

DRAFT SCIENTIFIC OPINION

**Scientific Opinion addressing the state of the science on risk assessment
of plant protection products for in-soil organisms¹**

EFSA Panel on Plant Protection Products and their Residues (PPR)^{2,3}

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ABSTRACT

Following a request from the European Food Safety Authority, the Panel on Plant Protection Products and their Residues developed an opinion on the science behind the risk assessment of plant protection products for in-soil organisms. The current risk assessment scheme is reviewed, taking into account new regulatory frameworks and scientific developments. Proposals are made for specific protection goals for in-soil organisms being key drivers for relevant ecosystem services in agricultural landscapes such as nutrient cycling, soil structure, pest control and biodiversity. Considering the time-scales and biological processes related to the dispersal of the majority of in-soil organisms compared to terrestrial non-target arthropods living above soil, the Panel proposes that in-soil environmental risk assessments are made at in- and off-field scale and field boundary levels. A new testing strategy which takes into account the relevant exposure routes for in-soil organisms and the potential indirect effects is proposed. In order to address species recovery and long-term impacts of PPPs, the use of population models is proposed.

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KEY WORDS

in soil-invertebrates, microorganisms, effects, pesticides, protection goals, risk assessment

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SUMMARY

The new regulatory framework for plant protection products (PPPs) laid out in Commission Regulation (EC) No 1107/2009 and Commission Regulation (EU) No 283/2013 and 284/2013 explicitly requires consideration of impacts on non-target species, on their ongoing behaviour and on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web. In view of this new legislative background and the new scientific developments, the European Food Safety Authority (EFSA) asked the Panel on Plant Protection Products and their Residues (PPR) to develop and update the guidance documents on terrestrial ecotoxicology (SANCO/ 10329/2002) under mandate M-2009-0002. The assessment of effects on biodiversity is not explicitly addressed under the existing guidance documents; appropriate risk-assessment methodology therefore needs to be developed. This scientific opinion has been written as a precursor to the guidance document on the risk assessment for in-soil organisms. Other terrestrial organisms as previously covered in the Sanco Guidance 10329/2002, such as birds and mammals, non-target arthropods, bees and non-target terrestrial plants are covered in other EFSA scientific documents (EFSA, 2009c; EFSA PPR Panel, 2015a; EFSA, 2013; EFSA PPR Panel, 2014a).

In-soil organisms are species that dwell primarily in the soil and soil litter. In-soil organisms are exposed to plant protection products (PPPs) from dermal and oral uptake routes of exposure in the surrounding soil compartment. A 'healthy' soil supports a range of ecosystem functions or services (such as nutrient cycling) that are essential for supporting the growth of crops as well as the organisms that depend on those crops. The working group of the PPR Panel reviewed the current environmental risk assessment, identified key drivers that sustain important in-soil, ecosystem services in agricultural landscapes and developed proposals for specific protection goal (SPG) options for in-field and off-field areas. The SPG options will then be discussed and agreed in consultation with Risk Managers. The working group developed proposals for exposure assessment and testing of effects as well as a method to calibrate the lower tier risk assessment steps.

The in-soil communities of invertebrates and microorganisms in and around crops are the most diverse part of the ecosystem. Yet, the current risk assessment at the first tier only examines a small selection of invertebrate species (e.g. *Eisenia fetida/andrei*, *Folsomia candida/fimetaria*, *Hypoaspis aculeifer*) and one microorganism-mediated process (N transformation). The current tests were reviewed in relation to the proposed in-soil SPG options and the available data. The Panel concludes that the current test battery with the use of an appropriate assessment factor might cover the intra and inter species variability in soil, with the exception of the toxicity of in-soil organisms when exposed *via* food and *via* litter. Note that the current trigger values are not calibrated. The Panel recommends adapting the test with *Hypoaspis aculeifer* to take the uptake of contaminated food into account, and to develop a standardized test with isopods, to take exposure *via* the litter into account. For microorganisms, the Panel proposed retaining and developing the N-transformation test, and adding a test with mycorrhizal fungi.

In a tiered approach, considering the possibilities for intermediate tier testing, the Panel considers that the Species Sensitivity Distribution (SSD) conceptual model is very useful, but that standard SSD methodology cannot yet be applied to in-soil organisms until further guidance on how toxicity data can be combined (e.g structural *vs* functional endpoints; toxicity data on different taxonomic groups of in-soil organisms, etc.) will become available. At higher tiers, the Panel recommends assessment of the response of *communities* of in-soil organisms to intended uses of PPPs, so that indirect effects on populations of key drivers can also be detected. Natural assemblages of in-soil organisms should be studied by using field tests or terrestrial model ecosystems (TMEs).

The Panel recommends that species recovery and other long-term impacts (including multiple stressors) at population level are best investigated using a combination of experimental data and population modelling, if these were including all relevant environmental and ecological parameters. However, since long-term impacts and indirect effects at community level cannot be assessed using population models, (semi)field studies with natural assembled communities are needed. It should be

77 noted that species recovery after impact of PPP intended uses might be demonstrated only later in
78 time than suggested in the SPG options, especially for key drivers with longer generation time.

79 It is suggested that assessment factors be derived on the basis of statistical modelling of the
80 relationships between effects for different species in the various possible lower tier tests and higher
81 tier field studies and the surrogate reference tier. In particular, a Bayesian network model can exploit
82 information from both experimental data and expert judgement and provides a relatively transparent
83 method for deriving assessment factors in order to ensure a high probability of acceptable effects for
84 uses that pass the risk assessment.

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1. INTRODUCTION

In 2008, the PPR Panel was tasked by EFSA to revise the guidance documents (GDs) for Terrestrial Ecotoxicology (SANCO/10329/2002 rev. 2 final) (EC, 2002), which is currently used in the routine environmental risk assessment for terrestrial non-target organisms (except for birds and mammals) exposed to active substances in plant protection products (PPPs). The replacement of Directive 91/414/EEC⁴ by Regulation (EC) No 1107/2009⁵ (hereafter referred to as “the Regulation”) in June 2011 called for revision of the existing GDs in order to include new elements in ERA, e.g. cut-off criteria and additional general protection goals.

It was decided to split the task and to address separately the risk for different groups of organisms, i.e. in-soil organisms, non-target arthropods (NTAs), amphibians and reptiles, and non-target terrestrial plants (NTTPs). For each group of organisms, the PPR Panel first summarises the science behind the respective risk assessment in a scientific opinion and, in a second step, EFSA will develop practical guidance on how to perform the risk assessment. The present Opinion is focussed mainly on in-soil invertebrates and soil microorganisms. Vertebrates such as moles or toads are dealt with in the Guidance for birds and mammals (EFSA, 2009a). Rooted plants are dealt with in the Opinion on non target terrestrial plants (EFSA PPR Panel, 2014a).

For the purpose of this Scientific Opinion and for consistency with the definitions as given in the recent Opinion of the PPR Panel on non-target terrestrial arthropods (NTA) (EFSA PPR Panel, 2015a), in-soil organisms are defined as species that dwell primarily in the soil and soil litter layer. In-soil organisms may become exposed to PPPs from dermal and oral uptake routes taking place in the surrounding soil compartment (EFSA PPR Panel, 2015a). The opinion is concerned with all non-target in-soil organisms, also considered beneficial because of their positive impact on soil functions and services while not causing harm to crops.

According to ISO 11074:2005, soil is defined as the upper layer of the earth’s crust transformed by weathering and physical/chemical and biological processes. It is composed of mineral particles, organic matter, water, air, and living organisms organized in generic soil horizons. Soil performs a multitude of key environmental, economic, social and cultural functions, and could be regarded as the most complex biological environment directly affected by PPPs. Soil, for example, provides food, biomass and raw materials and plays a central role as a habitat and gene pool (biodiversity). In-soil organisms, including macro, meso, microfauna and microorganisms, are extremely diverse and contribute to a wide range of ecosystem services, such as nutrient cycling, pest and disease control, natural attenuation of pollutants, soil formation and stabilization, etc. All these important ecosystem services could potentially be impacted by the intentional release of PPPs in the environment if the key drivers of the services were to be adversely affected by exposure to pesticides.

General protection goals are stated in European legislation but are not precisely defined. A precise definition is however crucial for designing appropriate risk-assessment schemes. Therefore, specific protection goal (SPG) options are presented, to be used in consultation processes with risk managers and stakeholders. It is the responsibility of risk managers to select the final SPG options that should be addressed in decision schemes of guidance documents. According to the PPR Panel Opinion (EFSA PPR Panel, 2010a), in-soil organisms from different functional groups (ecosystem engineers, microarthropods, nematodes, molluscs, microorganisms, etc.) have been identified as providers of important ecosystem services in the soil ecosystem. Specific protection goals have been developed considering six dimensions, namely ecological entity, attribute, magnitude of effects, temporal scale of effect, spatial scale of effect and degree of certainty.

⁴ Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market.

⁵ 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

Proposals for specific protection goals have been defined both for in-field and off-field as in-soil organisms also occur and are potentially exposed to PPPs outside the treated field. However, dispersal ability and biological characteristics need to be considered in the context of the pesticide risk assessment for these organisms. Some organisms move between fields and across field boundaries, so that recolonization processes from the off- to the in-field might take place in relevant time frames. Others move only a limited distance within a field and might pre-dominantly recover from PPP effects by processes that govern internal recovery.

Although managing landscape structure is not within the remit of this scientific opinion, the PPR Panel believes that modern ERA of PPPs for in-soil (and other terrestrial) organisms should consider landscape structure. This is because, although the risk to in-soil organisms on a very local scale and in short time intervals may depend almost exclusively on the exposure and toxicity of PPPs applied in crop protection programmes, the ultimate large-scale effects depend also on the existence, size and spacing of refuges where populations of in-soil organisms can survive any crop treatment. These refuges allow small populations of in-soil (and other) organisms to survive in off-crop areas and may provide an “organismal bank”, crucial for re-colonisation of cropped fields after in-crop populations have been severely affected by PPPs, or even driven to local extinction. Such organismal banks are also crucial for re-colonising abandoned fields and other plantations. The Panel recognises that such considerations depend on the scale of dispersal characteristic of a particular organism. Microorganisms and small fauna with a limited dispersal ability may only actively move a matter of centimeters or less and are, in effect, completely isolated from populations in other fields or field boundaries, apart from rare, random translocations. Ecological risk assessment, however, needs to take into account all important factors determining population dynamics, and the structure of the agricultural landscape can be one of the most crucial ecological elements for the dynamics of many species (Topping et al., 2015). Furthermore, some protection goals, e.g. food-web support, require the consideration of a certain spatial and temporal scale to characterize the availability of in-soil organisms as food, e.g. for birds and mammals.

In contrast to human toxicology, where individual health is protected by studies on several surrogate species, ecotoxicology is based on testing a limited number of species to make inferences about a much larger number of species. ERA for in-soil organisms is at the extreme end of the RA spectrum because the diversity of in-soil species is much greater than for any other group of organisms in an environment directly affected by PPPs, while the tests and test data available are few compared with, e.g., aquatic RA. This inevitably leads to a higher level of uncertainty.

This Opinion is structured to address key scientific aspects behind ERA for in-soil organisms, with a focus on in-soil invertebrates and microorganisms, including the major points resulting from the EFSA public consultation on the SANCO Guidance Document on Terrestrial Ecotoxicology. After a brief explanation on how the risk assessment for in-soil organisms is currently done, a section on the further elaboration of the proposed specific protection goal (SPGs) option is presented. Also, a general framework with an overview on the key aspects on the possible future risk assessment scheme for in-soil organisms is presented, followed by discussion on aspects of both exposure and effects assessment. For exposure of in-soil organisms, the focus is on the work developed by EFSA for spray application to annual crops under conventional or reduced tillage (development of exposure assessment for permanent crop is on-going and an update of the EFSA GD 2015 is foreseen by the end of 2017). On the effects-assessment side, existing and promising testing approaches are presented including tests for intermediate and higher tiers, how to deal with persistency and how to tackle recovery. These approaches may possibly be adopted in the future ERA scheme.

1.1. Background and Terms of Reference as provided by requestor

EFSA tasked the Pesticides Unit and the PPR Panel on the following activity, taking into consideration the legislative background, stakeholder comments and the recommendations and priorities identified by Member States.

Development of Guidance on risk assessment for in-soil organisms, with the following deliverables:

- Opinion addressing the state of the science to be delivered by the PPR Panel by April 2017
- Public consultation on the draft Opinion of the PPR Panel to be issued by the 1th quarter of 2016
- Guidance of EFSA to be delivered within 2 years after the agreement with risk managers on the specific protection goals
- Public consultation on the draft Guidance of EFSA

1.2. Legislative background

Active substances used in plant protection products (PPPs) are authorised in the European Union (EU) under Regulation (EC) No 1107/2009. The Regulation requires that ‘substances or products produced or placed on the market do not have any harmful effect on human or animal health or any unacceptable effects on the environment’. With respect to the environment, this includes, in particular, considerations of the impact on non-target species, including the ongoing behaviour of those species, and the impact on biodiversity and the ecosystem.

New Commission regulations laying down the data requirements for the dossier to be submitted for the approval of active substances contained in PPPs and the authorization of PPPs (Commission Regulation (EU) No 283/2013⁶ and 284/2013⁷) were published in 2013. Those documents provide information on the core data needed to assess active substances and PPPs. As a general requirement for substance approval, it is stated in Commission Regulation (EU) No 283/2013 that ‘the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web, shall be considered’.

Microbial PPPs have specific data requirements for active agents as well as formulated products; they are not specifically addressed in this opinion.

2. CURRENT RISK ASSESSMENT

2.1. Current risk assessment for in-soil organisms and other background documents

The state of the art regarding the risk assessment of pesticides to in-soil organisms is presented in the present chapter. In particular, an overview is given on the 1) current risk assessment approaches according to the SANCO/10329/2002 Terrestrial Guidance Document (EC, 2002); 2) main outcomes of EFSA public consultation on the SANCO/10329/2002 Terrestrial Guidance Document (EFSA, 2009b); 3) main outcomes of the workshop on semi-field methods for the environmental risk

⁶ 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. OJ L 93, 3.4.2013, p. 1-94

⁷ 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. OJ L 93, 3.4.2013, p. 85-152

assessment of pesticides in soil (PERAS workshop, Coimbra, 2007; Schaeffer et al., 2008; 2010) and 4) guidance for summarising earthworm field studies (De Jong et al., 2006).

2.1.1. Terrestrial Guidance Document SANCO/10329/2002

The current risk assessment for in-soil organisms is carried out according to the SANCO/10329/2002 Terrestrial Guidance Document developed under the Council Directive 91/414/EEC. This Directive was repealed in 2009 by the (EC) Regulation 1107/2009, while the Commission Regulations (EU) No 283/2013 and 284/2013 laid down the new data requirements for active substances and plant protection products (PPPs), respectively. Therefore, only the parts of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) covered by the regulations will be considered in the following paragraphs.

The risk assessment for in-soil organisms follows the principle of the risk assessment paradigm: 1) hazard identification, 2) hazard characterization, 3) exposure assessment, 4) risk characterization.

A tiered approach is used. The concept of the tiered approach is to start with a simple, conservative assessment and to go towards more complex evaluations (higher tiers), when necessary.

2.1.2. Exposure assessment

The exposure characterisation is represented by a comprehensive evaluation of fate and behaviour of the active substance and transformation products in soil of the treated area, including the estimation of predicted environmental concentrations (PECs). The initial PECs values after single or multiple applications and PECs plateau are calculated according to FOCUS (FOCUS, 1997). The choice of the relevant PECs to be used for risk assessment will depend on the characteristic of the active substance (e.g. persistence in soil) and on the intended uses.

2.1.3. Effect assessment

The effect assessment is represented by a comprehensive investigation of the dose-response relationships, in order to derive toxicity endpoints (e.g. LC₅₀, NOEC), which can be compared with the predicted environmental concentrations. According to the new data requirements, the studies listed below should be conducted and reported unless it is proven that the contamination of soil is unlikely. It is highlighted that the acute toxicity study on earthworms is no longer a data requirement.

- Test for sublethal effects on earthworms (*Eisenia fetida* or *Eisenia andrei*). The test is conducted according to the OECD guideline 222 (OECD, 2004) and information on the effects on growth, reproduction and behaviour of the earthworms should be reported. The relevant endpoint might be either EC₁₀, EC₂₀ or NOEC.

- Test on springtail *Folsomia candida* (OECD, 2009) and mite *Hypoaspis aculeifer* (OECD, 2008) for plant protection products applied directly to soil as soil treatments. For plant protection products applied as a foliar spray, data on soil invertebrates other than earthworms may be required in case concerns have been identified in the risk assessment of non-target arthropods, as data on both the hymenopteran parasitoid *Aphidius rhopalosiphi* and predatory mite *Typhlodromus pyri* may be used in an initial risk assessment. The relevant endpoint might be either EC₁₀, EC₂₀ or NOEC.

- Test on the impact of active substances and plant protection products on soil microbial activity in terms of nitrogen transformation (OECD, 2000). The test is done at two concentrations, the PEC (= maximum predicted concentration in soil) and double the PEC as the worst case. The results are reported as the ratio of the nitrate-formation rates at the PEC relative to the control, expressed as a percentage of the control rate.

In case further refinements of the risk are triggered, field studies reflecting the intended uses of the PPP, the environmental conditions likely to arise and species that will be exposed, should be conducted, as indicated in the Uniform Principles (Commission Regulation (EU) No 546/2011⁸). Field studies evaluate the effects on abundance and biodiversity, taking into consideration the likely level of effects, the species/groups affected, population recovery (within 1 year) as well as information on the application and fate of the PPP (EPPO, 2003). However, at present, there are few standardised higher tier protocols. The littebag test is one example mentioned in the terrestrial SANCO guidance document but this is more concerned with functional rather than structural endpoints.

The risk to in-soil organisms other than earthworms can be further refined using a more realistic test substrate or exposure regime.

2.1.4. Risk assessment

The risk characterisation is represented by the calculation of appropriate risk quotients. For earthworms and other soil macro-organisms, SANCO/10329/2002 recommends calculating the acute and chronic Toxicity Exposure Ratios (TERs). Only the chronic TER would be currently relevant, however, based on the new data requirement.

TERs are compared with trigger values defined in the Uniform Principles (Commission Regulation (EU) No 546/2011) to establish whether the risk is low (acceptable) or high (unacceptable). Triggers are sometimes described as “safety factors” that should take into account uncertainties in the intra- and inter-species variability and the extrapolation of toxicity endpoints from laboratory to field (including uncertainties with regard to the actual exposure in the field). For earthworms and soil macro-organisms, the current trigger value is 5. If the TER values are below 5, a high risk is identified. For soil microorganisms, the magnitude of effects is directly assessed in terms of risk. According to the Regulation 546/2011 a low risk to microorganisms is demonstrated if the percentage of effect is below 25% after 100 days.

2.2. Public consultation on SANCO terrestrial Guidance

In view of the revision of the current risk assessment for terrestrial organisms, in 2008, EFSA launched a public consultation on the SANCO Guidance Document on Terrestrial Ecotoxicology (EFSA, 2009b).

The aim of the public consultation was to collect issues and gaps identified by different stakeholders to be used as inputs in the revision of the terrestrial guidance.

A total of 33 comments were received from different stakeholders on the chapter about in-soil organisms (Chapter 6 of the SANCO guidance). The main comments concerned the following:

- Development of specific protection goals for in-soil organisms
- More clarity on the level of assessment (structure vs function)
- More guidance on persistent substances

⁸ 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. OJ L 155, 11.6.2011, p. 127-175

- More guidance on how to consider bioavailability when interpreting effect test results and need for more standardised test design (% peat, addition of feed, application of the test item, correction factor)
- Earthworm field studies: more guidance on the evaluation of effects and acceptability criteria (% effects based on total earthworm numbers, biomass, safety factor, etc.). The use of the guidance on how to summarise earthworm field studies was suggested.
- Introduction of semi-field tests (e.g. terrestrial model ecosystem, TME)
- More guidance on the interpretation of effects on soil microorganisms
- More guidance on the exposure assessment (measurement of the concentration in the test, selection of the appropriate PEC, persistence, etc.)

2.3. Other background documents – Semi-field methods for the environmental risk assessment of pesticide in soil

The main aim of the PERAS workshop (Schaeffer et al., 2008; 2010) was to identify suitable semi-field tests able to detect effects of PPPs on in-soil communities to be used in a tiered approach of pesticides risk assessment.

Different existing semi-field methods were reviewed. The semi-field study that was used most was identified as being the TME (Terrestrial Model Ecosystem) method. The TME method was considered as providing reliable and reproducible data concerning effects of pesticides on in-soil organisms. The TMEs are established by directly sampling intact soil cores in the field, e.g., from grassland or crop sites, with natural communities. Thus, TMEs are considered as representative of the field situation, provided that the abundance of sensitive species and their exposure to the test substance are realistic.

Table 1: Main features of Terrestrial model ecosystems (TMEs) (Schaeffer et al., 2010)

Guidelines	ASTM, 1993; UBA, 1994; USEPA, 1996; Knacker et al., 2004
Principles	Interaction of soil properties and the natural community of microorganisms, animals, plants
Species	Natural soil-organism community
Substrate	Undisturbed soils from field sites
Duration	Usually about 16 weeks
Parameter	Wide variety of fate and effect endpoints
Experience	Fungicides, contaminated field soil, or pharmaceuticals in dung

When conducting a TME study, there are aspects that need consideration in view of the problem formulation and, thus, its fitness for purpose. For example, the commonly used TMEs (soil cores 17cm in diameter) are more suitable for studying effects on in-soil micro and mesofauna (e.g. nematodes, micro-arthropods and enchytraeids) and microorganisms, whereas the more recently used ones (up to 50cm diameter) can be used to assess effects on earthworms. TMEs may be collected in crop areas or even forests but experience has shown that it is most suitable for grasslands. Soil moisture is one of the key parameters that needs to be measured and compensated regularly. This system has a limited time span of up to 1 year. Typically, the sizes of TMEs range between 10 and 50 cm in diameter and up to 40 cm in depth.

Recommendations were provided on how to conduct a TME study in order to have a standardized test method with regards to the soil community tested, the application of the test substance based on the persistence, choice of test concentrations, etc.

Gaps in knowledge were identified and recommendations for future research and developments for semi-field test and TMEs were given, in particular for: (i) the extrapolation of results across soil types, climatic and edaphic regions and biological communities; (ii) a classification scheme scaling the magnitude and the duration of effects, and the recovery; (iii) comparative studies to determine the variability of data within and between TMEs as well as those between TMEs with field tests. In addition, recommendations were provided to compare the sensitivity of in- and off-crop communities, if relevant from a risk-assessment point of view.

2.4. Other background documents - Guidance for summarising earthworm field studies

The Dutch platform for the assessment of higher tier studies developed guidance for summarising, reporting and evaluating field studies with earthworms (De Jong et al., 2006). The document did not provide guidance on the use of the results in risk assessment.

In the current practice, studies done according to several protocols can be submitted as part of a dossier for authorization of active substances. For field studies, the ISO guideline (ISO, 1999) and the papers by Greig-Smith (1992) and Sheppard et al. (1998) are cited in the SANCO terrestrial guidance. When an earthworm field study is included in a dossier for pesticide authorization, the Rapporteur Member State (RMS) has to make an evaluation report in which the data should be summarised in a concise and transparent way and the validity has to be discussed. According to De Jong et al. (2006), the reliability of the test should be evaluated by assigning a Reliability Index (Ri). The reliability scale goes from fully reliable (Ri1) to reliable with restrictions (Ri2) and not reliable (Ri3). The studies considered as not reliable are not used in risk assessment. It has to be remembered, however, that a reliable field study might not be useful for risk assessment even if it is defined as reliable.

Six different items for the description of field studies and three items for the reporting of results are proposed to be checked when summarising and evaluating field studies with earthworms:

Description

1. Substance (purity, formulation, vehicle)
2. Test site (location, field history, soil type/substrate, crop, general climatic conditions)
3. Application (mode of application, dosage, application scheme, climate/microclimate)
4. Test design (type and size, test date and duration, pre-treatment, negative control, positive control, replication, statistics, dose-response)
5. Biological system (test organisms, community)
6. Sampling (general features, actual concentration, biological sampling, climate/microclimate)

Results

7. Application (actual concentrations, conditions of application, weather)
8. Endpoint (type, value, verification of endpoint, pre-treatment, negative control, positive control, weather)

9. Elaboration of results (statistical comparison, presentation of results, dose-effect relationship, community level impact)

10. Classification of effects

A decision on reliability can be reached after checking all the above-listed items.

The interpretation of earthworm field studies is not always straightforward because of the several drawbacks in the field studies, such as small number of plots, identification of earthworm species, natural variation and low abundance in arable fields, variability in soil properties, plant cover, etc. Even when a field study represents a worst-case estimate for the representative uses of a specific substance, it is unclear what magnitude (%) of effects can be accepted.

A drawback of the ISO guideline 11268-3 and the SANCO terrestrial guidance (EC, 2002) is the lack of guidance on how to interpret the test results in terms of acceptability of effects. EPPO (2003) proposed a categorization that is, however, based on limitations of the ISO test rather than on objective acceptability criteria, as effects smaller than 50% are not usually measured with sufficient statistical power even in well performed field studies conducted according to the ISO guideline. Therefore, considering all the uncertainties, an alternative solution was proposed in the guidance by De Jong et al. (2006):

- Effects at dosage that is 10-fold the intended field dose <50%: low risk
- Else: high risk

The proposal was based on a field trial with benomyl. In addition, a one-year recovery option in field was still supported by the authors, in line with the EPPO guidance (EPPO, 2003).

3. IN-SOIL ORGANISMS

3.1. In-soil organisms in the scope of this Opinion

In-soil organisms are broadly separated into two groups: soil fauna and microorganisms (this division is also endorsed in this opinion). Soil microorganisms are a very diverse group of organisms that are generally not visible to the unaided eye (<100 µm body size). The major groups of soil microorganisms are bacteria, archaeans, fungi and protozoa, and they are unified by the lack of ability to form distinct tissues or organs. They operate on a spatial scale of a few millimetres and their generation time goes from hours to a few days. Soil fauna includes diverse organisms such as nematodes, potworms, earthworms, mites, springtails, beetles, ants, and termites. Fauna can be further divided by body size into macrofauna (>10 mm length, >2 mm width; e.g. earthworms, millipedes, centipedes, woodlice, termites, ants, beetles); mesofauna (0.2 to 10 mm length, 0.1 to 2 mm width; e.g. microarthropods and potworms) and microfauna (<0.1 mm length, <0.1 mm width; mainly nematodes) (see Figure 1).

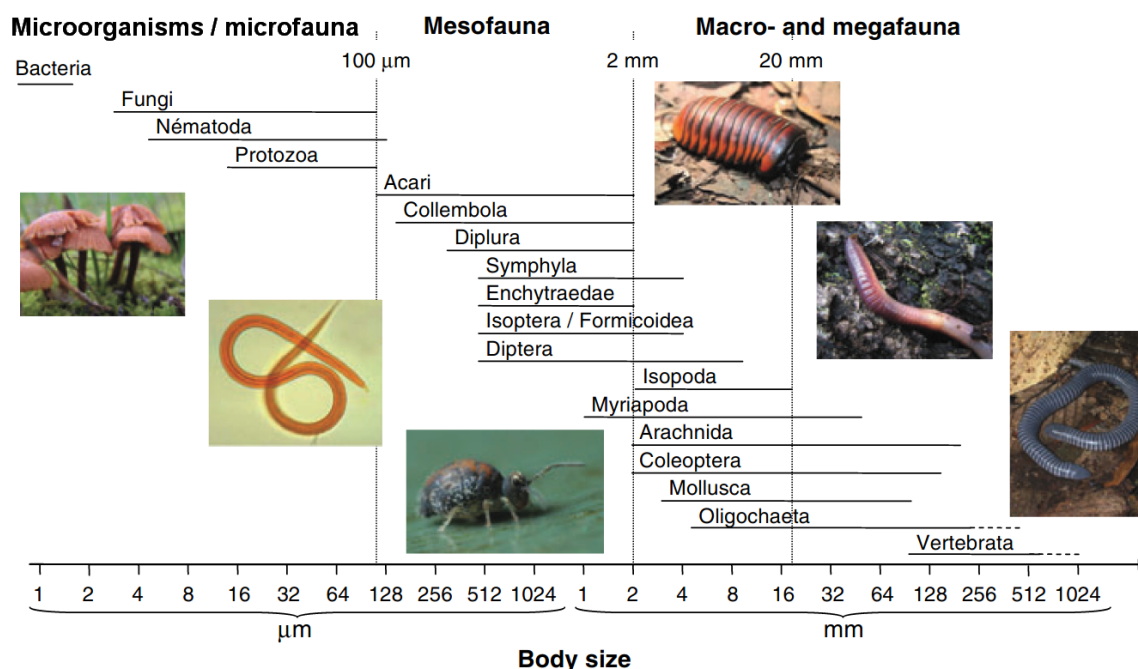


Figure 1: Representation of the main taxonomic groups of soil organisms on a body-size basis (after Swift et al., 1979) from Decaens (2010) and Barrios (2007) (all photo credits: Flickr, <http://www.flickr.com/>)

Macrofauna are relatively large organisms (see Figure 1) and include decomposers, herbivores, predators and the so-called ‘ecosystem engineers’. Most insects, spiders, isopods, myriapods and others belong to the ‘macroarthropods’. Other important macrofauna include soft-bodied, legless soil biota such as annelids and gastropods.

‘Ecosystem engineers’ have been defined as “organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In so doing they modify, maintain and create habitats” (Jones et al., 1996). Among soil fauna, earthworms, termites and ants have been identified as the most important soil engineers. Termites and ants are mentioned for completeness, even though these two groups are not covered in this Opinion. Termites mostly occur in the tropics, with only few species known from natural habitats in Europe, none being associated with agricultural fields. Termites are social insects organized into castes. They have the ability to digest wood and other lignocellulosic substrates thanks to their

intestinal microbiota (Dietrich et al., 2014). Termites are classified according to their feeding behaviour in different groups, e.g. wood-feeders, soil-feeders or humivores, fungus feeders, etc. (Donovan et al., 2001). Ants, together with other Hymenoptera, are covered by the scientific opinion on non-target arthropods (EFSA PPR Panel, 2015a). They are social insects that often nest in soil and consume a variety of foods, depending upon species such as microarthropods, decaying organic debris, seeds, plant secretions, and aphid secretions. Ants forming surface mounds are important in mixing soil from lower depths with surface soil. Therefore, they could modify soil chemical and physical properties by transporting food and soil materials during feeding, mound and gallery construction. Ants can affect plant productivity as they can have different types of relationship with plants (Gonzalez-Teuber et al., 2013).

Earthworms are well-known because of their ability to consume, excrete and organize mineral and organic constituents of soil. Two well-studied species are *Lumbricus terrestris*, which burrows vertically deep into the soil, and *Allolobophora caliginosa*, which dwells mainly at the soil surface. Burrows loosen soil, allowing roots and other soil animals to colonize this space. Earthworm casts are often enriched in organic matter, microbial populations, and nutrient content, which improves nutrient cycling.

Isopods are cryptozoans, i.e. surface soil dwellers under stones, bark, or litter layers, which emerge at night to forage and might show the ability of rolling into balls as a defense mechanisms and to avoid desiccation. They feed on roots, vegetation, and decaying plant litter, resulting in considerable fragmentation of organic matter.

Millipedes are saprophagous feeders, i.e. consuming dead or decaying organic debris, with a calcareous exoskeleton, and therefore important in calcium cycling.

Centipedes are elongate, flattened, and active predators of various microarthropods in soil and surface litter.

Some terrestrial organisms living in and on agricultural soil might spend only part of the life cycle in the soil. The following groups are considered to be non-target arthropods, even though they have life stages present in the soil matrix. Spiders are typically predators of insects in soil and surface-litter layers. Solitary wasps construct nests in soil and prey on other insects or spiders to feed developing eggs. Beetles are the most diverse insect family, and in the soil can be divided into predacious, leaf feeding, and saprophagous. Ground beetles, rove beetles, and tiger beetles are predators of other insects and are important pest-control agents in agroecosystems. Dung beetles feed typically on large-animal faeces. Wireworms are beetle larvae that feed on roots (Lavelle, 1996; Lavelle and Spain, 2005; Wall et al., 2012).

Mesofauna include mainly Acari, Collembola, Enchytraeidae, Tardigrada, Protura, and Diplura. Acari, or mites, are often the most abundant and the most species-rich group of the soil mesofauna. Mites inhabit air-filled soil pores and litter and have a hard body. Soil mites are a very diverse assemblage of Arachnida, divided into four major groups: oribatids, prostigmatics, mesostigmatics, and astigmatics. Oribatid mites are often most numerous in soils, are morphologically distinct between juvenile and adult stages, reproduce relatively slowly, and typically feed on detritus and fungi. Unlike most other microarthropods, oribatid mites have a calcareous exoskeleton. Prostigmatic mites feed on fungi, algae and other soil organisms. Mesostigmatic mites are not particularly abundant in soil and are mostly predators of nematodes and microarthropods. Astigmatic mites are the least common mites in soil and are found primarily in moist soil high in organic matter.

Collembola (or springtails) are small (0.2 to 5 mm in length), primitive hexapods. The number of Collembola species known is much lower than that of Acari, but they can reach the same abundances. Like Acari, Collembola live in air-filled soil pores and litter. Most Collembola eat decaying vegetation and fungi, although they have also been observed to consume nematodes and plant roots

throughout the soil profile. Collembola are opportunistic microarthropods, capable of rapid individual and population growth when conditions are favourable. They may be important biological control agents for crops by consuming pathogenic fungi. Eggs are laid in groups and, therefore, populations occur in aggregations rather than at random. Collembola can be important food sources for predacious mites, beetles, and ants.

Enchytraeidae (potworms) are similar to small earthworms, also belonging to Oligochaeta. They are most abundant in wet, organic soils and feed on detritus, algae, bacteria, fungi and other soil organisms. They live in the litter and in the upper few centimetres of soil because of their limited ability to move long distances. Some species can burrow in the sand while others can move vertically using earthworm casts (Lavelle and Spain, 2005).

Other mesofauna that are less numerous, but present in many soils, are Tardigrada (soft, plump body and eight poorly articulated limbs with claws), Protura (wingless hexapods lacking antennae and eyes that live near plant roots and litter), Diplura (elongate, delicate hexapods with long antennae and two abdominal cerci that feed either on decaying vegetation or predacious on nematodes, springtails, and potworms), pseudoscorpions (small arachnids that feed on nematodes, microarthropods, and potworms), symphylids (white, eyeless, elongate, many-legged invertebrates resembling centipedes that feed on vegetation and soft soil animals), and Pauropoda (another group of centipede-like, colourless arthropods with branched antennae, that feed on fungi and other soil organisms).

Microfauna are represented mostly by rotifers and nematodes. Rotifers can be found in soils that are continually moist, but are typically aquatic organisms and are therefore not considered important in-soil organisms.

Nematodes (also called roundworms, threadworms, or eelworms) are among the most abundant metazoan organisms in soil and are important components of the soil food web. Nematodes vary widely in their feeding strategies, being bacterivores, fungivores, plant feeders, pathogens of vertebrates and invertebrates, carnivores or omnivores. Nematodes proliferate in the water-filled pores of soil or plant roots. They have low motility, and they are often susceptible to stressors. Most nematodes are saprophytic, i.e., feeding on decaying organic matter. Nematodes are considered to be potentially holistic indicators of soil processes as they are active within the soil throughout the year. In addition, some species may be important biological control agents for crops by consuming pathogenic fungi and bacteria and being entomopathogenic agents (controlling some insect pests, and also molluscs). In agroecosystems, various nematode groups play critical roles in plant productivity. Bacterial- and fungal-feeding nematodes contribute to decomposition of organic matter and the release of nutrients for plant uptake (Ferris et al., 2004). Plant-feeding nematodes, however, can impair root function and act as nutrient sinks, thereby reducing crop yield (Luc et al., 2005). Predatory nematodes and some omnivorous nematodes prey on small invertebrates, including plant-feeding nematodes and may regulate populations of these pests (McSorley et al., 2008; Khan and Kim, 2007). Specific indices have been designed to assess soil quality using nematode communities (Yeates and Williams, 2001; Griffiths et al., 2002; Manachini et al., 2009; Okada and Harada, 2007). These indices are based on the concept that particular taxa differ in sensitivity to stressors or disruptions of the successional sequence because of their life history characteristics. Nematode taxa were rated along a colonizer–persister (c–p) scale of 1–5 according to Bongers and Ferris (1999), roughly equivalent to the range from extreme r- to extreme K-strategists. “Colonizer” nematodes at the lower end of the c-p scale are considered enrichment opportunists and therefore indicate resource availability; ‘persister’ nematodes at the high end of the scale indicate system stability, high food web complexity and high connectance. In addition to these, the metabolic footprint could also be estimated using nematodes. It is an estimator of nematode contribution to various ecosystem services and functions. Nematodes are considered as potential bio-indicators in assessing the impact of different toxicants, including plant protection products (PPPs), and other potential stressors on soil ecosystems and in general of soil health. They have been shown to be sensitive enough to indicate effects after exposure to relatively low concentrations of toxic chemicals (Kammenga et al., 2000; Shashikumar and Rajini, 2010).

In this scientific opinion, **microorganisms** (or “microbes”) collectively comprise single-celled organisms with heterotrophic organotrophic (saprophytes) or chemolithotrophic autotrophic lifestyle. Photosynthetic autotrophic microorganisms, i.e. cyanobacteria and eukaryotic algae can be also present in surface soil layers, but they play a smaller role in the majority of agricultural soils (although the N-fixing cyanobacteria can be important, for example, in rice plantations). Protozoans are eukaryotic, single-celled, non-photosynthetic organisms that are not fungi. The group is polyphyletic and, based on morphology, most protozoans are ciliates, flagellates or amoeboids. Unlike the bacteria and archaeans (lacking nucleus and other organelles and collectively referred to as prokaryotes) and fungi in which osmotrophic nutrition dominates, most protozoans are phagotrophic and ingest particulate matter (preferably bacteria) (Epstein, 1997). Not all microorganisms in soil occur as free-living organisms in pores of various dimensions, but many live in symbiosis with other organisms. For instance, specific microbial communities are associated with the gut system of soil-dwelling insect larvae (e.g. Andert et al., 2010) and collembolans (Thimm et al., 1998). Another example is the plant rhizosphere, which is characterised by high metabolic activity and harbours very diverse microbial communities (van der Heijden et al., 2006; Weinert et al., 2011; Kuzyakov and Blagodatskaya, 2015). Among microorganisms forming symbiotic interactions, mycorrhizal fungi have been identified in thousands of plant species among all major plant lineages including bryophytes, ferns, gymnosperms and angiosperms (Brundrett, 2009; Wang and Qiu, 2006). Six different types of mycorrhizal symbiosis have been identified (Kernaghan, 2005), although the most abundant and well-studied are the arbuscular mycorrhizal fungi (AMF), the ecto-mycorrhizal fungi (EM) and the ericoid mycorrhizae (ERM) (van der Heijden et al., 2008). The arbuscular mycorrhizae are abundant in grassland, savannah and tropical forests, they also associate with many grasses and are able to establish interaction with roots of 80% of plant families (Gianinazzi et al., 2010). Ectomycorrhizal (EM) fungi are widespread in temperate and boreal forest and comprise over 20,000 species forming root symbiotic with many long-lived perennial plants and trees (Finlay et al., 2008). Ectomycorrhizae are predominantly from the Basidiomycota and Ascomycota. Ericoid mycorrhiza are formed in three plant families, the Ericaceae, Empetraceae, and Epacridaceae, all belonging to the order Ericales (Finlay, 2008). Due to the low relevance of this group of mycorrhizae for crop plants, they are not further addressed in this Opinion. Bacteria belonging to the genera *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Bradyrhizobium*, and *Azorhizobium* (collectively referred to as rhizobia) grow in the soil as free-living organisms but can also live as nitrogen-fixing symbionts inside root-nodule cells of legume plants. The fundamental life-supporting roles that microorganisms have in nature are now considered an obvious component of ecosystem-services frameworks (Ducklow, 2008; EFSA PPR Panel, 2010a; Bodelier, 2011), and microbes are critical components in the discussion on soil health and ecosystem services of arable soils (Barrios, 2007; Kibblewhite et al., 2008).

3.2. Dispersal, recovery potential of in-soil organisms

Population recovery of in-soil organisms can be internal and/or external. Internal recovery depends upon surviving of individuals in the stressed ecosystem or upon a reservoir of resting propagules (e.g., seeds and ephippia) not affected by the use of pesticides or other environmental stressors. In contrast, external recovery depends on the immigration of individuals from neighbouring areas by active or passive dispersal. Species life-history traits are considered key elements in determining the rates of recovery of affected population. Important species life-history traits are the number of generations per year and related life-history strategies (r-K), the presence of relatively insensitive (dormant) life stages and the capacity of organisms to migrate actively from one site to another. For example, voltinism (pertaining to the number of broods or generations per year) may be an important property determining rates of population recovery of invertebrates, in particular (EFSA Scientific Committee, 2016a).

“Dispersal is a central ecological process that allows colonization of new habitats and exploitation of spatially and temporally variable resources (...). Active dispersal of animals (as opposed to passive dispersal, where individuals could be transported by an external agent and has not necessarily a cost for the individual) is the result of three successive behavioural stages. It involves the departure from a

breeding site, crossing to a new place, and settlement. It can occur at any life stage, at any spatial scales above the individual range and within more or less heterogeneous landscapes (...). It is assumed to depend on the balance between the costs and benefits of dispersal (...), which are strongly determined by both environmental conditions (e.g. habitat quality, habitat fragmentation, patch size, density, predation) and individual life traits” (Caro et al., 2013).

3.2.1. Potential for internal recovery

Soil fauna

Oligochaeta

As mentioned above, internal recovery depends upon the reproduction capacity and it is, therefore, linked to the generation time of a species. Thus, information on the life-cycle of species is considered crucial for understanding the recovery potential after toxic effects due to pesticides application. Earthworms have been proven to produce eggs during the whole year. The eggs are contained in cocoons which, for the majority of the species, are deposited on the soil surface or nearby, if the soil has required moisture. If the soil is too dry, the cocoons are deposited deeper. Although earthworms can potentially produce cocoons at any time of the year, seasonal variation in soil moisture, temperature, food supplies and other environmental factors can lead to changes in size and biomass of their populations (Edwards and Bohlen, 1996). At 75% moisture content, Gunadi et al. (2003) reported for *Eisenia fetida* the highest cocoon production per earthworm and hatchlings per cocoon (9.8 ± 0.1 cocoons produced over 30 weeks and 1.7 ± 0.1 hatchlings produced per cocoon) in cattle manure solids.

It is well reported in the literature that few cocoons are produced in the winter period while the highest number is produced in the period May-July. Generally, the number of cocoons decreases with decreasing temperature, but the relationship is different for different species since the influence of environmental factors on population dynamics differs among earthworms of different ecological categories. Epigeic earthworms, living and feeding mainly on the litter layer, may be more affected by seasonal temperature variations than endogeic or anecic species (those that inhabit permanent or semi-permanent burrow systems in the soil) (Monroy et al., 2007).

Venter and Reinecke (1988) attributed to the availability and the quality of food as well as the maintenance of optimal moisture conditions a great importance for the growth rate of the *E. fetida* at 25°C. As shown in Appendix A, *E. fetida* displays in comparison to other earthworm species a relatively short life cycle with a high reproductive rate. Appendix A lists the life-cycle parameter for 12 Lumbricidae species. Total time for development ranges from 38 to 74 weeks.

Enchytraeidae species can reproduce either by sexual reproduction or asexually. In species able to reproduce sexually, adults lay cocoons that are a sort of mucilaginous bag, containing from 1 to 48 eggs. Hatching rate is usually high and can range from 19 to 97%. Enchytraeidae can produce 4 to 10 immatures per adult per year with a developmental period of 4 to 12 months in British meadow. A total life cycle in the range of 60 to 120 days from cocoon hatching to maturity has been reported under optimal conditions (Lavelle and Spain, 2005).

Westheide and Graefe (1992) reported life-cycle data for 2 species of Enchytraeids: *Enchytraeus crypticus* and *Enchytraeus doerjesi*. Burgers (2012) reported data from 2 sources on *Enchytraeus albidus* (see table 2).

Table 2: Life-cycle data on 3 species of Enchytraeidae

Species	Embriological development (d)	Hatching to maturity (d)	Total life span (d)	Cocoon production (d ⁻¹)	No eggs in a cocoon	Mean No eggs (d ⁻¹)
<i>Enchytraeus crypticus</i>	9.06 ^a	8.3	81.6 ^a	0.62	7.6	4.6
<i>Enchytraeus doerjesi</i>	6.8	8.5	93 ^a	0.9	5.1	4.3
<i>Enchytraeus albidus</i>	-	44.5/21	68.3/261	0.22/0.40	4-5/1-35	

^a Average of different values obtained for populations originating from different localities

Asexual reproduction of Enchytraeidae occurs through fragmentation of individuals to form a few new ones. Individuals of *Enchytraeus fragmentosus* break into 3 to 14 fragments, each developing into a complete worm in 10 days. Individuals of *Cagnettia sphagnetorum* start to fragment when they have more than 42 segments. They divide into 2 or 3 fragments and they may take from 8 to 26 days to regenerate. Fragmentation occurs mainly in spring and summer, especially during dry periods (Lavelle and Spain, 2005).

Collembola and Mites

According to species and size, Collembola species can have a number of stages going from 4 to 50. Development through the reproductive instars can take 40 to 400 days and moulting occurs continuously over the entire life (Lavelle and Spain, 2005). The fecundity of collembolan females depends on the number of eggs laid in each clutch and the total number of clutches produced. A female of the species *Sinella curviseta* can produce an average of eight clutches with 50 eggs each during the entire life-cycle, under laboratory condition and with continuous access to a male. Similar results have been also reported for the species *Willowsia jacobsoni*. Overall, collembolan species have been reported to lay 100 to 600 eggs during the entire life time, which is around 1 year (Lavelle and Spain, 2005).

Embryo development takes about 10 days for the species *Tomocerus ishibashii*. For the species *Entomobrya nivalis*, egg development has been reported as taking 25 days at 9°C, 15 days at 13 °C and only 7 days at 20°C.

The maximum life span for a springtail under controlled conditions is 5-7 months for the species *Pseudosinella impediens*. However, under realistic conditions, some species can live longer, especially in stable cave environments. The complete life cycle of *Cryptopygus antarcticus* may take from 2 to 7 years, since in very cold climates growth and reproduction are much more slow.

Some species are univoltine while other can be multivoltine. For example, the species *Tomocerus cuspidatus* is univoltine with a short breeding period in spring, while the species *Entomobrya aino* is multivoltine.

Fountain and Hopkins (2005) reported for *Folsomia candida* an average life span for females of 240 days and 111 days at 15°C and 24 °C, respectively. The number of eggs laid by a female can decrease from 1100 to 100 going from 15 °C to 27 °C. An adult female may go through 45 moults in her lifetime with short reproductive instars (1.5 days) alternating to longer non-reproductive periods (duration 8.5 d).

At 20 °C, the average duration of the five juvenile instars is 3 days for *F. candida* and maximum 4 days for *F. fimetaria*. Sexual maturity is attained in the 6th instar occurring around age 15-16 days for *F. candida* and a few days later for *F. fimetaria*. Egg development for *F. fimetaria* took 9.5 days, hence similar to 9-11 days observed for *F. candida*. Reproduction may be parthenogenic or bisexual. Generally, it is reported that collembolans able to reproduce sexually, need fertilisation for every

reproductive instar. With that regard, Krogh (2008) reported the result of a study aiming at following the oviposition pattern of reproduction. In that study, 24 couples of 25-28 days old, 8th instar, *F. fimetaria* males and females, and 24 single females were isolated and followed for three weeks at 20° C. Single females did not produce any eggs and the couples produced 10 and 30 eggs in instars eight and ten, respectively, with a maximum clutch size of 60 eggs. In the same situation, for *F. candida*, 48 and 71 eggs were produced with a maximum clutch of eggs of 114.

