autumn)	UK	UN	39	0.9		0.06		Lennon et al., 2020
	Maize	The Netherlands	PrD	6	0.02	0.04			De Snoo and Luttik, 2004
	Maize	UK	PrD	20	1.12			1.88	Defra, 2010b
	Maize	US	PrD	7	0.04		0.03		Roy et al., 2019
	Onion	The Netherlands	PrD	5	0.06	0.05			De Snoo and Luttik, 2004
	Sugar beet	The Netherlands	PrD	6	0.02	0.04			De Snoo and Luttik, 2004
	Sugar beet	UK	PrD	20	0.05			0.2	Defra, 2010b
	Alfalfa	The Netherlands	SD	6	1.03	1.03			De Snoo and Luttik, 2004
	Flax	The Netherlands	SD	6	6.98	4.3			De Snoo and Luttik, 2004
	Pea	The Netherlands	SD	7	1.24	2.33			De Snoo and Luttik, 2004
	Oilseed rape	UK	PrD	16	1.8			3.3	Defra, 2010b
	Soybean	US	UN	17	0.6		0.2		Roy et al., 2019
Headland	Wheat (autumn)	The Netherlands	SD	8	41.6				De Snoo and Luttik, 2004
	Wheat (spring)	The Netherlands	SD	6	9.6				De Snoo and Luttik, 2004
	Wheat (autumn)	UK	SD	31	10.0		1.7		Pascual et al., 1999
	Wheat (autumn)	UK	SD	20	15.4			29.3	Defra, 2010b
	Barley (autumn)	UK	SD	20	15.1			38.6	Defra, 2010b
	Barley (spring)	UK	SD	20	23.9			47.8	Defra, 2010b
	Cereal (autumn)	Spain	SD,BC	28	43.4		5.5	98.1	Lopez-Antia et al., 2016
	Wheat (autumn)	UK	UN	39	3.7		0.4		Lennon et al., 2020
	Maize	The Netherlands	PrD	6	0.14			0.14	De Snoo and Luttik, 2004
	Maize	UK	PrD	20	0.83		1.68	0.83	Defra, 2010b
	Maize	US	PrD	7	0.1	0.06		0.1	Roy et al., 2019

Location in field	Crop	Study location	Drilling technique	N fields	Number of seeds per m^2				Reference
					Mean	SD	SE	90th percentile	
	Sugar beet	UK	PrD	20	0.41			1.26	Defra, 2010b
	Oilseed rape	UK	PrD	16	3.19			7.9	Defra, 2010b
	Soybean	US	UN	17	1.5		0.3		Roy et al., 2019

Drilling techniques: SD = Standard drilling, BC = Broadcasting, PrD = precision drilling, UN = Unknown. For cereals, the planting season is indicated in the table, for all other crops, the planting season was always spring.

Table P.2: Factors to consider when performing seed availability studies together with possible mitigation measures

Factors to consider	Characterisation		Mitigation measures (connected with the sowing)[1]
Drilling technique	Broadcasting/standard drilling/precision drilling/ direct drilling	All possible techniques normally used in a crop (across the EU MS where the crop is grown) have to be considered (Table P.3).	Precision drilling of seeds will lead to relatively fewer seeds remaining on the soil surface. Precision drilling is not the norm for some crops (cereals).
Field site	Headlands/field centre	Drilling efficiency is always lower in the headlands. Thus, the availability of seeds in the headlands must be calculated separately from the one in the field centre.	Avoid double sowing of the headlands.
Soil condition	Fine seedbed/cloddy seedbed	Drilling efficiency is reduced when large clods and stones are abundant.	Preparation of the seedbed (tillage). Remove bigger stones. Adjust drill settings and tractor speed to soil condition.

(1): As with any type of mitigation, the applicant should provide a risk assessment demonstrating that the suggested mitigation is sufficient to exclude a risk. Therefore, when performing studies investigating seed availability, it might be wise to also investigate seed availability when such mitigation has been used.