For acari the post-embryonic development can take several months. Acari belonging to the Mesostigmata group have only two immature stages before moulting to the adult form. The other three non-parasitic orders (Prostigmata, Astigmata and Cryptostigmata) have six different developmental stages. Inactive forms are very common in acarine population. For example Cryptostigmata can spend 30% or their annual cycle in moulting or resting stages. Most Cryptostigmata have one generation per year, although larger species or those living in boreal and arctic environments can take 2-3 years to complete their life-cycle. Reproduction is generally bisexual, although some species can reproduce *via* parthenogenesis. Cryptostigmata females may produce one to six eggs on average which hatch one to six weeks later. For Prostigmata, the number of eggs can vary from 10 to 100 depending on the species.

Oribatid mites are usually reported as having long life cycles, extended development, adult longevity, and iteroparity. The time for completion of an oribatid mite's life cycle is dependent on temperature, moisture, and the availability of food and can vary from five months to two years. In general, small oribatid mites in a warm climate will take less time to complete a life cycle. In field studies conducted in temperate climates, most oribatid mites have shown a generation time of one or two years (Jordan, 2001).

Acari of the species *Hyapoaspis aculeifer* (Acari: Mesostigmata), the standard test species, become sexually mature after 16 days (females) and 18 days (males). A life span between 48 and 100 days at 25 °C is reported (OECD, 226).

Nematodes

In several studies, pesticides contributed to the declining diversity and complexity of nematode communities as reported by different specific indices (Structural Index (SI), Enrichment Index (EI)). Moreover, specific nematode genera were indicated as sentinels for recovery and describing the impact of soil-management or land-use change. *Mesorhabditis* spp. was a consistent indicator of nutrient enrichment (Zhao and Neher, 2013); (Malherbe and Marais, 2015). The resilience of *Cephalobus* spp. to tillage and other agricultural practices was enhanced (Fiscus and Neher, 2002) and *Helicotylenchus* spp. were identified as a candidate soil-health indicator in the tomato agroecosystem studied (Malherbe and Marais, 2015). In general, species of larger body size, such as the longer living, K-selected predaceous nematodes that are somewhat slower moving, would require more time to recover from stress, e.g. PPPs exposure and a larger water film around soil particles (which could also depend on the PPPs applied) to maintain their activity, compared to nematodes in other trophic groups, such as smaller sized, faster moving bacterial feeders with r-selected life strategies (Yeates, 2002). Plant parasitic nematodes are rather reactive but can be either target or non target, depending on the PPP applied. Indirect measures of the resilience and natural attenuation of nematode communities are different traits of their ecological succession, including the fungivore to bacterivore ratio, maturity, and other ecological indices (Ferris et al., 2001).

Timper et al. (2012) found out that nematicides reduced numbers of all trophic groups compared to the control; for bacterial and plant feeders, there was also a consistent, lingering effect of the nematicides the following year at pre-fumigation. Interestingly, omnivores and predators were not severely impacted by the nematicide treatment; populations of both groups repeatedly recovered by the following spring from the yearly application of nematicides, with the exception of predators in some cases. The authors highlighted also that the nematicides may have altered the soil community to

allow a fungal, bacterial, or invertebrate antagonist of nematodes to increase in abundance, leading to an increase of suppressive service. In addition, although *C. elegans* is a bacterivorous nematode that exhibits exceptional resilience to adverse environmental conditions and different stress, protocols are now available to quantify its resistance to a variety of biotic and abiotic stressors (Keith et al., 2014). This could be a potential tool to estimate the potential recovery and consequent natural attenuation done by bacterivorous nematodes.

Slugs and snails

Most land snails are oviparous and lay their eggs in clutches at sheltered places (e.g. soil cracks or burrows, under stones, amongst herbage (Barker, 2001). The number of eggs laid per clutch is highly variable within but also between species. According to Barker (2001), small terrestrial gastropods show a particular low fecundity, tending to produce only few eggs throughout their life (e.g. six in *Punctum pygmaeum* (Draparnaud) (Punctidae) during an average life span of 170 days. Larger animals may deposit several egg clutches per season (Kerney et al., 1983) and there often is considerable variation in the number of eggs per clutch and size of eggs within species, depending on the size and age of the parent animal, but also on environmental factors such as competition, and seasonality in climate (Barker, 2004). Mortality during the early life stage of terrestrial gastropods is rather high and it is not unlikely that only 5% or fewer animals of one egg clutch reach sexual maturity. Many terrestrial gastropods reach sexual maturity after one year, while the largest terrestrial gastropod species (but also some small species belonging e.g. to the genera *Columella* and *Vertigo*) may take 2-4 years to reach sexual maturity (Kerney et al., 1983).

Overall, it is concluded that especially smaller terrestrial gastropod species that make up the greater part of terrestrial gastropod diversity (see e.g. Sturm et al., 2006) are likely to have a poor recovery potential due to their low number of produced offspring and also their generation time may be rather long.

Macroarthropods

Most temperate species of Isopods are seasonal breeders. However there is a large variation in the period and duration of the breeding season. While some species breed in spring, others breed during the fall. Most species from temperate and Mediterranean habitats have breeding seasons lasting four to eight weeks (Warburg, 1991), while others from tropical or temperate regions, the breeding season may last 3 to 6 months (Warburg, 1993). This is seen in tropical species *Orodillo maculatus* and sub-tropical species *Bethalus pretoriensis* that present a breeding season longer than for temperate species. The species *Porcellionides pruinosus*, however, represents an exception since it can breed continuously in tropical and temperate habitats.

Many woodlice species are iteroparous between years and they can produce more than one brood per year. Females of *Porcellio scaber*, for example, can produce up to three broods per year, whereas the species *Porcellio laevis* can produce up to six broods. This can vary also with the age of the individuals, and climate. In a warmer climate (California), first year females of *A. vulgare* produce one brood within a year, while second year females can produce two broods. However in East Anglia the same species was semelparous. Fecundity in woodlice is associated with body size (Alikhan MA, 1995). Larger *A. vulgare* females can produce two broods per seasons, compared to one brood of smaller females.

The number of broods during a female life time can vary across species, going from one brood in certain populations of *A. vulgare* and other Armadillidiidae to more than six in populations of the species *P. pruinosus* and *Porcellio laevis*.

Climatic parameters, such as temperature, can influence reproduction. Increased temperature shortened the development time for mancás of *Oniscus asellus* and accelerated the reproduction in *Armadillidium vulgare* (Warburg, 1993).

Soil microorganisms

Due to their specific traits and short generation time, it has been possible to study internal recovery of microbial populations or communities after exposure to PPPs relatively often. It has been demonstrated and reported (Puglisi, 2012) that microorganisms are often able to recover quite fast from toxic effects after exposure to pesticides. Those effects can be both at the structural and functional levels of the microbial community, as demonstrated by the heterogeneity in the measured and reported endpoints: abundance (number of cells or spores) and biomass (often re-calculated from respiration measurements), physiological parameters (e.g. CO₂ evolution, net nitrification or mineralization), measurements of enzyme activities, differences in the structure (PCR-DGGE, PLFA, etc.).

Recovery after pesticide application was reported as occurring from 28 days after application (effects on dehydrogenase) to 114 days (effects on colony forming unit for fungi) (see Appendix B.). Some studies have also reported an adaptive response of soil bacteria as shown by the faster recovery of enzymatic activity after repeated applications of a pesticide (Yu et al., 2006; Imfeld and Vuilleumier, 2012). This could be explained by the enhanced mineralization capacity acquired by the soil microbial community.

For mycorrhizae, little information is reported about their potential for internal recovery. Abd-Alla et al. (2000) investigated the effects of the pesticides pyrazophos (fungicide), bromoxynil (herbicide), paraquat (herbicide) and profenofos (insecticide) on arbuscular mycorrhizal (AM) spore number and root colonisation of the legumes cowpea (*Vigna sinensis* L.), common bean (*Phaseolus vulgaris* L.) and lupin (*Lupinus albus* L.). In the case of cowpea plants and common bean, the proportion of root length colonized by AM fungi was significantly decreased with all pesticides used 20 days after planting, but recovery from effects after the application of pyrazophos and bromoxynil was demonstrated after 60 and 40 days, respectively. However, root colonisation of lupin with AM fungi was significantly reduced with all pesticides. The number of AM spores sieved from the rhizosphere of cowpea was significantly decreased with all pesticides after 20 days, but the effect of paraquat had disappeared after 40 days. Except for pyrazophos after 20 days, all the other pesticides significantly reduced the number of AM spores collected from the rhizosphere of common bean after all experimental periods. AM spore formation in the rhizosphere of lupin was inhibited with all pesticides and after all experimental periods.

3.2.2. Potential for dispersal

Soil Fauna

Macrofauna

Dispersal of earthworms can be categorised as passive through anthropogenic or natural processes and active over the soil surface or through the soil. Both cocoons and adults can be dispersed passively by surface run-off, water currents, heavy rainfall, temporary inundation of a certain area, transported by other animals, e.g. birds or through plant materials and adhesion to soil particles. The active dispersal of earthworms can be triggered by various factors, such as increased earthworm density, low quality habitat or adverse conditions, like heavy rain or flooding, surface applications of irritating fluids, contamination with heavy metals or pesticides, in general, and copper compounds in particular, acid or highly alkaline soils or occurrence of roads and cabins. This was confirmed by Mathieu et al. (2010) who showed in a mesocosm study that dispersal can be reduced by: 1) high habitat quality including presence of litter; 2) low density; and 3) pre-use of the soil by conspecific individuals that are no longer present.

Data on dispersal rates of earthworms through soil are reported in table 3, showing mean horizontal movements ranging between 2.5 and 14 meters per year (m y⁻¹) (Eijsackers, 2011; Emmerling and Strunk, 2012; Dupont et al., 2015). For *A. caliginosa*, maximum dispersal of 72 m in eight years was reported. In the case of *L. rubellus*, a dispersal between 5 and 11 meters per year (m y⁻¹) has been

measured. Overall, in agricultural sites, limited variation has been reported in the dispersal rate between different species, and earthworm-population development started after an adaptation period in the range of 2-6 years after introduction.

All types of earthworm species show the ability to disperse over the soil surface by crawling at night. For example, *L. terrestris* has been shown to crawl 19 meters in one night and *A. longa* 23 meters. No directionality in crawling has been demonstrated. However, earthworms can detect and avoid adverse conditions as reported above and, thus, colonization by earthworms may not occur for years in the case of soil contaminated by persistent substances (Eijsackers, 2011).

Caro et al. (2013) recorded a high variability within each earthworm functional group concerning dispersal behaviours. Habitat quality significantly influences the dispersal rates of both anecic and endogeic species. In a homogeneous environment, anecics dispersed further and in greater proportion than the majority of endogeics. Overall, anecic species seemed to have a higher active dispersal inclination than most endogeic ones.

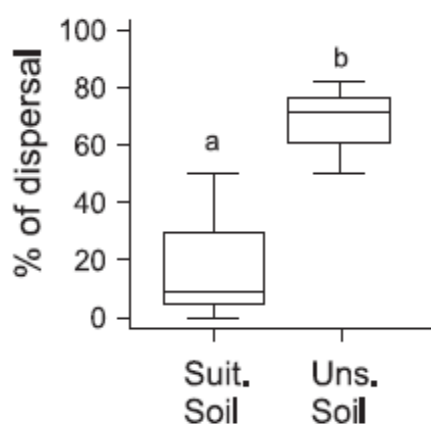


Figure 2: *Aporrectodea icterica* dispersal rates in response to soil properties. Suit: suitable soil (high pH, high org. matter); Uns: unsuitable soil (sandy soil, low pH). From Mathieu et al. (2010) with permission

Table 3: Mean dispersal rate of earthworms species in various habitats (from Eijackers (2011) and Emmerling and Strunk (2012))

Species	Land use/soil/environment	Dispersal rate (m y ⁻¹)
<i>Lumbriculus rubellus</i>	Grazed grassland	7-8
	Arable land	14
	Peat soil	>10
	Arable polder	14
<i>Aporrectodea caliginosa</i>	Grass strips orchards	6
	Grazed grassland	9-11
	Arable land	7
	Grassland	6
	Irrigated desert soil	3.5-5
	Pasture	10
	Grassland/reclaimed peat	2.5-10
<i>Allolobophora chlorotica</i>	Grass strips orchards	4
	Arable polder soil	7
<i>Aporrectodea longa</i>	Grazed grassland	5-8
	Grassland	6
<i>Lumbriculus terrestris</i>	Grazed grassland	4
	Grassland	1.5
<i>Octolasion cyaneum</i>	Grassland and arable soils	8

The greater part of terrestrial gastropod diversity comprises very small animals living as detritivores in the litter layer (< 1cm diameter in greatest dimension), some even have maximum diameters of < 1 millimeter (mm) (see e.g. Barker, 2001; Sturm et al., 2006). They occur in all kinds of agricultural habitats (as grassland, acres, specialized crops, seminatural habitats) and have often rather specific preferences in terms of habitat and environmental conditions (see e.g. Kerney *et al.* 1983).

Barker (2001) states that the dispersal abilities of terrestrial gastropods are so low that it can be assumed that mating will be predominantly driven by inbreeding at the level of the local population. Their ability to recolonize disturbed areas is low and affected by various environmental factors, such as the height of the corn (Wolters and Ekschmitt, 1997). For rape fields, the same authors reported that several snails only invaded about 3 meters into fields from woodlands and hedges. Hof and Bright (2010) found that the number of terrestrial gastropods significantly decreased with increasing distance from the field edge of arable fields.

Mesofauna

Dispersal is an important characteristic of mesofauna with implications for impacts and recovery of PPPs. Kattwinkel et al. (2012; 2015) reviewed the literature on recovery and concluded that Collembolan species reacted significantly differently to population perturbation (including the recovery pattern), meaning that a coarse taxonomic assessment might not be sufficient to detect adequately effects of pesticides. For example, when changing the same plots from the treated to untreated management, responses of individual species varied e.g. numbers of the species *Entomobrya nicoleti* remained close to zero, whereas the abundance of *Isotoma viridis* were the highest recorded during the study. They also concluded that unexposed field margins play a key role as source of recolonisation confirming the role of buffer zones for recovery for mobile surface dwelling collembolan.

There is a considerable body of evidence for the importance of dispersal. Rantalainen et al. (2005) reported the ability of various members of the detrital food web to colonise newly established habitat patches under field conditions, showing that the presence of habitat corridors promoted community diversity. However, rates of movement, although varied are generally low. Responses of soil organism communities after lindane application were investigated in a TME study (Terrestrial Model Ecosystem). Collembolans were adversely affected by moderate dosages of lindane in terms of total and species-specific abundance as well as the community endpoints (principal response curves, diversity measures). Recovery was observed within one year. Dispersal rates for the fungivore species of Collembola, *Onychiurus armatus*, using connected distinct patches of two different soil types covering a distance of 40 meters, ranged from 0.020 to 1.42 per day suggesting that on average species moved less than 10 centimeters per day (cm d⁻¹). Dispersal depended on population density, soil type and length of fungal mycelium, being inversely proportional to mycelial length, especially in a sandy soil. When a soil patch at 40 cm distance from the release point was enriched with a favoured food item, dispersal rate was increased by more than four times (Bengtsson et al., 1994). The role of hedgerows for external recovery (recolonisation) of springtails has also been investigated and demonstrated, especially in arable fields. Abundance of some species of Collembola (e.g. *Isotomurus* spp., *Bourletiella hortensis*) was consistently higher next to the hedgerow than within the field in a study conducted in Southern England in 1998 (Alvarez et al., 2000). Similar results were found by (Frampton, 2002) who showed that at the field edge, which was protected during insecticide applications by a 6-m unsprayed buffer zone, effects of pesticides were less severe, and were not persistent in the long term.

Other factors that can alter dispersal rates include reproductive strategy and pheromones. There is an indication that parthenogenetic species may colonise more quickly (Chahartaghi et al. 2009). Recovery and recolonisation of Collembola may be also enhanced by the existence of pheromones which induce aggregation. As mating in Collembola may be indirect, involving deposition of spermatophores by males and subsequent taking up by females, aggregation may increase the efficiency of reproduction (Verhoef et al, 1977, 1984).

Since dispersal is important but limited there are implications for the interpretation of field study data.

According to Duffield and Aebischer (1994), the recovery of invertebrate population also depended on the size of the treated plot. In addition, two different recovery patterns were identified: (i) recovery progressing from the edge to the centre of treated areas; (ii) more rapid recovery in the centre of the large treated areas. The first recovery pattern was mostly associated with the predatory groups such as Carabidae, Staphylinidae and Linyphiidae and it can be associated with a recolonisation of the pesticide-treated plots from the untreated surroundings. The second recovery pattern was associated with the prey groups such as Aphididae and Collembola. The recovery appeared to be faster in areas with less predation pressure. The results suggested, as also reported by Kattwinkel et al. (2012; 2015), that recovery assessment in small in-field areas might be a source of uncertainty since it could underestimate the pesticide effects on large predators, while overestimating them on microarthropods. This was extensively addressed by the EFSA PPR Panel (2015a) when dealing with the risk of intended uses of PPPs to non-target arthropods. Some patterns might be also valid for the larger Collembola species living on the soil surface and able to move significant distance between fields and edge-of-fields. One of the major threats to biodiversity is landscape fragmentation as it can result in the transformation of continuous (hence large) habitat patches into isolated (hence smaller) patches, embedded in a matrix of another habitat type. In turn, this leads to a loss of biodiversity, especially if species have poor dispersal abilities, such as Collembola. A recently created habitat might suffer from a reduced biodiversity because of the absence of adapted species that need a certain amount of time to colonize the new patch (e.g. direct meta-population effect). Thus, landscape dynamics leads to complex habitat, spatio-temporally structured, in which each patch is more or less continuous in space and time. Patches can also display reduced biodiversity because their spatial or temporal structures are correlated with habitat quality (e.g. indirect effects). Heiniger et al. (2014) demonstrated that habitat temporal structure is a key factor shaping collembolan diversity, while direction and amplitude of its effect depend on land use type and spatial isolation.

Soil microorganisms

For a long time, the “Baas-Becking hypothesis”, stating that “everything is everywhere, the environment selects” (Beijerinck, 1913; Baas-Becking, 1934; Fierer, 2008), made a strong imprint on thoughts and views regarding microbial biogeography. It is well known that microbial communities can exhibit spatial variability at scales ranging from millimeters to thousands of kilometres and that microbial community composition in natural environments can be influenced by a large number of biotic and abiotic environmental factors. However, the impact of specific aspects of the environment on the spatial patterns of microorganisms is still poorly understood. Typical features of microbial communities, such as large population sizes and short generation times, may result in biogeographic patterns. However, unlimited microbial dispersal may lead to constant turnover and increase in genes flow (Eisenlord et al., 2012). For example, many phylogeographical and population genetic studies on plant pathogenic fungi, but also on wood decay species, have reported efficient dispersal and gene flow at a regional or even continental scale. According to Finlay (2002) free-living microbial eukaryotes are probably sufficiently abundant to have worldwide distribution. Accordingly, prokaryotes, which are much smaller and several orders of magnitude more abundant, are even less likely than microbial eukaryotes to be restricted by geographical barriers.

In addition, in the case of fungal spores, for example, dispersal patterns can affect gene flow, population structure and community structure. Dispersal mode can vary among different fungi. While epigeous fungi are mainly transported by wind, the majority of hypogeous fungi are biotically dispersed since they have fewer opportunities than epigeous fungi for being passively dispersed. For example, fungivorous mammals and invertebrates may be an important dispersal agent for many ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) fungi that form sporocarps. It has been reported that arbuscular mycorrhizal fungal spores can remain viable after passing through digestive tracts of earthworms, sowbugs, and crickets. Besides ingestion, dispersal by adhesion to external

surfaces of in-soil organisms is another mode of dispersal for spores of soil fungi (Lilleskov and Bruns, 2005).

4. STEPS TO DERIVE SPECIFIC PROTECTION GOAL OPTIONS

Regulation (EC) No 1107/2009 defines general protection goals that aim at protecting biodiversity and ecosystems. It is thus necessary to define specific protection goals (SPGs) with the scope of implementing this general protection into explicit and viable mandates for risk assessors who need to know what to protect, where to protect it and over what time period. A procedure to define specific protection goals was developed by EFSA in consultation with stakeholders (EFSA PPR Panel, 2010a). Final decisions on the choice of specific protection goals need to be made in consultation with risk managers. In the PPR Panel Opinion (EFSA PPR Panel, 2010a), several steps are proposed in order to identify and to justify specific protection goals for aquatic and terrestrial organisms that may be affected as non-target organisms by the use of PPPs.

The role of EFSA's risk assessment is, therefore, to propose possible SPG options based on environmental and ecological criteria (and related exposure-assessment goals), acknowledging existing general protection goals described in the relevant EU Regulation or Directive and regulatory data requirements. These SPG options, as well as a description of the possible environmental consequences of each option, should be proposed and discussed with the risk managers. The role of risk managers is to select SPG options, or to amend SPGs proposed by risk assessors, that should form the basis of agreed ERA decision schemes (subsequently included in guidance documents). The choice by risk managers of DG SANTE and EU Member States is based on a cost-benefit evaluation, also using economic and political criteria and acknowledging consequences for human well-being (health and economic benefits) as well as environmental costs.

Based on the overarching ecosystem services concept, which was introduced in the so-called Millennium Ecosystem Assessment (MEA, 2005), the PPR Panel identified those ecosystem services that could potentially be directly or indirectly (e.g. via trophic interactions) affected by the normal agricultural use of plant protection products. The groups of in-soil organisms that are key drivers or service providing units (SPUs) for those ecosystem services were then identified. Specific protection goal (SPG) options have to be proposed for each combination of a key driver and ecosystem service.

The first step in the definition of SPGs is the identification of ecosystem services that are considered important and are provided by agricultural ecosystems. By means of describing services that mankind receives from ecosystem performance, the value of abstract ecological entities and processes becomes more explicit. Several classification schemes for ecosystem services have been proposed, e.g. MEA, 2005; CICES (<http://cices.eu/>) and TEEB (<http://www.teebweb.org/>). In this Opinion, in accordance with other Opinions and Guidance of EFSA on the topic (EFSA PPR Panel, 2010a; EFSA Scientific Committee, 2015a) a list of ecosystem services based on the MEA source has been used since it is widely recognised and adopted. The Millennium Ecosystem Assessment (MEA, 2005) noted, however, that 'modifications of ecosystems to enhance one service generally have come at a cost to other services due to trade-offs.' The impacts of these trade-offs should be clearly described also for ecosystem services in agricultural landscapes, so that risk managers can decide whether and to what extent costs of trade-offs should be tolerated. In this respect, MEA (2005) claims that 'many of the costs of changes in biodiversity have historically not been factored into decision-making'.

Seven ecosystem services were identified as being driven by in-soil organisms in the agricultural landscape. These services are:

- Genetic resources, biodiversity. In-soil organisms are extremely diverse and contribute highly to the biodiversity of agricultural landscapes.

- Education and inspiration, aesthetic values and cultural diversity. In-soil organisms support with their activity the formation of typical structures in agricultural landscapes, delivering aesthetic values, cultural heritage and sense of place. The aesthetic value of soils is widely acknowledged.
- Nutrient cycling. The cycling of nutrients in soils is the basis for terrestrial life. Dead organic matter from above- and below-ground is degraded by detritivores and finally mineralized by microorganisms. Mineralized nutrients can be then taken up by plants.
- Regulation of pest populations and of disease outbreaks. In-soil organisms are valuable antagonists of soil-borne pests affecting crop-plant species and have the potential to control the outbreaks of plant diseases.
- Soil remediation, natural attenuation. In-soil organisms degrade a variety of compounds in soils and contribute to the natural attenuation of xenobiotic soil pollution, including pesticides and their residues.
- Soil-structure formation, water retention and regulation. In-soil organisms are important drivers of soil-structure formation and maintenance. The activity of soil organisms modulates aggregate formation, alleviate soil compaction and regulate soil water-holding capacity.
- Food provision, food-web support. In-soil organisms are part of the below-ground food web and are the link to above-ground consumers. They are providers of secondary production and support biodiversity at higher trophic level.

The second step in the definition of SPGs is the **characterization of the main drivers** behind the ecosystem services deemed to be important in agricultural landscape. In the chapters dealing with the respective SPGs in the present Opinion, in-soil organisms species and/or groups have been identified as having, through their activity or presence, major influences on the service to be preserved. In the Draft Guidance of the Scientific Committee (EFSA Scientific Committee, 2015a), the definition of ‘key driver’ applies to ‘service providing unit’. SPUs are defined as the structural and functional components of ecosystems necessary to deliver a given ecosystem service at the level required by service beneficiaries (adapted from Luck et al., 2003; Vanderwalle et al., 2008).

Third step is the determination of the drivers’ ecological entity to be considered with respect to the ecosystem service assessed. The PPR Panel (EFSA PPR Panel, 2010a) suggested to differentiate between the ecological entities “individual”, “(meta)population”, “functional group” and “ecosystem”. The concept is based on the assumption that addressing organisms at one level of organization will protect those at a higher level of organization. For example, if the ecological entity to be protected is the “individual”, the entities “population”, “functional group” and “ecosystem” will implicitly be protected. The ecological entity addressed in the assessment is identified in the definition of every specific protection goal. In general, non-target organisms other than vertebrates are not protected at an individual level. In the case of SPGs for in-soil organisms, the ecological entities relevant to deliver different ecosystem services are either the populations of species or the functional group (see below).

Fourth step is the determination of the drivers’ attribute to be measured in the assessment. Changes in behaviour, on survival and growth, in abundance/biomass, in a process rate or in biodiversity are suggested by the PPR Panel (EFSA PPR Panel, 2010a) as possible measurements to be made for the different drivers considered. In the case of in-soil organisms, and according to the ecological entities considered in the previous step, the most reasonable attribute to measure will be abundance and/or biomass (see details below).

Fifth step is the determination of the magnitude of effect on the drivers that could be tolerated regarding the impact on the respective ecosystem service without affecting the general protection goal. In the following, a partitioning of magnitude of effects is proposed deriving from general effect classes in ecotoxicology. From these effect classes, the pertinent one is chosen, depending on the organisms’ traits that determine e.g. sensitivity, life cycle or recovery potential.

- 1144 Scaling of magnitude of effects on population/ functional group / biodiversity
- 1145 Large effects: pronounced reduction above 65%
- 1146 Medium effects: reduction between 35% and 65%
- 1147 Small effects: reduction above 10% and below 35%
- 1148 Negligible effects: reduction up to approximately 10% (corresponding to EC₁₀ / No
- 1149 Effect Level)
- 1150 Regarding the magnitude of effects on in-soil organisms arising from several years of PPPs exposure
- 1151 in an agricultural context, relevant measurement endpoints are still to be agreed in the scientific
- 1152 community. If the assessment of these effects is based on population models that address effects of
- 1153 PPPs on species, efforts should be made in order to identify those simulation endpoints that can be
- 1154 related to the magnitude of effects as defined above.
- 1155 Depending on the endpoints that will be chosen in future for assessment of PPP effects on population
- 1156 persistence, negligible, small medium and large effects will have to be defined. Since modelling
- 1157 endpoints integrated several years of PPP application ('system approach', see also section 7.3),
- 1158 tolerable effects might be of lower magnitude than those defined for community assessment at a local
- 1159 scale (e.g. in- or off-field). On the one hand, year on year decline in abundance should not be
- 1160 observed. On the other hand, negligible effects should also account for population-range restrictions:
- 1161 here, not only individual abundance but also range of occupancy should not be reduced by more than a
- 1162 level that will be considered negligible.
- 1163 In terms of this opinion, the definition of possible acceptable magnitude of effects as percentage
- 1164 reduction compared to a "control" applies to a defined context, i.e. agricultural systems supporting
- 1165 highest in-soil diversity of organisms that can be achieved in managed agricultural systems. In
- 1166 landscapes with lower in-soil diversity, the acceptability of effects might be at a far lower magnitude
- 1167 level. This applies to all proposed specific protection goal options for in-soil organisms.
- 1168 For services supported and provided by in-soil organisms, it is difficult to define effect thresholds
- 1169 marking tipping points for ecosystem functioning and the provision of the service of interest. This is
- 1170 due to the lack of knowledge on the detailed quantitative relationships between species and functions
- 1171 in soils. If no absolute threshold can be defined, maximum magnitudes of effects on drivers/SPUs are
- 1172 suggested marking the acceptable limits, in scientific terms, for the maintenance of the assessed
- 1173 service at a desired rate and ultimately for the general protection goal (EFSA PPR Panel, 2010a).
- 1174 This means that, if such limits are breached, severe consequences for the ecosystem functioning and
- 1175 for stakeholders who rely on certain services can be expected. These limits mark the upper range of
- 1176 the magnitude of effects in the different SPG options. The lower end of magnitude of effects in the
- 1177 SPG options is set where no or negligible effects are observed on in-soil drivers, with no or negligible
- 1178 impact on the provision of the specific ecosystem service.
- 1179 For in-field as well as off-field areas, the tolerable magnitude of effects should take multiple PPP
- 1180 applications according to typical PPP 'spray schedules'⁹ into account. This could suggest a lower level
- 1181 of tolerable effects for single PPP applications, especially in-field, if the intended use fits in an
- 1182 application scheme that includes several other PPPs with potential effects on in-soil organisms in the
- 1183 crop. Multiple applications of several PPPs in typical schedules should also be taken into account
- 1184 when addressing the recovery of in-soil organisms (please refer to section 7.4). This is currently not
- 1185 supported by the regulatory framework for approval of active substances/authorization of PPPs,
- 1186 however the Panel would strongly recommend that this aspect should be taken into consideration when
- 1187 setting SPGs.

⁹ Overall pesticide input and application patterns on a field

Sixth step is the determination of the temporal scale to be considered together with the magnitude of tolerable effects. This step is of particular importance when addressing effects other than negligible, since it implies that some effects might be tolerable as long as ecological recovery occurs within a specified period. As stated in the EFSA Guidance on the risk assessment for aquatic organisms (EFSA PPR Panel, 2013), when including “recovery to identify (un)acceptable effects, all relevant processes that determine population viability and the propagation of effects to the community-, ecosystem- and landscape-level are to be considered.” In this respect, multiple application of PPPs might pose a constraint to recovery processes in agricultural landscapes – in particular the consecutive PPP uses throughout crop-spraying schedules.

Considering the ecosystem services identified above in Step 1, their timely provision might be of central importance. In-soil organisms may display uni-, semi- or multivoltine life-history strategies (e.g. Lavelle and Spain, 2005). For univoltine and semivoltine species, full recovery from chronic effects might only be observed one year or more after PPP use. Therefore, the Panel considers time lapses of one year or more as relevant for the demonstration of e.g. long-term effects on in-soil species that may emerge after several year of PPP use or for the demonstration of recovery of species with a long life cycle. Therefore, the temporal scale of SPGs as assessment endpoints diverges from the time scale of measurement endpoints, which should cover also the life cycles of vulnerable species (see section 7.9).

Regarding the ecosystem services driven by in-soil and having an impact on other organisms – e.g. ‘pest control’ or ‘food web support’ – time ranges for full recovery that are greater than the growing season are most likely not adequate to satisfy the protection goals. The temporal scaling of effects on in-soil organisms drivers may be classified as follows:

>1 year:	not considered adequate to satisfy protection goals unless effects are negligible. Negligible effects are considered as no effect level
Months:	maximum of 6 months
Weeks:	up to 4 weeks
Days:	up to 7 days

5. IN-SOIL ORGANISMS AND ECOSYSTEM SERVICES IN AGRICULTURAL LANDSCAPES

5.1. In-soil organisms as drivers of the provision of biodiversity and genetic resources

The Introduction to this Opinion noted that the overall protection goal regarding the environment according to good agricultural practice is that PPP “shall have no unacceptable effects on the environment, having particular attention to [...] its impact on non-target species, including on the ongoing behaviour of those species; [and] its impact on biodiversity and the ecosystem” (Regulation (EC) No 1107/2009 on plant protection products).

The EFSA Scientific Committee gives guidance on how to set accurately (specific) protection goals that should cover the general protection goal ‘biodiversity’ laid down in the legislation of several regulated products using the framework of the ‘ecosystem service approach’ (EFSA Scientific Committee, 2015a). The ecosystem service approach has been introduced for environmental risk assessment by EFSA in 2010 (EFSA PPR Panel, 2010a) and has been successfully applied since then in several scientific outputs (e.g. EFSA PPR Panel, 2013, 2014a; 2015a, 2015b).

Biodiversity can be defined as the ‘variety of life, including variation among genes, species and functional traits’ (Cardinale, 2012). Several aspects of biodiversity can be assessed when it comes to ‘measuring’ the diversity of organisms in a specified system. For example, richness ‘is a measure of the number of unique life forms’, while evenness ‘is a measure of the equitability among life forms’, and heterogeneity ‘is the dissimilarity among life forms’ (Cardinale, 2012).

Some general aspects on the importance of wider biodiversity that are agreed upon in the scientific community and are relevant for ecosystem services in agricultural landscapes are as follows:

- there is unequivocal evidence that biodiversity loss reduces the efficiency by which ecological communities capture biologically essential resources, produce biomass, and decompose and recycle biologically essential nutrients.
- there is strong evidence that biodiversity provides stability of ecosystem functions over time.
- the impact of biodiversity on any single ecosystem process is non-linear and saturating, such that a change in process rates accelerates as biodiversity loss increases.
- diverse communities are more productive because they contain key species that have a large influence on productivity, and differences in functional traits among organisms increase total resource capture. Loss of diversity across trophic levels has the potential to influence ecosystem functions even more strongly than diversity loss within trophic levels.
- functional traits of organisms have large impacts on the magnitude of ecosystem functions, which give rise to a wide range of plausible impacts of extinction on ecosystem function.

The wide definition and some peculiarities of biodiversity measurements, however, make the assessment of this general protection goal challenging.

First, as reviewed by the PPR Panel (EFSA PPR Panel, 2015a), ‘normal operating ranges’ of biodiversity differ between different ecosystems. As a general rule, no single biodiversity value—whatever measurement endpoint is chosen—can be defined as being appropriate for all ecosystems. In fact, apart from some ecosystems claimed to be ‘highly diverse’, an increase in species diversity owing to the additional presence of generalists on top of specialists might be an indication of the onset of disturbance (e.g. Begon et al., 2006). In the framework of this Opinion, extensively managed organic farmed fields with comparatively low input of PPPs and high biodiversity could act as a reference system to derive appropriate ‘normal operating ranges’, especially for in-field areas in agricultural landscapes (e.g. Moreby et al., 1994; Hole et al., 2005; Tuomisto et al., 2012; literature review in Brühl et al., 2013).

Second, the evaluation of biodiversity relates to comparisons between areas or between time points, and different biodiversity measurement endpoints might apply and deliver different metrics (e.g. so-called Alpha-diversity at local scale, Beta-diversity between different fields or Gamma-diversity at landscape scale). No single measurement endpoint of biodiversity can be proposed as appropriate for all organisms because several ecosystem services driven by non-target organisms are important at the same time in agricultural landscapes and different spatial assessment scales might be relevant for different ecosystem services. The spatial scales defined for the assessment of potential effects on in-soil organisms after exposure to PPPs should be related to the traits of these organisms regarding their dispersal and recovery capacities. It is anticipated that assessment of local aspects of diversity of in-soil organisms in the in- and off-field areas might deliver the appropriate metrics (see Chapter 6.1).

As defined in the chapter above, the initial step to derive SPGs is the identification of relevant ecosystem services (ES) and of the so-called key drivers or service providing units (SPUs). The SPUs – as defined by EFSA Scientific Committee (2015a) and employed in the present Opinion – are “the structural and functional components of biodiversity necessary to deliver a given ecosystem service at the level required by service beneficiaries” (see also e.g. Vanderwalle et al., 2008).

EFSA Scientific Committee (2015a) points to the fact that several types and categories of ES (e.g. ES underpinning plant/animal production or other services relevant to society) have elements that either depend on or are influenced by biodiversity. In particular, the provisioning service ‘genetic resources’ can be also suitably defined in order to address parts of the general protection goal ‘no unacceptable effects on non-target organisms, biodiversity and the ecosystem’.

The relevant SPUs are those components of biodiversity that are ‘necessary’ for in-soil organism communities in the context of agricultural landscapes (EFSA Scientific Committee, 2015a). For this purpose, it was concluded that in-soil organism SPUs regarding the ecosystem service ‘biodiversity and genetic resources’ should address the following specific aspects:

- 1) the diversity level needed to support biodiversity of in-soil organisms as a general protection goal, i.e. the ‘intrinsic’ value of biodiversity as regulated good
- 2) the reliability of the performance level of other SPUs among in-soil organisms, with particular reference to changing environment and multiple stress, i.e. the supporting service of biodiversity for other ESs; and
- 3) an option value for biodiversity and genetic resources, i.e. the provisioning service of diversity in order to take advantage of ecosystem services also in the future.

Microorganisms pose particular challenges when it comes to defining diversity in terms of the entity ‘species’. Soil fauna and soil microorganisms will therefore be dealt with in separate sections.

Soil fauna

The first aspect of the ecosystem service ‘biodiversity and genetic resources’ addresses the *diversity level needed to support communities of in-soil organisms* in agricultural landscapes. In the discussion about definition of biodiversity as a general protection goal, EFSA (2014a) states that the species diversity *per se* is often defined as ‘structural biodiversity’ versus a so-called ‘functional biodiversity’. Structural biodiversity delivers through the magnitude of the different species’ traits the ‘functional biodiversity’. A functionally diverse system is also likely to host a higher structural diversity than one with low functional diversity. However, functional biodiversity focuses on the specific function that (a group of) species exerts in the processes of interest.

A clear assignment of a species to a functional group is, however, not possible. A single species has several functions, as several traits can be assigned to every species, and every species in turn is uniquely characterised by its trait configuration (e.g. Gardner et al., 2014). Given that the knowledge on the functions of the extremely diverse soil fauna in ecosystem processes is far from being comprehensive, it is not recommended to address ‘biodiversity’ at a merely functional level. This

helps in defining SPGs that might otherwise being considered vague and prone to be contested (Schroter et al., 2014; Kull et al., 2015; Büscher et al., 2014; Arsel and Buscher, 2012).

It is accepted that the diversity of species stands behind processes that might eventually result in desirable services (see above), but the species composition over time in one functional group might change drastically at the expense of the level of diversity. In an extreme scenario of species erosion, one function of interest may be performed by a single species, with far reaching consequences not only for the goal of protecting biodiversity, but also for the stability and resilience of the system.

Functional groups of in-soil organisms are hierarchically organized in soils (functional domains *sensu* Lavelle, 2002; Anderson, 1988). Species assigned to different functional domains interact with each other, especially at the lower trophic level. Interactions between the component of the soil food web, are of biological, biophysical and biochemical nature (Brussaard et al., 2007b and references therein). Their overwhelming complexity indicates that species shifts in one functional group might interfere with the structural diversity of other functional groups (e.g. Sheenan et al., 2007). The most difficult species' benefits to assess are those occurring via trophic cascades and the so-called 'consumer connections', since they might be wholly unanticipated or build up unexpectedly, with far reaching consequences even in species-rich communities (Gascon et al., 2015; Duffy, 2002; Mouillot et al., 2013).

Therefore, the SPUs for soil fauna addressing the level of genetic resources needed to support biodiversity of in-soil organisms as a general protection goal are the different species of soil invertebrates belonging to the micro-, meso- and macrofauna. Populations of different species are the relevant entities of these key drivers, independently of whether species belong to the same functional group or not (e.g. several earthworm species).

The second aspect that has been identified above, as relevant, to the ecosystem service 'biodiversity and genetic resources' and driven by in-soil organisms is the *impact of diversity on the reliability of the ecosystem functional responses* (stability and resilience) in a changing environment and under the impact of multiple stressors. As stated by Naeem and Li (1997), 'reliability refers to the probability that a system will provide a consistent level of performance over a given unit of time'. Here, we do not refer to the general relationship 'higher diversity, better ecosystem performance', but to the fact that agricultural fields (and often also their surrounding habitats) are hot spots of drastic environmental changes and repeated PPPs applications. Since a 'system that is functioning properly is one that will persist despite natural environmental fluctuations' (Palmer et al., 1997), we argue that the timely and simultaneous performance of different services requires here a different level of diversity than in systems with more constant environmental conditions.

There is a continuum of hypotheses linking soil species diversity to a better functional performance of ecosystems, with two opposite far ends. One is the 'rivet' hypothesis, which suggests that each species has a unique effect on ecosystem function, and the opposite is the 'redundant species' hypothesis, which suggests that only a minimum number of species is necessary for ecosystem function. Even if some studies seem to support the hypothesis that species are redundant and saturation of functions is reached at low level of species richness, Wolters (2001) argues that the potential effect of associated diversity implies that 'redundant species may gain functional significance by interacting with functionally important species'. These interactions between redundant species and keystone species, the loss of which might seem more critical in the first term (Gitay et al., 1996), is best explained in the figure below.

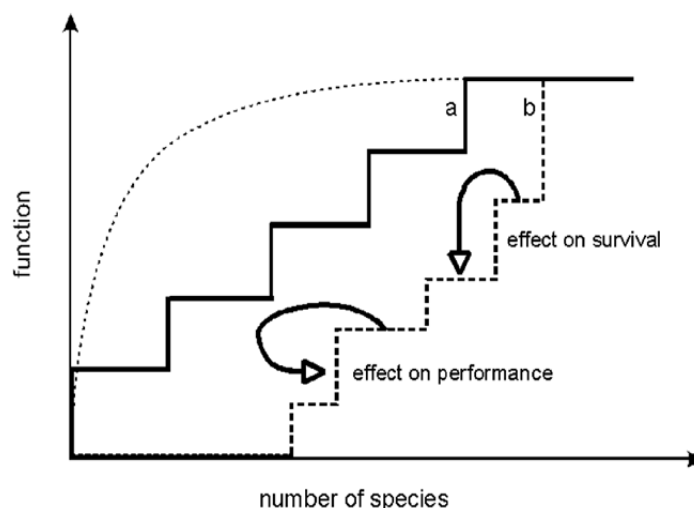


Figure 3: Average functional effect of the elimination of species from a soil biota community composed of a very rich group of redundant species and a small group of functionally important species. Graph a) no interaction between species; Graph b) effect of redundant species on survival and/or performance of functionally important species. From Wolters (2001) with permission.

Elimination of redundant species may affect the steepness of the relationship between species diversity and function. Feedback effects of functionally important species may accelerate this process; the same is true for disrupted facilitative or mutualistic interactions (Zhang and Zhang, 2015; Mod et al., 2015; Wall and Moore, 1999). The complexity of interaction between species makes the determination of the ‘number’ of species that a system can afford to lose extremely difficult. Some species loss can be compensated for, but, if the erosion process continues, a ‘tipping point’ for ecosystem functioning and ecosystem service provisioning is reached and the system may slip in a different status or definitively collapse (e.g. Lever et al., 2014). Therefore, the concept of species redundancy may be regarded as a ‘redundant concept’ (Gitay et al., 1996), above all in the case of heavily disturbed ecosystems as agricultural fields. Here, species redundancy is a valuable commodity, since each species tolerates only a limited range of climatic and biotic conditions and any change in environment beyond these conditions leads to poor performance or to extinction. As Brussaard et al. (2007a, b) pointed out, if there are several species in a functional group, some species are likely to survive any extreme event, ensuring continuation of the services provided (e.g. ‘insurance hypothesis’ Yachi and Loreau (1999); Naeem (1998)). The species diversity within a functional group expands the range of conditions over which ecosystem services are performed (Ferris and Tuomisto 2015). Agricultural soils undergo drastic changes compared with natural systems and soil communities do experience a range of physical and chemical management stressors. Therefore, the SPU for in-soil organisms addressing the supporting service of diversity for the reliability of the ecosystem functional responses under the impact of multiple stressors in agricultural areas are again the different species of soil invertebrates belonging to the micro-, meso and macrofauna. The relevant entities of these key drivers are the populations of different species, independently of whether species belong to the same functional group or not. Surely, also phenotypic erosion will have adverse effects on species diversity. In changing environments under high disturbance, high phenotypic variance cannot be sustained *ad infinitum* without external input (Norberg et al., 2001). This has to be considered when defining the magnitude of tolerable effects and the temporal scale of specific protection goal options for in-soil organisms.

The third aspect that we consider when addressing the ecosystem service ‘biodiversity and genetic resources’ of in-soil organisms in agricultural fields is the identification of SPUs to *deliver diversity and genetic resources as an option value*, i.e. a provisioning service in order to take advantage of ecosystem services also in the future.

Several studies have detected diverging effects of species with apparently very similar traits on measurable ecosystem processes at different levels of resolution (e.g. Cragg et al., 2001; Pieper & Weigmann, 2008). What is more, the unique importance of rare species to ecosystem function has been demonstrated also in species-rich ecosystems, where high functional redundancy is likely and should buffer the system against species loss (Mouillot et al., 2013; Gascon et al., 2015). Acknowledging these relationships for services that are currently known and measurable and top rated as valuable to mankind, means that similar diversity levels should be granted in order to preserve the option to use these or other services in the future. The *option value* should allow also in future the use of services like genetic resources that are provided by biodiversity. These services might be already known at present –but their attributed value change in future; or these are services that have yet to be quantified.

Summarizing the arguments on the three key aspects above, ‘maintaining multiple ecosystem processes at multiple places and times requires higher levels of biodiversity than a single process at a single place and time’ (Cardinale, 2012). The Panel identifies the SPU for ‘biodiversity and genetic resources’ of in-soil organisms as the different species of in-soil invertebrates belonging to the micro-, meso-, and macrofauna. Populations of different species are the relevant entities of in-soil key drivers, independently of whether species belong to the same functional group or not. This is in agreement with the draft guidance of the EFSA Scientific Committee (EFSA Scientific Committee, 2015a) that states the following: ‘when the aim is to maintain specific populations and biodiversity, structural endpoints need to be selected as the ecological entity’ to be protected.

Soil microorganisms

Collectively, microorganisms represent more of the genotypic diversity in the universal phylogeny of life on earth than all other organisms taken together (Pace, 2009). Both the phylogenetic and functional diversity of microorganisms and their numbers and biomass in soil are high, with e.g. one gram of comparatively unperturbed soil containing up to 10^{10} prokaryotic cells (bacteria and archaeans) representing several thousand of different genomes (Torsvik and Ovreas, 2002).

The use of the ecosystem-services framework for defining protection goals in environmental risk assessment has strong connections to the discussion of the value of specific organisms and biodiversity in general as a base for decisions concerning environmental conservation. The value that can be assigned to the genetic diversity of ecosystems can have different character, as explained above in this section (see also Swift et al., 2004; Cockell, 2005). Values that relate to the system-supporting functions that organisms perform generally in the biosphere or for direct human benefit have been termed their *instrumental* (or *utilitarian*) values. These values of microorganisms are not really a matter of dispute. In contrast, the *intrinsic* value represents the non-use value that biodiversity has on its own, regardless of any human benefits. Clearly, it is not possible to protect (individual) microorganisms at the same levels of attribute and ecological entity as for vertebrates or plants. Although the question whether microorganisms have intrinsic value is largely unresolved, there have been claims that microbes should have increased attention in biodiversity-conservation programs (Cockell and Jones, 2009; Bodelier, 2011).

It can be expected that the functional capacity of microbial communities in soil is somehow related to the genetic or structural diversity of the total assemblage of microorganisms. The biodiversity–ecosystem function relationship in microbial communities has lately received much attention (e.g. Griffiths et al., 2001; Swift et al., 2004; Fitter et al., 2005; Wertz et al., 2007; Hallin et al., 2009; Philippot et al., 2013; Krause et al., 2014; Singh et al., 2014). This structure/function relationship is quite complex, however, and Ducklow (2008) found that in the information available at that time there were roughly equal numbers of positive, negative or inconclusive relationships between functional properties and diversity of microbial communities. One reason for such variable results, in studies on the biodiversity–ecosystem function relationships, can be the functional redundancy in microbial communities, meaning that if organisms mediating a certain function are inhibited or eliminated, other organisms take over and fill the niche. Functional redundancy can be expected to contribute to the lack

of biodiversity–ecosystem function relationships mainly for common properties shared by many of the microbial populations; for specific functions carried out by fewer populations, the relationships are more direct and positive (Ducklow, 2008). Another factor of importance is that only a minority of publications on biodiversity–ecosystem function relationships report results from proper manipulation experiments, while the majority rely on designs where properties of different systems are compared (Krause et al., 2014). Additionally, it may be that although microbial genetic diversity *per se* is not strongly correlated with a particular functional capacity, the sizes of the concerned populations are (e.g. Hallin et al., 2009).