Table P.3: Seed size, type of drilling used and maximum and maximum common sowing rates for different crops (source: Lucchesi et al., 2016)

Crop	Driller type	Standard Drilling (SD)/Precision Drilling (PrD)	Maximum seed sowing rate		Maximum commonly used seed sowing rate		Average thousand grain weight (g)
			(kg/ha)	(no. seeds/ha)	(kg/ha)	(no. seeds/ha)	
Maize ZEAMX	1	PrD	70	150,000	30	110,000	160–420
Winter oil seed rape BRSNW	2	SD*	9	1,600,000	6	900,000	3–9
Spring Oilseed rape BRSNS	2	SD	12.6	2,600,000	10	2,000,000	2–10
Sunflower HELAN	3	SD/PrD	30	225,000	30	200,000	50–200
Winter wheat TRZAW	2	SD*	400	7,000,000	260	6,500,000	31–61
Spring wheat TRZAS	2	SD*	400	7,000,000	280	7,000,000	30–55
Spring Durum Wheat TRZAW	2	SD*	275	6,000,000	220	6,000,000	35–60
Winter barley HORVW	2	SD*	300	6,000,000	250	6,000,000	30–59
Spring barley HORVS	2	SD*	300	5,500,000	250	5,500,000	33–59
Winter rye SECCW	2	SD*	280	5,500,000	280	5,000,000	22–45
Spring rye SECCS	2	SD*	250	6,000,000	230	5,500,000	23–41
Rye hybrid cultivars	2	SD*	150	3,200,000	150	3,500,000	21–43
Winter triticale TTLWI	2	SD*	260	6,500,000	250	6,500,000	30–58
Spring triticale TTLSO	2	SD*	300	6,500,000	230	5,500,000	29–55
Winter oat AVESW	2	SD*	230	5,900,000	200	4,900,000	24–40
Spring oat AVESA	2	SD*	230	6,500,000	210	6,500,000	27–48
Spelt	2	SD*			250		
Sugar beet BEAVA	4	PrD	4	200,000	4	150,000	10–31
Linseed, flax LIUUT	5	PrD	180	30,000,000	180	30,000,000	2–10
Broad Bean VICFX	6	PrD	500	1,200,000	350	800,000	200–3000

Crop	Driller type	Standard Drilling (SD)/Precision Drilling (PrD)	Maximum seed sowing rate		Maximum commonly used seed sowing rate		Average thousand grain weight (g)
			(kg/ha)	(no. seeds/ha)	(kg/ha)	(no. seeds/ha)	
Pea PIBSX	6	PrD	350	1,500,000	350	1,400,000	88–432
Garden Beans PHSVX	6	PrD	214.5	600,000	180	420,000	100–700
Lupin LUPSS			500	1,400,000	250	1,100,000	111–400
Onion ALLCE	7	PrD	20	2,500,000	6	1,350,000	2.9–5
Carrot DAUCA	7	PrD	6	2,500,000	6	2,000,000	0.7–2.5
Head Brassicas (including Red cabbage BRSOR, Head cabbage BRSOL, Oxheart cabbage BRSON, Savoy cabbage BRSOS)	8	NA(1)	3	100,000	2,5	80,000	3–5
Leek ALLPO	7	PrD	8	1,200,000	3.5	250,000	2.5–3.5
Spinach SPQOL	9	SD (PrD upcoming)	125	5,000,000	60	4,000,000	6–15
Parnips					0.7		
Small radish RAPSR	7	PrD	60	4,000,000	30	2,600,000	7–7.5
Cucumber	7	PrD	8	60,000	8	60,000	20–35
Cucurbits ICUUG			2,5		2,5		
Spring oilseed turnip BRSRO			12,6	3,000,000	10,5	3,000,000	2,5–4
Buckwheat FAGES			80	4,500,000	80	4,500,000	
Red Beetroot BEAVD	7	PrD	10	860,000	10	550,000	
Soya GLXMA			175	700,000	120	600,000	120–250
Alfalfa MEDSA	10	SD or PrD		3,500,000		3,500,000	
Garlic ALLSA			1000		1000		
Lettuce (head) LACSC	8	NA(1)	2		2	120,000	
Lettuce (leafy) LACSA	8	NA(1)	3		3		
Lambs lettuce VLLLO	8	NA(1)		7,000,000		6,000,000	
Sorghum SORVU	1	PrD	30		30	500,000	28–33
Green pepper CPSAN	7	PrD	1.2		1.1		
Tomato LYPES	7	PrD	0.3		0.3		

*: It may be substituted by precision drilling in the future.
(1): Not applicable; only sown and raised to young plants indoors; later transplanted indoors or outdoors.