Although the discussion regarding the importance of microbial diversity for ecosystem functioning of soils and the role of functional redundancy goes on, it is clear that loss of microbial diversity can have an impact on function, at least for relatively specific functions performed by few populations (Philippot et al., 2013; Singh et al., 2014). Recently, several studies have suggested that introducing the concept of community–aggregated traits may lead to a better understanding in the future of the role of biodiversity in supporting functions and ecosystem services (Fierer et al., 2014; Krause et al., 2014).

For microorganisms, there is no scientific framework in place for making protection at the population (i.e. species) level possible. The major drawback is that the majority of soil bacteria and archaeans have largely unknown specific properties, since they have not been isolated and studied in pure form. Additionally, just to determine the biogeographical distribution and “rarity” of a microbial species is a big technical and scientific undertaking. In addition, since bacteria and archaeans grow as clones, the species concept is arbitrarily applied in these groups. Due to these factors, there is as yet no scientific basis for protecting single microbial species and they are not included in current red-listing of threatened species, with the exception of fungi producing different types of fruiting bodies. In the Opinion of the EFSA PPR Panel on sediment organisms (EFSA PPR Panel, 2015b) “functional groups” was used as the ecological entity for protection of SPU for microbes across different ecosystem services/specific protection goals.

Even if relationships between genetic microbial diversity in soil and the ecosystem functions they provide are complex and still discussed in the scientific community, clearly there must be some such relationships. At the moment, it seems reasonable for the majority of ecosystems services to use the same general ecological entity of microorganisms to be protected as in the EFSA sediment opinion (EFSA PPR Panel, 2015b), i.e. “functional groups”. However, for the genetic resources provision and biodiversity support, it is proposed to use the (entire) community as the entity to protect for bacteria, archaeans and fungi since at least some specific functions can only be performed by a restricted subset of the community.

It is proposed to use the diversity, i.e. the variety/richness, as the attribute. Diversity of fungal, bacterial and/or archaeal communities in soil can be described using both structural and functional terms and methods (see Appendix I. and chapter 6.3 for more details on microbiological tests) and both approaches are potentially useful for describing effects of PPPs on fungal, bacterial and archaeal communities.

For mycorrhizal fungi, although they are obligate symbionts and need to establish a close symbiotic association with plants in order to growth, it is proposed to also protect them at the community level as for other soil microorganisms. This is mainly due to: (i) absence of an absolute host-specificity; (ii) low species diversity for arbuscular mycorrhizae but high within-species genetic variability and trait plasticity (Koch et al., 2004; Munkvold et al., 2004); (iii) high species diversity for ecto-mycorrhizal fungi but lack of exhaustive taxonomic information makes the identification at the species level uncertain (Kõljalg et al., 2004); (iv) possible co-occurrence of multiple fungal species able of occupying different niches as well as to the possibility of co-occurring host species having distinct mycorrhizal communities (Dickie et al., 2007).

5.2. In-soil organisms as drivers in maintaining cultural services

According to MEA (2005), cultural services are those non-material benefits people obtain from ecosystems through spiritual enrichment, heritage values, inspiration, recreation, and aesthetic experiences. Many studies focusing on soil ecosystem services do not address ‘cultural services’ (Barrios, 2007; Daily et al., 1997; Lavelle et al., 2006; Wall et al., 2004; Weber, 2007); however, *a priori*, cultural services have no greater or lesser value than provisioning, regulating or supporting services. It has to be noted that the intrinsic value of biodiversity and species of conservation concern can be considered a ‘cultural service’, since the species-based approach to conservation considers biodiversity as a good that has a range of cultural values, such as aesthetic, recreational, and existence values.

Soil, as part of the landscape, supports vegetation, and across many cultures has been the source of aesthetic experiences, spiritual enrichment, and recreation. Comerford et al. (2013) associated cultural and spiritual ecosystem services with land and landscape and a “*sense of place*”. Agriculture provides cultural services as some farmers, for example, may maintain field-boundary vegetation or contribute in diversifying landscapes by planting hedgerows, shelterbelts or trees. Some farms also provide accommodation (Dominati et al., 2010), providing places for carrying out recreational activities and nature appreciation. Soil and land provide images that permeate our written and linguistic heritage and our art, such as painting, sculpture, photography, film-making, etc. According to Landa and Feller (2010), soil art combines the diverse roles and functions soil may have, being habitat, growth medium for plants, substrate for architecture, natural filtration system as well as basis for cultural identity. Soil art also explores the aesthetic value of soil with its colours and textures. Soil also plays a direct role in our cultural and religious traditions as a physical material and various cultural uses across the globe (Comerford et al., 2013; Dominati et al., 2010). In addition, soil and agricultural landscapes also play a key role in environmental education. The assessment of cultural services is *per se* difficult, as some contexts elicit aesthetic experiences that have traditionally been called ‘scenic beauty’, others may elicit different experiences, such as perceived care, attachment and identity (Gobster et al., 2007; Manachini, 2013).

The perception of fulfilled values is very personal and dependent on the social context. For example, significant differences in the perception of the cultural value of agricultural areas have been described for ‘farmers’, ‘naturalists’ and ‘students’, thus demonstrating that cultural services are not absolute values (e.g. Rogge et al., 2007; Natori and Chenoweth, 2008; Tempesta, 2010; Weyland and Laterra, 2014). Interestingly, if a general rule can be set up, Weinstoerffer and Girardin (2000) see in humans a general attraction for ‘diversity, which is source of pleasure, satisfaction or happiness’. The human perception and attraction for nature and biodiversity can also vary among different major groups of organisms. For instance, single individuals and species of plants, insects and other animals are often admired for their beauty. On the other hand, the attraction of bacteria and archaeans is much less obvious and can be more connected to their importance for creating living “healthy” topsoils which form excellent growth medium for plants. Even the typical “soil smell” of such healthy soils is perceived as attractive by many. A main reason that prokaryotes have received less attention than other organisms in this respect is that they are microscopic and not visible by the naked eye. Another contributing factor could be the fact that it is not possible for humans to identify with particular species of prokaryotes in the same way as animals or plants.

Soil structure, a result of soil formation processes including the activity by in-soil organisms, has been often taken up by visual and plastic arts (e.g. <https://soilarts.wordpress.com/>).

In order to help achieving the ‘desirable complementary relationship between aesthetic pleasure and ecological health’ (van Zanten et al., 2013), it is suggested in the framework of this Opinion to couple the service providing units for in-soil organisms providing cultural values to the SPU for genetic resources and biodiversity.

5.3. In-soil organisms as drivers of nutrient cycling

Soil fauna

Soil fauna is the assemblage of a very diverse groups of invertebrates, including e.g. earthworms, arthropods, gastropods, nematodes. Many representatives of soil fauna are important facilitators of organic matter decomposition and nutrient transformation. They can feed on litter material and excrete it partially decomposed as faecal pellets, thus increasing the surface area for microbial activity (Hasegawa and Takeda, 1995). They also act as dispersal agents of fungal spores and bacteria. Moreover, they exert a more direct effect on microbial communities by performing a selective grazing on archaea, bacteria and fungal hyphae (Lummer et al., 2012; Garcia-Palacios et al., 2013), promoting microbial (mainly fungal) succession in decomposing plant material, accelerating decomposition and enhancing nutrient mineralization (Ke et al., 2005; Cragg and Bardgett, 2001; Cole et al., 2005; Crowther et al., 2012).

Even though microorganisms are directly involved in the biochemical decomposition of organic matter and nutrient transformations, their activity is closely related to the presence and activity of soil invertebrates that act as catalysts of microbial activity and modulators of community composition of microorganisms (Lavelle and Spain, 2005). The role of soil fauna in organic matter decomposition and nutrient turnover can be direct, *via* feeding on litter material (fragmentation), or indirect, by creating favourable conditions for microbial communities. Many species composing the soil macrofauna (lumbricids, isopods, millipedes, ants, insect larvae) and mesofauna (collembolans, mites, enchytraeids) are actively involved in organic matter breakdown via their feeding activity, contributing to its efficient and fast decomposition and associated nutrient release (e.g. Ketterings et al., 1997; Schrader and Zhang, 1997; Filser, 2002; Dechaine et al., 2005; van Eekeren et al., 2008).

“Litter transformers” (e.g., isopods, millipedes, and some earthworm species) participate in the early phase of this process, promoting litter fragmentation and influencing microbial dynamics by altering substrate quality when excreting the vegetal material as faeces. They develop external mutualistic associations with microflora by contributing to an increase of substrate surface area accessible to microbial attack and to an increase of substrate pore volume and aeration, thus enhancing the overall microbial resource exploitation (Hassall et al., 1987; Kayang et al., 1996; Maraun and Scheu, 1996; Cotrufo et al., 1998). This may, ultimately, influence nutrient mobilization rates in the system (Anderson et al., 1983; Verhoef and Brussaard, 1990; Teuben and Roelofsma, 1990).

However, results from microcosm studies reported in the literature on the effects of “litter transformers” on microbial communities are diverse. Enhanced microbial activity induced by the presence of isopods or diplopods was observed for oak litter (Hanlon and Anderson, 1980; Anderson et al., 1983), black pine (Teuben and Roelofsma, 1990) and on ¹⁴C-labelled pondweed (Griffiths et al., 1989). In contrast, a decrease in microbial activity caused by macrofauna was reported for oak litter under high feeding intensity (Anderson et al., 1983), for poplar (Van Wensem and Adema, 1991; Van Wensem et al., 1991; Van Wensem et al., 1992) and for a mixture of deciduous leaves (Vink and van Straalen, 1999). When litters in different decomposition stages were analysed, contrasting effects were found in experiments with poplar (Van Wensem et al., 1997), black pine (Teuben, 1991) and beech (Maraun and Scheu, 1996). These results indicate that the type and magnitude of effects depend, among other factors, on the type and status of the substrate, namely its chemical composition and degree of processing by soil fauna.

Effects on nutrient mineralization may follow similar contrasting trends, and the observed diverging results have been also related to the nutrient status of the litter. To a certain extent, animal activity seems to act as a buffering factor, inducing an element of mineralization when basal nutrient contents are low and *vice-versa* (Teuben and Roelofsma, 1990; Teuben, 1991). Model simulations revealed that effects of isopods on nutrient pools in decomposing litter were dependent on the litter C:N ratio (Van Wensem et al., 1997), with available carbon and nitrogen levels increasing in the presence of woodlice in litter with medium to high C:N ratios. Not only the quality of litter or soil organic matter affects the

role of litter transformers on decomposition, microbial communities and nutrient release. In a microcosm experiment with isopods, millipedes and earthworms, evaluated either as single species or in different combinations, Heemsbergen et al. (2004) demonstrated that litter decomposition, microbial respiration and nitrogen mineralization were more influenced by community functional dissimilarity (different species with different traits and having different roles in the process) than by species richness *per se*.

Although “ecosystem engineers” are involved in litter consumption and nutrient cycling, their role in organic matter turnover is also exerted via their burrowing and casting activities, providing habitat for microbes and facilitating the availability of organic substrates, regulating their decomposition activities (e.g. Jegou et al., 2001; Smith and Bradford, 2003; Cole et al., 2006; Frouz et al., 2006; Postma-Blaauw et al., 2006). Brussaard et al. (2007a) postulate that the role of soil macrofauna on water and nutrient use efficiencies in crop areas might be better related to their influence on soil structure. The biogenic structures they produce (soil aggregates and pores) modulate the water and nutrient fluxes, with macroaggregates contributing to the stabilization of soil organic matter and to the storage of nutrients and their consequent slow release during their decomposition (Brussaard et al., 2007b; Mariani et al., 2007; Jimenez et al., 2003). However, their influence on N mineralization can be seen beyond the area of the burrows, as found out for *Lumbricus terrestris* by Amador et al. (2006), who observed an increase in soil nitrate in the surrounding of these structures in a mesocosm experiment. The role of earthworms in nitrogen mineralization seems to depend on the ecological group they belong to and the nutrient source. In a microcosm experiment analysing the influence of different combinations of earthworm species representing different life strategies, Postma-Blaauw et al. (2006) reported enhanced mineralization of crop residues in the presence of epigeic and anecic species (*Lumbricus rubellus* and *L. terrestris*, respectively), whereas the mineralization of SOM increased in the presence of the endogeic species *Aporrectodea caliginosa* in combination with the epigeic species *L. rubellus*. Both interactions resulted in a reduction of mineral N in soil, possibly due to its immobilization in microbial biomass. When the endogeic and anecic worms were present, an increase in microbial biomass was also observed with a decrease in total soil carbon. These results demonstrate that the effect of earthworms on nutrient mineralization depends on the traits of the different species present and can be modified by their interactions.

Regarding non-arthropod invertebrates belonging to the macrofauna, it is questioned whether defining different functional groups (here: anecics = vertical burrowers, endogeic = burrowing in soil matrix and epigeic = surface dwellers) as the ecological entities to be protected would be sufficient to address these key drivers. Having species representatives like e.g. *Lumbricus terrestris* as a key driver and ‘ecosystem engineer’, the populations of anecic earthworm species should be the main ecological entity addressed in the SPG definition.

Most land snail species are herbivorous and feed mainly on decaying or half-decayed plant material; some are predators (Burch and Pearce 1990) and possess a very different feeding strategy compared to e.g. earthworms. There is no known herbivorous snail species in Europe whose food spectrum is limited to particular plant species and a few snail and slug species are known as pest organisms in ruderal systems, often due to their preference for crop plants that show higher palatability than their wild forms (Gosteli 1996; Kerney *et al.* 1983). As for other organisms, some terrestrial snails and slugs can be, thus, considered both non-target organisms and pest species, depending on particular circumstances. Considering only ingestion, the average contribution of terrestrial gastropod species to litter input in temperate ecosystems seems to be lower than for other soil invertebrates (see e.g. Mason 1970, Jennings & Barkham 1976, Peterson and Luxton 1982), although data on environments other than woodlands are scarce. Comparing anatomical and physiological features, Wieser (1978) reported gastropods to be “both efficient digesters and assimilators”, whereas isopods can be considered “efficient digesters but usually inefficient assimilators”, which suggests that gastropods turn over a lower amount of organic material for the same gain of nutrients compared to isopods. Newell (1967) discusses the possible role of terrestrial gastropods in soil formation and states that terrestrial gastropods may have an important function by producing partially digested plant material and modifying their environment during crawling and with their feces. Faeces and mucus may provide a

suitable habitat for the proliferation of microorganisms as a starting point for decomposition processes, which Dallinger et al. (2001) consider to be probably their most important function in nutrient cycling in temperate ecosystems. Since terrestrial gastropods (mainly snails but also slugs) probably make a significant contribution to the fixation of calcium in the upper soil layer, they may have a strong impact on nutrient cycling in terrestrial ecosystems by diverting fluxes and changing availabilities of macronutrients in terrestrial ecosystems (Dallinger et al, 2001).

Many soil arthropods ingest large amounts of dead organic matter, fungal hyphae and bacteria. Although their role in direct plant-litter decomposition is probably minor, they significantly affect organic matter decay by a range of indirect effects. For example, several studies have shown that, at a moderate density of Collembola, litter enzyme activity, litter respiration and rates of nutrient release increase when compared with litter decomposing in the absence of these animals (Verhoef and Brussaard, 1990). The influence of springtails on nitrogen and phosphorus mineralization depends on the dominant species involved and their traits, on the climate and type of ecosystem (Filser, 2002; Cragg and Bardgett, 2001).

Interactions between microarthropods and nematodes have been reported to affect soil carbon (C) and nitrogen (N) cycles (Yeates, 2003; Osler and Sommerkorn, 2007). In addition, abundance of total, bacterivorous, and fungivorous nematodes were found to be positively correlated with net N mineralization rates. Neher et al. (2012) attempted to quantify the relative importance of specific faunal groups in the decomposition of organic matter and for the N availability in soils. Variation in soil N availability and decomposition rates were analyzed accounting for the contributions of two faunal communities: nematodes and arthropods. Nematode communities explained between 7-12% of the variation in NO_3^- and NH_4^+ availability, indicators of N mineralization, in disturbed and undisturbed forests. Microarthropod communities explained almost 15% of the variation in decomposition rates in forests. Therefore, alterations in soil food web structure can result in significant changes in decomposition processes (Setälä, 2002).

Considering that soil fauna includes a number of identified 'ecosystem engineers', the ecological entity holding different traits in terms of nutrient mobilisation and cycling in agricultural soils is the functional group. However, as illustrated above in the section on the role of species diversity for the long term performance of functional groups in strongly disturbed agricultural soils, the solely definition of SPU at the level of functional groups might lead under unfavourable conditions to a loss of function performance. Species loss within a functional group will lead to the erosion of trait diversity and to a reduced resilience and stability under changing environmental conditions. In order to support the long term performance of the functional role of soil fauna in nutrient cycling of agricultural soils it is therefore recommended to define the SPU as the abundance/biomass of species belonging to different functional groups.

Soil microorganisms

Soil microorganisms play a dominating role in the degradation of organic matter in soil, which results in mineralisation of C and the essential macronutrients N, P and S (Hopkins and Dungait, 2010; Hussain et al., 2009). The mineral forms can then be further transformed by specific groups of microbes (Prosser, 2007; Hopkins and Dungait, 2010). Since in crop-production soils the plant material is largely harvested and removed, the direct dependence on remineralisation of primary production in field is principally over-run. Resident soil microbial communities, however, still perform critical functions related to mineralisation and transformation of nutrients supplied as organic and inorganic fertilisers (e.g. Ninh et al., 2015).

The nitrogen cycle is particularly relevant to how in-soil organisms increase fertility and is one of the most studied processes. Nitrification and denitrification represent key processes determining the availability and forms of nitrogen (N) in soils. The ability to denitrify is widespread among various microbial taxa, including such phylogenetically diverse groups as bacteria, archaea and eukaryotes (Hallin et al., 2009; Szukics et al., 2010). Conversely, nitrification, including ammonium oxidation

and nitrite oxidation, was long believed to be accomplished by a small, specific group of bacteria, until the existence of an archaeal ammonium oxidizer was identified about a decade ago (Hu et al., 2014). Fixation of atmospheric nitrogen by diazotrophic bacteria is a significant N source in rice and legume cultures, and is thus critical for sustainable production in these systems. In rice paddies it can be performed both by heterotrophs and photosynthetic cyanobacteria (Choudhury and Kennedy, 2004; Wartiainen et al., 2008) and research is ongoing, aiming to increase the use of cyanobacterial inoculants in rice production (Choudhury and Kennedy 2004; Das et al., 2015). In legume crops, nitrogen fixation is performed by several genera of root-nodule forming bacteria (Graham, 2008). *Rhizobium*-legume symbioses massively contribute to biological nitrogen fixation entering soil ecosystems (Hussain et al., 2009) with over 100 agriculturally important legumes. Altogether, Rhizobia form symbiotic relationships with an estimated 15,000 legume species. The symbioses between *Rhizobium* or *Bradyrhizobium* and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume-based crop and pasture production than the application of fertilizer-N. It is estimated that N-fixing bacterial symbionts of the legumes can contribute up to 20% of all plant N. Actinorhizal interactions (*Frankia*-nonlegume symbioses) are major contributors to nitrogen inputs in forests, wetlands, fields, and disturbed sites of temperate and tropical regions.

Microorganisms also play a central role in the phosphorus cycle. Most agricultural soils contain large reserves of phosphorus. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants. A second major component of soil P is organic matter. Organic forms of P may constitute 30–50% of the total phosphorus in most soils, although it may range from as low as 5% to as high as 95%. To make this form of P available for plant nutrition, it must be hydrolyzed to inorganic P. Mineralization of most organic phosphorous compounds is carried out by means of phosphatase enzymes, such as acid phosphatases. Soil bacteria expressing a significant level of acid phosphatases include strains from the genus *Rhizobium*, *Enterobacter*, *Serratia*, *Citrobacter*, *Proteus* and *Klebsiella*, as well as *Pseudomonas* and *Bacillus* (Rodriguez & Fraga, 1999).

The provision and the regulation of primary production is one of the most important services delivered by soils. Plant growth and productivity is heavily influenced by the interactions between plant-roots and the surrounding soil, including the microbial populations within the soil. Thus, soil microorganisms have a strong impact on plant productivity. The main mechanisms for plant growth promotion driven by microorganisms include suppression of disease (biocontrol), enhancement of nutrient availability (biofertilization), and production of plant hormones (phytostimulation) (Pereg and McMillan, 2015). The service of pest regulation is indirectly related to the primary production, since such a control limits the loss of plants and plant products.

Plant uptake of water and mineral nutrients from the soil is greatly aided by mutualistic associations with mycorrhizal fungi, which grow into and extend out of the plant roots. Nutritional fluxes are bidirectional (Berruti et al., 2014). Nitrogen acquisition strategies are different in arbuscular mycorrhizae and ecto-mycorrhizal fungi (Rebel et al., 2013; Marschner and Dell, 1994) but both play a key role in providing plants with phosphorus, which is mainly available in soil as insoluble organic or inorganic forms that make it unavailable to plants (Jones et al., 1998; Berruti et al. 2014; Smith et al. 2011). Arbuscular mycorrhizal fungi have been shown to enhance plant productivity by improving P uptake by plants (van der Heijden et al., 2006) up to 90%. This is particularly important for legumes, for example, which have a high P-requirement. Also, a functional complementarity has been demonstrated between families of arbuscular mycorrhizae. For instance, *Glomeraceae* provides protection against fungal pathogens while *Gigasporaceae* enhances P uptake (van der Heijden et al., 2008).

It is known that morphological arbuscular mycorrhizae traits are rather well preserved within the same genus (i.e., hyphal length, fungal biomass, number and volume of spores, internal vs external mycorrhizal root colonization). However, the manifestation of those traits can be quite variable even within one species, being highly dependent from the host plant (and plant community) and the

symbiosis established. Among the potentially important ectomycorrhizal fungal response traits influencing their abundance in various communities are preference for N source, exploration morphotype and the deposition of melanin in cell walls (Koide et al., 2014). Fungi that form ectomycorrhizae are not a monophyletic group in contrast to arbuscular mycorrhizae which all belong to the monophyletic group, the Glomales. The ecto-mycorrhizae can belong to all of the phyla of true fungi (Zygomycota, Ascomycota, and Basidiomycota) (Horton and Bruns, 2001). Ecto-mycorrhizae communities are species rich, however there is still a lot of uncertainty on the composition of ecto-mycorrhizae community in terms of number and abundance of species mainly because ecto-mycorrhizae are not easily manipulated and cultivated in laboratory. One of the main elements of mycorrhizal symbiosis is foraging for nutrients and carbon. The foraging strategy may include: proliferation of hyphae, carbon and nutrient allocation within a mycelium and spatial distribution of the mycelium (internal mycelium for carbon and external mycelium for nutrients) (Olsson et al., 2002). It is well reported in the literature that foraging-related functional traits of hyphae are typically conserved at the genus level (Agerer, 2006, Aguilar-Trigueros et al., 2014), although it is also reported that significant within-species functional variability exists in ectomycorrhizal fungi (Koide et al., 2007).

Due to the complexity of soil fungal communities and the interaction plant-fungi also considering environmental variables, arbuscular mycorrhizae and ecto-mycorrhizae taxa have not been categorised by using specific criteria but a mix of taxonomic, morphological and physiological characteristics.

So, although these arguments could indicate that “population” (=species) would be the ecological entity to protect, the variation of trait expression within each arbuscular mycorrhizae species, being highly context-dependent and to the ubiquity of these fungi species in terms of geographical distribution (Davison et al., 2012) and ability to colonize many plant species, makes it difficult to base the risk assessment at population (species) level and rather focus on the functional group arbuscular mycorrhizae. The same conclusion can also be drawn for ecto-mycorrhizae because, although much has been learned about behaviour, physiological ecology and traits have been measured on individual species, particularly of *Suillus*, *Rhizopogon*, *Paxillus*, *Laccaria*, *Pisolithus*, and *Cenococcum*, in laboratory microcosms, meaningful extrapolations to species also depend on trait variability among individuals and populations, as well as on the adequacy of the species concept for fungi. In addition, it is not very clear how factors like host diversity, soil types, organic inputs, disturbance, and succession can affect the structure composition. Moreover, vegetative structures of these fungi (i.e. mycorrhizae and mycelium in the soil) occur largely below ground and are difficult to track and identify (Horton and Bruns, 2001).

Free-living microbes also increase nutrient availability for plants through breakdown of organic matter and releasing mineral nutrients to soil solution. For example, most of the N in soil is contained in complex insoluble polymers, such as proteins, nucleic acids and chitin, which are broken down and mineralized by soil microorganisms, eventually releasing mineral forms of N that are available to plants. In turn, free living N-fixing bacteria are able to fix significant amounts of N, thus contributing to the N budget in many ecosystems. Free-living microorganisms can also contribute to the availability of nutrients to plants by weathering the bedrock *via* exudation of organic acids and solubilisation of precipitated P. In addition, they can improve plant productivity by suppressing plant diseases, for example through the production of antifungal metabolites by *Pseudomonas* spp.

Soil microorganisms seem to be characterized by a redundancy of functions. However, functional redundancy is considered greater for functions that are performed by a large number of microorganism groups, such as litter decay, than for processes performed only by few specific microbial groups, such as decomposition of specific organic compounds or other processes requiring specific and rare biochemical pathways. In accordance, Singh et al. (2014) suggested that even modest loss of diversity can affect key, specialized functions. Hence, for microorganisms, in case of processes conducted by only a few species, it is more relevant to focus on abundance/biomass of the populations of these specific taxa rather than on biodiversity, respiration rate or biomass of whole microbial communities.

1791 For processes conducted by large number of species, such as litter decay, the activity and/or biomass
1792 of whole communities may be of interest.

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Table 4: Key drivers for the ecosystem service nutrient cycling. Main taxa, exposure routes, and examples of species with slow and fast dispersal ability.

Key drivers	Main taxa/groups	Main exposure routes	example species
Litter / organic matter fragmenters	Isopoda	Oral litter	<i>Porcellio scaber</i> , <i>Armadillium vulgare</i> <i>Philoscia muscorum</i> , <i>Porcelionides pruinosus</i>
	Diplopoda	Oral litter	<i>Julidae</i> , e.g. <i>Julus scandinavicus</i> Pill millipedes (?): <i>Glomeris marginata</i>
	Collembola	Oral litter/org. matter /microorganisms Contact soil / soil pore water Contact litter / litter water film	<i>Folsomia quadrioculata</i> , <i>Pseudosinella alba</i> <i>Entomobrya multifasciata</i> , <i>Lepidocyrtus cyaneus</i>
	Oribatida	Oral litter/org. matter /microorganisms Contact soil / soil pore water Contact litter / litter water film	<i>Oppeia nova</i> , <i>Tectocephus velatus</i> , <i>Punctoribates punctum</i> , <i>Scheloribates laevigatus</i>
	Nematoda	Oral litter / org.matter Contact soil / soil pore water Contact litter / litter water film	<i>Bacterial feeders</i> (Rhabditida) <i>Fungal feeders</i> , <i>omnivorous feeders</i> (e.g. <i>Dorylaimida</i> , <i>Diplogasterida</i>).
Ecosystem engineers	Lumbricidae	Oral soil Oral litter Contact soil / soil pore water Contact litter / litter water film	Anecic worms: <i>Lumbricus terrestris</i> , <i>Aporrectodea longa</i> Endogeic worms <i>Aporrectodea caliginosa</i>
	Enchytraeidae	Oral soil Oral litter/org. matter /microorganisms Contact soil / soil pore water Contact litter / litter water film	<i>Enchytraeus albidus</i>
Bacteria and fungi feeders, nutrient release		Contact soil / soil pore water Contact litter / litter water film	
Microorganisms	Bacteria	Contact soil/contact soil pore water	
	Mycorrhizal fungi	Contact soil/contact soil pore water	
	Fungi	Contact soil/contact soil pore water	

5.4. In-soil organisms as drivers of pest and disease control

This section treats the ability of in-soil organisms to act as natural competitors, predators, parasites or antagonists, and thereby as biological control agents for pest species or plant diseases. According to Cook et al. (1996), ‘pests are organisms at population densities that cause death or injury, or constitute a nuisance to crops, livestock, pets, people, or the environment. Pest refers to weeds, plant parasitic nematodes, arthropod pests of plants, animals, plant pathogenic viruses, prokaryotes (including bacteria, mycoplasma-like organisms [MLOs], and spiroplasmas), and fungi. Disease is a process that

results from a compatible interaction between virulent pathogen and susceptible plant. Plant disease refers to infectious diseases caused by plant pathogenic viruses, viroids, bacteria, mycoplasma-like organisms (MLOs), spiroplasma, fungi, and nematodes. Diseases, however, can also be caused by abiotic factors such as unfavorable soil properties, fertility imbalances, moisture extremes, temperature extremes, chemical toxicity, physical injuries (Kennelly et al., 2012).

Pest control has often been highlighted as an important ecosystem service provided by biodiversity and one that is threatened by modern agricultural practices (Wilby and Thomas, 2002). The natural enemies of insect pests are responsible for about 50–90% of the biological pest control occurring in crop fields (Martin et al., 2013). Soils provide habitat to beneficial species that regulate the composition of communities and thus can prevent proliferation of herbivores and pathogens. This service depends not only on the health of the soil, including its abiotic properties, but also on the biological processes driving inter- and intra-species interactions (symbiosis, competition, host–prey associations) (Aislabie and Deslippe, 2013). A healthy soil community has a diverse food web where beneficial organisms can contribute to suppressing pests and disease-causing organisms through competition, predation, and parasitism. Evidence from natural systems shows that low diversity of an ecosystem can be associated with a higher vulnerability to pests due to altered top-down and bottom-up control mechanisms. In agricultural fields, for example, the soil functioning is modified and, as a consequence, its equilibrium can be altered leading to outbreaks of crop pests (Turbé et al., 2010).

Soil fauna

As a rule, all species of animals are regulated by other living organisms (antagonists) that are not under manipulation by man but occur naturally in crop environments. Nematodes within agroecosystems provide numerous ecological services and economic benefits for pest and pathogen control. Predatory, entomogenous, and entomopathogenic nematodes (EPNs) and omnivorous nematodes consume insect pests, fungal and bacterial feeders control populations of fungal and bacteria pathogens of plants, as in the case of *Aphelenchus* spp. (Lagerlof et al., 2011), while plant-feeding nematodes can also affect weeds.

Entomogenous nematodes, i.e., nematodes associated (often parasitically) with insects, is a group of insect-killing nematodes. Some species are currently used for biological control or Integrated Pest Management (IPM). Entomopathogenic nematodes (EPNs) live parasitically inside the infected insect host, and so they are termed as *endoparasitic*. They carry bacteria that infect many different types of insects living in the soil, like the larvae of moths, butterflies, flies and beetles, as well as adult grasshoppers and crickets. EPNs have been found all over the world and in a range of ecologically diverse habitats. Nine families of nematodes (Allantonematidae, Diplogasteridae, Heterorhabditidae, Mermithidae, Neotylenchidae, Rhabditidae, Sphaerulariidae, Steinernematidae, and Tetradonematidae) include species that attack insects and kill or sterilize them, or alter their development. The most commonly studied entomopathogenic nematodes are those that can be used in the biological control of harmful insects: the members of Steinernematidae and Heterorhabditidae families. Entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae have proven to be the most effective as biological control agents (Kaya and Gaugler, 1993). They are soil-inhabiting organisms and can be used effectively to control soil-borne insect pests, but are generally not effective when applied to control insects in the leaf canopy. When considered as a group of nearly 30 species, each with its own suite of preferred hosts, entomopathogenic nematodes can be used to control a wide range of insect pests, including a variety of caterpillars, cutworms, crown borers, grubs, corn root worm, crane fly, thrips, fungus gnat and beetles. Dozens of different insect pests are susceptible to infection by entomopathogenic nematodes, yet no adverse effects have been shown against beneficial insects or other non-target animals in field studies (Georgis et al., 1991; Akhurst and Smith, 2002). In addition, EPN are often important for the potential control of alien insect species (Landi et al., 2009). Certain nematodes can also parasitize spiders, leeches, annelids, crustaceans and molluscs.

EPNs can be used as biological control agents to suppress a variety of economically important insect pests, especially in integrated pest management (IPM) and integrated production (IP) systems (Grewal, 2002; De Nardo and Grewal, 2003). The species that have been most studied in this context are those that have been introduced as biopesticides, while few data are available for native EPNs. Duncan et al. (2013) recorded a strong negative correlation between the density of native EPNs and the abundance of the root weevil pest of citrus, *Diaprepes abbreviatus*, highlighting that EPNs can have an important role in the control of the population of this insect pest. Indeed, when evaluating the potential of the use of chemicals integrated with biological control agents (e.g. EPNs), the International Organization of Biological Control (IOBC) developed a sophisticated approach based on a tiered hierarchy made up of threshold values for lethal and sublethal effects on non-target antagonists (Manachini, 2013). However some synergy of EPNs used together with chemical pesticides has been recorded. Control of larvae of *Diabrotica virginifera* was enhanced by such combination, resulting in a synergistic response and an increase in expected mortality of 24%; the combined effect of the insecticides plus EPNs was greater than either product applied on its own (Nishimatsu and Jackson, 1998). The mechanism for increased nematode efficacy when used together with chemical pesticides is not fully known, although it has been suggested that it could be due to a reduction in the host insect's immunity and activity even at sublethal dosages (Manachini, 2013). On the other hand, Campos-Herrera et al. (2008) have shown that natural EPN populations isolated from crop fields appeared less active against *Galleria mellonella* than those isolated from natural areas and field edges, suggesting that agronomic management could affect their natural activity, reducing their virulence and reproductive potential.

In order to support the long term performance of the functional role of soil fauna in control of pest and pathogens in agricultural soils, it is recommended to define the SPU as the abundance/biomass of species belonging to different functional groups. Nematodes can be allocated to functional groups to condense information efficiently and to determine their contribution to ecosystem processes, e.g. feeding groups as bacterivores, fungivores, plant feeders, vertebrate and invertebrate pathogens, carnivores or omnivores (see e.g. Bongers and Bongers, 1998). These functional groups could be used as indicators to interpret the nematodes' contribution to the ecosystem services 'pest and disease control'. Additionally, grouping according to the c-p scale (see section **Error! Reference source not found.**) could help to interpret changes in the community structure and to indicate a decrease in diversity and ecosystem stability.

Soil microorganisms

Agricultural systems host numerous microorganisms with the ability to control populations of pests and diseases (Persmark et al., 1995; Kasiamdari et al., 2002; Barrios 2007; Stewart et al. 2010). These are assumed to exert control under natural conditions, but also represent a resource that can be tapped for isolates for development of biological pest- and disease-control products. Microbial biocontrol agents include natural enemies and antagonists of pests and pathogens (Cook et al., 1996). Beneficial species include microbes that support plant growth through increasing nutrient availability and by outcompeting invading pathogens (Aislabie and Deslippe, 2013). Some microbes isolated from soil and other habitats have been developed into biocontrol agents and subsequently marketed as biocontrol products. Examples are antagonistic bacteria and fungi used against fungal plant diseases (Whipps and Gerhardson, 2007), pathogens of pest nematodes (Dong and Zhang, 2006; Wilson and Jackson, 2013), and entomopathogenic fungi and bacteria (Inglis et al., 2001). Species reported to act as biocontrol agents in compost-amended substrates include bacteria such as *Bacillus* spp., *Enterobacter* spp., *Flavobacterium balustinum* and *Pseudomonas* spp., and fungi such as *Penicillium* spp., *Gliocladium virens*, and several *Trichoderma* spp. (Litterick et al., 2004). These beneficial microorganisms can be released from the compost or the compost may provide nutrients that stimulate the proliferation of antagonistic bacteria and fungi in the rhizosphere (Noble and Coventry, 2005; Green et al., 2006). Four main mechanisms of suppression by the beneficial microbe of the pathogen have been described: competition for nutrients; antibiotic and enzyme production; parasitism and predation; and enhanced resistance to plant diseases (both induced systemic resistance and systemic acquired resistance). Several or all these different mechanisms of disease suppression may occur simultaneously through the activity of one or more beneficial microorganisms present in disease-

1981 suppressive soil or composts. For example, several different *Trichoderma* spp. can compete with
 1982 pathogens for nutrients and space, exhibit antibiosis, parasitize the pathogen and elicit induced plant
 1983 resistance. Fungi are the predominant pathogens of insects and play a significant role in the natural
 1984 regulation of soil-dwelling pests. Insecticidal toxins are produced by most entomopathogenic fungi
 1985 during pathogenesis. After successfully penetrating the insect cuticle, the fungi enter the haemocoel
 1986 where they have to overcome insect immune responses in order to colonize and to kill the host.
 1987 Generally, these toxins are bioactive secondary metabolites secreted during growth inside the insect.

1988 There is no doubt that soils harbour a high diversity of microorganisms that contribute to biological
 1989 population regulation and more specifically to natural control of pests and diseases. For practical
 1990 reasons, however, it is extremely difficult to estimate the total range of this control activity, and the
 1991 contribution of different microbial groups and species. For example, although there have been
 1992 estimates of the population densities in soil of the marketed biocontrol entomopathogens *Metarhizium*
 1993 spp. (Bidochka et al., 1998; Schneider et al., 2012) and *Bacillus thuringiensis* (Eskils and Lövgren,
 1994 1997; Guidi et al., 2011), very little is known regarding their effect on insect populations under natural
 1995 conditions.

1996

1997 The natural plant disease-control effect is for some diseases manifested as a general soil
 1998 suppressiveness and it has been known for a long time that certain soils are suppressive to specific
 1999 soil-borne plant pathogens (Bidochka et al., 1998; Weller et al., 2002; Termorshuizen AJ, Jeger MJ,
 2000 2008). It has been demonstrated that antagonistic micro-organisms play a decisive role for this
 2001 suppressiveness and for some diseases, the biological basis and contributing microbial groups have
 2002 been at least tentatively identified (Weller et al., 2002; Mendes et al., 2011).

2003 Organisms contributing to pest and disease control cannot be identified as a functional group, so this
 2004 ES should be identified at the (meta)population level (at relevant taxonomical level).

2005 Mycorrhizal fungi have also been reported among the groups of microorganisms showing antagonism
 2006 to pathogens, showing the potentials for use as biocontrol agents for soil-borne diseases. The role of
 2007 mycorrhizae in disease control has been observed both in arbuscular mycorrhizae and in
 2008 ectomycorrhizal associations. The most relevant aspects of ectomycorrhizae that make them efficient
 2009 for plant-disease control are: (i) efficient competition with the ubiquitous soil microflora, (ii) root
 2010 colonisation and mycorrhiza formation at rates faster than the pathogen invading the roots, (iii)
 2011 suppressive action against most pathogenic species. For arbuscular mycorrhizae, the possible
 2012 mechanisms involved in biocontrol are: i) enhanced plant nutrition, ii) biochemical changes in plant
 2013 tissues, iii) anatomical changes, iv) alleviation from stresses predisposing plants to disease, (v)
 2014 microbial changes in the rhizosphere (mycorrhizosphere), (vi) induced changes to the root-system
 2015 morphology, (vii) direct competition between the fungi and the pathogens for physical space or
 2016 resources, and (viii) induction of systemic resistance (Mukerji and Ciancio, 2007; Harrier and Watson,
 2017 2004).

2018 Regarding the entity to protect for these fungal groups, as mentioned above in section 5.3 (service of
 2019 nutrient cycling), the high range in trait expression within arbuscular mycorrhiza species, highly
 2020 dependent on the plant they establish the symbiosis with, and the high trait variability between
 2021 individuals in the case of ectomycorrhizae, as well as the lack of adequacy of the species concept for
 2022 this fungal group, makes it appropriate to base the risk assessment at the functional group level
 2023 (arbuscular mycorrhizal and ectomycorrhizal fungi).

Table 5: Key drivers for the ecosystem service pest control. Main taxa, examples of species, example of traits determining dispersal and exposure routes.

Key drivers	Main taxa	Main exposure routes	example species
Pest pathogens competitors and suppressors	Nematodes Microorganisms (e.g. fungi and bacteria)	Contact soil/contact soil pore water	Nematodes: Mermithidae Nematods: <i>Heterorhabditis</i> spp., <i>Steirnenema</i> spp. Fungi: <i>Bauveria bassiana</i> , <i>Metarhizium anisopliae</i> and <i>Isaria fumosorosea</i> Bacteria: <i>Bacillus thuringiensis</i> , <i>Pasteuria penetran</i>
Toxin dispersal antagonists	Lumbricidae	Oral soil Oral litter Contact soil	Anecic worms: <i>Lumbricus terrestris</i> , <i>Aporrectodea longa</i>
	Collembola	Oral litter/org. matter Contact soil	Collembolans: <i>Folsomia quadrioculata</i> , <i>Pseudosinella alba</i> , <i>Entomobrya multifasciata</i> , <i>Lepidocyrtus cyaneus</i>
Plant pathogens competitors and suppressors	Arbuscular and and Ecto-mycorrhizae, bacteria and fungi	Contact soil/contact soil pore water	

5.5. In-soil organisms as drivers of natural attenuation

Natural attenuation, also called intrinsic bioremediation, is an ecosystem service that helps keep the soil clean and suitable for, e.g., production of food and drinking water (National Research Council, 2000; Rittmann, 2004) and contributes to maintaining a healthy habitat for in-soil organisms, which support other ecosystem services (van Wijnen et al., 2012). The latest two definitions of natural attenuation are the following:

... “Reliance on natural attenuation processes (within the context of a carefully controlled and monitored site cleanup approach) to achieve site-specific remediation objectives within a time frame that is reasonable compared to that offered by more active methods. The natural attenuation processes that are at work in such a remediation approach include a variety of physical, chemical, and biological processes that, under favorable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater. These in situ processes include biodegradation; dispersion; dilution; sorption; volatilization; radioactive decay; and chemical or biological stabilization, transformation, or destruction of contaminants” (US EPA, 1999)

Three main types of process are involved in natural attenuation: (i) Transport processes including advection, dispersion, diffusion and sedimentation; (ii) Phase-transfer processes being responsible for movement between compartments such as sorption and volatilization; and (iii) Transformation processes being the only ones that effectively reduce the mass of contaminants whereas transport processes mainly affect concentration and exposure. Chemical alteration of contaminants may be due to both abiotic (e.g., dissolution, complexation, hydrolysis, precipitation, oxidation, and reduction) or biotic processes (e.g., biodegradation) (Ouvrard et al., 2013).

Soil fauna

Earthworms enhance soil aeration and moisture and contribute to improve its nutritional status via their activity as ecosystem engineers, within their sphere of influence (the drilosphere, including their burrows and casts). These earthworm activities are often limiting factors for soil microorganisms

responsible for the biodegradation of organic compounds (Hickman & Reid, 2008). In this way, earthworms can directly influence microbial communities playing a role in the natural attenuation of chemicals in soil. This has been shown for PAHs where the presence of earthworms (*E. fetida*, *E. andrei* and *L. rubellus*) enhanced the aerobic degradation of PAHs in soil (Ma et al. 1995; Eijsackers et al. 2001; Contreras-Ramos et al. 2006, 2008). This occurred mainly through their bioturbation activity (increasing soil aeration, facilitating the attack by microorganisms promoting aerobic degradation) but also *via* their ability to change the structure, biomass, and functioning of soil microbial communities (Sheehan et al., 2008; Natal da Luz et al., 2012), which may indirectly stimulate PAH biodegradation, predominantly dependent on microbial activity (Weissenfels et al. 1992; Shuttleworth and Cerniglia 1995). However, the role of earthworms on PAH degradation is not always evident since the rate of PAH biodegradation is highly variable and dependent not only on PAH structure but also on the intimate association with the soil matrix, determining bioavailability, and on the composition and activity of soil microbial communities (Shuttleworth and Cerniglia 1995).

The ability of earthworms to interact with microorganisms involved in pesticide degradation was shown by Liu et al. (2011) when the presence of *Aporrectodea caliginosa* stimulated an increase in activity and abundance of microbes degrading the herbicide MCPA, as well as an increase in the expression of *tfdA*-like genes (genes encoding oxygenases initiating aerobic MCPA degradation) on their burrows and on the 0-5cm soil. Increased herbicide mineralization catalyzed by earthworms has also been shown for Atrazine (e.g., Maharg, 1996), 2,4-D, Carbofuran and Metribuzin (Mallawatantri et al., 1996), and Isoproturon and Dicamba (Gevao et al., 2001). However, opposite results have been shown by other authors (see Hickman & Reid, 2008 for a more detailed analysis of the studies performed), especially in terms of compound sorption and release and the consequent effects on mineralization. Despite these contradictory results, there is evidence supporting the role of earthworms as agents of biodegradation of organic compounds, especially by creating favourable conditions for the activity of microbes responsible for their degradation. These types of interactions are to be assumed also for other groups of soil fauna enhancing microbial activity, e.g. microarthropods, even if no specific study on natural attenuation in terrestrial ecosystems by soil fauna other than earthworms is known.

Soil Microorganisms

Microbial degradation of chemical compounds in the environment is an important route for the removal of these compounds. The process of natural attenuation, i.e. processes driven naturally by indigenous soil microorganisms leading to dissipation through biological transformation, mainly depends on microorganisms that attack the pollutants enzymatically and convert them to innocuous products. The abundance of microorganisms and their high growth rates allow them to evolve quickly and to adapt rapidly to environmentally changing conditions (Diaz, 2004). Natural attenuation processes carried out by microorganisms can be divided into three general categories: 1) the target compound is used as a carbon source; 2) the target compound is enzymatically attacked but is not used as a carbon source (co-metabolism); and 3) the target compound is not metabolized at all but is taken up and concentrated within the organism (bioaccumulation). Although fungi participate in all three categories, they are often more proficient at co-metabolism and bioaccumulation than at using xenobiotics as sole carbon sources.

Bacterial activity is the major process involved in the hydrolysis of organic pollutants. Extracellular enzyme activity is a key step in degradation and utilization of organic polymers. Hydrolytic enzymes disrupt major chemical bonds in the toxic molecules, which may result in the reduction of their toxicity. This mechanism is effective for the biodegradation of oil spill, organophosphate, organochloride and carbamate pesticides, and especially performed by soil bacteria belonging to the genera *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Micrococcus* (Porto and Melgar, 2011). Mixed microbial communities are usually more efficient in biodegradation because the genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas.

Most filamentous fungi are unable totally to mineralize aromatic hydrocarbons; they only transform them into products of decreased toxicity and increased susceptibility to decomposition by bacteria, suggesting that the interaction among fungi and bacteria is profitable for petroleum-hydrocarbon mineralization (Joutey et al., 2013). For example, ligninolytic fungi, such as the white rot fungus *Phanaerochaete chrysosporium*, can degrade several xenobiotics such as pentachlorophenol and dioxin under co-metabolic conditions (Aislabie and Deslippe, 2013). However, fungi are also an important part of degrading microbiota because, like bacteria, they metabolize dissolved organic matter. Fungi are more efficient than bacteria especially in breaking down more recalcitrant material, like the natural polymeric compounds (e.g. lignin) thanks to extracellular, multi-enzyme complexes. They are also able to colonize and to penetrate substrates rapidly, and to transport and redistribute nutrients within their mycelium by means of their hyphal systems.

Both ectomycorrhizae and arbuscular mycorrhizae have shown the ability to detoxify toxic substances. It has been demonstrated that the process of remediation carried out by mycorrhizal fungi in conjunction with other microorganisms is usually faster and more efficient. Both the filamentous fungus, *Cunninghamella echinulata* and the bacterium, *Sphingomonas paucimobilis* have been used in conjunction with arbuscular mycorrhizae for the remediation of a soil polluted with petroleum hydrocarbon (Chibuikwe, 2013).

For natural attenuation to occur, microbes with the appropriate biodegradative ability must be present in sufficient numbers. This will depend in part on how long they have been exposed to the contaminant. The role of the community of indigenous microbiota is considered essential for an efficient natural attenuation of contaminated soils, because no single microbe has the metabolic potential to degrade all contaminants.

The reasons presented before to consider the “functional group” as the ecological entity to protect regarding both arbuscular mycorrhizal and ectomycorrhizal fungi for the ecosystem services “nutrient cycling” and “pest and disease control” are also valid for this ecosystem service “natural attenuation”.

Table 6: Key drivers for the ecosystem service natural attenuation. Main taxa, examples of species, example of traits determining dispersal and exposure routes.

Key drivers	Main taxa	Main exposure routes	example species
Soil non arthropod invertebrates	Lumbricidae	Oral soil Oral litter Contact soil and soil solution	Anecic worms: <i>Lumbricus terrestris</i> , <i>Aporrectodea longa</i> Endogeic worms. <i>Aporrectodea caliginosa</i>
Soil non arthropod invertebrates – small	Enchytraeidae	Oral soil Contact soil and soil solution	
Microfauna	Nematoda	Contact soil and soil solution	
Microorganisms	Bacteria	Contact soil/contact soil pore water	
	Mycorrhizal fungi	Contact soil/contact soil pore water	
	Fungi	Contact soil/contact soil pore water	

5.6. In-soil organisms as drivers of soil structure formation and water retention

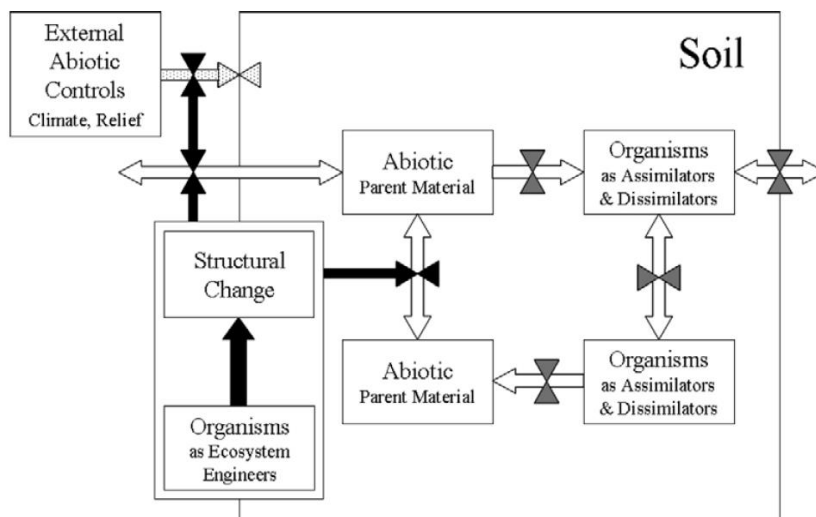
Soil structure consists of soil aggregates and the resulting pore spaces between them. One of the most relevant ecosystem services for the formation and stabilization of soil structure is soil aggregation (Rillig et al., 2015). The extent of aggregation, the stability of the soil aggregates and the pore space are considered important parameters when evaluating soil structure (Shukla, 2014). Soil structure is a key feature for plant productivity since: (i) it improves soil fertility, increasing agronomic productivity, enhancing porosity and decreasing erodibility; (ii) it can affect plant growth by influencing root distribution and the ability to take up water and nutrient; (iii) it enhances oxygen, water infiltration and water storage (Bronick and Lal, 2005).

Soil fauna

The importance of soil faunal activity for the formation of soil biophysical structures and for the development of soil horizons has been recognized since it was reported by Darwin (1881) in his book 'The formation of vegetable mould, through the action of worms, with observations on their habits'. There he stated that 'Worms have played a more important part in the history of the world than most persons would at first suppose. [...] The plough is one of the most ancient and most valuable of man's inventions; but long before he existed the land was in fact regularly ploughed, and still continues to be thus ploughed by earth-worms'. This was probably the first time that the concept of 'ecosystem engineers' was presented to the general public, even if the term was not defined until 1996. In the characterization by Jones and co-authors, ecosystem engineers 'directly or indirectly modulate the availability of resources (other than themselves) to other species by causing physical state changes in biotic or abiotic materials. In so doing, they modify, maintain, and/or create habitats' (Jones et al., 1996).

It is not only earthworms that act as ecosystem engineers in the soil compartment to modulate the availability of resources to other organisms or to modify their habitat. In fact, it is a feature of in-soil organisms in general to be strongly influenced by the characteristics of the medium soil around them, but in turn to be able to modify it to some extent according to their particular requirements – also termed 'niche construction' (Odling-Smee et al., 2003; Weigmann, 1998). Nevertheless, the outcome of the activity of soil micro- and mesofauna is not as remarkably visible at larger scales as the results of earthworms, ants or termites burrowing and 'engineering' the soil, but it has recently become a matter of attention (e.g. Maaß et al., 2015; Lehmann and Rillig 2015). The definition of ecosystem engineers is therefore not fully straightforward. Soil micro- and mesofauna are not able to burrow in the mineral soil horizons, and are largely confined to pre-existing voids in litter and surface soil horizons, especially if the soil is compacted (Lee and Foster, 1991; Roithmeier and Pieper, 2009).

The processes that can be allocated to in-soil organisms changing the physical environment around them are illustrated in the figure below by Jones et al. (2006), pointing at two fundamental pathways highly interrelated: assimilation/dissimilation (uptake, metabolism) and physical ecosystem engineering. The authors conclude that 'soils and sediments are probably the most highly physically engineered of all environments'.



Very comprehensive review papers have been published on the role and importance of soil organisms for the shaping of the soil physical environment and for processes of soil-horizon formation and hydrological properties. Please refer for details on the reported studies to the work of Lavelle (2002) and Lavelle et al., (2006), Bottinelli et al., (2015), Berke (2010), Blouin et al., (2013) and Bertrand et al., (2015). Some general patterns will nevertheless be outlined below.

It is important to note that the in-soil organisms' community shapes its environment at different spatial scales (Anderson, 2000; Lavelle, 2002), according directly to their action range and indirectly to the integrated structure/process on the next level. Moving up the hierarchy, successive levels might concern the same processes, but with slower dynamics and covering larger areas (Anderson 1988).

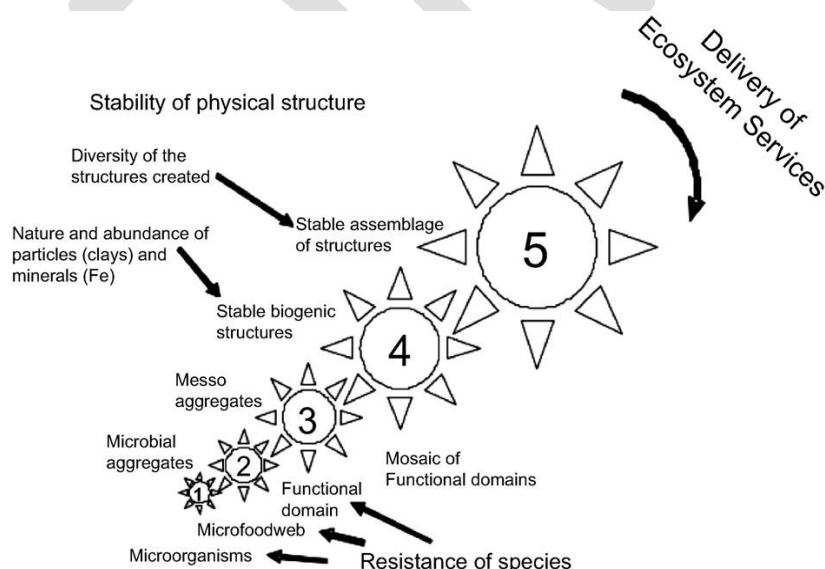


Figure 5: Self organizing systems in soils at different scales from microbial biofilms to the landscape. The stability of delivery of ecosystem services at scales >5 is supported by the resistance of species to disturbances and/or the stability of physical structures (from Lavelle et al., 2006).

2202 The smallest scale relates to effects of microbial biofilms on microaggregates or microtubules by
2203 fungal hyphae (see chapter below for more detail about soil microorganisms), followed by micro and
2204 mesofauna. The effects of small invertebrates, like enchytraeids or microarthropods, producing
2205 microgranular pellets structures in the upper soil centimetres are well documented, as are the effects of
2206 the three major groups of soil ecosystem engineers (scale 3) – ants, termites and earthworms. Large
2207 diversity of biogenic structures will provide the 4th scale, while scale 5 comprises effects of soil
2208 invertebrate engineers at the landscape scale, which have also been described (Jones et al., 2012).
2209 Table 5 provides a summary of the contribution of soil fauna to ecosystem services related to the
2210 physical soil structures.

Table 7: Contributions of soil invertebrates to the provision of ecosystem goods and services by soils (after Lavelle, 2002, modified).

Service types	Goods/ services	Ecosystem process	Invertebrate contribution	Indicator of soil fauna contribution
Production	Water supply	Infiltration and storage of water in soil pore systems	Building and maintenance of stable porosity through bioturbation and burrowing	Proportion and arrangement of biogenic structures in soil Water-holding capacity Water infiltration dynamics
Support	Soil formation	Pedogenesis	Bioturbation Surface deposition Particle selection	Development of horizons DNA and Near Infrared Spectroscopy analyses in biogenic structures
Regulation	Flood and erosion control	Regulation of water runoff Infiltration and storage of water in soil	Creation of surface roughness by biogenic structures Building and maintenance of stable porosity through bioturbation and burrowing	Production of biogenic structures Soil and humus morphology

Of particular importance in agricultural fields is the ecosystem service ‘soil formation and water retention’ since it addresses issues of solute transports in soils and possibly the movement of PPP in the soil profile. Soil fauna has been reported to increase the hydraulic conductivity of soils, to increase preferential flow but also to reduce run-off and to increase soil porosity (e.g. Bailey et al. 2015; Friend & Chan 1995, Shipitalo et al. 1994, Pieper et al. 2008, Laine-Kaulio, et al. 2015). The activity of soil fauna affects the formation and permanence of hydrophobic patches in soil that promote preferential flows. Earthworms have been proposed as a means of reclaiming heavily hydrophobic soils (Ritsema and Dekker, 2000; Mueller and Deurer, 2011).

It is important to discriminate between the actions of different functional groups of soil fauna when characterizing their effects and evaluating studies with contrasting outcome. Without prejudice to the difficulties of defining ‘true’ functional groups, behavioural and feeding traits of species ‘groups’ determine the structures impacted and/or created in soils. Anecic worms do burrow vertically in soils and live in almost permanent burrows even open to the soil surface, facilitating direct solute transport to deeper soil layers and reducing run-off events. Earthworms living mostly in the upper 15 cm of the soil, so called endogeics, refill the horizontal burrows they have channelled with the soil they have processed and therefore increase the water-holding capacity of soils and prevent the formation of stable hydrophobic patches. Even if not very well studied, the impact of soil micro and mesofauna on soil structures is deemed to resemble at smaller scales the activity of endogeic worms (e.g. Van Vliet et al. 1998).

Collembola contribute to soil aggregation. The soil in the hyphal compartment shows greater soil aggregation with larger mean weight diameter when Collembola are present, and a similar result was found in the presence of arbuscular mycorrhizal fungi compared to control treatments. Moreover, the combined presence of both arbuscular mycorrhizal fungi and Collembola results in a non-additive increase of soil aggregation. The study by Siddiky et al. (2012) clearly indicates that Collembola can enhance soil aggregation and can partially complement effects of arbuscular mycorrhizal fungi, and that these effects are independent of roots.

Degraded soil structure in agricultural soils might result in soil compaction, defined as a process that rearranges soil grains, resulting in decreased void space and increased bulk density (Soil Science

Society of America 2008). Compaction accounts for around 68 million hectares of degraded soil worldwide (Flowers and Lal, 1998). Compaction might result from e.g. intensive farming with heavy machinery, but also from animal grazing (Ferrero and Lipiec, 2000; Gayle et al., 2005). Amongst others, compacted soils are characterized by increased soil strength and less interconnected pores (Schjønning et al., 1998; Hamza and Anderson, 2005), by reduced soil aeration and drainage capacity (Larink et al. 2001). Plants suffer from hampered rooting ability and spatial access to nutrients and water (Dannowski 1993, Larink and Schrader 2003; Kuchenbuch and Ingram, 2004) and soil fauna is faced with the deterioration of living conditions through the loss of habitable space, with increased injuries and possible death (Wiermann et al., 1997; Larink and Schrader, 2003; Larink and Schrader, 1999; Brussard and van Fassen 1994; Horn et al., 1994). It has been reported that the abundance and activity of collembolan and enchytraeids negatively correlates in the field with increased soil bulk densities, probably due to a reduction in coarse pores as habitable pore space (Schrader and Zhang, 1997; Heisler and Kaiser, 1995; Langmaack et al., 1999; Dittmer and Schrader, 2000; Larsen et al., 2004).

As a feature of the ecosystem service ‘soil-structure formation’ by soil animals, burrowing soil fauna may however loosen compacted soil through bioturbation, e.g. by earthworms (Barros et al. 2001, Larink et al. 2001, Schrader et al. 2001). Also soil mesofauna as enchytraeidae are known to improve pore structure and connectivity (Didden, 1990; van Vliet et al., 1998), positively affecting gas exchange, water conductivity, and plant root growth through the soil (Schrader et al., 1995; Van Vliet et al. 1998; Larink and Schrader 2003).

Even if, ‘extrapolations of faunal activity detected at the microhabitat scale to the level of watershed without an explicit consideration of the multiplicity of structures may facilitate a conclusion of the functional redundancy of soil fauna’ (Heneghan and Bolger 1999), and considering soil micro- and mesofauna to have a scarcity of ‘ecosystem engineers’, the ecological entity holding different traits in terms of soil formation is the functional group. The attribute we are protecting – and in this case also measuring – is the abundance/biomass of species belonging to different functional groups. The difficulties in defining functional groups originate from the scarce knowledge in attributing specific function to species of microarthropods (compared to e.g. earthworm species). Nevertheless, also microarthropods have different traits regarding their role in soil processes.

Regarding non-arthropod invertebrates belonging to the ecosystem engineers, the ecosystem service ‘soil formation’ including retention function is provided by anecics (= vertical burrowers) and endogeic (= burrowing in the soil matrix) and epigeic (= dwelling in the organic matter at the surface) earthworm functional groups. It is however questionable, if the ecological entity to be protected should be defined as a functional group. Having often under field conditions in agricultural landscapes only single to few species belonging to e.g. anecic groups, the entities to be protected would be the populations of these earthworm species. Species diversity holds a key role for the long-term performance of functional groups in strongly disturbed agricultural soils. In these environments, the defining SPU solely at the level of functional groups might lead under unfavourable conditions to a loss of function performance. Species loss within a functional group will lead to the erosion of trait diversity and to a reduced resilience and stability under changing environmental conditions. In order to support the long-term performance of the functional role of soil fauna in soil structure formation, it is therefore recommended to define the SPU as the the abundance/biomass of species belonging to different functional groups.

Soil microorganisms

Microbial communities can be considered as architects of soils. Soil aggregation influences virtually all nutrient cycling processes and soil biota. Microorganisms are key organisms in aggregate stabilization and in decomposition of plant litter. Both fungi and bacteria contribute to stabilization of soil aggregates through deposition of extracellular polysaccharides binding soil particles and formation of degraded, aromatic humic materials (Umer and Rajab, 2012). The formation of humic substances by soil microorganism is catalysed by microbial exo-enzymes (Guggenberger, 2005).

2293 Fungal hyphae improve aggregate stability by reorientation of clay particles, binding particles with
2294 extracellular polysaccharides, and enmeshing particles. Hyphae also enmesh microaggregates to form
2295 macroaggregates suggesting that aggregation increases with hyphal density.

2296 Within the huge variety of soil microorganisms, mycorrhizal fungi play a crucial role in the formation
2297 and maintenance of soil aggregates and are considered one of the most important biotic factors
2298 influencing soil aggregation. A substantial contribution to soil aggregation and stability has been
2299 demonstrated for arbuscular mycorrhizae while the contribution of ectomycorrhizal fungi has not been
2300 comprehensively investigated yet (Graf and Frei, 2013). Arbuscular mycorrhizal fungi appear to be
2301 the most important mediators of soil aggregation for three reasons: (i) the extraradical hyphae of
2302 arbuscular mycorrhizal fungi represent a substantial component of soil microbial biomass, making up
2303 to 50% of fungal mycelia in soil (Gianinazzi et al., 2010); (ii) they are independent of the limiting
2304 carbon supply in bulk soil on which saprophytic fungi depend, by directly tapping into carbon
2305 resources of the plant; (iii) their hyphae appear to have a longer residence time in soil, allowing for a
2306 less transient contribution to soil-aggregate stabilization than saprobic hyphae. Additionally, these
2307 fungi presumably act as a long-term soil-binding agent through the production of a stable putative
2308 glycoprotein, called glomalin. Glomalin is present in soils at high concentrations and is an important
2309 factor in stabilizing aggregates and its concentration in aggregates (Wright and Upadhyaya, 1998) and
2310 soil (Rillig et al., 2001) correlates with the percentage of water-stable aggregates Rillig et al. (2002)
2311 reported a much stronger effect of glomalin on soil aggregation than the direct effect of arbuscular
2312 mycorrhizal fungi hyphae themselves, suggesting the high importance of the protein in hypha-
2313 mediated mechanism of soil aggregate stabilization. Thus, the extensive networks of arbuscular
2314 mycorrhizal fungal hyphae play important roles in soil physical processes, particularly with regard to
2315 macroaggregates (>250 µm), while glomalin is tightly correlated with soil aggregate stability (Peng et
2316 al., 2013).

2317 Hyphal networks of arbuscular mycorrhizae have an impact on the soil structure and plant-community
2318 composition and are therefore important below-ground carbon sinks (Soka and Ritchie, 2014). Wilson
2319 et al. (2009) observed that a loss of hyphal abundance of arbuscular mycorrhizae led to a concomitant
2320 cost in soil aggregation for which no other processes compensated. Similarly, increases in soil hyphae
2321 of arbuscular mycorrhizal were correlated with an increased proportion of macroaggregates.
2322 Disturbance can affect the occurrence of arbuscular mycorrhizal fungi in both agricultural and natural
2323 ecosystems in several ways. First, it may change the physical, chemical or biological environment of
2324 soil leading to either direct effects on arbuscular mycorrhizal fungi or indirect effects operating *via*
2325 effects of disturbance on plant growth. Changes in symbiotic activity may be critical because a
2326 reduction in extramatrical mycorrhizal hyphae networks is likely to impact soil structure, soil C and N
2327 storage, and soil food webs. Wilson et al. (2009) showed a highly significant linear correlation
2328 between hyphal abundance of arbuscular mycorrhizal fungi and soil aggregation, and C and N
2329 sequestration with an experimental field study, involving long-term diverse management practices of
2330 native multispecies prairie communities. This suggested serious consequences to the loss of arbuscular
2331 mycorrhizal fungi from ecosystems.

2332 By forming a complex intra- and extra-radicular mycelial network arbuscular mycorrhizae confer a
2333 higher plant/soil adherence and contribute to soil stabilization (e.g., Turrini and Giovannetti, 2012). In
2334 fact, together with the release of cementing agents, the ability to form an extra radicular mycelium and
2335 the ability to render surfaces hydrophobic, are among the most important arbuscular mycorrhizal traits
2336 contributing to soil stabilization (Rillig et al., 2015). Long-term field experiments revealed arbuscular
2337 mycorrhizal fungi abundance as the key factor explaining soil aggregation (Wilson et al., 2009).

2338 In terms of the ecological entity to protect for this fungal group for this service, the risk assessment
2339 should be based on the functional group “arbuscular mycorrhizal fungi”. This can be justified by the
2340 reasoning presented above for the other services. Moreover, Rillig et al. (2015) advocates the use of a
2341 trait-based approach to assess and understand the role of arbuscular mycorrhizae in soil stabilization,
2342 which favours the adoption of “functional group” as ecological entity to protect.

Table 8: Key drivers for the ecosystem service soil structure formation. Main taxa, examples of species, example of traits determining dispersal and exposure routes. For underlined species, test protocols are available

Key drivers	Main taxa	Main exposure routes	example species
Macropores creators	Lumbricidae	Oral soil Oral litter Contact soil	Anecic worms: <i>Lumbricus terrestris</i> , <i>Aporrectodea longa</i>
Soil mixers	Lumbricidae	Oral soil Contact soil	Endogeic worms <i>Aporrectodea caliginosa</i>
Litter / organic matter fragmenters	Oniscidae	Oral litter	Isopods: <i>Porcellio scaber</i>
	Lumbricidae	Oral soil Oral litter	Anecic worms: <i>Lumbricus terrestris</i> , <i>Aporrectodea longa</i>
	Collembola	Oral litter/org. matter Contact soil	<i>Folsomia quadrioculata</i> <i>Pseudosinella alba</i> , <i>Entomobrya multifasciata</i> , <i>Lepidocyrtus cyaneus</i>
	Oribatida	Oral litter /org. matter Contact soil	Oribatid mites: <i>Oppeia nova</i> , <i>Tectocephus velatus</i> , <i>Punctoribates punctum</i> , <i>Scheloribates laevigatus</i>
Aggregates stabilizers	Fungi and bacteria	Contact soil/Contact soil pore water	
Glomalin producers	Arbuscular mycorrhizal fungi		

5.7. In-soil organisms as drivers of food web support

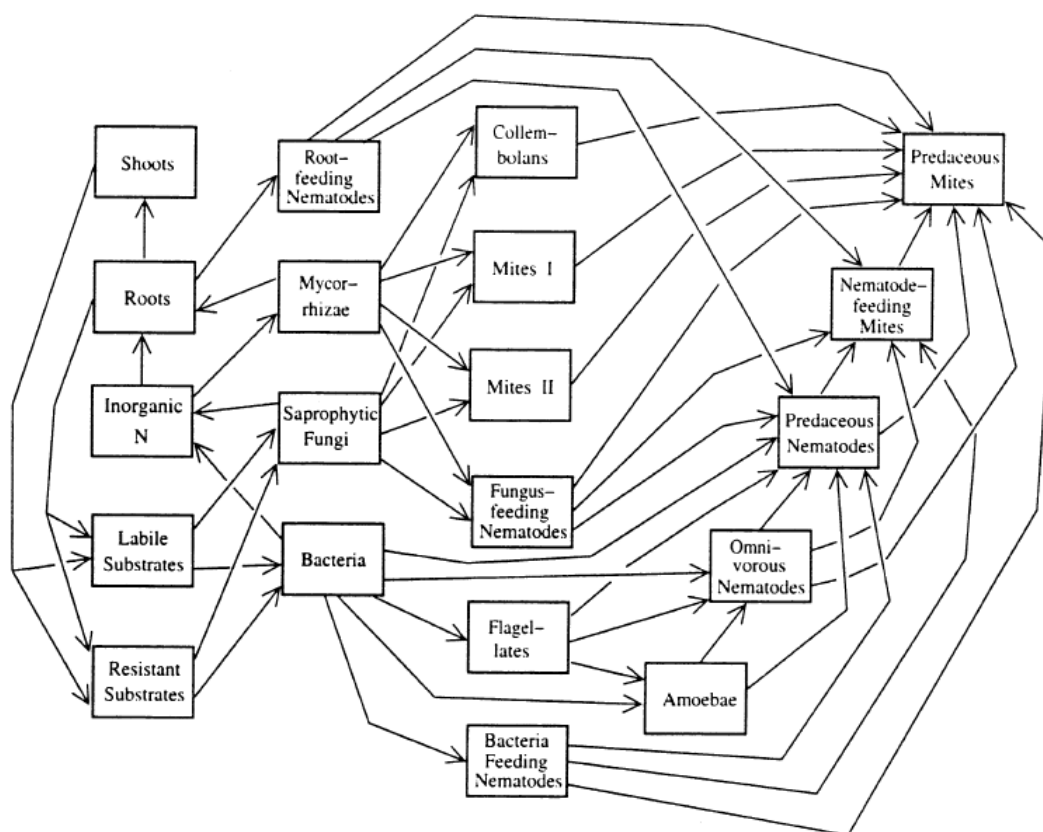
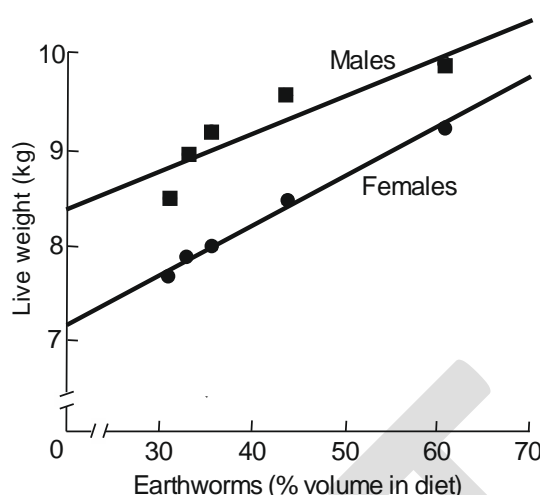


Figure 6: Soil food web (Hunt and Wall, 2002)

Soil fauna

Although the overall importance of the in-soil invertebrate fauna to terrestrial food chains is rather poorly recognized, there are several species and food-chain links that have been relatively well studied. Most of them show unequivocally that at least certain in-soil invertebrate species are of crucial importance for supporting terrestrial food chains. Among the best recognized links between the soil environment and terrestrial fauna are those mammals and birds for whom earthworms constitute an important food resource. The prime and long-known example is the Eurasian badger (*Meles meles*) with up to ca. 90% of food biomass consumed in the spring made up of earthworms (Goszczynski et al., 2000). The authors noted also an important difference between pristine forest habitats and farmlands. In forests, earthworms constituted on average 62% of the badger diet, while in farmlands and pastures earthworms and plant material were equally important (34% each). These differences are probably not without an effect on badgers: Kruuk and Parish (1985) found that badger body weight is positively correlated with earthworm consumption and suggested that long-term differences in earthworm availability affect badger population size. During their studies the average badger biomass decreased gradually between 1978 and 1981, by 14% in males and 17% in females, and the trend was strongly correlated with the proportion of earthworms in the diet (see figure 7). The authors hypothesised that the observed decrease in earthworm population size and availability to badgers resulted from changes in pasture management. Unfortunately, they did not study whether it was related to the use of any plant protection products. Nevertheless, these data clearly show the importance of earthworms for maintaining healthy populations of badgers.

2374

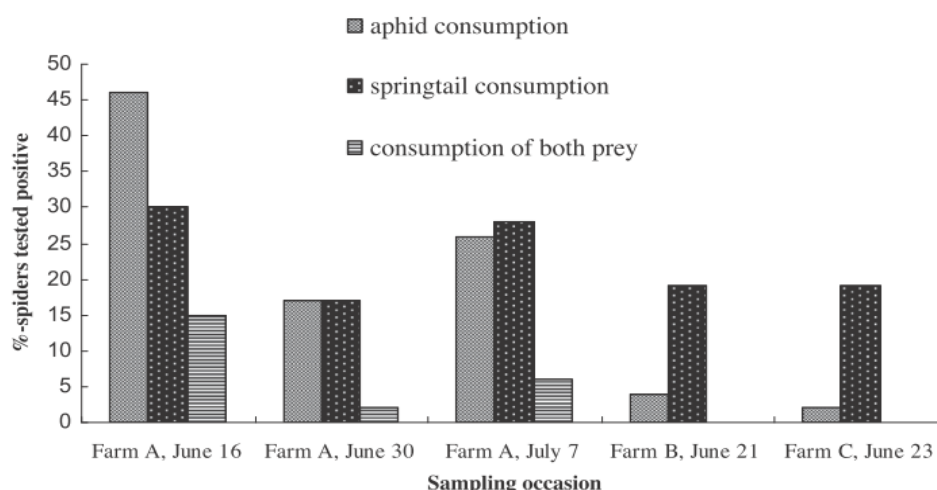


2375

2376 **Figure 7:** Relation between relative volume of earthworms in badger diet and the live weight of
 2377 badgers during March-June: males: $r=0.89$, $P < 0.05$; females: $r=0.99$, $P < 0.001$.
 2378 Redrawn after Kruuk and Paris (1985).

2379 Earthworm- availability was also the main factor besides altitude determining the spatial distribution
 2380 of Eurasian Woodcock (*Scolopax rusticola*) at the landscape scale in Cantabrian Mountains in Spain
 2381 (Brana et al., 2013). Indeed, according to Hoodless and Hirons (2007), earthworms can provide 50-
 2382 80% of food biomass for this species.

2383 While earthworms represent an important food resource for birds and mammals, many smaller in-soil
 2384 invertebrates support a number of ground dwelling and aboveground invertebrate predators. For
 2385 example, *Pardosa* spiders, which predate on both above-ground (e.g., Diptera) and small soil
 2386 invertebrates, seem to prefer aphids when they are abundant but springtails prevail at low aphid
 2387 densities (Kuusk and Ekbom, 2010) (see figure 8). The authors conclude that the abundance of
 2388 springtails may help in maintaining spider populations and, in consequence, enhance spider predation
 2389 on pests.



2390

2391 **Figure 8:** Percentage of *Pardosa* spiders collected in spring-sown cereals that tested positive for
 2392 aphid consumption, springtail consumption, or consumption of both prey by PCR
 2393 (Polymerase Chain Reaction)-based gut-content analysis. From Kuusk & Ekbom (2010).

2394

Springtails are also the most numerous and important prey of epigeal linyphid spiders. Even if the spiders consume also substantial quantities of other prey, with aphids as the main component, Romero and Harwood (2010) noted that a diet consisting of aphids alone is not sufficient for spider development. Similarly to Kuusk & Ekbom (2010), the authors stress the importance of spiders in controlling pest populations, especially aphids. At the same time, they found that spiders were severely prey limited and hypothesized that this is due to the impact of adverse climate conditions and agricultural practices on prey. If the latter is the case, the study by Romero & Harwood (2010) provides an important link between protection goals for in-soil invertebrates and non-target, above-ground arthropods (NTA). In the recently published “Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods” (EFSA PPR Panel, 2015a), the crucial importance of landscape structure for the maintenance of NTA biodiversity has been stressed. The work by Romero & Harwood (2010) indicates that landscape structure may be of crucial importance in maintaining the important ecosystem function that small and less mobile soil invertebrates have in supporting higher trophic levels in a food chain.

A recent extensive study across four European countries, Sweden, UK, Czech Republic and Greece, showed that increasing land-use intensity negatively affects species richness of earthworms, springtails and oribatid mites and reduces mean body mass of soil fauna (Tsiafouli et al., 2015). A comparison of invertebrate prey abundance for birds between organic and conventional arable farms showed that earthworm abundance was 2 – 4 times higher on the former than on the latter (Kragten et al., 2011). Having in mind the trophic relationships described briefly above, this can also mean negative changes at higher trophic levels, including populations of important invertebrate and vertebrate carnivores.

Many birds, mammals, reptiles and insects are known to include gastropods in their diet, but there is a lack of studies about the ecological significance of malacophagy (Barker, 2004). As reviewed by Nyffeler and Symondson (2001), it has been shown for around 20 genera of spiders that they include gastropods in their diet. For harvestmen, gastropods are a particularly significant component in their diet, and some genera, mainly *Trogulus* and *Anelasmoecephalus*, specialize in these prey. Their diet includes larger snails (e.g. family Helicidae) but also smaller sized species (e.g. families Vallonidae, Cochlicopidae, Pupillidae, Enidae, Endontonidae, see Nyffeler and Symondson (2001)). Carabid beetles of the tribe Cychrini, of which in Europe only the genus *Cychrus* occurs (Freude et al. 2005), principally feed on snails and slugs (Larochelle, 1972), and some species of the genus *Carabini* have specialized in eating snails and slugs (Freude et al., 2005).

Snail shells might be a vital calcium source for organisms at higher trophic levels. It has been shown for birds on poor soils in Dutch forests that a decline of snail densities resulted in eggshell defects in the great tit *Parus major* caused by calcium deficiency (Graveland and Wal 1996). Graveland and Van Gijzen (1994) showed that forest passerines need to ingest calcium-rich items, e.g. snail shells, additionally to their normal food to satisfy their calcium requirements. It cannot be excluded that birds occurring in the agricultural landscape also require snails as a source of lime, but this topic requires further study.

Nematodes are important food sources for several species of other nematodes or soil microarthropods (Huhta et al., 1998), and opportunistic scavenging of dead invertebrates is common. Isotomid, entomobryid, tomocerid, and hypogastrurid collembolans have been found to correlate negatively with wood-decomposition rate, suggesting that direct feeding on fungi by these organisms may contribute to this negative relationship. Collembola form a significant proportion of the diets of ground-dwelling spiders, ground beetles (Carabidae), rove beetles (Staphylinidae), mesostigmatid mites, ants, diplurans, and other predacious arthropods (Hopkin, 1997; McBrayer and Reichle, 1971). Data on their importance as food supply for vertebrates are not available but, due to the small size of nematodes, they are probably negligible as direct food for larger animals.

The positions and roles of soil animals in the terrestrial food webs are extremely diverse. The ecological entity to be protected should be attributed therefore to functional groups in terms of position in the food web, but the functional groups are yet to be defined. From the point of view of

present knowledge on food-web relationships, as described above, the following groups may be identified as crucial prey for different groups of animals: annelids, nematodes, gastropods, and arthropods. It should be also noted that the food-web relationships may be complex and multi-level, so that specific functional/taxonomic groups serve as food to other in-soil invertebrates which, in turn, are preyed upon by other animals, representing domains other than soil.

Soil microorganisms

Biotic interactions within soil food webs, can be both bottom-up and top-down. Bottom-up interactions can be based on, for example, fresh root material for plant feeders, dead roots, root exudates and litter in the case of primary decomposers or prey in the case of secondary decomposers and predators. Top-down effects are mainly driven by predation (Turbé et al., 2010). Top-down regulation at higher trophic levels may appear marginal, considering that microbial biomass comprises the majority of the total biomass in soil. However, bottom-up interactions can be mainly ascribed to the prokaryotes and fungi (Mulder et al., 2005).

Prokaryotes, fungi and protozoa are considered key players in the soil food web, being at the bottom of the detritus food web. Thus, they are mainly eaten by all the other in-soil organisms including nematodes and protozoans. For example, the diet of earthworms mainly consists of organic material in various stages of decay. Dead plant tissue comprises the bulk of the organic matter consumed, but living microorganisms, nematodes and other microfauna, mesofauna and their dead remains are also ingested. It has been reported that earthworms are usually food generalists with a preference for dark pigmented fungi (Dematiacea), which often comprise up to 60% of fungal isolates from soil and are generally of high food quality. In addition to fungi, protozoa and algae may constitute a significant part of the diet of earthworms.

Most oribatid mite species appear to feed preferentially on certain genera of dark pigmented fungi, such as *Alternaria* and *Ulocladium*, but the food spectrum varies among oribatid mite species. Oribatid mites have also shown feeding preference for ectomycorrhizal fungi (Schneider et al., 2005). It has been reported, however, that some species can also be bacteria feeders, detritivorous species and some of them feed on carrion (Behan-Pelletier, 1999).

Nematodes can be classified according to their feeding behaviour in either bacterial, fungal or protozoan feeders. Like nematodes, protozoans can also be classified according to their feeding behaviour in bacterial or fungal feeders. Collembola have also been considered as generalist in their feeding behaviour feeding both on fungi and bacteria.

As presented above, soil microorganisms make up a taxonomically diverse yet single functional group in terms of food-web support. Even if some organisms show preferential feeding on certain groups of microorganisms, none is fully selective and generalists prevail. Hence, for SPGs, soil microorganisms should be regarded as one functional group, indispensable to support viable populations of a range of in-soil organisms.

Table 9: Key drivers for the ecosystem service food web support. Main taxa, examples of species, example of traits determining dispersal and exposure routes.

Key drivers	Examples of taxa	Main exposure routes	Dispersal
			‘slow’ ‘fast’
Soil non arthropod invertebrates	Lumbricidae	Oral soil Oral litter Contact soil	Anecic worms: <i>Lumbricus terrestris</i> , <i>Aporrectodea longa</i> Endogeic worms. <i>Aporrectodea caliginosa</i>
Soil non arthropod invertebrates – small	Enchytraeidae	Oral soil Contact soil	
Litter macroarthropods	Isopoda		
Litter microarthropods	Collembola	Oral litter/org. matter Contact soil	Collembolans: <i>Folsomia quadrioculata</i> <i>Pseudosinella alba</i> , <i>Entomobrya multifasciata</i> , <i>Lepidocyrtus cyaneus</i>
	Oribatida s	Oral litter /org. matter Contact soil	Oribatid mites: <i>Oppiella nova</i> , <i>Tectocephus velatus</i> , <i>Punctoribates punctum</i> , <i>Scheloribates laevigatus</i>
Microfauna	Nematoda	Contact soil	
Decomposers	Bacteria		
	Fungi including Mycorrhizal fungi		
Grazers	Protozoan		

6. SPECIFIC PROTECTION GOAL OPTIONS FOR IN-SOIL ORGANISMS IN AGRICULTURAL LANDSCAPES

6.1. Spatial scale in ERA for in-soil organisms

The spatial scale considered in soil ERA cannot be divorced from a temporal scale. Given the slow rate of movement of many in-soil organisms, external recovery (by immigration) is slow and therefore consideration of long-term effects requires a large spatial scale covering multiple habitats. However, space may be important at small scales too since internal recovery *via* dispersal from more suitable refugia (microhabitats not affected by the stressor) towards impacted areas after the reduction of the stressor can occur. At the local in-field scale, the landscape may be described by a scale at which heterogeneity of one or more factors important for determining the population health of the organisms being assessed is considered (e.g. moisture, soil type, soil compaction, toxic chemicals). Between these two scales in both space and time we may need to consider boundary conditions between in-field and off-field. As a result, we need three definitions of spatial scale with appropriate time scales:

1) Field scale (in- and off-field) – a local scale expressing heterogeneity of one or more habitat factors at a scale that determines local differentiation in population densities. Processes occurring over short to medium time scales of less than one season.

2) Field-boundary scale – expressing variation between in-field and off-field habitat conditions and a time scale representing external recovery from off-field habitats to in-field to occur. Processes occur over a timescale of more than one season.

3) Landscape scale – representing habitat diversity at regional scales and capturing the spatio-temporal dynamics of in-soil organisms as they are affected by management and colonise or recolonise areas over long time periods. Processes occurring over a timescale of many years.

Solid data on effects of landscape structure on in-soil organisms are next to non-existent, yet some conclusions about its importance for species richness can be drawn from the few studies on organisms that at least partly can be considered “in-soil organisms”. One such group is the ground beetles (Carabidae) in which at least three stages (eggs, larvae and pupae) live in soil. This is also one of the most widely studied groups of terrestrial invertebrates, offering some insight into factors determining their abundance and diversity in different environments. For example, Trichard et al. (2013) showed that landscape structure explained four times more of the total variation in the abundance of granivorous carabids than local agricultural practices. Probably the most extensively studied group among all soil invertebrates is earthworms and there are some, albeit rather limited, data on landscape effects on species richness and biomass of this group. Vanbergen et al. (2007) showed that earthworm abundance and species richness were positively correlated with landscape-scale habitat richness, and Hof and Bright (2010) found that the presence of grassy field margins increases the abundance of earthworms (and carabids). Similarly, in the study by Nuutinen et al. (2011), field margins appeared crucial for colonisation of cultivated fields by the earthworm *Lumbricus terrestris*. The authors stressed the general importance of field margins in the dispersal ecology of earthworms in arable landscapes in the long term. It has to be mentioned, however, that at least one study showed that under certain circumstances the earthworm biomass is actually higher in cultivated land than in small non-cultivated areas in Northern Europe. Lagerlof et al. (2002) found that when soil cultivation is moderate and organic matter is added, large populations of most earthworm species can be found in cultivated fields. Nevertheless, they also found that some epigeic species, such as *Lumbricus rubellus* and *L. castaneus*, are more abundant in non-cultivated areas from where they can migrate to the fields in the long term. The authors stressed that their findings are restricted to fields with moderate cultivation intensity and that, if heavy cultivation is used with soil compaction and intensive pesticide use, non-cultivated areas are crucial for preserving earthworm populations in agricultural landscapes.

Yet another aspect worth taking into account in landscape-level risk assessment is the importance of soil invertebrates for terrestrial food chains. Kauhala and Ihalainen (2014) proved that the higher the landscape diversity the higher was the diversity of diet of the badger (*Meles meles*) and the raccoon dog (*Nyctereutes procyonoides*). It is generally accepted that high diet diversity is beneficial for animals, so the study indirectly shows that maintaining diverse landscapes promotes healthy populations of mammal omnivores by securing a diverse diet. For more information on food-chain effects see section 5.7 of this opinion.

Considering the time-scales and biological processes related to the dispersal of the majority of in-soil organisms compared to terrestrial non-target arthropods living above soil, the Panel proposes that in-soil environmental risk assessments are made at local (1) and field boundary (2) levels only. For NTAs, the primary justification for making assessments at a landscape scale was the need to take ‘action at a distance’ into account (see Topping et al., 2015, EFSA PPR Panel, 2015a). This effect will occur when spatial-dynamic processes operate over a timescale whereby depletion of source populations can occur. In the case of most in-soil organisms, the rate of internal recovery (driven by reproduction) is likely to be much higher than any change in local density driven by dispersal (see section 3.2.1 and 3.2.2). As a result, ‘action at a distance’ is not expected to be important for most in-soil organisms, and thus landscape-level assessment is not needed. In addition, timescales for

landscape-level processes are long since the dynamics considered at that scale are related to recovery following long-term agricultural use of soils. Nevertheless, assessment of year on year effects on in-soil organisms is considered important, since small effects from exposure to PPPs in the field within one season might accumulate over multiple years of PPPs use (Liess et al., 2013).

The proposed different options for Specific Protection Goals for in-soil organisms in agricultural landscape are derived by combining the knowledge summarized in the sections above regarding the determined key drivers with their pertinent ecological entities (Service Providing Units or SPU according to EFSA Scientific Committee, 2015a) and the traits of the SPU in terms of their recovery and dispersal potential (see section **Error! Reference source not found.** above). These data are integrated to derive a magnitude of effects by intended PPP use that might be acceptable without compromising the delivery of the ecosystem services of interest.

In the trade-off between crop production and protection of biodiversity and ecosystem services, the Panel might propose a higher level of effects on diversity in-field than off-field. In doing this, the Panel acknowledge that in-field crop protection might be ‘rated’ higher in term of provisioning service than biodiversity and other ecosystem services. This is not the case for off-field SPG Options.

As discussed in the sections above, species diversity plays a key role for the long term performance of functional groups in strongly disturbed agricultural soils. When identifying the ‘functional group’ as SPU for the maintenance of certain ecosystem services, it should be kept in mind that the definition of SPU solely at the level of functional groups might lead under unfavourable conditions to a loss of function performance. Species loss within a functional group will lead to the erosion of trait diversity and to a reduced resilience and stability under changing environmental conditions. In order to support the long-term performance of the functional role of in-soil organisms in several ecosystem services in agricultural soils, it is recommended to define the SPU as the abundance/biomass of the populations of species belonging to the different functional groups.

For the off-field non-target areas it is proposed that only negligible effects on the abundance / biomass of in-soil organisms populations can be tolerated.

In terms of this Opinion, the definition of possible acceptable magnitude of effects as percentage reduction compared to a “control” applies to a defined context, i.e. agricultural systems supporting highest diversity of in-soil organisms that can be achieved in sustainably managed agricultural systems. In landscapes with lower in-soil diversity, the acceptability of effects might be at a far lower magnitude level. This applies to all proposed Specific Protection Goal options for in-soil organisms.

For in-field as well as off-field areas, the tolerable magnitude of effects should take multiple PPP applications according to typical PPP ‘spray schedules’ into account. This will possibly implicate a lower level of tolerable effects for single PPP applications, especially in-field if the intended use fits in an application scheme that includes several other PPPs with potential effects on in-soil organisms in the crop. Multiple applications of several PPPs in typical schedules should also be taken in consideration when addressing the recovery of in-soil organisms.

6.2. SPG Options for Earthworms

The maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal is suggested to be small effects up to 35% on the ecological entity ‘populations of different earthworm species’. This magnitude of effect is deemed to allow for internal recovery of earthworm populations so that biodiversity levels and the provision of the ecosystem-services in agricultural field soils is assured in relevant time frames. For earthworm populations, medium effects up to 65% would not result in internal recovery in relevant time frames.

2597

2598 **Table 10:** Specific Protection Goal Options for Earthworms

Earthworms			
	ecological entity	attribute	magnitude/ temporal scale
<i>In-field</i>			
Biodiversity, genetic resources, cultural services	- population	- abundance/ biomass	- small effects (10%<35%) up to months
Nutrient cycling	- long term persistence of functional groups →population	- abundance/ biomass	- small effects (10%<35%) up to months
Pest control	- minor importance		
Natural attenuation	- minor importance		
Soil structure	- long term persistence of functional groups →population	- abundance/ biomass	- small effects (10%<35%) up to months
Food web support	- functional groups	- abundance/ biomass	- small effects (10%<35%) up to months
<i>Off field</i>			
Biodiversity and all ecosystem services	- population	- abundance/ biomass	- negligible effects (<= 10% or NEL) / not relevant

2599 **6.3. SPG Options for Enchytraeids**

2600 The maximum initial magnitude of effect that might be tolerated in-field without impairing the general
 2601 protection goal is suggested to be small effects up to 35% for months on the ecological entity
 2602 'populations of different enchytraeid species' or medium effects up to 65% for weeks. These
 2603 magnitudes of effects are deemed to allow for internal recovery of enchytraeids populations, so that
 2604 biodiversity levels and the provision of ecosystem services in agricultural field soils is assured in
 2605 relevant time frames.

2606 **Table 11:** Specific Protection Goal Options for Enchytraeids

Enchytraeids			
	ecological entity	attribute	magnitude/ temporal scale
<i>In -field</i>			
Biodiversity, genetic resources, cultural services	- population	- abundance/ biomass	- small effects (10% <35%) up to months - medium effects (35% <65%) up to weeks

Nutrient cycling	- long term persistence of functional groups →population	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Pest control	- minor importance		
Natural attenuation	- minor importance		
Soil structure	- long term persistence of functional groups →population	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Food web support	- functional groups	- abundance/ biomass	- small effects up to months - medium effects up to weeks
<i>Off field</i>			
Biodiversity and all ecosystem services	- population	- abundance/ biomass	- negligible effects ($\leq 10\%$ or NEL) / not relevant

6.4. SPG Options for Microarthropods

The maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal is suggested to be medium effects up to 65% for weeks on the ecological entity 'populations of different microarthropod species' or small effects up to 35% for months. These magnitudes of effects are deemed to allow for internal recovery of microarthropod populations, so that biodiversity levels and the provision of ecosystem services in agricultural field soils is assured in relevant time frames.

Table 12: Specific Protection Goal Options for Microarthropods

Microarthropods			
	ecological entity	attribute	magnitude/ temporal scale
<i>In -field</i>			
Biodiversity, genetic resources, cultural services	- population	- abundance/ biomass	- small effects (10% <35%) up to months - medium effects (35% <65%) up to weeks
Nutrient cycling	- long term persistence of functional groups →population	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Pest control	- long term persistence of functional groups →population	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Natural attenuation	- minor importance		
Soil structure	- long term persistence of functional groups →population	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Food web support	- functional groups	- abundance/ biomass	- small effects up to months - medium effects up to weeks

<i>Off field</i>			
Biodiversity and all ecosystem services	- population	- abundance/ biomass	- negligible effects ($\leq 10\%$ or NEL) / not relevant

2615

2616 6.5. SPG Options for Macroarthropods (e.g Isopods)

2617 The maximum initial magnitude of effect that might be tolerated in-field without impairing the general
 2618 protection goal are suggested to be medium effects up to 65% for weeks on the ecological entity
 2619 'populations of different macroarthropod species' or small effects up to 35% for months. These
 2620 magnitude of effects will allow for internal recovery and recolonization by macroarthropod species, so
 2621 that biodiversity levels and the provision of ecosystem services in agricultural field soils is assured in
 2622 relevant time frames.