Driller type codes: (1) 90% pneumatic vacuum principle. (2) mechanical and pneumatic seed drill equipment, pneumatic with vacuum principle upcoming. (3) both mechanical and pneumatic with and without vacuum technique are possible. (4) Pneumatic or mechanical precision drilling equipment. (5) mostly mechanical seed drill equipment, pneumatic with vacuum principle upcoming. (6) Pneumatic (mainly vacuum technique) or mechanical precision drilling equipment. (7) Pneumatic precision drilling equipment. (8) not applicable; only sown and raised to young plants indoors; later transplanted indoors or outdoors. (9) mainly mechanically drilled, pneumatic equipment upcoming (both vacuum and gauge pressure principle). (10) both mechanical and pneumatic (vacuum) are possible.

Appendix Q – Background information for the Tier 1 risk assessment for granular application

The present appendix reports the data considered for the suggestion of default parameters in the Tier 1 risk assessment for the scenario 2: Ingestion of granules as grit. For further details on how to conduct the risk assessment for products applied as granules, see chapter 8.

Number of grit per day in birds

The number of grit/day that are currently indicated in the guidance are extracted from de Leeuw et al. (1995).

In EFSA (2009), only number of grit in the gizzard of granivorous birds are considered and only grit with a size > 0.5 mm. Smaller particles were considered to be consumed by accident as it was not considered likely that those small particles may be actively selected by birds.

However, in a more recent work (Moore et al., 2010), it is reported that mean grit size may vary (0.3–2.9 mm) and may overlap with the size of pesticide granules which were categorised into coarse (1.0–0.5 mm) and medium-sized (0.5–0.25 mm). Gionfriddo and Best (1995) reported an overall mean size of 0.5 mm in the grit of house sparrow but 24% of grit were smaller.

Based on the available information, the WG considered as not justified the exclusion of grit with a size below 0.5 mm.

Additionally, from the work by de Leeuw et al. (1995) which is the one used in EFSA (2009), only data on predominantly granivorous were considered. Data on omnivorous and non-granivorous were excluded, although the highest number of grits in the gizzard were available for omnivorous species, like skylark. According to a report by Bennett et al. (2011), grit have been found in all the gizzards of granivores and omnivores while the occurrence of grit for insectivorous species was much lower.

Gionfriddo and Best (1996) also reported data on grit content in the gizzard. Those data were apparently not considered in the EFSA (2009).

Based on the available data, the WG considered neither justified the exclusion of data on smaller grit from the paper by de Leeuw et al. (1995) nor the exclusion of data on omnivores species. Moreover, the WG also considered the data from Gionfriddo and Best (1996) including data on non-European species. This was considered appropriate since the data set is far from been extensive and the additional data may make it more robust, covering information on species not considered in the original data set. It is noted that species from Gionfriddo and Best (1996) with an occurrence of grit in the gizzard below 50% were excluded (see Table Q.1).

The number of grit per bird have already been normalised for the body weight. Information on bird body weight were taken from the following website https://www.bto.org/understanding-birds/birdfacts.