2623 **Table 13:** Specific Protection Goal Options for Macroarthropods

Macroarthropods (e.g. Isopods)			
	ecological entity	attribute	magnitude/ temporal scale
<i>In -field</i>			
Biodiversity, genetic resources, cultural services	- Population	- Abundance/ - biomass	- small effects (10% <35%) up to months - medium effects (35% <65%) up to weeks
Nutrient cycling	- long term persistence of functional groups →population	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Pest control	- minor importance	-	-
Natural attenuation	- minor importance	-	-
Soil structure	- minor importance	-	-
Food web support	- functional groups	- abundance/ biomass	- small effects up to months - medium effects up to weeks
<i>Off field</i>			
Biodiversity and all ecosystem services	- population	- abundance/ - biomass	- negligible effects ($\leq 10\%$) / not relevant

2624

2625 6.6. SPG Options for terrestrial Gastropods (slugs and snails)

2626 Small effects < 35% for months on the ecological entity population of different gastropod species are
 2627 suggested as the maximum initial magnitude of effect that might be tolerated in-field without
 2628 impairing the general protection goal. For gastropod species, medium effects over period of weeks
 2629 does not appear suitable to enable internal recovery in relevant time frames (see 3.2.1).

2630

2631 **Table 14:** Specific Protection Goal Options for terrestrial gastropods

Terrestrial gastropods (slugs and snails)			
	ecological entity	attribute	magnitude/ temporal scale
<i>In -field</i>			
Biodiversity, genetic resources, cultural services	- population	- abundance	- small effects (10% <35%) up to months
Nutrient cycling	- long term persistence of functional groups → population	- abundance	- small effects up to months
Pest control	- minor importance		
Natural attenuation	- minor importance		
Soil structure	- long term persistence of functional groups → population	abundance	small effects (10% <35%) up to months
Food web support	- functional groups	- abundance	- small effects (10% <35%) up to months
<i>Off field</i>			
Biodiversity and all ecosystem services	- population	- abundance	- negligible effects (<= 10% or NEL) / not relevant

2632

2633 **6.7. SPG Options for Nematodes**

2634 The maximum initial magnitude of effect that might be tolerated in-field without impairing the general
 2635 protection goal are suggested to be medium effects up to 65% for weeks or small effects up to 35% for
 2636 months. Regarding the maintenance of biodiversity levels close to normal operating ranges for
 2637 agricultural field soils in relevant time frames, the ecological entity to be assessed should be
 2638 'community structure of nematodes'. In addition, the allocation of nematodes to trophic groups helps
 2639 to condense information efficiently and to determine their contribution to ecosystem services.

2640 **Table 15:** Specific Protection Goal Options for Nematodes

Nematodes			
	ecological entity	attribute	magnitude/ temporal scale
<i>In -field</i>			
Biodiversity, genetic resources, cultural services	- population /community structure	- abundance	- small effects (10% <35%) up to months - medium effects (35% <65%) up to weeks

Nutrient cycling	- functional group	- abundance	- small effects up to months - medium effects up to weeks
Pest control	- functional group	- abundance	- small effects up to months - medium effects up to weeks
Natural attenuation	- minor importance		
Soil structure	- minor importance		
Food web support	- functional group	- abundance	- small effects up to months - medium effects up to weeks
<i>Off field</i>			
Biodiversity and all ecosystem services	- population /community structure	- abundance	- negligible effects (< = 10% or NEL) / not relevant

2641

2642 6.8. SPG Options for Mycorrhiza, other fungi and protozoa

2643 Fungi have a short generation time and good dispersal ability (see text above), which allows them to
 2644 recover quite fast. The maximum initial magnitude of effect that might be tolerated in-field without
 2645 impairing the general protection goal is medium effects (35% < 65%) up to weeks on the ecological
 2646 entity population or functional group of different fungi, including mycorrhizal fungi and protozoan
 2647 species, depending on the ecosystem service. For the maintenance of biodiversity levels close to
 2648 normal operating ranges for agricultural field soils in relevant time frames, the ecological entity to be
 2649 assessed should be the 'community' and the attribute structure (phylogenetic or functional). The
 2650 proposed magnitude of effect is deemed to allow for internal recovery so that biodiversity levels and
 2651 the provision of the ecosystem service genetic resource in agricultural field soils is assured in relevant
 2652 time frames.

2653 **Table 16:** Specific Protection Goal Options for Mycorrhiza, other fungi and protozoa

Mycorrhiza, other fungi and protozoa			
	ecological entity	attribute	magnitude/ temporal scale
<i>In -field</i>			
Biodiversity, genetic resources, cultural services	- community	- structure	- small effects (10% <35%) up to months - medium effects (35% <65%) up to weeks
Nutrient cycling	- functional group	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Pest control	- functional group	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Natural attenuation	- functional group	- abundance/ biomass	- small effects up to months - medium effects up to weeks
Soil structure	- functional group	- abundance/ biomass	- small effects up to months - medium effects up to weeks

Food web support	- functional group	- abundance/ biomass	- small effects up to months - medium effects up to weeks
<i>Off field</i>			
Biodiversity and all ecosystem services	- community	- structure	- negligible effects ($\leq 10\%$ or NEL) / not relevant

2654

2655 6.9. SPG Options for Soil Bacteria and Archaea

2656 Soil bacterial and archaean communities may recover from disturbance quite fast, mainly because their
 2657 generation time is short, their rates of passive dispersal and recolonisation are comparatively high, and
 2658 because of functional redundancy. For services connected with genetic resources and biodiversity it is
 2659 suggested that the ecological entity should be microbial community and the attribute diversity
 2660 (phylogenetic or functional). For other ES, the proposed ecological entity to be protected is functional
 2661 group. It is suggested that the maximum magnitudes of effect that might be tolerated in-field are large
 2662 effects up to days, medium effects up to weeks and small effects up to months.

2663 **Table 17:** Specific Protection Goal Options for Soil Bacteria and Archaea

Soil Bacteria and Archaea			
	ecological entity	attribute	magnitude/ temporal scale
<i>In -field</i>			
Biodiversity, genetic resources, cultural services	- Microbial community	- diversity	- small effects (10% <35%) up to months - medium effects (35% <65%) up to weeks - large effects (> 65%) up to days
Nutrient cycling	- functional group	- abundance/ biomass / activity	- small effects up to months - medium effects up to weeks - large effects up to days
Pest control	- functional group	- abundance/ biomass / activity	- small effects up to months - medium effects up to weeks - large effects up to days
Natural attenuation	- functional group	- abundance/ biomass / activity	- small effects up to months - medium effects up to weeks - large effects up to days
Soil structure	- minor importance	- abundance/ biomass / activity	- small effects up to months - medium effects up to weeks - large effects up to days
Food web support	- functional group	- abundance/ biomass / activity	- small effects up to months - medium effects up to weeks - large effects up to days
<i>Off field</i>			
Biodiversity and all ecosystem services	- Microbial community	- diversity	- negligible effects ($\leq 10\%$) / not relevant

2664

2665 **6.10. Consequences of choosing different Specific Protection Goal Options for in-soil organisms key drivers**

2666 Limits of functioning for the key drivers are given in the sections above (tables 10-17) and chapter 4.

Ecosystem service	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Negligible effects	Option: Limit of functioning	Option: Above limit of functioning
Biodiversity, genetic resources, cultural services	<ul style="list-style-type: none"> - The upper level of the normal operating range for in-soil organism communities in agricultural landscapes is sustained. Species-specific interactions, food-web structure and ecosystem processes are unaffected by the intended PPP use. - General protection goal ‘<u>no unacceptable effect on biodiversity and the ecosystem</u>’ set out in Regulation (EC) No. 1107/2009¹⁰ is fully achieved. - Support of the target “<u>Increase the contribution of agriculture to maintaining and enhancing biodiversity</u>” (3a) of the EU 2020 Biodiversity Strategy¹², which has shown no significant progress so far. - This Option contributes to Action 10 of the EU 2020 Biodiversity Strategy¹²: “The Commission and Member States will encourage the uptake of agri-environmental measures <u>to support genetic diversity in agriculture and explore the scope for developing a strategy for the conservation of genetic diversity</u>”. - The aims of Council Directive 92/43/EEC¹¹ on the conservation of natural habitats and of <u>wild fauna and flora</u> are achieved. 	<ul style="list-style-type: none"> - The limit of functioning identified in the SPG tables marks a tipping point for the normal operating range of in-soil key drivers delivering genetic resources and cultural services and supporting all ecosystem services. - Reduction in species diversity reduces the efficiency with which ecological communities capture biologically essential resources, produce biomass, decompose and recycle biologically essential nutrients. - Biodiversity is supported to a degree that insures the long term functioning of agricultural system, even if sensitive species are affected in the short term and species-specific interactions might be disrupted. - General protection goal ‘no unacceptable effect on biodiversity and the ecosystem’ set out in Regulation (EC) No. 1107/2009 is still achieved if off-field areas of pertinent size in a diversified landscape sustain the upper level of biodiversity normal operating range. 	<ul style="list-style-type: none"> - Species loss above a tipping point may force ecosystems to move to a different (locally) stable state or to collapse. - Loss of biodiversity will weaken the ability of agricultural ecosystems to respond to external changes such as climate change (loss of stability and resilience). - Biodiversity losses will lead to disruption of valuable ecosystem functions thereby reducing delivered services. Cultural services will be reduced if vulnerable species decline or disappear. - General protection goal ‘no unacceptable effect on biodiversity and the ecosystem’ set out in Regulation (EC) No. 1107/2009 is not achieved. - The target “Increase the contribution of agriculture to maintaining and enhancing biodiversity” (3a) of the EU 2020 Biodiversity Strategy¹² will most probably not be met. - The aim of halting of biodiversity loss by 2020 is not achieved: ‘Halting biodiversity loss constitutes the absolute minimum level of ambition to be realised by 2020’ (2009/2108(INI)¹² and 2011/2307(INI)¹³. - UN sustainable development goals (SDG)¹⁴ Sustainable Goals 2.4 and 15.5 are jeopardized. These goals are: <ul style="list-style-type: none"> “By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality” and “Take urgent and significant action to reduce the degradation of natural habitats, halt the loss of biodiversity and, by 2020, protect and prevent the extinction of threatened species”

¹⁰ European Union: Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. 21 October 2009. Official Journal of the European Union L 309, 24 November 2009, 50 pp.

¹¹ Council Directive 92 /43 /EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

¹² 2009/2108(INI) Report on the implementation of EU legislation aiming at the conservation of biodiversity.

¹³ 2011/244(INI) Communication: on our life insurance, our natural capital: an EU biodiversity strategy to 2020 Committee on the Environment, Public Health and Food Safety.

Ecosystem service	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Negligible effects	Option: Limit of functioning	Option: Above limit of functioning
Nutrient cycling	<ul style="list-style-type: none"> - Upper limit of the normal operating range of soil organisms as drivers of organic matter decomposition and nutrient cycling is supported. - The aims of the EU thematic strategy for soil protection¹⁵ to “<u>protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms</u>” are fully supported. - UN sustainable development goals (SDG)¹⁴ Sustainable Goals and 2.4 and 12.2 are supported These goals are: “By 2030, ensure <u>sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems</u>, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality” and “By 2030, achieve the <u>sustainable management and efficient use of natural resources</u>” 	<ul style="list-style-type: none"> - This limit of functioning marks the lower threshold of the normal operating range for soil organisms in the decomposition of dead organic matter and the delivering of nutrients. - The General Protection Goal ‘no unacceptable effect on biodiversity and the ecosystem’ of Regulation (EC) No. 1107/2009 and the goal of the the EU thematic strategy for soil protection to “protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms” are still met in the long term. - Nutrient availability and plant productivity are not impaired in the long term, even if vulnerable species in functional groups might be affected in the short term. To ensure this, off-field areas of pertinent size in a diversified landscape should deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonization of vulnerable soil organisms in the middle and long term. 	<ul style="list-style-type: none"> - Reduced nutrient availability might reduce plant productivity. The requirements for external inputs of nutrients to maintain crop yield will increase. - Disruption of trophic networks can occur, impairing the ecological equilibrium of the system. - The aims of the EU thematic strategy for soil protection¹⁵ to “protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms” may not be met. - UN sustainable development goals (SDG)¹⁴ 2.4 and 12.2 are jeopardized. These goals are: “By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality” and “By 2030, achieve the sustainable management and efficient use of natural resources”

¹⁴ United Nations General Assembly (2015): Resolution adopted by the General Assembly on 25 September 2015. Transforming our world: the 2030 Agenda for Sustainable Development. Distr. General, 21 October 2015. Seventieth session, Agenda items 15 and 116, A/RES/70/1, 35pp.

¹⁵ COM/2006/0232 final (2006): Proposal for a Directive of the European Parliament and of the Council establishing a framework for the protection of soil and amending Directive 2004/35/EC.

Ecosystem service	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Negligible effects	Option: Limit of functioning	Option: Above limit of functioning
Pest and pathogen control	<ul style="list-style-type: none"> - Control of specific pest and pathogens by soil organisms is at the upper level of the normal operating range for agricultural soils. - Aims of Directive 2009/128/¹⁶for achieving a sustainable use of pesticides are fully supported: ‘Member States shall establish or support the <u>establishment of necessary conditions for the implementation of integrated pest management</u>. In <u>protection and enhancement of important beneficial organisms</u>, e.g. by adequate plant protection measures’ 	<ul style="list-style-type: none"> - Resilient organisms will still deliver the service of pest and pathogen control in agricultural soils. - However, control of specific pathogens by vulnerable soil organism key drivers might be reduced in the short term. - The General Protection Goal ‘no unacceptable effect on biodiversity and the ecosystem’ of Regulation (EC) No. 1107/2009 and the aims of Directive 2009/128/¹⁶for achieving a sustainable use of pesticides are still implemented, as long as off-field areas of pertinent size in a diversified landscape should deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonization of vulnerable soil organisms in the middle and long term. 	<ul style="list-style-type: none"> - Enhanced proliferation of pest and pathogens through the disruption of intra- and inter species interaction within the soil community (competition, predation, and parasitism) might finally lead to reduced plant productivity. - Pests and pathogens may increase both numerically and in geographical spread, leading to greater reliance on chemical pesticides and further reduction of biodiversity. - Aims of Directive 2009/128/¹⁶for achieving a sustainable use of pesticides are not implemented: - “Member States shall establish or support the establishment of necessary conditions for the implementation of integrated pest management. In protection and enhancement of important beneficial organisms, e.g. by adequate plant protection measures”.

¹⁶ 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

Ecosystem service	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Negligible effects	Option: Limit of functioning	Option: Above limit of functioning
Natural attenuation	<ul style="list-style-type: none"> - The aims of the EU thematic strategy for soil protection¹⁵ to “<u>protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms</u>” are fully supported. - UN sustainable development goals (SDG) 6.3 and 15.3 are supported. These goals are: “By 2030, <u>improve water quality by reducing pollution</u>, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally” and “By 2030, combat desertification, <u>restore degraded land and soil</u>, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world 	<ul style="list-style-type: none"> - The upper limit of the normal operating range for soil organisms to perform natural attenuation of contaminants in agricultural soils are reached. - The General Protection Goal ‘no unacceptable effect on biodiversity and the ecosystem’ of Regulation (EC) No. 1107/2009 and the aims of the Water Framework Directive (WFD)¹⁷ that commits European member states to achieve a good ecological and chemical status for surface waters and a good quantitative and chemical status for groundwater is still supported, even if the degradation of specific compounds by specialized vulnerable soil organisms might be hampered in the short term. 	<ul style="list-style-type: none"> - Slower removal and attenuation of contaminants from soil. - Reduction in soil fertility through microbial primary catabolic role in the degradation of plants and animal residues in the cycling of the organic, inorganic nutrients content of soil - Potential leaching of contaminants to groundwater and run-off/drainage entry into surface water - The aims of the Water Framework Directive (WFD)¹⁷ that commits European member states to achieve a good ecological and chemical status for surface waters and a good quantitative and chemical status for groundwater may not be fulfilled. - The aims of the EU thematic strategy for soil protection to “protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms” may not be met. - UN sustainable development goals (SDG) 6.3 and 15.3 are jeopardized. These goals are: “By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally” and “By 2030, combat desertification, restore degraded land and soil, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world

¹⁷ Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

Ecosystem service	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Negligible effects	Option: Limit of functioning	Option: Above limit of functioning
Soil structure formation and water retention	<ul style="list-style-type: none"> - Soil <u>aggregate stability</u> is increased, organic matter is incorporated into the soil profile and soil profile development is supported. Stabilization of organic matter and <u>carbon sequestration</u> in soil aggregates will protect carbon-rich detritus from microbial degradation. - Soil structure formation and support will <u>prevent water logging</u>, oxygen depletion and increased denitrification - Movement of soil organisms in the soil profile will <u>reduce hydrophobic patches</u> formation. - The aims of the EU thematic strategy for soil protection to “<u>protect soil and to preserve its capacity to perform</u> its functions in environmental, economic, social and cultural terms” is fully supported. - UN sustainable development goal (SDG) 2.4 is jeopardized. This goal states: “By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that <u>help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality</u>”. 	<ul style="list-style-type: none"> - In-soil key drivers of soil structure formation and water retention will deliver these services at the lower limit of the normal operating range. - Vulnerable key drivers might be affected by PPP use in the short term. - The General Protection Goal ‘no unacceptable effect on biodiversity and the ecosystem’ of Regulation (EC) No. 1107/2009 and the aims of EU thematic strategy for soil protection¹⁵ are still implemented, as long as off-field areas of pertinent size in a diversified landscape deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonization of vulnerable soil organisms in the middle and long term. 	<ul style="list-style-type: none"> - Soil structure disruption may lead to soil compaction in vulnerable soils, which is not broken up by soil organism key drivers. Less macropores will increase waterlogging in vulnerable soils, less connecting micropores to lower water holding capacity. - Increased surface run-off and erosion may lead to contaminant and nutrient entries into surface waters. Risk of floods increases. - The aims of the Water Framework Directive (WFD)¹⁷ to achieve a good ecological and chemical status for surface waters and a good quantitative and chemical status for groundwater may not be fulfilled. - The aims of Council directive 91/676/EEC¹⁸ concerning the protection of waters against pollution caused by nitrates from agricultural sources may not be fulfilled. - Agricultural areas may be classified as “areas facing natural constraints” according Regulation (EU) No 1305/2013¹⁹, which might cause additional costs to the European Community in the form of payments to farmers to maintain agriculture in these areas. To receive direct payments in the context of the EU Common Agricultural Policy (CAP), farmers shall maintain land in good agricultural and environmental condition. Soil degradation may lead to increased effort to maintain the reference conditions or even to the loss of direct payments. - UN sustainable development goal (SDG) 2.4 is jeopardized. This goal states: “By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality”.

¹⁸ Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources.

¹⁹ Regulation (EU) No 1305/2013 of the European Parliament and of the Council of 17 December 2013 on support for rural development by the European Agricultural Fund for Rural Development (EAFRD) and repealing Council Regulation (EC) No 1698/2005

Ecosystem service	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Negligible effects	Option: Limit of functioning	Option: Above limit of functioning
Food web support	<ul style="list-style-type: none"> - Structure and functioning of the soil food web in agricultural soils is preserved and <u>the support of all above-ground terrestrial food webs</u> is achieved. - Vulnerable species at higher trophic level, e.g. <u>farmland birds, that are highly dependent on invertebrates for chick growth and survival will be supported.</u> - The aim of halting of biodiversity loss by 2020 is fully supported: 'Whereas the <u>disappearance of species may break the food chain that is key to the survival of other animal and plant species of vital importance for food production, adaptation to climatic conditions, resistance to external agents and the preservation of genetic values</u>' (e.g. 2009/2108(INI) and 2011/2307(INI)) 	<ul style="list-style-type: none"> - Disruption of trophic networks can occur when vulnerable soil organisms are affected by PPP intended uses in the short term, impairing the ecological equilibrium of the system - The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009; the aims of Council Directive 79/409/EEC²⁰ on the conservation of wild birds and of Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora are still achieved in the long term -as long as off-field areas of pertinent size in a diversified landscape deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonization of vulnerable soil organisms 	<ul style="list-style-type: none"> - Vulnerable species at higher trophic level, e.g. farmland birds, that are highly dependent on invertebrates for chick growth and survival, will decline further and may become extinct. - Diverse income-earning activities such as game-bird shooting may recede, leading to reduced financial viability of farms. - General protection goal 'no unacceptable effect on biodiversity and the ecosystem' set out in Regulation (EC) No. 1107/2009 is not achieved. - Aims of Council Directive 79/409/EEC on the conservation of wild birds and of Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora are not achieved. - The aim of halting of biodiversity loss by 2020 is not achieved: 'Whereas the disappearance of species may break the food chain that is key to the survival of other animal and plant species of vital importance for food production, adaptation to climatic conditions, resistance to external agents and the preservation of genetic values' (e.g. 2009/2108(INI) and 2011/2307(INI))

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²⁰ Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds

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2672 **6.10.1. Do persistent substances need additional requirements?**

2673 The SPGs as set up have a spatial and a temporal aspect, and they consider the agricultural context.
2674 The question is whether particularly persistent substances can result in unexpected or unwanted effects
2675 on the long-term, even when the SPGs are met. From a point of view of sustainable land use, the use
2676 of PPPs should not hamper the use of soil for other functions in future, such as changing from
2677 traditional to organic ('ecological') farming, or taking agricultural land out of production. As soon as
2678 the use or the function has changed, there is a possibility that residues of substances may not comply
2679 with the SPGs for the new situation - if this possible change in function was not addressed in the
2680 authorisation procedure. It is proposed during the development of the guidance document on the risk
2681 assessment for in-soil organisms to do some example calculations with persistent substances in order
2682 to find out whether it could be necessary to include additional requirements for persistent substances.
2683 In Appendix F. an example is given of a possible approach to include persistence into the risk
2684 assessment.

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7. GENERAL FRAMEWORK

This chapter presents the opinion of the PPR Panel on how in principle the environmental risk assessment (ERA) for in-soil organisms exposed to intended uses of PPPs should be conducted in the future, based on the best scientific knowledge available.

Besides some general words on the principles of tiered ERA schemes, this chapter will focus on the selection of the surrogate reference tier (SRT) and how to deal with the different level of uncertainty at the different tiers, including the calibration of lower tiers. A section on the identification of the vulnerable species/groups and on how to link exposure and effects will also be included. Finally, a flowchart is presented with a proposal on how to conduct ERA.

7.1. The principles of a tiered approach

The guidance document for aquatic risk assessment (EFSA PPR Panel, 2013) provides an overview of the principles of the tiered approach and the need to adopt them when assessing environmental risks of PPPs to optimize costs and increase the efficacy of the assessment. According to Boesten et al. (2007) and Solomon et al. (2008), the general principles of tiered approaches are:

- lower tiers are more conservative than higher tiers;
- higher tiers aim at being more realistic than lower tiers;
- lower tiers usually require less effort than higher tiers;
- in each tier, all available relevant scientific information is used;
- all tiers aim to assess the same protection goal.

Thus the tiered system needs to be (i) appropriately protective, (ii) internally consistent, (iii) cost-effective, and (iv) it needs to address the problem with a higher accuracy and precision when going from lower to higher tiers (see Figure 9).

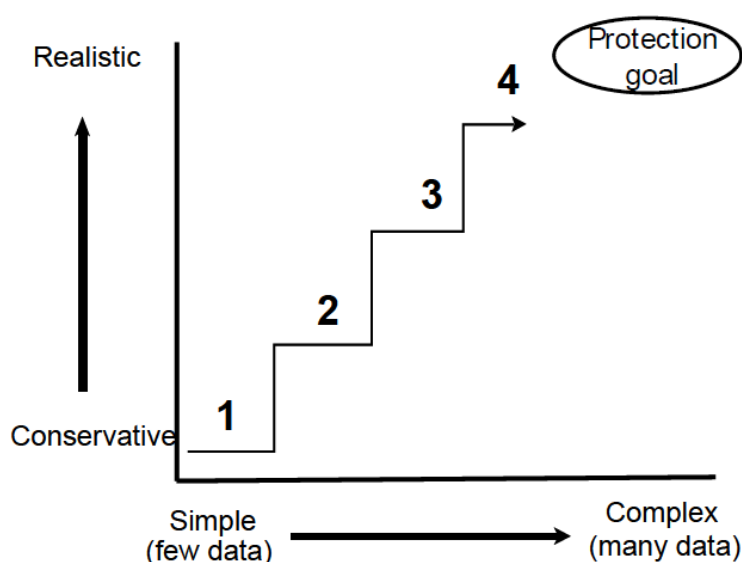


Figure 9: Tiers in the risk assessment process, showing the refinement of the process through the acquisition of additional data (redrafted after Solomon et al., 2008)

7.2. Tiered approach in the risk assessment for in-soil organisms and definition of (surrogate) reference tier

A tiered approach implies the existence of a surrogate reference tier (SRT), i.e., a representation, as accurate as possible, of the real situation in the field (i.e. the reference tier). This SRT should link the assessment being performed and the specific protection goals (see Figure 10). A SRT is a compromise between what would be desirable and what is practical. The SRT should be used to calibrate the lower tiers properly in order to make them sufficiently protective, taking into account the level of protection defined in the SPGs.

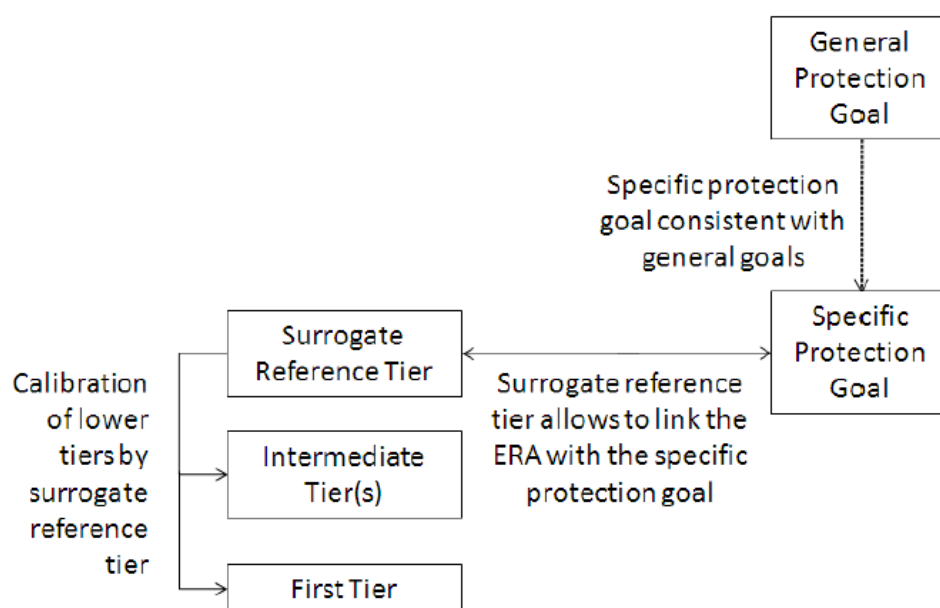


Figure 10: Illustration of the relationship between tiers of the risk-assessment process and protection goals, in the approach used by the PPR Panel (EFSA PPR Panel, 2010a)

The risk assessment of PPPs for in-soil organisms should be performed over different spatio-temporal scales. This is in contrast to what is currently done, where only the “in-field” area is considered. Since an “action at a distance” is not expected to occur for in-soil organisms on larger scale (see section 6.1), a landscape-scale assessment may not be necessary. For many in-soil organisms, recovery will be driven mainly by small-scale migrations and reproduction within the field (see section 3.2). Nevertheless, for some groups, external recovery from adjacent off-field areas and from field-boundaries may be of relevance for the assessment. The risk to in-soil organisms should be assessed both in-field and off-field and assessment may consider processes at the field-boundary. So, the actual reference tier for in-soil organisms (for organisms with either high or low dispersal ability) would be the soil-organism community present at the field scale and influenced by temporal and spatial processes at the field-boundary scale.

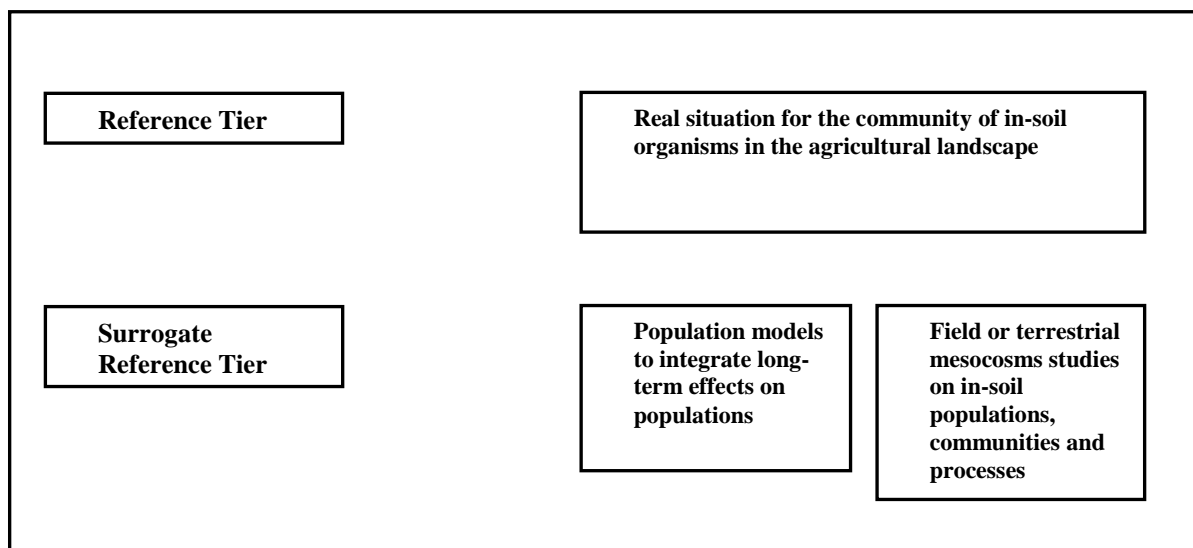


Figure 11: Reference tier versus surrogate reference tier in the risk assessment of in-soil species

In the current risk-assessment scheme, the highest available tier is represented by a full fauna field study performed at a local scale. However, in-soil organisms may be exposed to multiple stressors due to sequential as well as simultaneous use of different pesticides and other agricultural practices, which cannot be exhaustively studied in field experiments. Therefore, a combination of assessing the effects both at the local scale - through testing the toxicity of PPPs on in-soil communities - and at a larger scale covering field-boundaries and adjacent off-field areas - through modelling long-term exposure of single species populations integrating all relevant stressors - is proposed as a SRT for in-soil species to assess population-level effects (Figure 11). Even though a larger-scale approach (landscape) is not needed for the majority of in-soil organisms with a range of movement that is very restricted compared with field size, for relevant groups, the movement between field and boundary may need to be included in the risk assessment.

In this context, it can be assumed that a suitable field study or a mesocosm study (e.g., a TME with adequate dimensions) can act as a SRT for the assessment of community effects at a field scale. These studies address community composition, population dynamics, indirect effects (predation or competition effects), chronic exposure (eventually repeated exposure), interactions between and within species and exposure mimicking the actual field situation. Please refer to section 9.7 for recommendations on how to set up of appropriate field test and semi-field mesocosms (e.g. TME).

Long-term dynamics at population level over one or more seasons, embracing both population growth and spatial dynamics in-field, as well as recolonization, should be tackled by modelling approaches (combining spatial and temporal population models). The rate parameters of these processes may depend on the field size, the spatial configuration of the crop, the PPP application scenario (in rows or over the entire area) and the existence and dimension of field boundaries and adjacent off-field areas. To assess population-level effects, models for different ecological and agricultural practice scenarios should be developed for relevant key species, with different vulnerability components, and further validated.

7.3. Surrogate reference tier (SRT) and the systems approach

Current practice in prospective ERA is to conduct the exposure and effect assessment for one PPP at a time. An important question is whether the chemical-by-chemical approach in the current prospective ERA for PPPs is sufficient also to prevent cumulative risks from exposure to different PPPs, as well as to predict ecological recovery. To determine this, the impact of multiple stressors on the state of the population needs to be taken into account when assessing a particular PPP impact. Thus, due to the complexity of ecological systems and the need to evaluate direct and indirect effects and recovery in spatial and temporal dimensions, a systems approach is considered appropriate by EFSA (EFSA

Scientific Committee, 2016a). In this context, a *systems approach* is defined to mean taking into account the range of factors considered to potentially interact and affect the result of the risk assessment. For example, this would include multiple applications and non-chemical stressors as they might affect the organisms considered in the assessment. It may also include indirect effect and abiotic factors. The surrogate reference tier (SRT) for this type of assessment would thus be a fully implemented ecological-model system including the important factors identified.

In many other systems (e.g. non-target arthropods, aquatic systems) the *systems approach* is needed owing to the impacts of both spatial and temporal drivers of population change. Spatial drivers, in particular ‘action at a distance’ are relevant for these groups of organisms (EFSA PPR, 2015a; EFSA Scientific Committee, 2016a). In soil, the scales and rates of movements are smaller and thus the primary drivers considered are temporal drivers of population change, i.e. the vital rates. This means that recovery would be primarily driven by internal population growth rather than external migrations (see section 3.2), and that the measurement endpoint in focus is the long-term population trajectory.

In order to adopt a systems approach and to integrate this into the risk assessment, several steps need to be taken:

1. Focal taxa and focal cropping systems need to be identified to create relevant scenarios. These species need to cover those where population impacts and recovery can be related to the SPGs;
2. The normal operating range of focal taxa needs to be identified (bearing in mind that this may vary in time and between different ecosystems). This is used to establish a baseline against which the system with the addition of the regulated pesticide can be assessed. This baseline would need to be established for the range of scenarios needed to represent the range of conditions for which the assessment should cover (e.g. low input and high input agro-ecosystems);
3. Good mechanistic effect models, which are both manageable and realistic enough, will need to be developed. To assess effects on other species in an ecological network requires food-web modelling (De Ruiter et al., 2005). However, the use of food-web models for assessment would require that they are predictive, and that their predictive quality has been proven in independent experiments. Hence, although food-web models are conceptually suitable and appropriate, parameterization and uncertainty of predictions are challenges in their application in risk assessments (see e.g. Baird et al., 2001; Traas et al., 2004; De Ruiter et al., 1998; De Ruiter et al., 2005; De Laender et al., 2011). For community-level assessment, recourse must therefore be made to field studies (see section 9.7). Note also that the longer time-frame for field-study assessment provides the potential to detect delayed community or life-history effects e.g. as a result of reproductive impacts. However, in terms of understanding the case-specific results of field studies, food-web models may play an important role. In contrast, population models are relatively easy to develop and require fewer case-specific data. Hence, for assessment of long-term impacts the use of population models is proposed.

For in-soil organisms it is advised to take the aspects that affect vital rates into account, rather than large-scale spatial dynamics. For some organisms, vertical movements in the soil profile might be of relevance to assess exposure to PPP. Therefore, for in-soil organisms there are only a limited number of aspects to consider in terms of their impact and timing. These are:

- The regulated stressor of interest and its intended use;
- Abiotic conditions, e.g. temperature and moisture, as they affect population-growth rate;
- The reproductive profile within a season of the focal taxa;

- 2816 • The mortality profile within a season of the focal taxa;
- 2817 • Individual growth and development;
- 2818 • Individual vertical movement within the soil profile, if relevant;
- 2819 • Individual toxicokinetics and toxicodynamics of the active substance, in combination with
- 2820 varying exposure in the soil profile;
- 2821 • Impacts of non-regulated stressors, probably primarily impacts of agricultural management;
- 2822 • Other regulated stressors, i.e. other pesticides, GMO crops, and biocides, as relevant.

2823 The models to be developed do not need to take every possible management scenario into account. In
 2824 edge-of-field surface waters there are typically 2-3 pesticides dominating the mixture in terms of toxic
 2825 units (see e.g. Belden et al., 2007; Liess and von der Ohe 2005; Schäfer et al., 2007; Verro et al.,
 2826 2009). Consequently, when addressing cumulative stress of pesticides in ERA, it seems cost-effective
 2827 to focus on those pesticides that dominate the exposure in terms of toxic units in the relevant medium
 2828 (e.g. > 90%). However, it is important that a range of scenarios altering potential vulnerability of
 2829 populations is taken into account (e.g. highly stressed populations may be more vulnerable to further
 2830 stressors).

2831 Information on the distribution of crops in agricultural landscapes and frequently occurring pesticide
 2832 combinations may be derived from existing databases (e.g. databases under the EU subsidies scheme
 2833 and databases from EU pesticide usage as collected within the frame of the Sustainable Use Directive,
 2834 Garthwaite et al., 2015). This information may be important input for population models to evaluate
 2835 effect periods and recovery times following pesticide stress in a realistic agricultural landscape context
 2836 (e.g. Focks et al., 2014; Topping et al, 2016).

2837 When systems models (i.e. the surrogate tier) are sufficiently developed, their behaviour when used
 2838 for standard toxic reference pesticides could be used as the basis for calibrating the lower tier tests (see
 2839 section 7.5). This is important because currently there are no standards for determining acceptable
 2840 population-level impacts. Model development is therefore a prerequisite for the assigning of an
 2841 assessment factor for lower tiers. These models may also form a basis on which higher tier
 2842 refinements might be based if the basic model or scenarios do not include relevant case-specific
 2843 information.

2844 **7.4. Recovery**

2845 Recovery can be assessed at the levels of individuals, populations, communities, or functions. In broad
 2846 terms, recovery can be thought of as the return of an ecological entity (e.g. structure such as
 2847 abundance, or function such as an ecosystem service) to its normal operating range (sometimes
 2848 referred to as baseline properties), having been perturbed outside that range by a stressor (or multiple
 2849 stressors). In order to assess recovery, it is first necessary to define what the normal operating range of
 2850 the ecological entity and/or process is (EFSA Scientific Committee, 2016a).

2851 Recovery can be classified into two main types, depending upon whether it occurs *in situ* (internal
 2852 recovery) or *via* dispersal (external recovery). Both types of recovery may be exhibited by the same
 2853 ecological entity (e.g. at different stages in a species' life-history). However, those organisms more
 2854 dependent on external recovery will require larger scale (in both time and space) to represent their
 2855 systems adequately.

2856 EFSA recommends a systems approach in the cases where recovery is assessed (EFSA Scientific
 2857 Committee, 2016a). This is due to the need to consider spatial dynamics resulting in action at a
 2858 distance; hence, evaluating recovery at a too small a scale may result in erroneous conclusions

(Topping et al., 2014). The systems-level approach takes changes in time and space over a larger scale into account, thus subsuming recovery under the long-term impacts on the overall system state (e.g. represented by population size). If initial effects are considered tolerable, recovery can be considered as an essential and integral dynamic of any system subject to regulated stressors, but may not need to be taken into account explicitly if long-term system state is used for ERA.

According to (EFSA Scientific Committee, 2016a), for any (combination of) experimental or modelling approach to show that there will be actual recovery under realistic conditions of use, this approach needs to consider:

- (i) the properties of the types of potential stressors (including the timing of applications relative to life-history stage, the number and frequency of applications of the same PPP and the cumulative risks of exposure to multiple PPPs)
- (ii) direct and indirect effects (species interactions)
- (iii) the relevant (focal) species and their traits, e.g. related to demography, dispersal and foraging behaviour as well as adaptation to potential stressors
- (iv) the specific features of the landscape, i.e. variations in land use, and the types, spatial distribution and connectivity of habitats

Due to lack of mobility, most microorganisms and many soil invertebrates will primarily depend on internal recovery, and assessment of external recovery will therefore be unnecessary. However, in a few cases landscape effects (see section 6.1) will need to be taken into account, and the use of small plots for experimentation needs to be critically assessed as to whether it meets the criteria listed above.

The tools used to develop the systems approach are mechanistic models for prediction, experimentation, monitoring, and expert elicitation. Experimentation usually involves semi-field and field studies, which are primarily used for evaluating community interactions, and experimentation and monitoring are employed as a reality check and to guard against unexpected effects.

There exists a number of potential modelling approaches to assess recovery (please refer to EFSA Scientific Committee, 2016a). However, employing these approaches to develop systems models entails a high demand for data and expert skills for both the development and validation of potential models, especially in cases where external recovery is an important part of the dynamics. Population models that do not need to take spatial dynamics into account will therefore be easier to develop and test, and could be applied more easily to less mobile in-soil organisms, to incorporate the long-term effects and multiple stressors.

7.5. Calibration and treatment of additional uncertainties

This section is concerned with the closely related tasks of calibration and treatment of additional uncertainties. Both tasks are about addressing uncertainty. Calibration refers to the problem, when only lower tier data is available, of addressing uncertainty about what the surrogate reference tier (SRT) outcome would be. Even when SRT data are available for an assessment, however, there are likely to be additional uncertainties that need to be addressed, for example sampling variability for a field study/mesocosm or uncertainties affecting the population model(s) being used.

The EFSA Scientific Committee (2015b) draft “Guidance on Uncertainty in EFSA Scientific Assessment” provides specific guidance on the treatment of uncertainty when standardised assessment procedures are being developed. In particular, it is necessary to identify and to describe all the uncertainties that affect assessments for which a standardised procedure is being developed. Methods are provided to assist with this task. The standardised procedure should include allowance for as many sources of uncertainty as is feasible. This reduces the burden for subsequent applications of the procedure as those applications need only consider uncertainties that were not already taken into account.

In current, lower tier risk assessment for in-soil organisms (see section 2.1.4), a trigger value is used to address uncertainties relating to toxicity assessment. The risk assessment works by calculating the toxicity-exposure ratio (TER) and comparing it to the trigger value. The Panel is not aware that a transparent rationale has been provided for the current trigger values e.g. five for chronic effects.

Computing the TER and comparing it to the trigger value of e.g. five is equivalent to dividing the TER by an assessment factor (AF) of five and comparing the result to one. This is also equivalent to the approach used in aquatic assessment (EFSA, 2013): the toxicity measurement is divided by the AF to obtain the “regulatory acceptable concentration” (RAC), which is then compared with the predicted exposure. The rest of this section uses the language of assessment factors but it should be understood that the resulting overall AF to be applied can always be implemented instead as a trigger value. The core problem for calibration and treatment of additional uncertainties is to decide how big the overall AF should be and how the AF should change as more information becomes available to the assessor.

When surrogate reference-tier (SRT) data are available, the overall AF only needs to address the additional uncertainties. When standard tier 1 data are available, the overall AF also needs to address uncertainty about what the SRT outcomes would be if the SRT were carried out (please see section 7.8 for a schematic risk-assessment flow chart). This is the calibration problem and has two components: uncertainty about the outcome of the field study or mesocosm part of the SRT and uncertainty about the outcome of population modelling specific to the substance being assessed. If laboratory tests are available for additional species or some intermediate tier test data are available, uncertainty about the first component is expected to be reduced and this may lead to a change to the overall AF.

7.5.1. Probabilistic modelling of uncertainty as the basis for assessment factors

One approach to obtain an AF addressing multiple sources of uncertainty and variability is to determine a suitable AF for each source and then to multiply them to obtain the overall AF. This has the apparent advantage that toxicity and exposure can be separated and addressed by separate AFs and that those AFs may themselves be obtained by multiplying individual AFs addressing particular sources of uncertainty. EFSA PPR Panel (2015a) discusses the problems with this approach: it lacks a sound theoretical basis and tends to lead to an overly conservative result.

Probabilistic modelling of variability and uncertainty is suggested by EFSA Scientific Committee (2015b) as the best approach to deriving AFs. WHO/IPCS (2014) provides an example of the approach in the context of human risk assessment for chemicals. For in-soil risk assessment, variability of exposure is addressed by targeting the 90th percentile (see chapter 8). In this approach, interspecies and interchemical variation should be described by a statistical model. The available data and knowledge should then be used to determine a probability distribution that represents the overall uncertainty about parameters in the statistical model. From the model and distribution for the parameters, a probability distribution could be deduced that would quantify risk, for a substance being assessed. That distribution quantifies how much the TER might change if the uncertainties were resolved, and the probability of each possible change for each possible changed value for the TER; the distribution says how likely that value is. The overall AF would then be the quantile of that probability distribution that provides sufficient certainty that allowance has been made for uncertainties. The decision about what quantile to use is primarily a risk-management issue. The probability distribution representing combined uncertainty is most easily obtained by first determining a probability distribution representing each individual uncertainty and then combining those distributions using the mathematics of probability, taking account of any dependence between uncertainties. The practical tools for determining distributions for individual uncertainties are statistical modelling and expert-knowledge elicitation (see EFSA, 2014b). Bayesian graphical models (for example, Gelman et al, 2013) provide a framework for expressing the relationships between uncertainties and Monte Carlo is then the natural practical tool for computing distributions for combinations of uncertainties.

7.5.2. Calibration of lower tier assessments against field study or mesocosm component of surrogate reference tier (SRT)

In order to make this kind of calibration when developing the guidance, it will be necessary to have first decided exactly what criteria would be applied to the data from the field study or mesocosm component of SRT if such were to be available for the substance being assessed. The purpose of those criteria would be to assess whether or not the relevant SPGs were achieved (or whether or not they were achieved to a sufficient degree).

The problem is then to decide how much the outcome (as a concentration) of some battery of lower tier tests might differ from the highest concentration that would lead to acceptable outcomes from the field study or mesocosm component of the SRT. Uncertainty about the ratio between the two concentrations would be represented by a probability distribution and the distribution would depend on which tests were included in the battery. For example, the battery might consist simply of the standard required tier 1 tests or it might also include similar tests for additional species or some more sophisticated testing. The distribution would also depend on what method is used to derive the single concentration representing the outcome of the battery of tests. For example, this might be minimum concentration from all the tests or it might be some other statistic such as the geometric mean if that were found to be a better predictor for the field study or mesocosm outcome.

The approach advocated here and in EFSA PPR Panel (2015a) is to use a statistical model to obtain the distribution representing uncertainty about the ratio of lower tier outcome to acceptable concentration in field study or mesocosm. The fundamental basis of this statistical modelling approach is the “random chemicals” viewpoint of Cooke (2010): the substance being assessed is modelled as though it were randomly selected from a population of relevant chemicals. The inter-chemical variability of the ratio is a primary source of uncertainty for a new substance because the location of the substance in that distribution is unknown. The statistical model describes how the ratio varies between substances and learns about that variability from relevant data and/or expert judgement. Residual uncertainty about the variability makes an extra contribution to uncertainty about the ratio for the new substance.

In practice, there are several reasons why the statistical model needs to do more than just describe variability of the ratio. First, data from field studies/mesocosms will not actually provide the highest concentration that leads to acceptable outcomes. Instead the data will provide information about some aspect of dose-response. The statistical model therefore needs to include a dose-response component so as to be able to make a relationship between the data from a study and the highest acceptable concentration. Second, because of the need to address dose-response, the model must include components for intra- and inter-species variability. Third, rather than build a different statistical model for each battery of tests, it is preferable to build a single statistical model that is capable of quantifying uncertainty for many batteries of tests. Consequently, the model needs to include components corresponding to the different tests and species that may be tested. An advantage of including a component for inter-species variability in the model is that there is no need for separate species-sensitivity distribution (SSD) modelling and calculations. The model itself will show how the AF should change as additional species are tested.

The way in which, for example, interspecies variation would enter into the statistical model would be through variation in dose-response parameters between species. Similarly, differences between lab and field would be handled by modelling differences between lab and field dose-response parameters. EFSA PPR Panel (2015a), in particular section 5.5.6 and figure 19 therein, provides more detail on statistical modelling of relationships between dose-response parameters for different species and tests.

Each of the components in the model will vary between substances and the model needs to describe this variability. By describing variability of each component, the model also describes variability of the ratio of lower tier outcome to acceptable concentration in field study or mesocosm.

This kind of modelling is a natural extension of the basic SSD model (for example, Aldenberg and Jaworska, 2000), which addresses inter-species variability. EFSA (2006) provided a simple way to incorporate inter-chemical variability into the SSD model. EFSA PPR Panel (2015a) summarises subsequent developments and recent further developments incorporating dose-response and dynamics into models of interspecies and interchemical variability are provided by Kon Kam King et al. (2015a,b). All these models are specific instances of the wider class of hierarchical random effects models. In that class of models, Bayesian networks and graphical models (for example, Gelman et al., 2013) have particular potential for this kind of application. They provide a formal mechanism, supported by theory, for combining expert judgments with data and quantify uncertainties using probability. This is advantageous for decision-making applications of statistical modelling.