Table Q.1: Data used for the estimation of Number of grit per kg bw/day

Species	Dietary class	Body weight (g)	N° animals	Grit size (mm)	Number/ bird	Number normalised bw	Grit size (mm)	Number/ bird	Number normalised bw	Number of grit per kg bw/day
Medium/Large Species										
Grey partridge	G	390	25	0.25–0.5	378	969	> 0.5	676	1,733	
Wood pigeon	G	450	20	0.25–0.5	4.9	11	> 0.5	208	462	
Pheasant (de Leeuw et al., 1995)	G	1,190	16	0.25–0.5	97.6	82	> 0.5	214	180	
Pheasant (ring-necked) (Gionfriddo and Best, 1996)	G	1,190	37				> 0.5	151	127	
Mourning Dove (Gionfriddo and Best, 1996)	G	140	40				> 0.5	10	71	
Northern Bobwhite (Gionfriddo and Best, 1996)	O	155	75				> 0.5	49	316	
Rock Dove (Gionfriddo and Best, 1996)	O	300	15				> 0.5	69	230	
Blue Jay (medium weight 55–110 g) (Gionfriddo and Best, 1996)	O	170	20				> 0.5	35	206	
American Crow (Gionfriddo and Best, 1996)	O	468	64				> 0.5	49	105	
Common Grackle (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	O	110	47				> 0.5	10	91	
geomean (G+O)						95			212	**142**
90th perc (G+O)						792			589	**683**
Small species										
Species			**N° animals**	**Grit size (mm)**	**Number**	**Number normalised bw**	**Grit size (mm)**	**Number**	**Number normalised bw**	
Greenfinch	G	28	8	0.25–0.5	46.6	1,664	> 0.5	95	3,382	
Chaffinch	G	24	8	0.25–0.5	26.3	1,096	> 0.5	65	2,704	
Linnet	G	19	2	0.25–0.5	11.4	600	> 0.5	100	5,237	
Twite	G	16	1	0.25–0.5			> 0.5	122	7,625	
Brambling	G	24	1	0.25–0.5	8.5	354	> 0.5	188	7,813	
Goldfinch	G	17	1	0.25–0.5	20.3	1,194	> 0.5	43	2,506	

Species	Dietary class	Body weight (g)	N° animals	Grit size (mm)	Number/ bird	Number normalised bw	Grit size (mm)	Number/ bird	Number normalised bw	Number of grit per kg bw/day
House sparrow (Gionfriddo and Best, 1996)	G	34	146				> 0.5	281	8,265	
Fox sparrow (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	G	35	5	0.5	102	2,914				
Savannah sparrow (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	G	26	21				> 0.5	41	1,577	
American Tree Sparrow (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	G	24	8				> 0.5	267	11,125	
House Sparrow (Gionfriddo and Best, 1995)	G (by the author)	34	245	0.5	580	17,059				
House sparrow	O	34	11	0.25–0.5	217.5	6,397	> 0.5	179.3	5,274	
Song sparrow (Gionfriddo and Best, 1996)	O	32	14				> 0.5	14	438	
Skylark	O	38	6	0.25–0.5	422.7	11,124	> 0.5	217	5,711	
Tree sparrow	O	24	1	0.25–0.5	44.3	1,846	> 0.5	76.6	3,192	
Chipping Sparrow (Gionfriddo and Best, 1996)	O	12	20				> 0.5	12	1,000	
Vesper Sparrow (Gionfriddo and Best, 1996)	O	24	125				> 0.5	12	500	
Lark Sparrow (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	O	28	5				> 0.5	42	1,500	
Horned Lark (Gionfriddo and Best, 1996)	O	31	69				> 0.5	11	355	
Hermit Thrush (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	O	30	6	0.3	14	467				
Indigo Bunting (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	O	14	21				> 0.5	35	2,500	

Species	Dietary class	Body weight (g)	N° animals	Grit size (mm)	Number/ bird	Number normalised bw	Grit size (mm)	Number/ bird	Number normalised bw	Number of grit per kg bw/day
Red-winged Balckbird (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	O	55	82				> 0.5	17	309	
Brown-headed cowbird (Gionfriddo and Best, 1996) (marginal occurrence in Europe)	O	42	175				> 0.5	10	238	
geomean (incl omniv)					52	1,933		53	2,072	**2,002**
90th perc (incl omniv)					423	11,124		222	7,858	**9,349**

Grit retention time

In EFSA (2009), in order to estimate the daily grit intake, the number of grits is multiplied by a conversion factor which is equal to 1 divided by the grit retention time. A conversion factor of 4.2 is recommended. This value was derived considering the grit retention time (0.24 days) estimated in Fischer and Best (1995). In this paper, house sparrows (*Passer domesticus*) were fed with silica granules mixed with a canned dog food and a grit retention time.

The grit retention time of 0.24 days represents one of the shortest reported retention times in the literature. In Stafford and Best (1999), however, the conversion of 4.2 was already questioned for the following reasons: (i) it was based on a single species and the granules were intermixed with dog food and thus the birds consumed them unintentionally; (ii) the administration of granules mixed with canned dog food limited the bird requirement for grit since the diet was considered atypically soft.