Available data relating lower-tier test outcomes to each other and to field study/mesocosm outcomes would be used to “train” the model, in effect, to learn about the inter-chemical variation. The data providing information, directly or indirectly, about the inter-chemical variability of any particular component need to be matched in terms of substances in order to obtain real information about inter-chemical variability for the component. If enough such data are not available, it may be necessary to obtain further information by expert knowledge elicitation

It is not clear that sufficient data are currently available with which to undertake the full calibration process. Mesocosm data are available for only a few substances. It is anticipated that earthworm field-study data exist for a sufficient number of substances, together with matching tier 1 effects data, to model statistically the relationship between earthworm tier 1 and field studies. It may be possible to fill some data gaps. If sufficient data are not available, it may be necessary to obtain further information by expert-knowledge elicitation but it would be preferable to have more data, especially mesocosm data for many more substances.

The trained statistical model could then be used to make a probabilistic quantification of uncertainty, for any battery of tests and choice of statistic to compute from the test outcomes, about the ratio of lower tier outcome to acceptable concentration in field study or mesocosm for a new substance. In principle, a suitable statistical model could also be extended to take into account any knowledge about chemical properties, mode of action, and pathways to effects of the substance being assessed, which is judged to be informative about the effects to be expected, for example.

7.5.3. Calibration of lower tier assessments against population modelling

In order to make this calibration when developing the guidance, it will be necessary first to have decided exactly what criteria would be applied to the data from population model(s) in order to assess whether or not the relevant SPGs were achieved (or whether or not they were achieved to a sufficient degree). Uncertainties relating to the population model(s) themselves and that are not substance-specific should be addressed at this stage.

The outcome of the population model(s) would be dependent on parameter values used for toxicity and other chemical properties in combination with the relevant use and environmental scenarios (the regulatory scenario (EFSA PPR Panel, 2014b)). Provided that the model(s) were easy enough to run, it should in principle be possible to establish the highest toxicity input that would lead to acceptable outcomes for any particular substance. Uncertainty about the relationship between the outcomes of lower-tier tests and the toxicity parameters needed by the model should be addressed, either by a fixed assessment factor or by a probability distribution for uncertainty.

For a lower-tier assessment, it would not usually be possible to run the model(s). Then it would be necessary to have a way to determine a lower bound for the concentration that would have resulted from modelling. A computer-simulation study exploring dependence of outcomes from the population model(s) on toxicity and other chemical parameters could be used to determine an assessment factor to be applied to lower-tier test results. The assessment factor might need to be dependent on some chemical parameters, either by table look-up or formula. The simulation study would need to be for a

large number of configurations of the parameters and the configurations would need to be chosen by applying standard principles for design of such computer experiments.

7.5.4. Addressing uncertainties affecting the surrogate reference tier (SRT)

As well as addressing the calibration problems as described in sections 7.5.2 and 7.5.3, an assessment factor can also be used to address additional uncertainties that relate to the SRT and that are anticipated at the time when the assessment factor is being determined. For example, these might include known weaknesses of field study/mesocosm protocols. In principle, these might be addressed when determining the criteria for evaluating the acceptability of field study/mesocosm outcomes. For example, if those outcomes were subject to significant statistical uncertainty, one might choose to use an upper confidence limit for effects if such a limit was available. However, this would be analogous to choosing an AF for this particular source of uncertainty and it might be preferable to address the problem by adding a suitable component to the statistical model used for calibration described in section 7.5.2. For the example given, one would include an inter-study component in the statistical model.

7.5.5. Uncertainties specific to a particular assessment

In principle, uncertainties common to all assessments should have been addressed by sections 7.5.2, 7.5.3 and 7.5.4. There may exist additional uncertainties for any particular assessment, and provision should be made in the guidance for addressing them. Consideration should also be given to providing, in the guidance, a checklist of recognised, possible additional uncertainties. The checklist would make it easier for assessors to evaluate the need to address additional uncertainties of this kind.

7.6. Linking Exposure and effects

7.6.1. The criss-cross model

EFSA PPR Panel, (2010a) described the framework for the risk assessment for aquatic and terrestrial organisms. The risk assessment requires two parallel, tiered flow charts, one for the effect assessment and one for the exposure in the field. Considering in more detail the interactions between the flow charts for field exposure and effects, there are only arrows from field-exposure to effect tiers (Figure 12). EFSA PPR Panel, (2012) mentioned that all options for delivery of field-exposure assessments to effect tiers are possible (called the ‘criss-cross’ model).

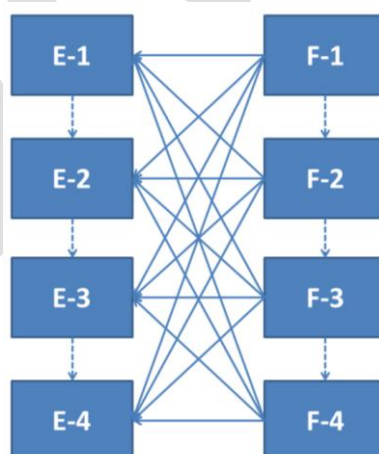


Figure 12: Flow charts for possible routes through the combined effect and field-exposure. The boxes E-1 to E-4 are four effect tiers and the boxes F-1 to F-4 are four tiers for assessment of exposure in the field (‘F’ from ‘field’). Dashed arrows indicate movement to a higher tier. Arrows from right to left indicate delivery of field-exposure estimates for comparison with effect concentrations in the effect flow chart.

The criss-cross model works well for species with a limited mobility at the landscape level such as in-soil organisms. Some species, such as carabid beetles, however, live on the soil surface at the adult stage but become soil dwelling as larvae. For adult life stages of those species, landscape-level approaches are proposed for higher tiers of the risk assessment in the Opinion of the PPR Panel on Non-target Arthropods (EFSA PPR Panel, 2015a). Such a higher-tier assessment would involve simulating the intended use (i.e. good agricultural practice, GAP) at the relevant spatial scale for in-soil organisms and including fate and exposure, as well as the ecotoxicological and ecological data as directly simulated components (Topping and Odderskaer, 2004; Topping et al., 2015). Lower-tier exposure assessments are typically based on a single realistic worst-case scenario (EFSA, 2010; EFSA PPR Panel, 2010b; 2012). It is obvious that such lower-tier exposure scenarios cannot be used in combination with landscape-level approaches, since such scenarios are not spatially explicit (EFSA PPR Panel, 2014b). If modelling approaches were to include TK/TD modelling to take into account vertical movement of PPPs in soil as suggested in section 9.9.3, then this criss-cross concept would similarly be invalidated. It has to be noted, however, that there could be some limitations in the applicability of the criss-cross model. Therefore, it needs to be further elaborated during the development of the in-soil guidance.

Figure 13 shows in detail how the interaction between exposure and effect assessment theoretically works for an arbitrary combination of an effect and a field-exposure tier (by zooming in on an arbitrary combination of an effect and field-exposure tier from Figure 12). The standard procedure in soil ecotoxicological experiments is to use a range of concentrations to derive a concentration–response relationship. Toxicity endpoints within effect tiers have to be expressed in terms of the same type ERC as the endpoints of the field-exposure tiers. This implies that there are two equally important types of exposure assessments required for the risk-assessment procedure. The first assessment (in the field-exposure box in Figure 13) involves estimating the exposure (in terms of a certain type of ERC) that will occur *in the field* resulting from the use of the PPP in agriculture. This is part of the field-exposure flow chart (see EFSA PPR Panel, 2012 and Chapter 8 for details) and is referred to as the Predicted Environmental Concentration. The second exposure assessment (in the effect box in Figure 13) is a characterisation of the exposure (defined in terms of the same type of ERC) to which the organisms were exposed in the ecotoxicological experiments. This second exposure assessment is part of all tiers in the effect flow chart and is the metric that is used to express the effect (the “C” in the NOEC or EC_x estimate).

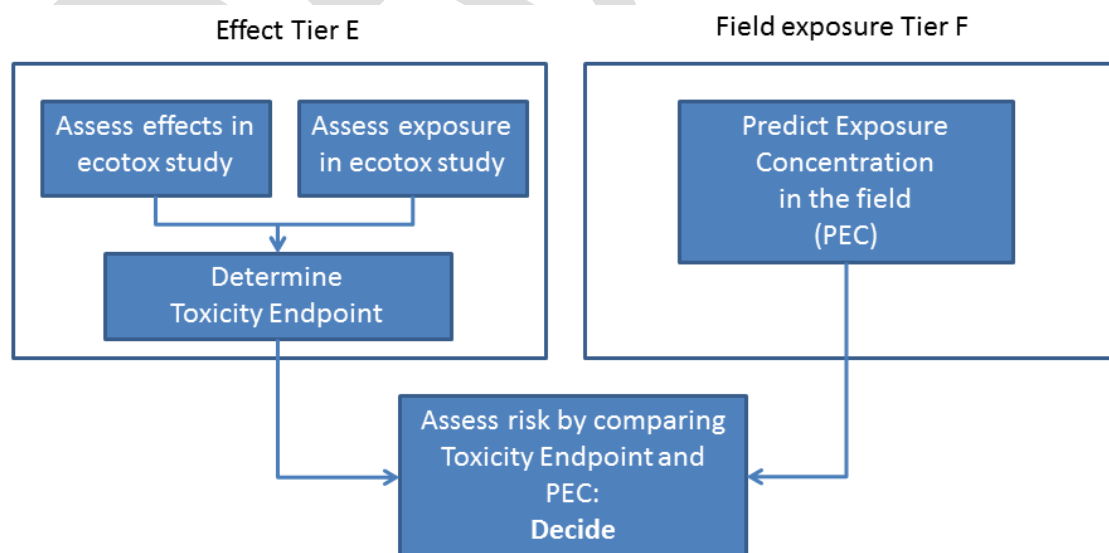


Figure 13: Schematic representation of the two types of exposure assessments that are needed in any combination of tiers of the effect and field-exposure flow charts (adapted from Boesten et al., 2007).

7.6.2. Exposure routes of key drivers

In-soil organisms are exposed to PPPs via a variety of pathways. This is modulated mainly by their morphology (e.g. their body form or the structure of the epidermis), physiology (e.g. the way they take up water, food and oxygen) and behaviour (where they live and move in soil) (Peijnenburg et al., 2012). Moreover, these pathways may vary during the life cycles of some species. The relative relevance of these uptake routes for the body burdens are also dependent on the properties of the chemical (e.g. hydrophobicity) and environmental conditions like soil properties and climate.

The major uptake routes considered for soil organism are:

- Contact with soil, soil pore-water and litter (so diffusion into the body via the ‘skin’);
- Ingestion of food (soil organic matter, litter, bacteria, fungi, prey), of soil particles and soil water;

A usual distinction is made between the so called “soft-bodied” and the “hard-bodied” organisms. The former include earthworms, enchytraeids, nematodes, some collembola and insect larvae, whereas the latter are composed by collembolans living in the upper soil profile, mites, insects and the epigeic detritivores - isopods and millipedes - and predators like spiders and centipedes.

For “soft-bodied” organisms, where water and oxygen is taken up mainly via the skin, soil pore-water is considered the most important uptake route for chemicals (Belfroid et al., 1994; EFSA, 2009c; De Silva et al., 2010, Šmídová et al., 2014; Diez-Ortiz et al., 2015). Ingestion of contaminated food and soil particles and the subsequent absorption of chemicals via the gastrointestinal tract, however, can play a significant role as well (Šmídová et al., 2014, Katagi and Ose, 2015). According to Belfroid et al. (1995), laboratory studies performed with earthworms and a range of PPPs showed that the uptake deviates by a factor lower than two when compared by model predictions based on Equilibrium Partitioning Theory (EPT). However, differences between species with different life-forms should be taken into account. Belfroid et al. (1994) found higher accumulation factors for *Eisenia andrei* (an epigeic species feeding mainly on humic material) than for *Lumbricus terrestris* (an anecic species feeding mainly on plant litter), which could indicate that uptake *via* soil could be of less importance for anecic species. On the other hand, anecics do ingest large amounts of soil when burrowing and looking for food, and evidence of temporary increase of internal concentration of HCB was found in *Lumbricus terrestris* when placed in a new contaminated soil and after the creation of new burrows (Beyer, 1996). Jager et al. (2003) and Katagi and Ose (2015) present two-compartment models for earthworms, including the adsorption of contaminants from the food *via* the gut. Jager et al. (2003) points to the fact that the contribution of the gut route increased with increasing hydrophobicity of the chemical. For the tested substance with highest Kow-values, the gut route clearly dominated. Moreover, measured concentrations in the worms exceed equilibrium with the soil and bioconcentration increased more with higher Kow than did soil sorption. Interestingly, the rate constant for exchange across the skin in the soil environment is much higher for hydrophobic compounds than an exchange in a ‘water-only’ situation would predict.

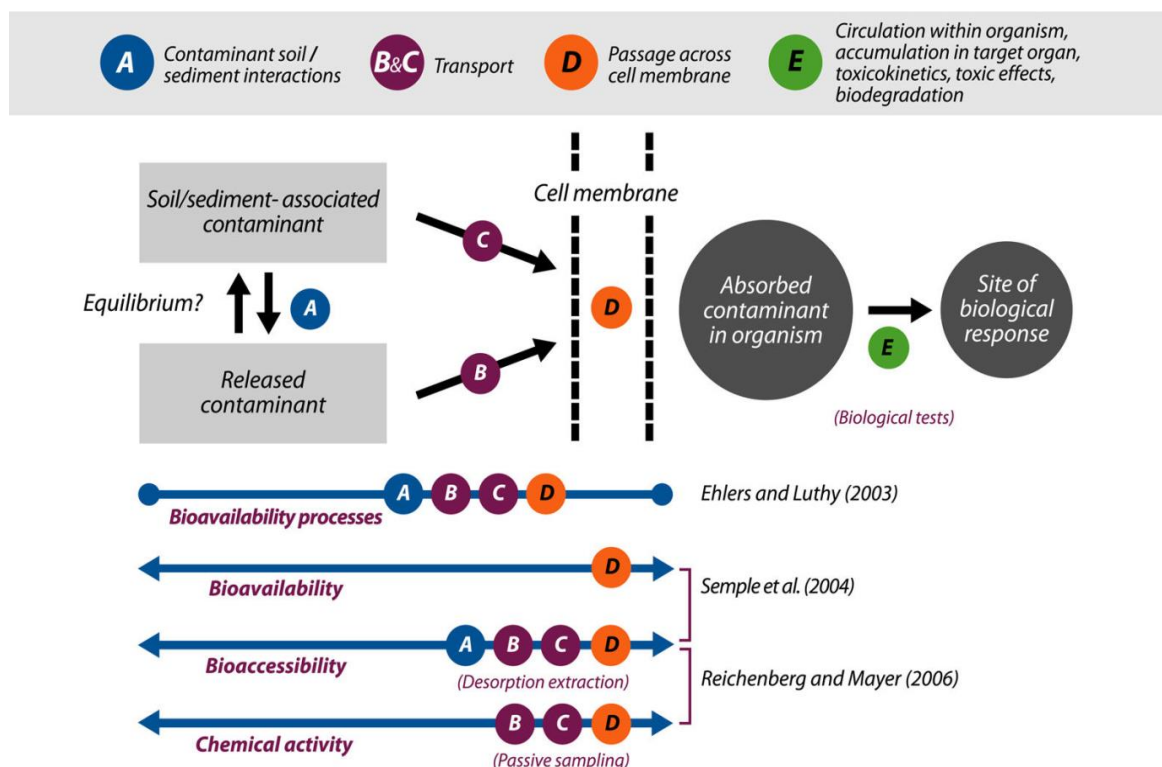


Figure 14: Scientific concepts on the bioavailability of organic chemicals. (from Ortega-Calvo et al. 2015, with permission, citing Ehlers and Luthy, 2003, Semple et al. 2004 and Reichenberg and Mayer 2006). Bioavailability can be examined through chemical activity, the potential of the contaminant for direct transport and interaction with the cell membrane (processes B, C, and D), or bioaccessibility measurements, which incorporate the time-dependent phase exchange of the contaminant between the soil/sediment and the water phase (process A). Depending on biological complexity, the passage of the contaminant molecule across the cell membrane (process D) may represent multiple stages within a given organism before the site of biological response is reached (process E).

“Hard-bodied” organisms take up water and oxygen via special organs. For these organisms ingestion of food is also a relevant exposure pathway. However, as expected, assimilation rates of the different food items play a key role in modulating the actual uptake in the digestive tract, and unfortunately not many studies exist on this topic, especially when dealing with microbivores (mainly collembolan and mites that are fungal and bacterial feeders) and predators (especially predatory mites). “Hard-bodied” organisms can also take up chemicals via contact with soil and/or soil pore-water, as demonstrated for collembolans (Gyldenkaerne and Jorgensen, 2000, Fountain and Hopkin, 2005; EFSA, 2009c) and isopods (e.g. Sousa et al., 2000; Santos et al., 2003). The former authors found that uptake from soil pore-water was the major route for pyrethroids and dimethoate. Regarding isopods, Sousa et al. (2000) reported that internal concentrations of lindane in *Porcellionides pruinosus* exposed *via* soil were 25 times higher than when exposed through food, which were due to different degradation kinetics of the active substance in the two matrices. Santos et al. (2003) found that internal body burdens in the same species and also for lindane were better correlated with concentration in soil extracts than in bulk soil. Similar findings were reported by Belfroid and van Gestel (1999) for slugs, where accumulation of DDT *via* soil was 10 fold higher than *via* food. Nevertheless, uptake *via* feeding on the litter layer should not be excluded as an important exposure pathway, especially for very hydrophobic compounds (Van Brummelen et al., 1996). Furthermore, exposure in the litter layer does not occur only *via* feeding but also *via* contact with the litter and its water-film. The results mentioned above are based on laboratory studies and generalizations to a real field scenario should be made carefully, as

3188 should generalizations to other species, even within the same group of organisms, due to the low
3189 number of species tested.

3190 Taking a pragmatic approach in terms of conducting a risk assessment, a major uptake route for “soft-
3191 bodied” organisms (e.g., earthworms, enchytraeids, soft bodied collembola, nematodes and some
3192 insect larvae) seems to be the uptake from soil pore water. Moreover, the concentration in pore water
3193 is also considered to be the driving factor for uptake and toxicity of pesticides for microorganisms.
3194 However, the relative importance of other uptake routes depends on the properties of the assessed
3195 active substance and will increase with increasing hydrophobicity of the chemical.

3196 Regarding “hard-bodied” organisms (e.g. some collembolans, mites, isopods), although contact with
3197 soil plays a role, evidences of the importance of soil pore water as an important exposure route to
3198 these organisms does also exist. This stresses the importance of considering exposure of in-soil
3199 organisms to pore water as well, particularly for compounds that have high water solubility. The
3200 Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field
3201 surface waters (EFSA PPR Panel, 2015b) states that the freely dissolved fraction in pore-water of
3202 sediment-associated PPPs most likely is the main exposure route for these organisms, although dietary
3203 exposure might also play a role.

3204 Regarding uptake *via* food, namely for those organisms feeding on fungi, bacteria, soil organic matter,
3205 and for predatory organisms, not many data are available and the derivation of robust concentrations in
3206 these matrices is, for the moment, a difficult task. Furthermore, evidence exists that this uptake route
3207 has high relative relevance for compounds with a high K_{ow} .

3208 Particular attention should be given to the litter layer (EFSA PPR Panel, 2010c). Even if the definition
3209 of a ‘proper’ litter layer includes a certain stability over time, dead organic matter as plant debris is
3210 located on the soil surface of most crop systems during the vegetation period and after harvest. Plant
3211 debris constitutes an important food source for anecic earthworms and litter dwelling organisms also
3212 in non-permanent crops, with consequent relevance as an uptake pathway for active substances, and
3213 there is also potential uptake *via* contact with the litter material. This would be more common in
3214 annual crops with low or no tillage and permanent crops. Exposure from the litter should therefore, be
3215 taken into consideration, whether from a litter layer or from plant debris on the soil surface.

Table 18: Exposure routes of key drivers belonging to the in-soil organisms

Group affected		Exposure route	
		via contact	via food
<u>Litter dweller</u>		litter layer / water film	
<i>Feeding type</i>	<i>Key drivers</i>		
litter fragmenters, detritivores	macroarthropods (e.g. isopods, millipeds) gastropods (snails and slugs) non-arthropod invertebrates (e.g. earthworms, enchytraeids)		(fragmented) litter
predators	macroarthropods (e.g. centipeds) microarthropods (e.g. mites)		prey
(micro-) detritivores, grazers, browser	microarthropods (e.g. collembola, mites), nematodes		fragmented litter, fungi & bacteria
decomposers	microorganisms (fungi, bacteria)		(fragmented) litter
<u>Soil dweller</u>		soil and soil pore water	
<i>Feeding type</i>	<i>Key drivers</i>		
(micro-) detritivores, grazers, browser	microarthropods (e.g. collembola, mites), nematodes		dead organic matter, fungi & bacteria
detritivores	non-arthropod invertebrates (e.g. earthworms, enchytraeids)		soil, dead organic matter, fungi & bacteria
decomposers	microorganisms (fungi, bacteria)		dead organic matter
predators	macroarthropods (e.g. beetle larvae) microarthropods (e.g. mites), nematodes		prey
herbivores	invertebrates (e.g. snails, nematodes)		living plants

7.6.3. Temporal and spatial exposure profiles for in-soil organisms

In-soil organisms are exposed to a range of different concentrations in the medium where they dwell, when active substances reach the soil after intended uses of PPP. Exposure gradients for in-soil organisms toward the active substance applied are present since the very first entry time of the a.s. on or in the soil and these gradients evolve through time. One gradient is spatial, related to the vertical distribution of the active substance in the soil profile; the other gradient is temporal, related to the fraction of the active substance that is (bio)available over time.

Since soil animals move (some more, some less) through the soil profile in a circadian/seasonal rhythm that is modulated by light, temperature, humidity and food and predator presence, they are

exposed to different a.s. concentrations over time in the short, medium and long term, ranging from hours to the whole lifespan of the organism. Organisms dwelling in a specific soil layer also experience a temporal gradient of exposure towards the active substance as it dissipates, degrades, is being sorbed and desorbed to the soil matrix or leached deeper in the soil profile through time.

Temporal exposure profiles

In-soil organisms face exposure profiles varying over time. Since concentrations vary over time, it could be considered whether an average exposure concentration (e.g. time weighted average, TWA) might give a good descriptor of observed ecotoxicological effects or not. In order to determine the correct average exposure concentration related to observed effects, several criteria should apply.

- First, observed toxic effects should directly depend on the product of concentration (intensity) and time (duration). This so-called reciprocity concept (Giesy and Graney, 1989) would apply if the toxic outcome in organisms exposed to higher concentrations for short time periods would be similar to the effects observed in organisms exposed to lower concentrations but for longer time spans.
 → Some experimental results with aquatic organisms show a good prediction of chronic effects based on time-weighted average concentrations when extrapolated to other exposure regimes resulting in similar mean concentrations (same 'area under the curve' as integrated exposure concentration). Several study results, however, do not support linear reciprocity assumptions, showing that higher pulsed exposure to an active substance has stronger effects than longer exposure to lower concentrations of the same substance Please refer to EFSA PPR Panel, (2013) for references to specific studies.
 → For in-soil organisms, results of ecotoxicological tests with different exposure regimes varying concentration and time span by keeping the same integrated exposure 'area under the curve' are not known.
- Second, the determination of time to onset of effects (TOE) should be possible, since knowledge of this time span determines the chosen time window over which concentrations are averaged.
 → In tests with in-soil organisms, TOE are commonly not determined. For measurement endpoints like reproduction, studies with differently timed but constant exposure pulsed would be required.

Averaged concentrations over time should *not* be used when the following conditions apply (adapted with specific reference to in-soil organisms from EFSA PPR Panel, (2013); Brock et al (2010)):

- In the assessment of chronic risk, when effect concentrations are to be used resulting from a) tests in which the exposure has not been maintained over time, b) concentration decline of the a.s. in the test system was relatively fast and c) the toxicity estimate has been expressed in terms of nominal or initially measured concentration.
 → These conditions apply almost consistently to all endpoints determined in tests with soil organism, since a) the concentrations are not kept constant throughout the test duration, b) the a.s. might adsorb to different extent to the soil matrix and c) toxicity estimates are often expressed as nominal concentration and - if occasionally measured – to initial concentrations.
- When the effect endpoint in the chronic test is based on a developmental process during a specific-sensitive life-cycle stage and evidence exists that the exposure may occur when the sensitive stage is present
 → For soil fauna, knowledge on sensitive life stages for specific developmental processes is scarce. Exposing juveniles or young adults in test systems designed to detect effects on chronic endpoints (e.g. reproduction, see chapter 9) results most likely in the exposure of sensitive life stages.
- When the effect endpoint in the chronic test is based on mortality occurring early in the test (e.g. in the first 96-hours)

→ does not apply frequently in the risk assessment for in-soil organisms, since current data required for the assessment of effects of active substances or PPP at lower tier are chronic data on reproductive performance. In higher tier studies with (severalfold) intended rates applied, however, this might occur.

- If latency of effects (delayed effects) occurs, resulting from delays in the chain of events between exposure and expression of effects.
→ In experiment with in-soil organisms, the observation of effects at different time intervals (and even beyond the exposure time in a clean environment) to demonstrate that latency does not occur are not performed. Delayed effects resulting from e.g. initial high concentrations cannot be ruled out.

It is concluded from the above that further research and development of test protocols with in-soil organisms is needed to assess whether underlying assumptions for an employment of time-weighted average concentrations might be given. Effect concentrations for in-soil organisms that have been determined in tests with currently standard set-ups do virtually exclude the use of time-weighted average concentrations to describe the exposure that has elicited the observed endpoints.

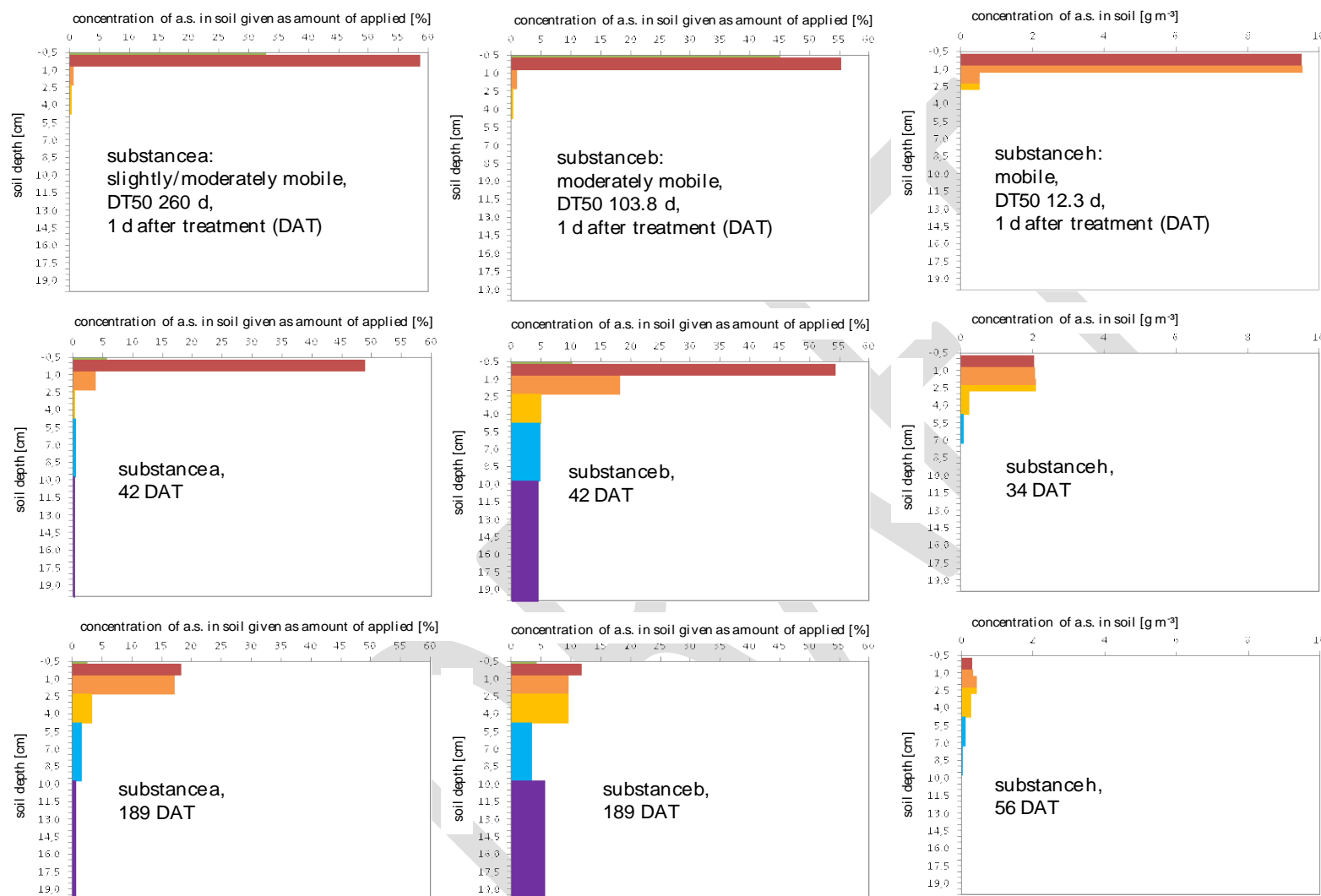
Therefore, maximum concentrations in the relevant matrix (see Table 20) are suggested as best first descriptors for acute and chronic toxic effects elicited in in-soil organisms exposed to active substances or formulated PPP. For very mobile organisms with a depth profile reaching deeper in the soil, the temporal and spatial exposure profiles might be jointly addressed in exposure models that predict degradation, dissipation and movement of the active substance and deliver spatially explicit concentrations profiles over time (please see next section and section 7.7). In order to compare the outcome of different tests – especially laboratory tests with field tests – the comparability of the time course of the concentrations in the different soils is essential. However, degradation, dissipation and movement of active substance in the field soils will differ from the degradation/dissipation in a standard artificial soil (please refer to section 7.7).

Spatial exposure profiles

Vertical distribution of the active substance

The highest concentration of an active substance that is sprayed and/ or reaches the soil *via* drift or run-off from the treated field is generally found shortly after application and in the uppermost soil or litter layer. For PPP that enter the soil as granules or on treated seeds or *via* dripping in deeper soil profiles, highest concentrations are to be found in the relevant application depth (see EC, 2012).

Several field and semi-field studies in which fate and behaviour of PPP active substances were tightly traced over a season or a year at very high spatial resolution have been reviewed. These studies provide vertical concentration gradients of the compounds in soil horizons of one or few centimeter thickness (Anderson et al., 2010; Fent et al. 1999; Possberg et al., 2014; Toschki et al., 2014, 2015; Egerer et al., 2015). The distributions in the soil profile of substances with different physico-chemical properties and from different experiments are depicted by way of example in Figure 15. The findings of studies available with very high vertical sample resolution over time corroborate the statement that, immediately after entering the soil compartment, the active substance is located with maximum concentrations in the upper soil centimetre (or less), independently of substance properties. The vertical pattern with the highest measured concentration in the uppermost layer may persist over a certain time, despite differences in substances properties (e.g. Boesten 1986, Poßberg 2014, Fent 1999, Evans 2003). The influence of both degradation and sorption properties of the a.s. on the attenuation of the vertical stratification will increase with time.



3320 **Figure 15:** Distribution of three different active substances (a, b, h) in the soil profile over time (modified according to Boesten, 1986, Possberg et al. 2014,
3321 Toschki et al. 2015). The different colored bars indicate the concentrations of a.s. in the different sampled soil depths.

For further details on the distribution of active substances in the soil profile, see also Egerer et al., (2015). If the concentration in the upper soil layer is high enough to elicit acute or chronic effects, a relevant share of the in-soil organisms community will be exposed to it, either because they live principally in the upper centimeters or because of vertical movement of in-soil organisms or a combination of both. In the field, lateral and/or vertical transport of contaminated water might also occur. If the concentration in the upper soil layer is so low that only a longer permanence of the organisms there would let internal concentrations reach levels with adverse outcome, then the behaviour and the individual spatial range of the organisms in the soil profile would be of importance for the expression of toxicity. The following paragraph will clarify these issues.

Functional groups and vertical distribution

Vertical gradients of matter and energy are manifold in the soil compartments. Nutrients, light and water are unevenly distributed. Often, these gradients correlate, with high input channels entering the soil from the soil surface, also from above-ground ecosystem compartments. In-soil organisms are adapted to these environments, shape it with their own activity and coexist *via* niche differentiation in strongly structured space with unexpected high species diversity. Specific adaptations to the different environments in the soil profile have led to different, more or less typical, life-form types, which are often roughly categorized as above-ground/surface dwellers (epigeic/epiedaphic species), mixed surface and soil dwellers (hemiedaphic species), soil dwellers (endogeic/euedaphic species) and ‘migratory’ species (anecic worms) that feed on the soil surface and dwell in the soil. These categories are however only indicative. They describe typical trait assemblages and preferences for some soil environments, but do not imply that species belonging to different groups as defined above will be only to be found in their ‘preferred’ soil layer, if any is known.

For microarthropods, species with different life-form traits can be found more or less stratified in soil, depending on the degree of pronounced differences between soil horizons. But even in soil profiles with a distinct litter layer, soil and litter-dwelling species change their mean depth during different seasons. Also the distribution of individuals belonging to one species between different depths (‘depth deviation’ *sensu* Usher 1970) will be narrow in some seasons and more even in others, reaching over the whole accessible profile. Usher (1970) has investigated the seasonal and vertical distribution of Collembola species and found numerous combinations of species with different mean depths and depth deviations. These distributions will change with seasons and change also during lifetime, since juveniles show distinct pattern compared to adults. In contrast to Usher (1970), Detsis (2000) analyzed the vertical distribution of collembolan in southern climates. Also here the majority of animals was found in the upper soil layer when the climatic conditions were favourable. In dry summer periods, a vertical migration of all species was observed, and ‘only minute differences, if any, were observed in the vertical distribution pattern of the most abundant species, irrespectively of the life form they belong to’ (Detsis, 2000). Similar patterns have been observed for oribatid mites (e.g. Mitchell 1978), where different species were predominant in different (micro-)horizons. Detailed analyses showed also for this group that unique and also more even vertical distribution patterns varied in time. As for Collembola, juvenile mites have vertical distributions that are different from the adult forms: being more vulnerable, the juveniles react more strongly and therefore earlier in the different seasons to unfavourable, but also to favourable conditions.

Even though specialized life forms might therefore typify successive layers, there is a considerable vertical migration in most soils (e.g. Lange et al., 2012). Next to the yearly phenology of species’ distribution patterns, short term changes in critical factors such as moisture regime or food supply can also initiate rather quick vertical migration.

Berthet (1964) and Wallwork (1970) found that so-called hemiedaphic oribatid species would regularly move into and from the epigeic zone, driven by the actual humidity fluctuations. One of the most striking migrations patterns in soils is shown by desert organisms (Whitford et al., 1980, Wallwork, 1970), which display an ephemeral burst of surface activity in the dew-moist morning hours, contributing significantly to litter degradation in the dry season, and then within one hour are

back again in deeper soil layers. Such vertical migratory movements have been experimentally induced by drying-rewetting experiments with e.g. oribatid mites (Metz 1970).

Hassal et al. (1986) showed experimentally that these vertical migrations are not only a consequence of a better accessibility of former unfavourable environments but are directed movements, induced by new food sources. In this work, more than 30% of the population of the studied onychiurid collembola moved to the very top surface within half a day – but only if palatable food was offered. According to several authors, vertical migration is a way of maintaining a balance between the possible higher mortality in upper layers (drought, predation) and reduced reproductive output resulting from less favourable feeding conditions in the lower layers (Bengtsson et al., 1991). This trade-off might even attract microarthropods of deeper layers to light when becoming increasingly starved (Dromph 2003).

Summarising, changes in humidity and temperature alter the vertical distribution of soil animal species (Krab et al., 2010 and 2015), also in the very short term, often independently from defined life form types. The ecological plasticity can be used by species to respond to soil conditions (Edwards 2004). The needs of in-soil organisms and their perception of the soil matrix will change during their lifetime. While e.g. neonates macrofauna might depend on water films and have a restricted mobility to existing pores, their role in shaping the soil environment will change with age (Demon and Eijsackers 1985). Therefore, vertical movements especially directed to the soil upper layers should be accounted for when characterizing the ecotoxicologically relevant type of exposure concentration for in-soil organisms.

Functional groups of in-soil organisms in agricultural fields

The soil biocoenosis of treated areas in agricultural fields resembles for a variety of faunal groups a more or less impoverished grassland biocoenosis in terms of species diversity and individual densities (e.g. Römbke et al., 2010, 2012). Given distressing environmental conditions at the soil surface over several seasons (e.g. drought in some Mediterranean areas), anecic oligochaete key drivers might not be present or be replaced by more robust organisms (EFSA PPR Panel, 2010d). In general, however, the upper centimetres of the soil represent the principal habitat for invertebrate organisms in agricultural fields. Römbke et al., (2010) have analyzed the dominance of different earthworm ecological groups in grassland and crop sites in Central Europe, reporting a very similar relative group distribution. For collembola, species numbers and dominance of different ecological groups in grasslands and in crops are reported in Table 19. Epigeic and euedaphic species show comparable shares in the in-soil organisms' community of arable and grassland sites. Also Enchytraeids in agricultural fields might display similar but species-impovertised communities compared with grassland sites (Figure 16).

In summary, unless environmental conditions are very harsh, which may apply only to a relatively small area in Europe that is devoted to agriculture, and/or to a seasonal phenomenon, epigeic, anecic and hemiedaphic species are regularly present in agricultural fields, inhabiting the uppermost centimetres of the soil profile as well as microorganisms and fulfilling important ecosystem services (see chapter 5).

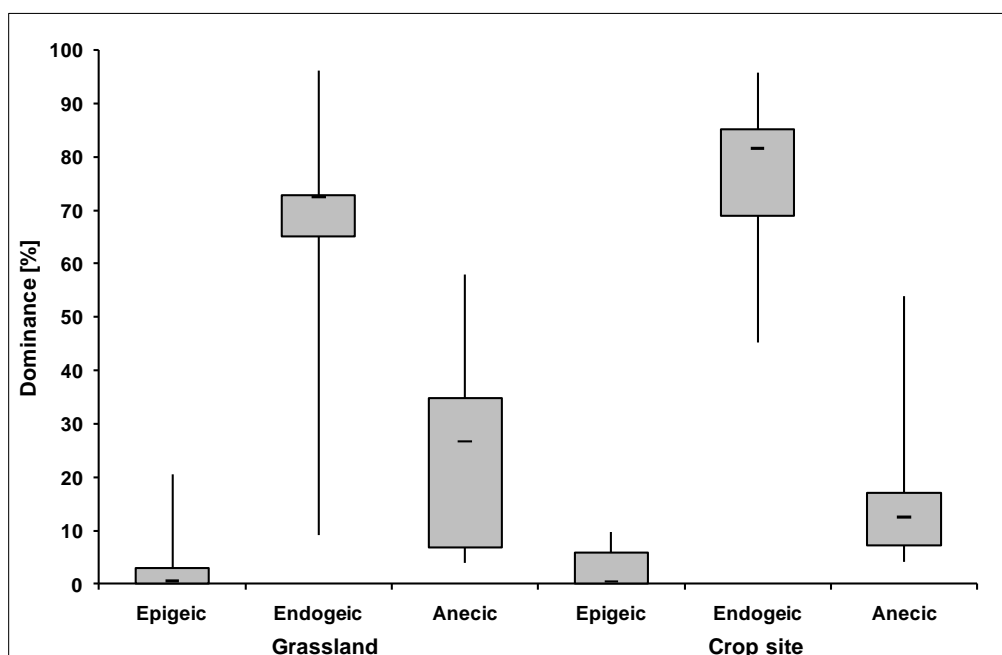


Figure 16: Dominance of the three ecological groups of earthworms at grassland and crop sites in Central Europe (Römbke et al., 2010)

Table 19: Average values (and limits) for the number and percentage of Collembola species in each life-form class for crop and grassland areas at Central European sites (adapted from Römbke et al., 2010)

	Euedaphic species fast dispersal species, living in soil surface	Hemiedaphic species medium dispersal species, living down to 2.5cm layer	Epigeic species species with very low dispersal ability, living down to 5cm layer
Crop areas (N=12)			
Species (N)	5 (1 – 14)	11 (0 – 24)	13 (1 – 29)
Species (%)	24 (9 – 50)	34 (0 – 67)	42 (17 - 57)
Grasslands (N=8)			
Species (N)	5 (0 – 15)	8 (2 – 16)	11 (4 – 24)
Species (%)	15 (0 – 31)	34 (24 – 50)	51 (44 - 71)

Vertical spatial heterogeneity and toxicity

As shown in the previous paragraph, in-soil organisms for which a preference for slightly deeper soil horizons is known (e.g. endogeic worms, euedaphic collembolan) might also be exposed to active substances located in the upper soil centimetres, resulting from vertical movements in the soil profile e.g. in the search of food or moisture after raining events.

The movements of endogeic earthworms are shown by way of example in the figure 17 below (from Capowiez et al., 2006; *Allolobophora icterica*, b). While the anecic earthworm species *Aporrectodea nocturna* (a) burrows permanent tunnels that often open to the soil surface, the endogeic species (b) processes a wider amount of soil in shallow and deeper layers. With increasing PPP concentration, the activity decreases and reduces to the topsoil.

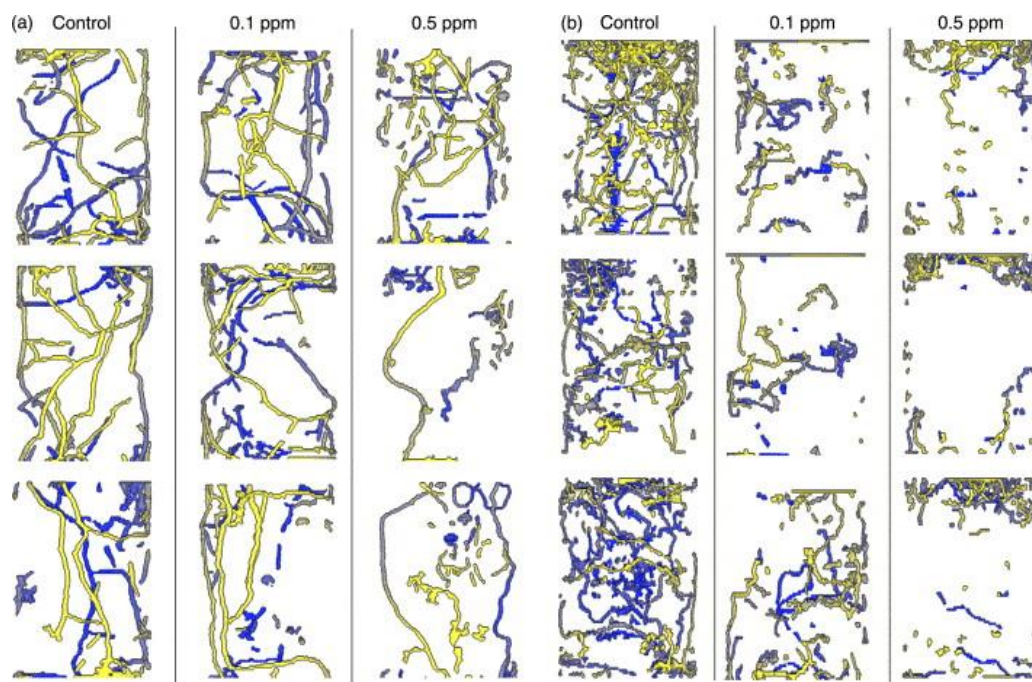


Figure 17: 3D-Reconstructions of the burrow systems made different earthworm species and increasing imidacloprid concentrations. Colours range from light to dark according to the distance from the point of observation. (a) *Aporrectodea nocturna*, anecic and (b) *Allolobophora icterica*, endogeic. From Capowiez et al. 2006, with permission

Differences in behaviour between species (e.g. the amount of burrowing) may affect exposure to soil-incorporated chemicals and surface foraging to surface applications (Curl et al. 1987, in Edwards and Coulson, 1992). The endogeic *Aporrectodea caliginosa* and the anecic *Lumbricus terrestris* were exposed to the active substance cypermethrin incorporated in the soil. Bioconcentration factors for *A. caliginosa* and *L. terrestris* were x30 and x8, respectively. This pattern is consistent with their behaviour, since *L. terrestris* has permanent burrows and forages on the soil surface, while *A. caliginosa* burrows through the soil when condition are suitable and ‘digests’ the ingested soil matrix.

Such observations in the laboratory were also supported by several field studies. The effects of a strobilurin fungicide, which is highly toxic to earthworm, illustrate especially the importance of considering both the distribution of the toxic compound in the soil profile and the behaviour of different species. The compound is located after spraying in the first centimetre of the soil and persists there for a considerable time without vertical movement (Evans 2003 analytical report). On the one hand, the mean compound concentration for the investigated substance calculated for a soil-horizon thickness of 5 cm was by far not sufficiently high to explain the observed mortality effects on earthworms. Exposure to the active substance and effects on soil organisms matched only if smaller horizon increments (1 cm) were considered for the exposure calculation (Evans, 2003). On the other hand, high mortalities of *L. terrestris* and of juvenile worms of several species were detected in the short term after irrigation, when the earthworms came to the soil surface. In the mid-term, however, the endogeic worm *A. caliginosa* showed the highest effects after a few weeks.

In an experiment with the insecticide dimethoate, Krogh (1995) set up a series of microcosms with different structured microhabitat and food-supply regimes for the collembolan *Folsomia candida*. Two of the six variants are of special interest in this context, for they addressed the question of whether an uncontaminated soil layer beneath a contaminated one reduces the observed effects on collembolan reproduction. The toxic effects of the active substance were almost identical in the two variants, leading the author to the conclusion that the collembolan species is not able to avoid dimethoate and

that “[...] *F. candida* prefers eating in a contaminated soil instead of starving in an uncontaminated zone without food”.

Prinzing et al. (2002) investigated whether tolerance of disturbance in oribatid mites correlates with species traits. Two of the hypotheses tested, which stated that high tolerance to a single application of diflubenzuron was to be expected in species (a) ‘with short generation time, because they can recover quickly after the disturbance’; and (b) ‘which feed on fresh macrophyte detritus, because it was less altered by the disturbance than fungal microphytes’, were corroborated in the study. A third hypothesis, stating that high tolerance would be found in species (3) ‘which prefer the topsoil because they are less exposed to the disturbance than species that prefer the litter layer’ could not be confirmed. Contrary to expectations, also of van Straalen and Løkke (1997), the disturbance tolerance of species did not correlate with their preference for the soil layer, even if the total diflubenzuron concentrations in the soil layer was 60 times lower than in the litter.

The experiments above illustrate the difficulties that in-soil organisms might encounter when it comes to avoiding exposure to an applied active substance. Pelosi et al. (2014, and references therein) describe the usefulness of avoidance responses of in-soil organisms to contaminated soil, which can be tested according to agreed standards (ISO 17512-1, 2008). There are, however, several examples demonstrating that this ability should not be taken as given and might be specific for organisms/substance combinations (e.g. Hodge et al., 2000; Prinzing et al., 2002; Krogh, 1995).

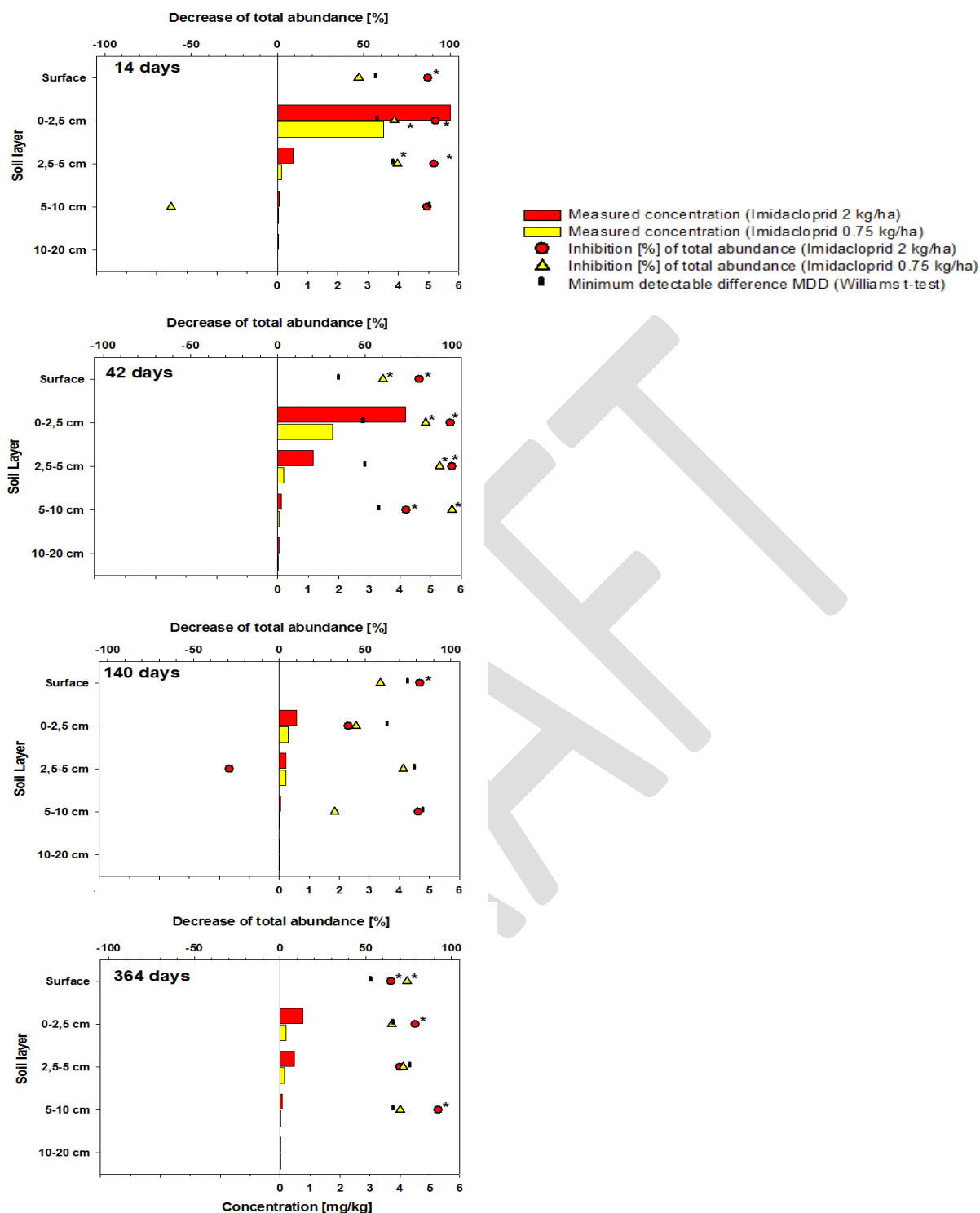
In Scholz-Starke (2016) an evaluation of chronic studies submitted to authorities for product authorization performed with *Eisenia fetida* and PPPs in artificial soil is presented. The working hypothesis was that mixing the substance to be assessed into the soil would lead to significantly lower effect concentrations than applying it on the soil surface, since the earthworms would dwell in the contaminated matrix. Interestingly, when substances were not specifically differentiated, test designs with sprayed chemicals onto the soil surface delivered significantly lower effect concentrations than the tests with substances mixed into the soil. When pairs of tests with the same substance were compared with each other, then the effect concentrations were similar or lower in the test variants with sprayed application compared to mixed application. Since the test protocol requests weekly feeding of the earthworms on the soil surface, the animals in the test system with sprayed substances were forced to pass through the uppermost soil layer, which contained the highest proportion of active substance.

In semi-field terrestrial model ecosystems (TME), Toschki et al. (2014, 2015) investigated the distribution of three active substances with different properties (imidacloprid, lindane and carbendazim) applied to replicate soil monoliths in two different concentrations. A second set of experiments was run in the laboratory, also with soil monoliths from the same site but with ¹⁴C labelled substances. Here, depth increments of 1 cm could be analyzed, which was not feasible for all soil faunal samples in the outdoor mesocosms without losses in the statistical power of the assay. Analyses of the applied chemicals in high spatial resolution showed that, as expected, the largest proportion of the active substances were located in the upper soil centimetre of the soil profile during approximately the first three months after application.

The effects of the applied substances on earthworms, enchytraeids, collembola and oribatid mites were also monitored over time with vertical differentiated sampling. A subset of the reported results is depicted in figures 18. Shortly after application (14 days), effects e.g. on earthworms in the carbendazim treatments or on Collembola in the imidacloprid treatment reached deep in the soil profile, where the respective compound could not be detected. Interestingly, organisms that are known to prefer deeper soil horizons were also affected shortly after application. During the time course of the experiment, effects on in-soil organisms were found at depths where total concentrations detected were not high enough at any sampling date to explain the observed effects. The experiments were designed with dosages high enough to elicit effects on different groups of organisms with high certainty. Even if the total concentrations detected often matched best the observed effects when related to the soil horizon thickness in which they were measured, not all comparisons of exposure and effect concentrations in the uppermost layer could be performed, since cases had to be excluded in which the concentrations were far above the medium effect concentration for short time exposure,

In the TME experiments described by Toschki et al. (2014; 2015), total concentrations were assessed according to current practice and measurement of pore water concentration was not performed. The distribution of the three chemicals investigated showed pronounced vertical gradients and the information value of averaged concentrations over the soil monoliths was low compared with the actually measured total high concentrations in the top layer. Since the observed direct effect on organism's survival result from exposure to the substances applied, the movement of soil animals towards the uppermost soil layer is most likely. Some anecic worms were included in the soil monoliths and therefore biopores open to the soil surface might have been present, increasing the possibility of preferential flow reaching lower layers. However, no unexpected high increase of substance residues over time was observed either in lower soil layers or in the leachate outflow. In any case, the preferential flow loading would be driven by the high concentrations in the upper layer and not by an averaged concentration over all depths.

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3523 **Figure 18:** Decrease of total abundance of Collembolan species in the imidacloprid-treatments 0.75
 3524 kg a.s./ha and 2.0 kg a.s. /ha (5 replicates each) for the different soil layers in
 3525 comparison to the control (10 replicates). Columns show the measured total
 3526 concentration for the two treatment concentrations at the respective sampling date. *:
 3527 significant difference according to Williams t-test; bars showing the minimum
 3528 detectable difference (MDD) as value for the specific possible statistical resolution.
 3529 MDD values higher than 100 % are not shown.

7.7. Ecotoxicologically Relevant Concentrations

Table 18 lists the major exposure routes of key drivers, specifically for each organism group. For the single exposure routes, ecotoxicologically relevant types of concentrations (ERC) are listed in Table 20 below. The proposed ERC are based on the knowledge about the major exposure routes for in-soil organisms and the considerations about the temporal and spatial exposure profiles the organisms are exposed to.

From the data referred to above, it emerges that in-soil organisms inhabiting the upper soil layer or feeding there will be exposed to the highest concentration of the applied PPP after spraying. For these organism groups, the possible uncontaminated layers below the uppermost one do not deliver a definite shelter, since other vertical gradients - e.g. of food and water - attract the animals to the surface and in-soil organisms are not able to detect and to avoid all active substances.

Averaging of exposure between high concentrations in the top layer and the low concentrations in deeper soil layers would deliver a 'space weighted average', which, as the 'time weighted average' concentration, could be used only under specific preconditions (e.g. reciprocity) and only for specific measurement endpoints (see chapter 7.6.3 for details).

Organisms with a greater mobility in the soil and having a geophagous feeding mode, though, will also dwell frequently in the uppermost soil layer, but not necessarily continuously and for longer periods. If the concentrations in the uppermost soil layers are sufficiently high, then a short permanence there will also elicit acute or chronic effects. If the concentrations in the uppermost soil layer are such that only a long permanence there would lead to acute or chronic effects, then moving to uncontaminated deeper layer and feeding on uncontaminated matter would decrease the animal chemical burden. For these organisms, no single soil depth, or in many cases type of exposure, would be relevant. The choice of one, single ecotoxicologically relevant type of concentration could possibly deliver unreliable exposure metrics. To deal with this situation, exposure and effects need to be integrated over time. This could be achieved by linking following components:

- Reliable models of movement for endogeic earthworms, within the soil profile
- Dynamic models of exposure providing soil and pore-water concentrations at all relevant soil depths and varying with time. Ideally, these would be linked to the systems models proposed for population assessment (see chapter 7.3).
- Toxicokinetic-toxicodynamic models capable of integrating both internal concentrations and toxicological effects with time (see section 9.9).

Currently there is no available systems model combining all three components, although technically this is considered to be feasible. Ideally, this combined system would be included in the systems model used to develop the population-modelling 'surrogate reference tier' (see section 7.5.3).

Since both exposure routes *via* total soil or pore-water concentration are considered to be relevant, and will have different relative importance for different substances and species, it is recommended to assess both exposure routes for in-soil organisms (see table 18). Further research should clarify which exposure metrics are pertinent in different soil type/species/substance contexts.

3573 **Table 20:** Ecotoxicologically relevant concentrations for in-soil organisms exposed to active
3574 substances in PPPs via different exposure routes.

Exposure route	Reciprocity is not demonstrated and adverse outcome can be elicited by initial concentrations	Reciprocity is demonstrated and adverse outcome due to delayed effects of initial concentrations is excluded	Adverse outcome can be related to the (time course of) internal concentrations modulated by behaviour
Contact soil microarthropods, nematodes, epigeic and anecic earthworms, soil gastropods, microorganisms	Maximum concentration in upper soil centimeters including accumulation (mg/kg soil dw)	Time weighted average concentration in upper soil centimeters including accumulation (mg/kg soil dw) – time frame depends on endpoint	-/-*
Contact soil endogeic earthworms	Maximum concentration in upper soil centimeters including accumulation (mg/kg soil dw)	Time and space weighted average concentrations including accumulation (mg/kg soil dw) – time frame depends on endpoint – spatial resolution depends on vertical distribution of species	Spatially explicit concentrations at different time points matching the resolution of measured/modeled internal concentrations (mg/kg soil dw)
Contact pore water microarthropods, nematodes, epigeic and anecic earthworms, soil gastropods, microorganisms	Maximum concentration in upper soil centimeters including accumulation (µg/L)	Time weighted average concentration in upper soil centimeters including accumulation (µg/L) – time frame depends on endpoint	-/-
Contact pore water endogeic earthworms	Maximum concentration in upper soil centimeters including accumulation (µg/L)	Time and space weighted average concentrations including accumulation (µg/L) – time frame depends on endpoint – spatial resolution depends on vertical distribution of species	Spatially explicit concentrations at different time points matching the resolution of measured/ modeled internal concentrations (µg/L)
Oral soil organic matter microarthropods, nematodes, soil gastropods, microorganisms	Maximum initial concentration in upper soil centimeters including accumulation (mg/kg OM/soil dw)	Time weighted average concentration in upper soil centimeters including accumulation (mg/kg OM/soil dw) – time frame depends on endpoint	-/-
Oral soil organic matter endogeic earthworms	Maximum initial concentration in upper soil centimeters including accumulation (mg/kg OM/soil dw)	Time and space weighted average concentration including accumulation (mg/kg OM/soil dw) – time frame depends on endpoint – spatial resolution depends on vertical distribution of species	Spatially explicit concentrations at different time points matching the resolution of measured/modeled internal concentrations (mg/kg OM/soil dw)
Contact litter layer litter dwelling organisms and epigeic and anecic earthworms	Maximum initial concentration in the litter layer including accumulation (mg/kg litter dw)	Time weighted average concentration in the litter layer including accumulation (mg/kg litter dw) – time frame depends on endpoint	-/-

Oral litter layer litter dwelling organisms and epigeic and anecic earthworms	Maximum initial concentration in the litter layer including accumulation (mg/kg litter dw)	Time weighted average concentration in the litter layer including accumulation (mg/kg litter dw) time frame depends on endpoint	-/-
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*-/-: The relevance of the impact of vertical movements for soil organisms living in the upper centimetres is deemed to be low

7.7.1. Overall assessment of the exposure based on the different routes

As mentioned above, exposure by contact and oral uptake are both relevant. These two exposure routes require different exposure concentrations because the ecotoxicologically relevant concentration is different (Table 20). Developing a worst-case exposure scenario that considers both routes would require a model that integrates both exposure routes, e.g. a TK/TD model (see also EFSA PPR Panel 2014b for deriving effects based environmental scenarios). Since such models are not yet available for regulatory purposes at the European level, it is not possible to assess the relative contributions of individual exposure routes to effects. Measured effects result from the combined exposure of both routes. Extrapolation from lower tier to the field situation is done by assessment factors (Section 7.5.2) which address uncertainties: this includes uncertainty about the relative contributions of different routes of exposure.

Currently available exposure data in test systems probably do not distinguish between the various exposure routes and the results are then to be seen as lumped over two routes (contact *via* pore water and *via* bulk soil and oral uptake). Available data should therefore be carefully checked and attributed to one of the two exposure routes or marked 'lumped'. Appropriate values should be used for estimating exposure according to the two specified routes (see next section).

7.7.2. Using consistent concentrations in the exposure and effects assessment

For the scheme in section 7.6.1 to work, the same type of concentration should be used in the effect assessment and the exposure assessment. The type of concentration depends on the properties of the substance, the organism (e.g. soft-bodied or hard-bodied, see (EFSA, 2009c) and the intake route (Section 7.6.2) and is either the bioavailable concentration in total soil or the concentration in pore water. Both types of concentrations are delivered by the exposure assessment; however, different scenarios are used for the concentration in total soil and for the concentration in pore water (chapter 8).

The most commonly used tests in the effect assessment are the earthworm, collembolan and predatory mite reproduction test (OECD 222, 232 and 226). The OECD guidelines recommend using artificial soil (70% sand, 20% kaolin clay, 10% or 5% coarse ground *Sphagnum* peat and pH adjusted to 6), but give also some indications to the use of natural soils. Artificial soil has the advantage that it is relatively well reproducible (Van Gestel, 1992). However, *Sphagnum* peat has different properties than organic matter in arable soils. The type of the organic matter influences sorption and hence bioavailability (EFSA PPR Panel, 2015c). Therefore, a standardised arable soil with properties closer to the scenarios in the exposure assessment would be preferred over an artificial soil with *Sphagnum* peat. However, developing a new standardised test would require extensive research to assess the suitability of the soil(s) selected to allow the survival and the reproduction of the test organisms in acceptable levels. For this reason, it is assumed that the OECD tests will be run with artificial soils in the nearest future until better alternatives are available and tested. The Panel recommends to investigate the use of feasible, alternative natural soils for standardized test systems and to perform a sensitivity analysis for a comparison of tests performed in natural soils to tests run with artificial soil.

7.7.3. Measuring exposure in test systems

In most test guidelines for in-soil organisms, the tested substance is incorporated into the soil; either in a solution or with sand. Present test systems can be adapted in order to test effects of soil fumigants, treated seeds and granules. In the past, tests were also often performed with the substance applied to

the soil surface. The disadvantage of this application is that it introduces uncertainty about the actual exposure of the in-soil organisms. Mixing though the soil is proposed in almost all recent test guidelines. in order to avoid this uncertainty.

A litter layer is not included in current, first-tier laboratory tests. Exposure *via* the litter layer is, however, a relevant route of exposure for some soil invertebrates, like macroarthropods, slugs and snails. Furthermore, exposure *via* food uptake is not included in the standard laboratory tests, since normally uncontaminated food is provided. This may lead to underestimation of internal exposure due to dilution.

As mentioned above, either the concentration in total soil or the concentration in pore water is to be used for linking exposure and effects. Total concentrations in laboratory tests are currently commonly expressed as nominal concentrations, i.e. the total mass of chemical added to a certain mass amount of dry or wet soil. During laboratory handling procedures of the spiked soils, however, possible losses of the pesticides due to volatilization, degradation, and sorption to e.g. the glass matrix of vessels used, may occur. The Panel therefore recommends that the exposure concentration be measured as a function of time regardless of the metric chosen (see also (EFSA, 2009c). Measuring the concentration increases the certainty about exposure, and could deliver information about formation of metabolites.

Exposure could be measured using the two-step extraction procedure that is proposed in EFSA PPR Panel, (2015c). This consists of a 24-hour extraction with a 0.01 M CaCl_2 solution to characterise the pore-water concentration and a solvent extraction to characterise the total extractable mass (OECD, 2002). In principle, centrifugation would also deliver the pore-water concentration. It was, however, observed that variability was larger and therefore the CaCl_2 extraction is preferred. Instead of the currently used harsh solvent extraction, a mild organic solvent would be preferable because this fraction generally correlates better with ecologically relevant endpoints like uptake in organisms (see Appendix 1 in EFSA, 2009c for an overview). However, no standard protocol is currently available for mild chemical extractions in relation to bioavailability testing (see also EFSA, 2009c; EFSA PPR Panel, 2015c). The Panel therefore recommends developing a protocol for characterising the fraction that is available for uptake *via* pore water.

In order to compare the outcome of different tests – especially laboratory tests with field tests – the comparability of the time course of the concentrations in the different soils is essential. However, degradation, dissipation and movement of active substance in the field soils will differ from the degradation/dissipation in a standard artificial soil. Hence it is recommended that the concentrations of active substances in soils is measured at least twice, depending on the degradation of the active substances and the length of the test. It should be insured that the time course of exposure in the tests covers the predicted exposure profile in the field. The time course of the exposure profile could be use in models that consider toxicokinetics /toxicodynamics (to be developed) to estimate toxicity based on internal body concentrations.

7.7.4. Calculating the exposure concentration in test systems

The Panel does not recommend calculating the pore-water concentration from the concentration in total soil using the partitioning coefficient. As indicated in EFSA PPR Panel, (2012), the partitioning coefficient (K_d) is measured in at least four different soils and the variability of K_d -values between these four soils is generally more than 25%. This would give a high uncertainty of the calculated exposure concentration in the test system. However, in current ecotoxicological studies (legacy studies), only the initial nominal concentration is known. For such legacy studies, the initial pore-water concentration could be calculated when instantaneous sorption equilibrium is assumed and the water content and the sorption coefficient for the test soil are known. This can be done using the equation:

$$c = \frac{M_t}{V_{soil} + M_{soil} m_{OM} K_{OM}} \quad (1)$$

where c (kg/L) is the concentration in pore water, V_{soil} (L) is the total volume of liquid in the system, M_{soil} (kg) is the total mass of soil in the system and M_t (kg) is the total amount of substance applied, m_{om} (kg/kg) is the mass fraction of organic matter in the soil and K_{OM} (L/kg) is the coefficient of equilibrium sorption on organic matter.

The equations above are based on the assumption that sorption is linear. In reality, sorption is non-linear and because the exponent is usually <1 , the concentration in pore water will be overestimated when linear sorption is assumed. Overestimation of the pore-water concentration in the effects study would underestimate toxicity and therefore the Panel recommends using the Freundlich equation for calculating the pore-water concentration. Notice further that the equations above only give the initial concentration of a parent substance. If a time-weighted average concentration is needed, it could be calculated assuming first-order degradation kinetics. For this purpose, the Panel recommends developing a dedicated version of the PERSAM model (EFSA PPR Panel, 2012). This tool could also be helpful for characterising the exposure concentration of metabolites in the test system. Specific attention is needed for parameterisation of this model so that it will lead to conservative estimates of the pore-water concentration (Van der Linden et al., 2008).

7.7.5. Scaling of the toxicity endpoint to account for bioavailability

It is now common practice to divide the obtained toxicity endpoint (LC_{50} or NOEC) of lipophilic substances ($\log(Kow) > 2$) for in-soil organisms (except microorganisms) by a factor of two. This is done because the organic matter content of the artificial substrate of the earthworm laboratory tests is higher than that of many natural soils (Van Gestel, 1992). The scaling factor was needed because concentrations were only available as nominal concentrations (Section 7.7.5), while toxicity endpoints generally correlated better with pore-water concentrations. This was demonstrated because differences between soils almost completely disappeared when LC_{50} values in mg/kg were recalculated towards values in mg/l pore water concentrations (Van Gestel and Ma, 1988, 1990, see also EFSA, 2009c).

Scaling of the toxicity endpoint is no longer necessary (and even not justified) if the appropriate exposure concentration in the test system is either measured (Section 7.7.3) or calculated (section 7.7.4). When only the nominal concentration is available (legacy studies) and when the ERC is expressed in terms of a pore-water concentration, scaling of the toxicity endpoint is justified (Table 20). Notice, however, that the factor of two has no scientific basis for assessments at the European level because it is based on the ratio of the organic matter content of the test medium (10%) and the organic matter content of the old Dutch standard soil (4.7%) and not on that of the proposed exposure scenarios described in Chapter 8. So instead of the default factor of two, scaling should be done based on the following equation:

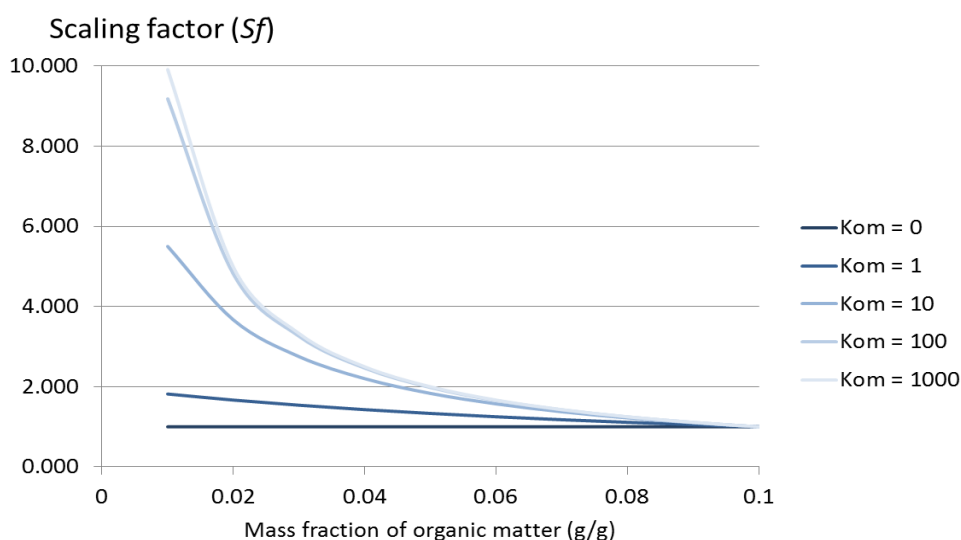
$$S_f = \frac{V_{soil} + K_{om} OM_{test} M_{soil}}{V_{soil} + K_{om} OM_{scenario} M_{soil}} \quad (2)$$

If in the equation V_{soil} is neglected, equation 2 reduces to:

$$S_f = \frac{OM_{test}}{OM_{scenario}} \quad (3)$$

Figure 19 gives the scaling factor calculated with equation 2 for a range of values of K_{om} . In this example, the mass fraction of organic matter in the test medium is 0.1 g/g (or 10%). The figure indicates that the scaling factor is substance-dependent, which is caused by the effect of V_{soil} . For substances with a $K_{om} > 10$ the equation yields the traditional factor of two when the organic matter

3709 content of the test medium is 10% and the organic matter content of the scenario is 5%. For lower
3710 values of K_{om} , ignoring V_{soil} would overestimate the scaling factor, and would therefore give a
3711 conservative estimate of the toxicity endpoint.



3712

3713 **Figure 19:** Scaling factor of the toxicity endpoint as a function of the mass fraction of organic
3714 matter of the exposure scenarios for a range of values of K_{om} . In this example, the mass
3715 fraction of organic matter in the test medium is 0.1 g/g (or 10%).

3716 **Table 21:** Recommended scaling factor to account for differences between the concentration used
3717 in the effects study and the ecotoxicologically relevant concentration.

Type of concentration measured or calculated in the effects study	Ecotoxicologically Relevant Concentration*	Scaling factor for toxicity endpoint
Concentration in total soil	Concentration in total soil***	1
Concentration in pore water	Concentration in pore water	1
Concentration in total soil	Concentration in pore water	S_f calculated with Equation 2**

3718

3719 * See section xx for a description of the ecotoxicologically relevant concentration
3720 ** OMtest (%) is the organic matter content of the test medium and OM scenario is the organic matter content of
3721 the selected exposure scenario.

3722 *** The Panel recommends using the concentration in total soil also when effects correlate best with a mild organic
3723 fraction instead of the total concentration, which is the case for e.g. oral uptake.

3724 The implicit assumption of this scaling is that organic matter is the main adsorbent (Van Gestel,
3725 1992). The latter is true for the majority of PPPs; however, in some cases, PPPs show affinity for other
3726 soil constituents such as clay minerals or sesquioxides (see EFSA PPR Panel, 2015c) for an overview
3727 of sorption processes). Clearly, in those cases, the scaling procedure is not valid and the pore-water
3728 concentration should be calculated or measured. Van Gestel (1992) further mentions that for
3729 substances with a $\log(Kow) < 2$ differences in soil moisture content should be taken into account when
3730 extrapolating toxicity data. However, current practice at EU-level is not to scale the toxicity endpoint
3731 at all for such substances. This is not in line with the original description given by Van Gestel (1992).

3732 If the PEC is derived from a Tier 1 or 2A assessment, the organic matter content of one of the standard
3733 concentrations in total soil scenarios should be selected. If the PEC is derived from a Tier 3
3734 assessment (substance or crop specific exposure scenarios) the organic matter of this Tier 3 scenario

should be used. Notice that organic matter content is not delivered when running a Tier 2B/C exposure assessment. It can however easily be obtained by running Tier 3B. Notice that the scaling factor is less than one when the organic matter content of the selected exposure scenario is higher than 10%, which is the case for some of the scenarios in the Northern zone.

7.8. The risk assessment flow chart

The risk posed by PPPs and their active substances to in-soil organisms – soil fauna and microorganisms – is currently assessed separately for each ‘group’ of the in-soil organisms’ community (EC, 2002). Macrofauna (earthworms), mesofauna (collembola and mites) and microorganisms are considered independently in their own risk-assessment schemes from lower to higher tiers, depending on which group was indicated to be at risk at the first assessment step. Since even at the highest tier in field trials (surrogate reference tier, see section 7.3) it is currently seldom attempted to assess the risk for the soil community as a whole – rather, only earthworms or collembola are investigated – the interactions between the components of the soil biocoenosis are not addressed so far, and the detection of possible indirect effects of use of PPPs is not possible.

The proposed risk assessment flowchart (see Figure 20) does account for the fact that the evaluation of indirect effects is part of the data requirements (EU 283/2013 and 284/2013, see also chapter 2). Accordingly, it is advised that higher assessment tiers (surrogate reference tier) should address interactions between species and indirect effects via food-web disruption - besides effects on species/groups that are not tested at lower assessment tiers but are believed to be at risk (see Chapter 9).

At the first risk assessment step, the effects of active substances or PPPs on in-soil organisms are investigated in simple laboratory tests. According to the current data requirements (EU 283/2013 and EU 284/2013), tests are currently performed with an earthworm species, a collembola, a mite species and a test addressing the nitrogen transformation capacity of in soils by microorganisms (see chapter 2 for further information), assuming that these are appropriate surrogate species and processes. Additionally, to the assessment of active substances or PPP effects on these organisms, the Panel advises to investigate effects on mycorrhiza fungi and on litter feeding organism (e.g. isopods). Please refer to the sections on specific protection goal options (chapter 6) and effect assessment (chapter 9) for the rationale behind these proposals and for the choice of the appropriate tests, respectively.

If the comparisons of the detected effects in the lowest assessment step with the predicted environmental concentration meet the respective acceptability criteria, no further assessment is needed. For the derivation of pertinent assessment factors that do address the uncertainties existing when extrapolating from effects detected in the lab to effects on the community of in-soil organisms in the reference tier, please refer to chapter 7.5.

A further assessment step intends to address the effect of year on year application of PPPs in a so-called ‘system approach’ with appropriate population models. Experience in the implementation of population modelling in the risk assessment of PPP is still lacking. Therefore, the Panel recommends further research activities in order to develop relevant population models for in-soil organisms and to relate model outcomes to other measurement endpoints in the risk assessment for in-soil organisms (see section 7.3). The assessment factors for the extrapolation of modelling results to effects at community level could diverge from the standard assessment factors, since these approaches address different uncertainties and will be calibrated accordingly (see section 7.5).

If the relevant trigger values are not met at the lowest assessment step, the risk may be refined by (i) refinement of exposure (see chapter 8) or/and (ii) further ecotoxicological testing, which might improve the description of the risk for soil organisms and address specific uncertainties present at the lowest assessment steps. It is pointed out that the results of modelling approaches assessing the effects of year on year application of PPP on in-soil organisms in a so-called system approach can only be refined to a very limited extent with further ecotoxicological testing at higher tier (e.g. toxicity data for

other species). Accordingly, information from higher tier testing with soil-organism communities will be of limited use to refine the risk indicated by population models, since up to now these approaches address different uncertainties in the risk assessment.

Although outside the remit of this Opinion, it is acknowledged that appropriate risk mitigation measures and inclusion of management options might lead to the indication of acceptable risk under realistic conditions. For further information on risk-mitigation measures, please refer to the outcome of the workshop on mitigation measures in agricultural land (MAGPIE - Mitigating the risks of plant protection products in the environment).

In the proposed risk assessment flowchart (Figure 20), two types of test set ups are highlighted that may be performed to address specific uncertainties of the assessment before performing a field or semi-field tests with intact soil communities exposed to active substances or PPPs.

One type of experiment can be performed to address the possible differences in sensitivity of species belonging to one group. Testing singularly more species of microarthropods might reduce the uncertainties regarding the sensitivity of *Folsomia candida* as a surrogate species towards the tested substances. Please refer to chapter 9.5, for discussion of the pros and cons of species sensitivity distributions (SSD). Regarding microorganisms, recent developments of functional tests (biology, microresp, see section 9.3.2) give more detailed information on the capacity of microorganisms to degrade different types of compounds, and the effect of pollutants on degradation.

Another type of experiment studies effects on communities assembled in a generic microcosm set-up. Here, interactions between species and groups of in-soil organisms and the structural and functional responses of endpoints for microorganisms' communities may be jointly investigated. Please refer to chapter 9, for the characterisation of these types of tests. However, since experience with this type of assembled terrestrial communities is so far almost completely lacking – and therefore the proper calibration of the outcome of these experiments with the reference tier could be very difficult – this step of the proposed risk-assessment scheme is considered to be conceptually relevant but problematic in the implementation until further experience is available. This is indicated by the light shading of this refinement step in the box displaying the 'intermediate tier with assembled communities'. If the relevant trigger values indicate high risk for in-soil organisms at these intermediate assessment steps (e.g. with SSD or with SSD and microcosms), exposure-refinement options and possible risk management measures might be considered as in the lowest-tier assessment step. If a low risk is indicated, no further refinement steps are needed.

In the case that more information is needed to conclude on low risk for in-soil organisms exposed to intended PPP uses, higher-tier assessment steps might be performed. Please refer to chapter 9.7.1, for the description of higher-tier effect-assessment test options. Given the high natural variability of measurement endpoints regarding in-soil organisms in the field, it is recommended to select test set ups with pertinent statistical power.

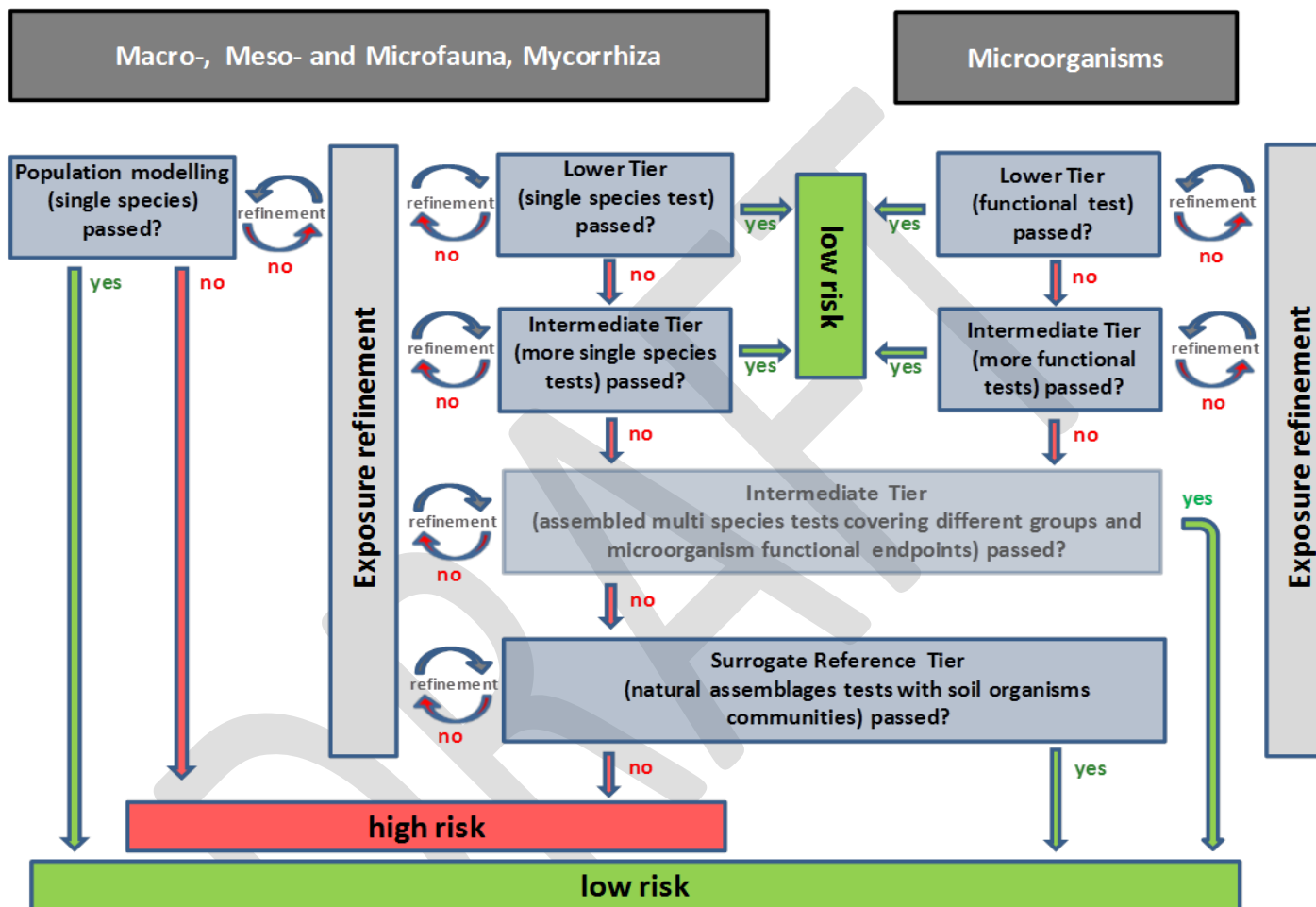
Field or semi-field tests are performed with the aim of characterizing the effects of the active substance in PPPs on the communities of in-soil organisms under realistic conditions. It is recommended to assess jointly populations of macro-, meso- and microfauna and structural/functional responses of microbial communities, in order to be able to detect indirect effects of intended uses of PPPs on in-soil organisms, i.e. disruption of food webs or significant shifts in niche occupancy.

If, on the basis of such a surrogate reference tier assessment, it is indicated that the risk is high, exposure refinements or realistic management options might be considered to reduce the risk for in-soil organisms. If such options cannot be implemented, it cannot be concluded risks are low for in-soil organisms under the intended use of the assessed PPP.

This flowchart is valid for the assessment of in-field and off-field areas in agricultural landscapes. Till the implementation of an integrated 'system approach' in the assessment of the risk from PPP use for

3830 non-target organisms (see EFSA Scientific Committee, 2016a), it is recommended to assess
3831 communities of in-soil organisms with limited mobility present in-field and off-field, – particularly
3832 since different SPG options have been proposed for target and non-target areas. Different levels of
3833 acceptable effects in-field and off-field might result as trade-offs from agricultural use, making
3834 unreliable an *a priori* decision whether the in-field assessment does also cover the off-field
3835 environment.

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3836

3837 **Figure 20:** Illustrative risk-assessment flowchart for in-soil organisms exposed to (active substances in) plant-protection products. In-soil organisms like
3838 macro- meso and microfauna are currently addressed with single species test in the lower assessment step. For microorganisms, functional
3839 responses are assessed. It is recommended to jointly assess effects on different groups of in-soil organisms at higher tier, in order to detect
3840 possible indirect effects of PPP intended uses. Population modelling with single species might indicate that a high risk could not be excluded
3841 e.g. year on year PPP use.

3842

3843 7.9. Identification of vulnerable species/focal trait groups

3844 The information on the traits of in-soil key drivers determining their vulnerability to intended PPP
3845 uses is summarized in the following section. The vulnerability of a species to specific plant protection
3846 products and other potentially toxic chemicals depends on four major aspects as described by De
3847 Lange et al. (2010): probability of being exposed to the toxicant, intrinsic sensitivity to it, possibility
3848 of suffering from indirect effects, and ability to recover after a direct or indirect effect. These aspects
3849 are closely linked to a number of species traits that could be relevant for the identification of
3850 vulnerable species or focal trait groups.

3851 In a field situation, the first step of vulnerability is the exposure of an individual to a toxicant. In
3852 general, in the soil environment three routes of exposure are most important: (i) *via* food, (ii) *via* direct
3853 contact with contaminated soil and/or litter, and (iii) through direct contact with soil solution. Which
3854 one of these three routes is most important for a particular species depends primarily on the following
3855 three groups of traits:

3856 (1) The prevailing living environment – surface-living vs. soil dwelling vs. inhabiting water-filled
3857 soil cavities and pores.

3858 Generally, surface-living organisms can be considered more vulnerable to direct pesticide sprays as
3859 they are exposed at the very moment of pesticide application and before any degradation or
3860 leaching takes place. Because of this, the exposure rate can be similar as for target pest species but
3861 the exposure time *via* this route might be relatively short. In contrast, soil-dwelling organisms, such
3862 as earthworms, microarthropods, millipedes, centipedes or woodlice, are thought to be protected
3863 against direct spray by litter and by the top soil layer. However, the exposure time can be much
3864 longer than in the case of surface-dwelling animals, depending on the properties of the compound
3865 in soil (e.g., persistence, leaching potential) and on the life-form of the organisms. Moreover, the
3866 species' behaviour in the profile can lead to vertical movements between soil layers (see section
3867 7.6.3). For many of these species, both the direct contact with litter, soil or soil solution and food
3868 are routes of exposure. The balance between them depends on body cover and size of an organism
3869 (see below), with small earthworms belonging probably to the most exposed in-soil organisms due
3870 to huge quantities of soil passing through their digestive tract and, at the same time, the direct
3871 contact with soil and soil solution. (see below). In turn, many smallest soil animals (e.g. nematodes
3872 and potworms), most protozoans and bacteria, inhabit small soil cavities filled with water. Many of
3873 them can be actually considered aquatic organisms as they cannot live for a prolonged time outside
3874 the water-filled soil pores. As they are small, they also have a large surface-area-to-volume ratio
3875 (see below) and, hence, the main exposure route is similar to regular aquatic species. For these
3876 groups the main exposure route is probably via the soil solution and transfer of toxicants through
3877 the body surface (see section 7.6.2).

3878 (2) Body cover – hard-bodied vs. soft-bodied organisms.

3879 From this point of view, in-soil organisms can be divided into three groups: hard-bodied
3880 invertebrates, such as some mite and collembolan species, woodlice, centipedes or millipedes,
3881 protected with a solid chitin exoskeleton; soft-bodied invertebrates, e.g. earthworms, potworms and
3882 nematodes and microarthropods; and microorganisms, which due to their mostly single-celled
3883 structure do not have body cover in the strict meaning of the term (see section 7.6.2). Body cover
3884 determines to a large extent the contact exposure through soil or pore water. For earthworms,
3885 which pass large amounts of soil through their digestive tract, plus in anecic species that feed on
3886 the leaf litter in the soil surface, the exposure through food is at least equally important.
3887 Microorganisms are exposed almost exclusively through body surface as there is not much to
3888 protect them against absorbing chemicals from the environment and for many this is also the main

way of feeding (although many protozoans are phagotrophic, that is ingest small food particles, such as single-celled or filamentous algae, bacteria, and microfungi).

(3) Size – the smaller the organisms the larger the surface-area-to-volume ratio.

Surface-area-to-volume ratio is especially important for soft-bodied organisms exposed mostly through the contact of body surface with pore water. In such organisms, the larger the surface-area to-volume ratio, the higher the bioconcentration rate. Bioconcentration defines the accumulation rate of a chemical by an organism from its environment. High bioconcentration rate means that in a short time a chemical can reach high concentration in the body of an organism. In case of certain chemicals, in particular organic pesticides, this can lead to body concentrations a few orders of magnitude higher than in the environment. In some organisms this can directly result in fast acute toxicity; whether this happens depends, however, on organism's physiology and biochemistry (see below) – for example, some microorganisms are able to utilize organic pesticides as an energy source.

Once a species is eventually exposed to a toxicant, its vulnerability is further determined by the next set of traits, linked either to its intrinsic sensitivity to the toxicant or to its recovery potential:

(1) Inherent species sensitivity to a particular toxicant or a class of toxicants.

The inherent toxicological sensitivity of a species to a plant protection product is the ultimate and most important trait defining its vulnerability. This trait is intimately related to the toxic mode of action of the compound and to the mechanisms dictating the toxicokinetics (especially its metabolism and excretion components) and the toxicodynamics processes at the individual, i.e., chemical-receptor interactions and the propagation of effects through molecular networks and over different levels of biological organisation (e.g. cell, tissue, organ, and individual) (EFSA Scientific Committee, 2016b).

(2) Life history – e.g., semelparity vs. iteroparity; short vs. long life span; low vs. high reproductive rate, etc.

The ultimate effect of a toxicant on population depends heavily on inherent population parameters defining its dynamics in the environment. In general, species with high reproductive rates, such as some insects that are able to lay few hundred eggs per season, are considered less vulnerable to environmental perturbations due to their ability to rebuild large populations even within a year (= fast recovery). Most such species are short-lived and semelparous. As such, they die within a season anyway and whether a pesticide treatment has any effect on the population depends more on the timing of the treatment (before or after the reproduction) than on adult mortality. Under normal circumstances, larval mortality is also very high, hence even substantial pesticide-driven mortality in this stage does not necessarily bring a proportional decrease in population size. Such populations are considered to have relatively high 'buffer capacity' for increased mortality as their survival rate is strongly density-dependent. On the other hand, species like some centipedes or earthworms, which are typical iteroparous organisms, can live for a few years, with relatively low annual reproduction. Their intrinsic population growth rate is low and application of pesticides may have much longer-lasting consequences. Moreover, due to their long life span, such species may be exposed to toxicants many times in their lifetime and either toxicants themselves or toxicant-driven damage may accumulate in the body.

(3) Dispersal potential – i.e., ability to disperse from field-boundary areas to in-field and within in-field

Dispersal potential of a species or a trait group, together with the spatial structure of field and off-field areas (see section 3.2.2 and 6.1), determine to a large extent the external recovery potential of its population or community at a certain crop site. Although soil invertebrates have a lower

dispersal ability than other invertebrate groups (e.g., NTAs), movements from semi-natural areas like hedgerows and field boundaries towards the in-field treated area have been shown for spiders and even for organisms with low dispersal ability as collembolans, showing that external recovery also plays a role in population/community recovery in the long term. The ability to disperse is also relevant in internal recovery since species/trait groups that were able to keep resistant populations in suitable refugia less affected by the pesticide at the field area (e.g., between-rows in permanent crops or annual crops when pesticide granules are used). This holds true if the animals do not habitually move from contaminated to uncontaminated soil areas (or layers).

To sum up, specific combinations of traits can make a species more or less vulnerable to a plant protection product. In the first step, the inherent sensitivity of different taxonomic groups to a particular pesticide should be assessed. This can be done taking into account the mode of action and, if available, toxicodynamic data of the pesticide. Once this is done, the sensitive groups (species) should be evaluated for potential vulnerability considering the traits described above. Generally, specific combination of traits can be suggested that make a species or group of species particularly vulnerable to a pesticide to which it is sensitive: e.g. soft-bodied, small and living in topsoil, has relatively long life span and low intrinsic population growth rate; it's dispersal potential is small and it tends to stay in arable areas throughout a year. Additionally, predatory species may be particularly vulnerable due to the biomagnification phenomenon.

8. EXPOSURE ASSESSMENT

8.1. Introduction

In the European Union, PPP exposure assessment for in-soil organisms was traditionally based on the FOCUS methodology on persistence in soil (FOCUS, 1997; EC, 2000). However, during a general consultation of Member States on needs for updating existing Guidance Documents and developing new ones, a number of EU Member States (MSs) requested a revision of the SANCO Guidance Document on persistence in soil (SANCO/9188VI/1997 of 12 July 2000). The consultation was conducted through the Standing Committee on the Food Chain and Animal Health.

An EFSA Guidance (EFSA, 2015) has recently been published laying down a new exposure assessment for plant protection products (PPPs) and their transformation products for in-soil organisms according to Regulation EC No 1107/2009 of the European Parliament and the Council (EFSA, 2015). The document provides methodologies for calculating all types of concentrations that could be relevant for assessing ecotoxicological effects, including the concentrations in total soil and concentrations in pore water, both averaged over various depths and time windows.

The recommended exposure-assessment procedure consists of five tiers. User-friendly software tools have been developed to facilitate efficient use of the tiered approach in regulatory practice (the simple analytical model PERSAM and the more complex numerical models EFSA PEARL and EFSA PELMO; see EFSA, 2015). The first tier is based on the total area of annual crops in a regulatory zone, assuming that crop interception of the substance does not occur. In higher tiers of the exposure assessment, crop interception and subsequent dissipation at the crop canopy may be included. The models that simulate these processes were harmonised. Additionally, an easy to use table for the fraction of the dose reaching the soil has been developed, which should be used at higher tiers in combination with the simple analytical model. With respect to substance-specific, model inputs, this guidance generally follows earlier documents; however, new guidance is included for some parameters, including the rapidly dissipating fraction at the soil surface.

Although the standard output of the exposure models is a limited number of maximum and time-weighted average concentrations, the numerical models PEARL and PELMO are at least able to deliver concentration-time profiles in high resolutions. This additional information could be used for further modelling, for example as input into TK/TD models to estimate time-dependent, internal concentrations in the bodies of the species.

The calculation of soil concentrations can be performed separately for the three regulatory zones in accordance with Regulation EC No 1107/2009 of the European Parliament and the Council (Figure 21).

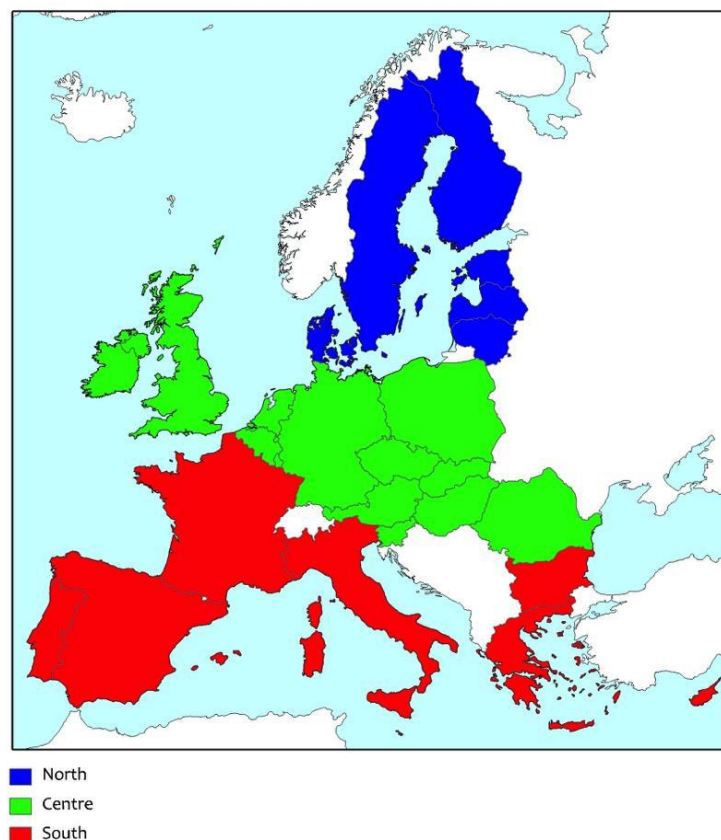


Figure 21: Map of the three regulatory zones according to Regulation EC No 1107/2009 of the European Parliament and the Council.