In general, grit retention time may vary depending on grit availability, being relatively short when grit availability is not limited (Bennett et al., 2011).

Table Q.2: Available data on grit retention time (Bennett et al., 2011)

Reference	Species	Type of grit	Grit retention time (d)
Mateo and Guitart (2000)	Mallard duck	Calcareous and commercial diet	1.4 (median) (95% CI 0.91–1.87)
Trost (1981)	Mallard duck	Quartzite and different diets in different experiment	3.1 (median)
Gionfriddo and Best (1995)	House sparrow	quartz	0.5 (median)
Best and Stafford (2002)	House sparrow and Bobwhite quail	Silica grit	1 (mean)
López-Calleja et al. (2000)	Refous-collared sparrow	Various	1 (mean)

By considering the available information, the WG considered a grit retention time of 1 day as more appropriate mainly because this was derived by experiments where birds had a more typical seed diet.

Estimation of Gdensity

As reported in section Table 13 and Section 8.2, the $G_{loading}$ is the mg a.s./granule. Normally this value should be available as part of the GAP. However, there may be situation where this information is missing. Reported below is an approach on how to estimate the $G_{loading}$.

For the $G_{density}$, defined as the number of granules at soil surface per m^2, when according to the GAP the granules are incorporated, the default values reported in the EFSA Opinion (2008) can still be used (see Table Q.3 below). Those values are, however, not very recent and include data from Canada and USA. Therefore, if those are not considered appropriate for the conditions of use, broadcast may be assumed and the approach reported in Table Q.4 below may be used to estimate the $G_{density}$.

Table Q.3: Default percentages to be used for the proportion of incorporated granules left on the soil surface after application (EFSA, 2008)

Crop	Planting conditions	Mid field	End row (no shut-off mechanism)	End row (shut off mechanism)	End row (cross seeded in headland)
Maize	Surface banding – press wheel only to incorporate	37			
	Surface banding – tines or chains to incorporate	3.7–40			
	In furrow application	0.4–0.8	52	21	
	Sub-surface band (T-band)	6.5	8.1	21	
Various	Broadcast with field-wide incorporation	2.2–4.2			1.7–2.3
Rapeseed	Various drill types	0.31–14			0.13–15

Table Q.4: Estimation of $G_{density}$ for broadcast granule

Parameter	Unit	Reference
pour density of the granule formulation:	g/mL	Point 2.3 of Regulation 284/2013
Mean diameter of one granule	μm	Point 2.8.5.1 of Regulation 284/2013
Volume of one granule: $4/3 \times r^3 \times \pi$	μm^3	Calculate Π = pi = 3.14 r = radius of the granule
Convert volume of one granule in μm^3 to mL (divide by 1×10^{12})	mL	Calculate
Calculate number of granules/mL 1/volume of one granule in mL	Number of granules/mL	Calculate
Calculate weight of a single granule = (Pour density of granules (g/mL) × 1,000)/number of granules/mL	mg/granule	Calculate
Calculate number of granules per g (Number of granules/mL/pour density × 1,000)	Number of granules/g	Calculate
Calculate g product/m^2 (i.e. g product/ha/10,000)	g product/m^2	Calculate
Calculate granules/m^2 (G product/m^2 × Number of granules/g)	Granules/m^2	Calculate

Appendix R – Food web models in regulatory ERA for birds and mammals

Introduction

A food web is a network of species connected by trophic (feeding) links, representing multiple pathways (intersecting food chains) through which energy and matter flow through an ecosystem (Chiu, 2013). Consequently, food webs allow linking population dynamics to community dynamics by illustrating direct and indirect trophic interactions within a community or between interconnected ecosystems. Food web models aim to capture these trophic links by mathematical expressions. In ecotoxicology they are often used to model trophic transfer of contaminants and secondary poisoning, to estimate the consequences of chemical stress on food webs including indirect effects due to shifts in interactions of populations, and to link ecosystem structure and function (Baird and Burton, 2001; Solomon et al., 2008).

Use of food web models to refine risks due to secondary poisoning

In this revised Guidance Document for Birds & Mammals, with a focus on toxic effects due to dietary exposure, food web models may play a (future) role as a Tier 4 tool to refine the assessment of the potential risk of secondary poisoning (see chapter 10).

Food web models to assess the bioaccumulation potential of toxicants within terrestrial food webs are far less developed than for aquatic ecosystems (e.g. Carbonell et al., 2000; Arnot and Gobas, 2004; Alonso et al., 2008). Aquatic bioaccumulation food webs have been developed to assess the risk of secondary poisoning to humans (through the consumption of commercial fish and shellfish) and to terrestrial wildlife species linked to the aquatic food web. Terrestrial food web bioaccumulation models can be divided in agricultural-foodstuff food webs and wildlife food webs. The former includes transfer of chemicals from air and soil to plants, from plants to herbivore (cow/livestock), from cow to milk and meat, followed by consumption by humans. Wildlife food web models deal with bioaccumulation of chemicals from air and soil up to herbivorous and carnivorous vertebrate wildlife species.

According to Gobas et al. (2016), the application of food web models is currently limited to chemicals that undergo a passive diffusion transport mechanism where lipid is the main storage compartment within the organism, since chemical-specific biotransformation rates and other parameters are so far missing for other than lipid-stored chemicals. Models for ionic and ionogenic chemicals (including some pesticides) are lacking. In addition, field data sets for developing and testing terrestrial bioaccumulation models are limited, standardised modelling scenarios are unavailable and the species-taxa coverage within the existing models is poor. Consequently, the use of food web models to address secondary poisoning to birds and mammals largely is a research activity to date.

EFSA PPR Panel (2015) published a schematic presentation of a food web model to address the potential risks of secondary poisoning of birds and mammals through pesticide exposure via the benthic and pelagic aquatic food chain, but detailed guidance how to develop and interpret food web models was not given.

Before their use in regulatory ERA, it is important that food web bioaccumulation models and their documentation are further developed, following the comprehensive suggestions of EFSA PPR Panel (2014) and Raimondo et al. (2021), as described for population models (refer to Section 7.3).

Use of food web models to assess consequences of indirect effects

Food web models also have great potential to provide mechanistic understanding of the consequences of the combination of direct and indirect effects on birds and mammals. Important indirect effects are due to pesticide-induced shifts in food availability in agricultural landscapes, e.g. a decline of arthropods due to insecticide application or a decline in weeds due to herbicide application (refer to protection goal section). Addressing potential risks of these indirect effects for birds and mammals, however, requires an integrative approach in guidance development for terrestrial organisms (particularly in concert with that of weeds, non-target arthropods and soil organisms).

Appendix S – Background consideration of risk to birds and mammals via consumption of residues in puddles

EFSA (2009) recommended that a risk assessment was performed for birds and mammals taking contaminated water from puddles containing residues of pesticides and/or metabolites, formed in the field after a heavy rainfall event. A quantitative risk assessment was only needed for those substances which did not 'pass' a screening step as follows:

- For substances with a K_{oc} < 500 L/kg, if the ratio of effective application rate (g/ha) to the relevant endpoint did not exceed 50 then no quantitative risk assessment was needed.
- For substances with a $K_{oc} \geq 500$ L/kg, if the ratio of effective application rate (g/ha) to the relevant endpoint did not exceed 3,000 then no quantitative risk assessment was needed.

The effective application rate was determined by use of a MAF value in the case of multiple applications.

In cases where the screening step did not pass then a quantitative risk assessment was needed. Such assessment accounted for the daily water intake for birds and mammals and the concentration in puddles. However, it was questioned (EFSA, 2020) whether there is need for a risk assessment for birds and mammals as experience had shown that the resulting TERs usually exceeded the assessment factor by a notable margin.

Moreover, the methodology for calculation of the concentration of substances in puddles (PEC_{puddle}), proposed in EFSA (2009), has been criticised (e.g. EFSA PPR Panel, 2012a). Two approaches for estimating PEC_{puddle} have been suggested over the past years by EFSA. The first method is to use the concentration in the run-off water from the four FOCUS surface water scenarios (R1, R2, R3, R4) as a surrogate for PEC_{puddle} (EFSA PPR Panel, 2012a). The second approach is to use the pore water PEC value in the top 1 cm of soil (EFSA, 2017). Whilst neither approach would give precise concentrations in puddles, the WG considered that both approaches to be reasonable for the purposes of a scoping assessment. The concentration in run-off water is not a standard output of FOCUS surface water modelling and would require a more in-depth consideration. Since pore water PEC can easily be obtained following the approach described in EFSA (2017), this method is selected for the purposes of the scoping assessment.

To understand the need for a risk assessment for birds and mammals from consumption of contaminated water in puddles a scoping assessment was performed by performing a risk assessment for 9 substances considering the concentration in puddles calculated according to the methodology in EFSA (2009) and also assuming the PEC in puddles is equivalent to the PEC in pore water (1 cm) determined according to the methodology in EFSA (2017). The resulting assessments are summarised in Tables S.1 and S.2.

Table S.1: Scoping assessment for birds for 9 active substances

Substance	RA using PEC Puddle using the methodology in EFSA (2009)				RA using PEC Puddle from Tier 3 PEC pore water according to EFSA (2017)			
	PEC Puddle mg/L	Residue intake via puddles mg a.s./kg bw per day	Acute TER	Reproductive TER	PEC Puddle mg/L	Residue intake via puddles mg a.s./kg bw per day	Acute TER	Reproductive TER
Alpha-Cypermethrin	0.000005	0.000002	631,629,087	7,430,930	0.00003	0.00001	96,098,218	1,130,567
Benflualin	0.006660	0.003064	652,778	2,187	0.08106	0.03729	53,635	180
Benthiavalicarb	0.165517	0.076138	49,594	1,379	0.66156	0.30432	12,408	345
Benzovindiflupyr	0.002900	0.001334	985,803	18,742	0.01719	0.00791	166,324	3,162
Bifenazate	0.050699	0.023322	4,545	99	0.24094	0.11083	956	21
Diflufenican	0.003007	0.001383	1,554,577	66,406	0.03036	0.01397	153,953	6,576
Flufenacet	0.075312	0.034643	12,528	271	0.64547	0.29692	1,462	32
Fludioxonil	0.000230	0.000106	18,914,783	593,924	0.00089	0.00041	4,859,603	152,592
Linuron	0.110658	0.050903	2,381	273	1.27844	0.58808	206	24

Table S.2: Scoping assessment for mammals for 9 active substances

Substance	RA using PEC Puddle using EFSA, 2009, methodology				RA using PEC Puddle from Tier 3 PEC pore water according to EFSA, 2017			
	PEC Puddle mg/L	Residue intake via puddles mg a.s./kg bw per day	Acute TER	Reproductive TER	PEC Puddle mg/L	Residue intake via puddles mg a.s./kg bw per day	Acute TER	Reproductive TER
Alpha-Cypermethrin	0.000005	0.000001	31,155,724	8,011,472	0.000031	0.000	4,740,139	1,218,893
Benflualin	0.006660	0.001599	3,127,894	3,441	0.081	0.019	2,57,003	283
Benthiavalicarb	0.165517	0.039724	125,868	503	0.662	0.159	31,491	126
Benzovindiflupyr	0.002900	0.000696	79,027	9,771	0.017	0.004	13,333	1,648
Bifenazate	0.050699	0.012168	410,920	123	0.241	0.058	86,468	26
Diflufenican	0.003007	0.000722	6,929,315	49,198	0.030	0.007	686,224	4,872
Flufenacet	0.075312	0.018075	32,587	2,069	0.645	0.155	3,802	241
Fludioxonil	0.000230	0.000055	90,633,333	3,625,333	0.00089	0.000	23,285,598	931,424
Linuron	0.110658	0.026558	112,961	30	1.278	0.307	9,778	**3**

Further consideration of the calculation of PEC_{puddle}

Puddles can be formed in the soil surface of a field after a rainfall event. To estimate PEC_{puddle} in EFSA (2009) it was assumed that these concentrations would be equal to concentrations in the pore water diluted by rainfall and then a simplified model, without water outflow routines, was proposed to calculate PEC_{puddle} as a function of the application rate and the organic carbon normalised adsorption coefficient (Koc) of the substance.

The starting point was the following equation:

$$C_{pw} = \frac{X_{pw} \times AR/10}{V_{pw}}$$

where
C_{pw}: a.s. concentration in pore water (mg/L)
X_{pw}: fraction of the a.s. partitioning to pore water (see below)
AR: application rate (g a.s./ha) divided by 10 to achieve mg a.s./m^2
V_{pw}: pore water volume considering dilution due to runoff (see below)

The term X_{pw}

The fraction of the substance partitioning to pore water (X_{pw}) was calculated using the following equation:

$$X_{pw} = \frac{V_{pw}}{V_{pw} + V_s \times d \times K_d}$$

where
V_{pw}: pore water volume (m^3_{water}/m^2) considering dilution due to run-off (see below)
V_s: volume of soil per field area, equal to 0.05 (m^3_{soil}/m^2) when considering the first 5 cm of soil
d: soil density, default value of 1.5 (kg/L)
K_d: distribution coefficient of the substance

The term V_{pw}

Some assumptions were taken into account to derive the pore water volume considering dilution due to runoff (V_{pw}):

- the field capacity was set as equal to 0.4 (m^3_{water}/m^3_{soil}) and the moisture content to 50%, which leads to a volume of 0.2 (m^3_{water}/m^3_{soil}). The first 5 cm of soil were considered, and then the volume of soil per square meter of surface is equal to 0.05 (m^3_{soil}/m^2).

  Therefore, the expected volume of water ('pore water') per square meter when accounting for the first 5 cm of soil is equal to:

$$\text{pore water} = 0.2\ \frac{m^3 water}{m^3 soil} \times 0.05\ \frac{m^3 soil}{m^2} = 0.01 \frac{m^3 water}{m^2}$$

- a rainfall of 10 mm was considered to trigger runoff without diluting too much the pesticide concentration:

$$10\ \text{mm rainfall} = 10\ \frac{L\ water}{m^2} = 0.01\ \frac{m^3 water}{m^2}$$

Therefore, the pore water volume considering dilution due to runoff (V_{pw}) is equal to:

$$V_{pw} = 0.01\ \frac{m^3 water}{m^2} + 0.01\ \frac{m^3 water}{m^2} = 0.02\ \frac{m^3 water}{m^2}$$

Final equation

Putting the equation for X_{pw} into the main equation, the outcome is:

$$C_{pw} = \frac{\frac{V_{pw}}{V_{pw}+V_s \times d \times K_d} \times AR/10}{V_{pw}} = \frac{AR/10}{V_{pw} + V_s \times d \times K_d}$$

To pass from (m^3/m^2) to (L/m^2) the terms V_{pw} and X_{pw} should be multiplied by 1,000, and then:

$$C_{pw}\left[\frac{\text{mg a.s.}}{L_{water}}\right] = \frac{\frac{AR}{10}\left[\frac{\text{mg a.s.}}{m^2}\right]}{1000 \times \left(V_{pw}\left[\frac{L_{water}}{m^2}\right] + V_s\left[\frac{L_{soil}}{m^2}\right] \times d\left[\frac{kg_{soil}}{L_{soil}}\right] \times K_{oc}\left[\frac{L_{water}}{kg_{soil}}\right] \times f_{oc}\right)}$$

This equation was simplified in EFSA (2009) as:

$$C_{pw}\left[\frac{\text{mg a.s.}}{L_{water}}\right] = PEC_{puddle}\left[\frac{\text{mg a.s.}}{L_{water}}\right] = \frac{AR/10}{1000 \times (w + K_{oc} \times s)}$$

where

$$w = V_{pw} = 0.02\ \frac{m^3 water}{m^2}$$

$$s = V_s \times d \times f_{oc} = 0.05\ \frac{m^3 soil}{m^2} \times 1.5\ \frac{kg_{soil}}{L_{soil}} \times 0.02 = 0.0015$$

Annex A – Refinement of residue decline in food items

Annex B – Summary of Generic Model Species Parameters

Annex C – RUD Database

Annex D – Deposition Values

Annex E – Extrapolation tables

Annex F – Critical Appraisal Tool for ecological field studies

Annex G – Critical Appraisal Tool for effect field studies

Annex H – Outcome of the public consultation

Annex A–H can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2023.7790