The purpose of the exposure assessment is to consider the total area of the crop where the PPP is intended to be applied. For any specific crop assessed, the spatial statistical distribution of the exposure concentrations would be different. Therefore, in Tiers 1 and 2A, default scenario-adjustment factors are needed because the scenario that represents the required percentile for a specific crop could differ from the scenario that represents the required percentile scenario for all arable land.

Based on discussions with stakeholders, it was a boundary condition that the exposure assessment can be applied by taking median or average substance properties from the dossiers. Such substance properties are uncertain and inclusion of this uncertainty leads to probability density distributions that show greater spread. As a consequence, this boundary condition led to the need to base the exposure assessment procedure on the spatial 95th percentile concentration instead of the spatial 90th percentile.

Unfortunately, the soil-exposure scenarios are currently limited to annual field crops under conventional and reduced tillage. However, scenarios for the selection and parameterisation of scenarios for permanent crops and row crops on ridges are under development (see Beulke et al., 2015).

8.2. Overview of the tiered approach

For the exposure, a tiered assessment scheme is used that is more conservative at lower tiers and less conservative at higher tiers. All tiers, however, aim to address the same protection goal (i.e. at the 90th percentile concentration within the area of intended use of a PPP). This principle allows a direct assessment at higher tiers without performing assessments for all lower tiers. The scheme can be found in Figure 22.

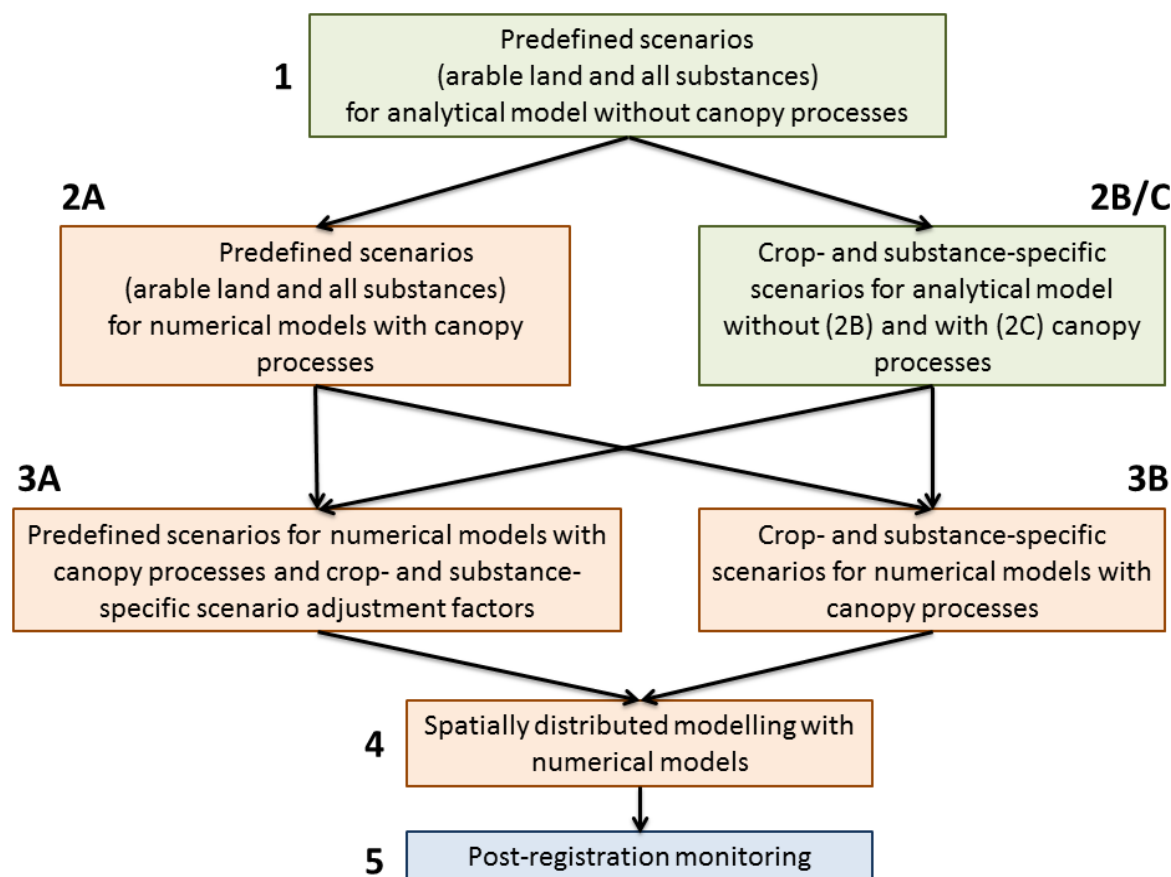


Figure 22: Tiered scheme for the exposure assessment for both the concentration in total soil and the concentration in pore water. Tiers 1, 2 and 3 are all based on one PEC for each of the regulatory zones, North, Central and South, and allow for one or multiple applications every one, two or three years. At Tiers 1 and 2B/C (green) the analytical model in the software tool PERSAM is used. At Tiers 2A, 3A, 3B and 4 (orange) modelling is carried out with numerical models (taken from EFSA, 2015)

The predefined scenarios in Tier 1 and Tier 2A are based on the total area of annual crops in a regulatory zone. Furthermore, Tier 1 and Tier 2B are based on the assumption that crop interception of the substance does not occur. In Tiers 2A, 2C, 3A, 3B and 4, crop interception and subsequent loss processes at the plant canopy can be included. This can be carried out by simulations with the numerical models at Tier 2A, 3A or 3B, or by using a table with default soil loads depending on crop development at Tier 2C.

To be able to perform the exposure assessments, the following software tools should be used:

- Tiers 1, 2B, 2C and 3A: The analytical software tool PERSAM
- Tiers 2A, 3A, 3B and 4: The numerical models PEARL or PELMO

More detailed information on how to perform calculations at different tiers can be found in EFSA (2015).

8.3. Properties of the six predefined soil-exposure scenarios

Tiers 1, 2A and 3A are based on one predefined scenario per regulatory zone (North–Central–South) for each of the two types of Ecotoxicologically Relevant Concentration (ERC) as defined in chapter 7.7: the total content in soil and concentration in pore water. The properties of these six scenarios are summarised in (Table 22) and their position is shown in Figure 23.

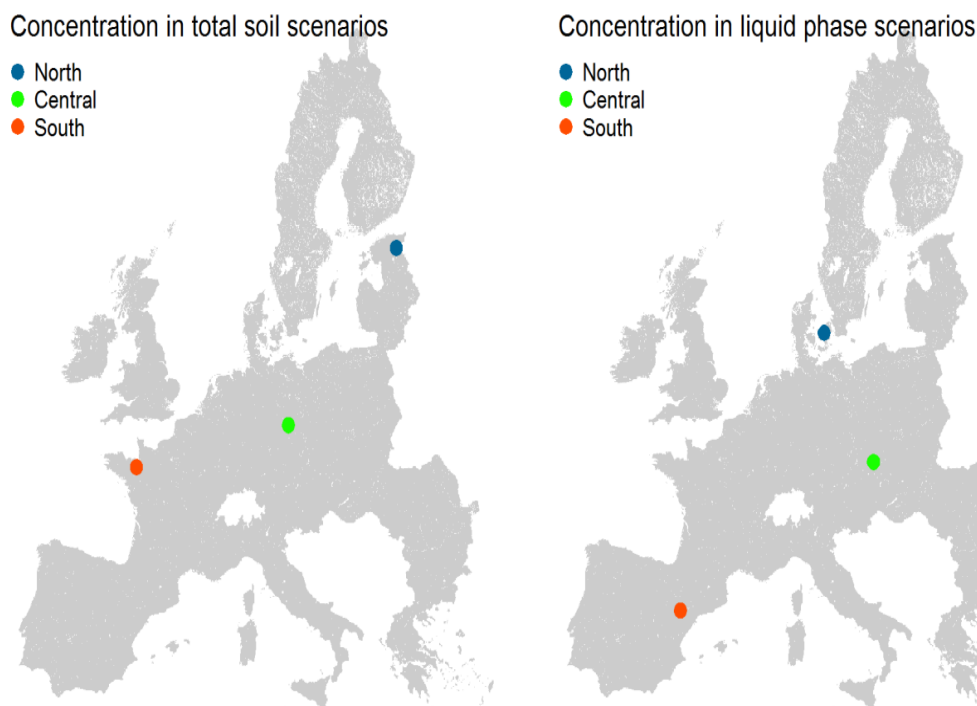


Figure 23: Position of the six predefined scenarios for carrying out Tier 1, 2A and 3A soil exposure assessments. Left-hand panel: Scenarios for the concentration in total soil. Right-hand panel: Scenarios for the concentration in pore water

Table 22: Properties of the selected predefined scenarios used at Tier 1, Tier 2A and Tier 3A for the concentration in total soil and pore water as given in EFSA (2015).

Zone	Code	Country	T_{arit} (°C)	Texture	f_{om} (%)	θ_{fc} (m ³ m ⁻³)	ρ (kg dm ⁻³)
North	CTN	Estonia	4.7	Coarse	11.8	0.244	0.95
Centre	CTC	Germany	8.0	Coarse	8.6	0.244	1.05
South	CTS	France	11.0	Medium fine	4.8	0.385	1.22
North	CLN	Denmark	8.2	Medium	2.3	0.347	1.39
Centre	CLC	Czech republic	9.1	Medium	1.8	0.347	1.43
South	CLS	Spain	12.8	Medium	1.1	0.347	1.51

T_{arit} is the arithmetic mean annual temperature

f_{om} (–) is the organic matter content (calc. based on organic carbon content, conversion factor 1.724)

θ_{fc} (m³ m⁻³) is the water content at field capacity

ρ (kg dm⁻³) is the dry bulk density of the soil.

8.4. Scenario and model adjustment factors

The scenarios in Table 21 were based on the 95th spatial percentile considering the total area of annual crops in each regulatory zone. However, the purpose of the exposure assessment is to consider the total area of the crop where the PPP is intended to be applied. For any specific crop assessed, the spatial, statistical distribution of the exposure concentrations would be different. Therefore, in Tiers 1 and 2A default scenario-adjustment factors are needed because the 95th percentile scenario for a specific crop could differ from the 95th percentile scenario for all arable land. The new scenario-adjustment factors are listed in Table 23. They are based on the different spatial distributions of the total area of annual crops compared with the distribution of the worst-case individual crop (see EFSA, 2010).

Table 23: Scenario-adjustment factors (f_s) to be used when performing an assessment for the three regulatory zones at Tier 1 or Tier 2A for the concentration in total soil and for the concentration in pore water.

Zone	Scenario adjustment factors to be used for the	
	concentration in total soil	concentration in pore water
North	3.0	2.0
Centre	2.0	1.5
South	2.0	1.5

The simple analytical model used in lower tiers does not consider dynamic soil moisture.

Since it cannot be guaranteed *a priori* that the simple analytical model is conservative enough when compared with the more realistic numerical models used in Tiers 2A, 3A, 3B and 4, additional model-adjustment factors are needed in all the tiers that use the analytical model. Factors of x2 and x4 are applied for concentrations in total soil and pore water, respectively. The same model-adjustment factors are used for all regulatory zones.

8.5. Crop-canopy processes

Tiers 1 and 2B are based on the assumption that crop interception of the substance does not occur. In Tiers 2A, 2C, 3A, 3B and 4, interception may be included. Ever since the introduction of the FOCUS groundwater scenarios, it has been common practice to reduce the application rate by the fraction that is intercepted by the crop canopy and to apply this reduced fraction to the soil. As described by EFSA PPR Panel, (2010b), this approach cannot be defended since there is insufficient evidence that wash-off from the crop canopy can be ignored. So the effect of dissipation at the crop canopy and foliar wash-off should be included when the substance is applied to the crop canopy.

Crop-canopy processes and foliar wash-off can be simulated by PEARL and PELMO in Tiers 2A, 3A, 3B and 4. The analytical model used at Tier 2C cannot simulate plant processes. For this reason, a table of the fraction of the dose reaching the soil depending on the crop-development stage was created based on simulations with PEARL and PELMO. This table should be used only when performing an assessment at Tier 2C.

Table 24: Fraction of the dose reaching the soil (f_{soil}) including wash-off to be used at Tier 2C considering crop-interception and canopy-dissipation processes as a function of crop-development stage. The figures are rounded to the nearest 0.05. Please note that this table is based on the weather conditions in the current six scenarios and should therefore not be used for groundwater assessments (EFSA, 2015)

Crop	BBCH code ^{1,2}				
	00-09	10-19	20-39	40-89	90-99
Beans (vegetable and field)	1.00	0.85	0.85	0.70	0.50
Cabbage	1.00	0.90	0.85	0.60-1.00 ³	1.00 ³
Carrots	1.00	0.85	0.75	0.45-1.00 ³	1.00 ³
Cotton	1.00	0.90	0.90	0.55	0.30
Linseed	1.00	0.80	0.70	0.70	0.70
Maize	1.00	0.85	0.80	0.65	0.40
Onions	1.00	0.95	0.90	0.80-1.00 ³	1.00 ³
Peas	1.00	0.75	0.70	0.65	0.60
Oil seed rape (summer)	1.00	0.75	0.65	0.65	0.55
Oil seed rape (winter)	1.00	0.75	0.60	0.65	0.40
Sugar beets	1.00	0.90	0.75	0.55-1.00 ³	1.00 ³
Soybeans	1.00	0.85	0.80	0.75	0.65
Strawberries	1.00	0.80	0.80	0.65	0.45
Sunflowers	1.00	0.90	0.80	0.75	0.40
Tobacco	1.00	0.70	0.70	0.70	0.60
Tomatoes	1.00	0.80	0.80	0.70	0.70

Crop	BBCH code ⁴				
	00-19	20-29	30-39	40-69	70-99
Spring cereals	1.00	0.90	0.70	0.65	0.60
Winter cereals	1.00	0.90	0.65	0.60	0.60

1. The BBCH code is a decimal code ranging from 0 to 99 to characterise the crop development stage (Meier, 2001).

2. BBCH 00-09: bare to emergence; BBCH 10-19: leaf development; BBCH 20-39: stem elongation; BBCH 40-89: flowering; BBCH 90-99 Senescence and ripening

3. Since these crops are harvested at BBCH 50, the higher value of 1.00 should be used for BBCH code 50-99.

4. BBCH 00-19: bare to leaf development; BBCH 20-29: tillering; BBCH 30-39: stem elongation; BBCH 40-69: flowering; BBCH 70-99 Senescence and ripening

8.6. Row crops

As mentioned earlier the exposure scenarios are currently limited to annual field crops under conventional and reduced tillage and scenarios for the selection and parameterisation for row crops on ridges are still under development.

In contrast to normal annual crops, applications in row crops (e.g. potatoes) cannot be generally considered as homogeneously distributed over the whole agricultural field. Applications may be targeted to the rows whereas the area between the rows are exposed dependent on the distance to the rows. This is in principle a similar situation as in off-crop areas. If the area between the rows is also not irrigated, degradation could be affected, which may result in accumulation of residues at higher concentrations than expected from the relatively lower exposure at the time of application.

8.7. Litter layer

According to Beulke et al. (2015) data on the litter layer is scarce and it is suggested that no litter layer is present in the majority of the permanent crops. Litter might, however, become more important in the future as good soil-management practices are promoting the presence of organic matter on the soil, so there may be a shift to a more sustainable management of this litter layer. In addition, although there is usually no permanent litter layer in current agriculture, there may be litter debris in the field temporarily, which could be a significant exposure route for some species. Unfortunately, the exposure models used presently in registration are not able to simulate the fate of pesticide in litter and no scenarios have been defined so far to cover this exposure route. However, a simple estimation of the peak concentration in litter could be based on the current soil scenarios considering the application rate, the crop interception at the time of application, and an appropriate density of litter. Nevertheless, the development of more advanced computer models that also consider additional processes (e.g. the uptake of pesticide *via* the plant roots) would be necessary to describe this exposure completely.

8.8. Exposure routes in the off-crop area

The presence of PPPs on off-field non-target surfaces (plants, non-target arthropods, etc.) is a combination of three processes during and after the application of the compounds in the field: (i) the emission of the applied product out of the field by spray drift and runoff, (ii) the deposition of the emitted amounts onto the off-field surfaces, and (iii) dissipation processes from the non-target surface. Drift is currently considered to be the most important factor for off-field emissions to non-target surfaces. However, dependent on the meteorological conditions shortly after application, losses due to surface run-off may also contribute to the contamination of non-target, terrestrial ecosystems in the neighbourhood of agricultural areas. Other emission routes such as leaching and drainage are not considered as direct emission routes. Drift is defined as droplet drift but vapour drift and dust drift are also considered to be important emissions in particular cases. "Deposition" on non-target surfaces is defined as the entry path for transport of airborne or waterborne substances from the air to the non-target surface, i.e. to an aquatic or terrestrial compartment or directly to non-target plants, arthropods, bees, etc. Dry and wet depositions should be considered separately because they are subject to different atmospheric and physical processes.

As mentioned in EFSA PPR Panel, (2010b), the exposure estimate should preferably apply to a given percentile of the concentration distribution (usually the 90th percentile) of the treated fields. Developing an exposure scenario for a given percentile requires simulating the concentration distribution in the entire target area (e.g. EFSA, 2015). The model for simulating this concentration distribution should preferably include all relevant exposure routes (i.e. spray-drift deposition, vapour-drift deposition, dust-drift deposition and surface run-off). Since such models are not yet available for regulatory purposes at the European level, the simplifying assumption is made that the individual exposure routes can be assessed separately.

8.8.1. Spray drift/deposition

Spray drift is defined as that part of the applied product that leaves the treated field through air because of air current during the application of the plant protection product. These spray drift emissions do not include emissions by volatilisation. Droplet drift is considered to be a short distance process (0-30 m) and occurs only during and shortly after application (i.e. within a few minutes actually defined by the time between spraying and collection of samples during drift experiments)

Spray drift is not compound-specific but mainly dependent on droplet size, wind speed, wind direction and crop and spray-boom height during spraying. The spray drift is calculated on the basis of spray-drift tables, which give the deposition as a percentage of pesticide-application rate deposited at a given distance from the last crop row as a function of crop type (arable crops, fruits, grapes, hops and vegetables), the crop stage (early or late), and the spraying technique. Different spray-drift curves are available (Rautmann et al., 2001; Southcombe et al., 1997). The most recent publication on the topic is

4159 a report that presents joined spray-drift curves from German and Dutch experiments (Zande et al.,
4160 2015).

4161 However, the Guidance on tiered risk assessment for plant protection products for aquatic organisms
4162 in edge of field surface waters (EFSA PPR Panel, 2013) recommends estimating the drift deposition
4163 on surface water with the spray-drift values reported by FOCUS (2001), which are based on Rautmann
4164 et al. (2001) and apply to 90th percentile conditions. Nevertheless, it could be more appropriate to
4165 base further assessments on the most recent publication by Zande et al. (2015). Independently of the
4166 dataset, all measurements were obtained from deposit measurements on artificial receptors (e.g. filter
4167 paper strips) on bare soil. The use of these datasets also for the off-crop area can therefore be
4168 considered a worst case.

4169 Generally, the risk assessment for the off-field area could consist of two steps. In the first step, the
4170 exposure could be based on the standard spray-drift curves in-field risk assessment, i.e. the drift
4171 deposition would be 100% of the application rate. If the protection goal for the off-field area would
4172 not be met in this step, risk-mitigation options would have to be assessed in a follow-up step. Options
4173 to mitigate spray-drift deposition to off-field areas include (i) the use of spray-drift reducing
4174 techniques, and (ii) the establishment of non-spray buffer strips, with or without crop. Since spray-
4175 drift deposition decreases with both distance and drift-reducing technique class, spray-drift mitigation
4176 options could be evaluated using a matrix. Spray-drift deposition could for example first be evaluated
4177 for the standard spraying technique, second for drift-reducing techniques and measures, and third for
4178 all spray techniques with stepwise wider buffer strip.

4179 Spray -drift deposition differs between crop types (grass and bare soil, field crops, fruit crops, vines
4180 and hops) and crop-development stage. For this reason, a spray-drift deposition curve and hence
4181 evaluation matrix is needed for each combination of crop type and crop-development stage, or classes
4182 of these. For estimating spray-drift deposition onto surface waters, spray-drift deposition curves were
4183 developed by the FOCUS Surface Water work group (FOCUS, 2001) for many major crops.
4184 Harmonised European drift curves are currently only available for bare soil, grass and fully developed
4185 arable field crops (van de Zande et al., 2015); however, spray-drift curves for fruit crops are expected
4186 to become available in the near future. For vine and hops, no updated values are foreseen in the near
4187 future. In this situation, the PPR Panel recommends that the efficiency of new spray-drift curves is
4188 evaluated when they become available and that the spray-drift assessment methodology is revised
4189 accordingly. For the time being, the current assessment based on FOCUS (2001) can be used.

8.8.2 Vapour drift

4191 Vapour drift can occur by (1) evaporation of the solvent from small spray droplets that are still present
4192 as “drift” after application and (2) post-application evaporation of the spray deposits from treated
4193 plant/soil surfaces. Vapour-drift deposition is usually short to medium range (0 to 1000 m). Most
4194 emission by volatilisation occurs during the first 24 hours after application and is caused by spray-
4195 droplet evaporation. However, evaporation from deposits may continue for several days or weeks after
4196 treatment (Bedos et al., 2002). Evaporation of the leaf/soil deposits is dependent on the active
4197 ingredient properties, such as volatility, and interaction with leaves. Volatilisation from plant surfaces
4198 is one of the main pathways of pesticide emission to the environment and normally is greater than
4199 volatilisation from soils because plants have fewer sorption sites than soil.

4200 The main factors controlling pesticide volatilisation are the physicochemical properties of the pesticide
4201 (in particular vapour pressure), agricultural practices (time and type of application), soil or plant
4202 physical properties and meteorological conditions (during and after application). Several models for
4203 vapour-drift emissions were evaluated by the FOCUS Air working group (FOCUS, 2008). They
4204 concluded that none of the models available completely fulfilled the requirements for use within a
4205 regulatory context. For pragmatic reasons, FOCUS (2008) recommended using the EVA 2 model for
4206 calculating the deposition after volatilisation for short-range transport. Later, the PPR Panel has
4207 evaluated this model and came to the conclusion that the recommendations regarding the use of the

EVA 2 model are scientifically not robust enough (EFSA, 2007). The PPR Panel further came to the conclusion that the recommended model does not give realistic worst-case exposure estimates. Therefore, the PPR Panel recommends improving the estimation of vapour-drift deposition by the EVA 2 model and also to investigate the option to use alternative modelling approaches since these have now become available.

FOCUS (2008) stated that volatilisation is only relevant for compounds with a vapour pressure higher than 10^{-4} Pa when applied to the soil and for compounds with a vapour pressure higher than 10^{-5} Pa when applied to the crop. In this context, it is worth noting that, whereas it is possible to minimise droplet-drift emission to the off-crop area using appropriate application techniques (e.g. drift-reducing nozzles, buffer zones), this does not apply to volatilisation, since this process is mainly driven by pesticide and crop properties. The relative contribution of vapour-drift deposition is demonstrated below. These tables summarise calculations with the EVA 2 model for a compound with medium volatility (vapour pressure of 5×10^{-3} Pa) assuming various crop-interception fractions in the field. The consideration of crop interception is necessary, since volatilisation from the crop canopy is estimated to be three times higher than volatilisation from the soil surface. For the crop-interception values, the most recent numbers are recommended (EFSA PPR Panel 2014a).

Table 25: Spray Drift and Volatilisation deposits for arable field crops calculated with EVA 2*

Distance (m)**	Droplet Drift ($\mu\text{g}/\text{m}^2$)	Cumulative volatilisation deposits over 24 h ($\mu\text{g}/\text{m}^2$) dependent on crop interception in field			
		no interception	25% interception	70% interception	90% interception
1	2770	518	778	1244	1451
3	943	465	697	1116	1302
5	570	417	625	1000	1167
10	290	318	476	762	889
15	200	242	363	580	677
20	150	184	276	442	516
30	100	107	160	256	299
50	60	36	54	86	101
100	30	2	4	6	7

*Application dose 1 kg/ha, vapour pressure 5×10^{-3} Pa

**From last row of treated crop

4228

4229 **Table 26:** Spray Drift and Volatilisation deposits in orchards (early) calculated with EVA 2*

Distance (m)*	Droplet Drift ($\mu\text{g}/\text{m}^2$)	Cumulative volatilisation deposits over 24 h ($\mu\text{g}/\text{m}^2$) dependent on crop interception in field			
		no interception	25% interception	70% interception	90% interception
1	-	1814	534	548	670
3	29200	1627	479	492	601
5	19890	1459	429	441	539
10	11810	1111	327	336	410
15	5550	846	249	256	313
20	2770	645	190	195	238
30	1040	374	110	113	138
50	300	126	37	38	46
100	60	8	2	2	3

4230 *Application dose 1 kg/ha, vapour pressure 5×10^{-3} Pa

4231 **From last row of treated crop

4232

4233 It should be noted that the depositions of vapour drift in the tables are the cumulative exposure over 24
 4234 h. They are compared with the deposition of spray drift, which can be considered as an instantaneous
 4235 event actually defined by the time between spraying and collection of samples during drift
 4236 experiments, usually 15 minutes. Further information about the equations in the model is given in
 4237 EFSA PPR Panel, (2014a).

4238 If the deposition rate exceeds the maximum acceptable dose, risk assessors might want to establish a
 4239 buffer strip. Similar to the calculation of drift deposits, EVA does not directly allow for the calculation
 4240 of the width of buffer strip necessary to meet maximum acceptable deposits. However, this is possible
 4241 when transforming the original equation as described in EFSA PPR Panel (2014a).

4242 The next table shows examples for different volatility classes when 1 kg/ha was sprayed and the
 4243 maximum acceptable load was calculated to be 0.01 kg/ha.

4244 It has to be noted that the numbers in Table 25 and 26 are based on a very volatile compound, while
 4245 the numbers in Table 27 are based on a range of substance with different volatility.

4246

4247 **Table 27:** Necessary buffers (m) to prevent non-acceptable volatilisation deposits*

Vapour pressure range at 20 °C	relative volatilisation from canopy, deposit at 1 m (% of application dose)	necessary distance (m) no interception (in field crop)	necessary distance (m) 100% interception (in field crop)
vp < 10 ⁻⁵ Pa (plant) vp < 10 ⁻⁴ Pa (soil)	0.00	No buffer	No buffer
10 ⁻⁴ Pa > vp > 10 ⁻⁵ Pa	0.09	No buffer	No buffer
5 × 10 ⁻³ Pa > vp ≥ 10 ⁻⁴ Pa	0.22	No buffer	No buffer
vp ≥ 5 × 10 ⁻³ Pa	1.56	No buffer	9.17

4248 *application dose 1 kg/ha, maximum acceptable load: 0.01 kg/ha

4249
4250 In the table a buffer zone was calculated only for the compounds having vapour pressures above
4251 5 × 10⁻³ Pa and when the application was targeted fully at the (target) canopy. That demonstrates that in
4252 most situations deposition caused by droplet drift will be the dominant entry route rather than
4253 volatilisation deposits.

4254

4255 8.8.3. Particulate drift

4256 Particulate drift can occur due to (1) application of dust from dustable powder formulations (e.g.
4257 sulphur dusting in vineyards), (2) dust formation during non-spray applications (NSA), e.g. granules
4258 (fertilizer-herbicide combinations for application in lawns) and treated seeds, or (3) soil dust with
4259 adsorbed pesticide deposits. However, the latter emission is not considered to be a direct emission
4260 route.

4261 Particulate drift happens generally over a short range and in short periods after application and is thus
4262 comparable to droplet drift. The main driving force is the particle size/weight of the dust particles.

4263 The EFSA Opinion on non-spray applications (EFSA, 2005) gives guidance for the exposure
4264 assessment of NSAs (non-spray applications). The main conclusions and recommendations for dust
4265 drift are:

- 4266 1. Dust in NSA is a relevant route of exposure for surface water.
- 4267 2. Broadcast granular applications even with subsequent incorporation can form dust drift that
4268 can have comparable effects as spray drift.
- 4269 3. Broadcast application of treated seeds (with and without subsequent incorporation) is also
4270 considered to be a relevant route of exposure.

4271 According to the EFSA Opinion on non spray applications (EFSA, 2005), dust drift can be handled by
4272 FOCUS surface-water models with adjustment of the normal default inputs in such a way that an
4273 evaluated dust-drift value is entered. For default values, adapted spray-drift models can be used to
4274 estimate dry deposition from dust by taking into account a number of specific, underlying criteria.

4275 As there is currently an increasing concern with regard to dust drift and seed treatments, the European
4276 Commission recently prepared a document that includes experimental data from dust-drift deposition
4277 for different crops (EC, 2014). There is, however, a problem when considering the experimental

studies on dust-drift deposition in this document. In contrast to the standard FOCUS values, the evaluation performed in EC (2014) does not give dependencies between the deposition and the application dose, which would be necessary to define safe distances dependent on the application rate.

8.8.4. Run-off entries

This section deals with the assessment of pesticide movement to surface water caused by run-offs and its links to the terrestrial compartment. The assessment of pesticide movement to surface water caused by run-off is currently a key process in European risk assessment. The recommended methodology as described by FOCUS (2001) follows a tiered approach. Run-off occurs after heavy rainfall events, which may transport residues of the active substance or transformation products either dissolved in the water or sorbed to the eroded sediment particles to the non-target area. If mitigation measures for run-off entries reaching surface waters are used (e.g. vegetated buffer strips), this will be connected with deposition of residues to the terrestrial ecosystems.

For the estimation of run-off and erosion losses leaving the edge of field, several models are available, e.g. the models used in the different tiers of FOCUS surface water (FOCUS, 2001). At tier II, pesticide losses by run-off as summarised in Table 28 are considered.

Table 28: Step 2: pesticide losses by run-off and soil erosion according to FOCUS STEP 2

Region/season	% of soil residue
North/Centre* Europe, Oct. - Feb.	5
North/Centre* Europe, Mar. - May	2
North/Centre* Europe, June - Sep.	2
South Europe, Oct. - Feb.	4
South Europe, Mar. - May	4
South Europe, June - Sep.	3

*according to FOCUS (2001) the number also reflects the situation in Northern France

For pragmatic reasons, the losses due to run-off at step 2 were defined by FOCUS independently of sorption properties of the compound. According to FOCUS, they have been calibrated against the results of tier III calculations. The key model for the estimation of run-off in FOCUS at tier III is PRZM. Reichenberger et al. (2007) made a probabilistic analysis of losses caused by run off and erosion using the Pesticide Root Zone Model (PRZM) and analysed losses dependent on sorption. For run off, the maximum losses were found for compounds with KOC values in the range of 100–200 L/kg. For losses by soil erosion, the maximum numbers were found for compounds with maximum KOC values. The results were evaluated by the German federal environmental protection agency and, meanwhile, were also implemented into their model EXPOSIT 3.0 used in German risk assessment for estimating pesticide losses caused by run-off (Umweltbundesam, 2011). Currently, this analysis is of use only in the central European zone, since only German environmental conditions were considered. However, it is recommended that the dependencies between important pesticide properties and run-off losses for all European zones be analysed in order to improve the information given by FOCUS (2001) (Table 29). As mitigation measures for run-off entries reaching surface waters, the EFSA PPR Panel (2013) recommends the use of vegetated buffer strips taken from FOCUS (2007). These mitigation measures for surface water are directly connected with deposition of residues to the respective terrestrial ecosystems (vegetated buffer strips). In the following table, the reduction factors are re-calculated to give the reduction that is occurring at the respective distance only.

4317

4318 **Table 29:** Deposited fraction dependent on the position of the buffer strip

Buffer width (m)	Run-off fraction deposited (%)	Erosion fraction deposited (%)
0-5	37	55
5-10	23	25
10-15	12	10
15-20	8	5

4319

4320 It is not possible to describe the deposition of pesticide in water by single exponential functions.
 4321 However, EFSA PPR Panel (2014a) gives exponential functions that could be used to derive deposited
 4322 fractions for any distance for the water phase:

4323 Table 30 shows examples for the acceptable distance in different seasons when 1 kg/ha was applied
 4324 and the maximum acceptable load was calculated to be 0.01 kg/ha.

4325 Substances with very high sorption constants ($KOC > 5000$ L/kg) are mainly transported *via* the
 4326 sediment. For these substances, sorption *via* the particulate sediment phase (soil erosion) has to be
 4327 considered with additional equations.

4328 **Table 30:** Example of necessary run-off buffers (m) in different regions/seasons *

Region/Season	% of soil residue leaving the field**	KOC (L/kg)	necessary distance (m)
North Europe, Oct. - Feb.	5	100	19.7
North Europe, Mar. - May	2	100	7.3
North Europe, June - Sep.	2	100	7.3
South Europe, Oct. - Feb.	4	100	16.5
South Europe, Mar. - May	4	100	16.5
South Europe, June - Sep.	3	100	12.3

4329 * application dose 1 kg/ha, maximum acceptable load: 0.01 kg/ha, org Carbon in soil 2 %, concentration of
 4330 suspended particles in run-off 0.01 kg/L

4331 ** degradation in soil before run-off event not considered

4332

4333 FOCUS (2007) recommended the reduction factors as being 90th percentile worst-case values for the
 4334 reduction efficiencies of the buffer strips. Consequently, the related deposition fractions in the
 4335 vegetated area should be considered as minimum deposited fractions. It is therefore recommended to
 4336 re-evaluate the existing information on the efficiency of vegetated buffer strips with regard to worst-
 4337 case situations for off-crop areas.

4338

9. EFFECTS ASSESSMENT

9.1. Introduction

As outlined in chapter 6, SPGs have been developed for the following groups/species of in-soil organisms:

- Earthworms
- Enchytraeids
- Microarthropods
- Macroarthropods
- Terrestrial gastropods
- Nematodes
- Soil Bacteria and Archaea
- Mycorrhiza, other fungi and protozoa

This chapter describes the available study protocols for assessing the toxicity of PPPs to in-soil organisms. The aim is to evaluate whether the current methodology for effect assessment is appropriate to address the specific protection goals (SPGs) as reported above.

9.2. Choice of standard laboratory test methods for in-soil invertebrates

The objective of environmental risk assessment is to evaluate the likelihood that adverse effects may occur in the environment following exposure to a chemical. Standard laboratory tests aim at establishing the toxicity levels of a chemical on tested organisms. By characterising the toxicity, the way a chemical may interact with organisms and cause adverse effects should be taken into account. This means that, theoretically, all the potential routes of exposure (i.e. contact and oral) should be considered, depending on the physical and chemical properties of the substance and its mode of action. The test organisms can be considered “models” and should be representative and sensitive enough for extrapolating the results to other species, when these results are used for risk assessment.

Many toxicity tests have been proposed and reviewed over the last decades (e.g. Lokke and Van Gestel (1998); Van Gestel, (2012); and Pelosi et al., (2013)). Criteria for selecting an appropriate test for soil ecotoxicology have been proposed by Van Gestel (1997) and reported in Van Gestel (2012). Although these criteria were proposed for invertebrates, they concern general requirements that can be considered valid for microorganisms as well.

The criteria among others include:

1. **Practicability**, referring to the feasibility and cost-effectiveness of a test;
2. **Acceptability**, including aspects like standardization, reproducibility and statistical validity of a test method as well as its broad chemical responsiveness;
3. **Ecological meaning**, including sensitivity and ecological realism of the test method.

The following, additional criteria need to be taken into account in order to obtain a balanced battery of tests (Van Gestel, 1997):

4. **Representativeness of the ecosystem to protect**: this includes e.g. the representation of organisms having different life histories, representing different functional groups, different taxonomic groups and different routes of exposure;
5. **Representativeness of responses**, to make sure that responses measured really are relevant for the protection of populations and communities;
6. **Uniformity**, which refers to the possibility of applying all tests in a battery to the same test media.

4383 The most relevant of these criteria for the purpose of this opinion are considered to be 1) the
4384 representativeness of the ecosystem to protect and 2) the representativeness of responses (points 4 and
4385 5 above). The uniformity of the test systems with regard to test media and the possibility of scaling the
4386 toxicity endpoint to account for the bioavailability in different test media are discussed in chapter 7.

4387 In chapter 6, SPGs have been developed using the overarching concept of Ecosystem Services, to
4388 identify key driver organisms that need to be protected in order to provide soil-ecosystem services that
4389 can be affected by the use of PPPs. Key drivers are therefore considered to be representative of
4390 ecosystems to protect in terms of different functional and taxonomic groups and of different biological
4391 traits.

4392 For invertebrates, a list of available laboratory-test methods was compiled according to the taxonomic
4393 groups of the key drivers (Appendix C.). Appendix C includes formally standardized test protocols,
4394 but also protocols from open literature. It should be noted that the information presented has been
4395 compiled by the working group experts from public literature but has no claim of being exhaustive.

4396 For microorganisms, functional endpoints are often used. Numerous methods are available for
4397 microorganisms; only the standard test (mentioned in the current data requirements) for nitrate
4398 formation is mentioned in Table 31, and other methods are referred to in Appendix E.

4399 From Table 31, it can be noted that at least one laboratory test is available for key drivers in the main
4400 groups of invertebrates. The choice of a particular test species is discussed in Lokke and Van Gestel
4401 (1998). The authors showed that every test organism mentioned has an important role in the food web.
4402 Sensitivity of organisms to PPPs depends on the life stage, different life histories etc. These aspects
4403 are discussed in Lokke and Van Gestel (1998) and for earthworms by e.g. Pelosi et al., (2013). It is
4404 also clear that choice of the test species and methods is a trade-off between the theoretically best
4405 species and practical aspects, like availability of an accepted guidance and handling in the lab.

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Table 31: Overview of laboratory test methods for the identified key drivers (for soil ecosystem services potentially affected by pesticides). **Bold** indicates the key drivers as defined in chapter **Error! Reference source not found.** Standard laboratory tests according to EU Regulation 283/2013 and 284/2013 are made **grey** in the table. Test protocol: 1 = standardised test protocol available; 2 = test method available, not formally standardised.

Taxonomic group	test organism/process	Ecosystem service						Habitat	Test	Exposure					Endpoints/ life stages
		Biodiversity and genetic resources	Nutrient cycling	Pest control	Natural attenuation	Soil structure	Food web support			duration (d)	Soil contact	Soil pore water	Food	Litter	
Prokaryotes Bacteria and Archaea		X	X	X	X	X	X	X			X	X		X	
	N transformation	X	X					X	1	28 - 100	X	X			Nitrate formation
Eukaryotic micro organisms Mycorrhiza other fungi and protozoa		X	X	X	X	X	X	X			X	X			
	<i>Glomus mosseae</i>	X	X	X	X	X	X	X	1	14	X	X			Number of spores and germinated spores
Nematoda Nematodes		X	X	X			X	X			X	X	X		
	<i>Caenorhabditis elegans</i>	X	X	X			X	X	1	1					Mortality
Mollusca Terrestrial gastropods		X	X			X	X	X			X	X	X	X	
	<i>Helix aspersa</i>	X	X			X	X	X	1	28	X	X	X		Survival, growth
Annelida Lumbricidae Earthworms		X	X			X	X	X			X	X	X	X	
	<i>Eisenia fetida fetida / andrei</i>	X	X			X	X	X	1	28 + 28	X	X	X		Reproduction
Enchytraeidae Enchytraeids		X	X			X	X	X			X	X	X		
	<i>Enchytraeus albidus</i>	X	X			X	X	X	1	42	X	X	X		Reproduction
	<i>Cognettia sphagnetorum</i>	X	X			X	X	X	2	70	X	X	X		Reproduction
Arthropoda Micro arthropods		X	X	X		X	X	X			X	X	X	X	
	<i>Folsomia candida</i> and <i>F. fimetaria</i>	X	X	X		X	X	X	1	21-28	X		X		Survival, reproduction

Arachnida: Laelapidae	Mites	<i>Isotoma viridis</i>	X	X	X	X	X	X	2	56	X		X	X	Survival, growth	4409
		<i>Hypoaspis aculeifer</i>	X	X	X	X	X	X	1	14	X		X		Survival Reproduction	
		<i>Platynothrus peltifer</i>	X	X		X	X	X	2	70	X		X		Reproduction	
		<i>Oppia nitens</i>	X	X		X	X	X	2	28	X		X		Reproduction	
Arthropoda	Macro arthropods		X	X		X		X	X		X	X	X	X		
Chilopoda: Lithobiidae	Centipede	<i>Lithobius mutabilis</i>	X	X		X		X	2	28-84	X	X	X		Survival, sublethal effects	
Diplopoda: Polydesmidae	Millepede	<i>Brachydesmus superus</i>	X	X		X		X	2	70	X	X			Survival, sublethal effects, reproduction	
Crustacea: Isopoda	woodlice	<i>Porcellio scaber</i>	X	X		X		X	2	4 survival growth) , 10 (repr.)				X	Survival, growth, reproduction	
		<i>Porcellionides pruinosis</i>	X	X		X		X	2	14	X	X	X		Survival, reproduction	

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Standard tier 1 testing according to current data requirements (Reg EU 283/2013 and 284/2013) includes earthworms (i.e. *Eisenia fetida/andrei*), microarthropods (i.e. the collembolan species *Folsomia candida* / *Folsomia fimetaria* and the mite *Hypoaspis aculeifer*) and soil microorganisms (i.e. nitrogen-transformation test). This means that mycorrhiza, nematodes, terrestrial gastropods, enchytraeids and macro-detritivores are not explicitly represented by the current, standard test protocols. It is therefore discussed below for in-soil invertebrates and in 9.3 for soil microorganisms, whether present standard tests are representative for other key drivers.

As discussed above, the representativeness of the ecosystem to protect and the representativeness of responses are considered to be the key criteria for the selection of the standard test species/test system. The representativeness of the ecosystem to protect is evaluated based on the traits of the species/communities and the representativeness of responses based on available toxicity data. Considering the proposed SPGs, toxicity data on different species identified as key drivers have been compiled, when possible, trying to understand the representativeness of the current standard species and their use as model test species.

9.2.1. Representativeness of *Eisenia fetida*

Eisenia fetida is one of the current standard species for evaluating the toxicity of PPPs to in-soil organisms. It is evaluated below whether *E. fetida* can be considered a suitable model species, in terms of sensitivity, and can therefore represent in-soil non arthropod invertebrates (i.e. other earthworms, enchytraeids, molluscs and nematodes) in the first-tier risk assessment.

E. fetida compared to other earthworms

E. fetida is an epigeic earthworm species, that lives in places rich in organic matter and it is mainly associated with compost and manure such as dung heaps. Endogeic earthworms like *Aporrectodea caliginosa* have different habitat, feeding strategy and life cycle. Differences in life cycle are one of the reasons why endogeic earthworm species are more difficult to maintain under laboratory conditions (Lokke and van Gestel, 1998).

Regarding the suitability of *E. fetida* as a sensitive indicator species with regard to toxicity of PPPs on earthworms, Frampton et al. (2006) reported that *E. fetida* was mostly less sensitive than other Lumbricidae, based on an analysis of a rather limited dataset (13 substances) of acute toxicity endpoints. Pelosi et al. (2013) suggested *A. caliginosa* as an indicator species after demonstrating a higher sensitivity of *A. caliginosa* and *Lumbricus terrestris* than *E. fetida* to PPPs. However, the analysis focused only on acute toxicity data since reproductive studies were not considered comparable (i.e. same earthworm development stage, active substance, type of substrate, pesticide addition, applied organic matter, and experimental duration). According to Daam et al. (2011), whose evaluation included both acute and chronic toxicity data from the US EPA ecotox database, the sensitivity of *E. fetida* appeared to be similar or slightly greater (for herbicides) compared with other Lumbricidae. There was no analysis available specifically comparing chronic sensitivity of different earthworm species.

Pelosi et al. (2014) reviewed public literature on acute and chronic effects of pesticides on earthworm including effects in the laboratory for authorized pesticides. The review done by Pelosi et al. (2014) allows a comparison of the chronic sensitivity of some earthworm species, since some studies were done under comparable lab conditions (listed in table 32).

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Table 32: Comparison of chronic sensitivity of different Earthworm species based on published literature for pesticides registered in Europe (adapted from Pelosi et al., 2014)

Reference	Species	Studied parameters	Active substance	Substrate	Organic material	Application method	Main Results
Bauer and Rombke (1997)	<i>Eisenia. fetida</i> , <i>Aporrectodea longa</i> <i>Lumbricus terrestris</i>	Mortality, biomass and reproduction	Amitrol/ diuron	Natural and artificial	Cow manure and dried leaves	Sprayed and mixed	No evidence of any effects
Kreutzweiser et al. (2008)	<i>E. fetida</i> , <i>D. octaedra</i>	Survival, weight and cocoon production (and leaf consumption)	Imidacloprid	Litter	Litter and leaf material	Sprayed and mixed	<i>D. octaedra</i> , more sensitive than <i>E. fetida</i> . Significant weight losses among survivors of <i>D. octaedra</i> at 3 mg/kg. No effects on cocoon production among survivors at 3 mg/kg. <i>E. fetida</i> , significant weight losses at 14 mg/kg
Ma and Bodt (1993)	<i>A. caliginosa</i> , <i>A. longa</i> , <i>E. fetida</i> , <i>Eisenia veneta</i> , <i>L. terrestris</i> <i>Lumbricus rubellus</i>	Mortality and reproduction	Chlorpyrifos	Natural and artificial	Sphagnum peat or leaves of alder	Mixed	Sensitivity <i>Eisenia</i> < <i>Aporrectodea</i> < <i>Lumbricus</i> . Reproduction more sensitive than survival. Substrate influence species sensitivity. Acute and chronic differences in sensitivity within one order of magnitude.
Viswanathan (1997)	<i>E. andrei</i> <i>L. terrestris</i>	Biomass, cocoon production, excretion	Terbuthylazine	Artificial	Alfalfa	Mixed	No reduction of juvenile number for <i>L. terrestris</i> at high doses. For <i>E. andrei</i> , reduction of cocoon production and biomass, increase of cocoon sterility with increasing duration and pesticide concentration
Dittbrenner et al. (2012)	<i>E. fetida</i> , <i>A. caliginosa</i> , and <i>L. terrestris</i>	HSP (Heat Shock Protein) 70 protein level and avoidance behaviour	Imidacloprid	Reference test soil		Mixed	HSP protein quantity is not a good biomarker of imidacloprid toxicity. <i>E. fetida</i> showed the strongest response of HSP protein levels. Significant avoidance behavior but different between species.

The few references allowing a direct comparison of chronic sensitivity of different earthworm species do not allow us to draw any robust conclusion about the sensitivity of *E. fetida* compared with other species. Pelosi et al. (2013) suggested that the more rapid excretion and higher metabolic rate of *E. fetida* could be the reason for a potentially lower sensitivity of *E. fetida* compared with other earthworm species, according to available literature.

In conclusion, data from the literature indicate that *Eisenia* species may not be the most sensitive species compared to other earthworms. Further research is, however, required in order to reach a robust conclusion on this issue, especially considering chronic sensitivity. Therefore, the widely used standardized test with the model species *E. fetida* is, for the time being, still recommended, taking into account that it is easy culturable and it is considered as covering potential adverse effects of PPPs on other earthworm species.

***Eisenia fetida* compared to Enchytraeids**

Regarding the suitability of *E. fetida* as a sensitive indicator species also covering potential effects on Enchytraeids, Jarratt and Thompson (2009) discuss that Enchytraeids might be more suitable as standard test organisms than Lumbricidae, since tests on Enchytraeids have practical advantages. Their comparison of acute and chronic toxicity of different chemicals to earthworms and Enchytraeids, however, indicates that Lumbricidae were either similarly or more sensitive than Enchytraeids overall, based on mortality and reproductive endpoints. In their comparison there was also a small number of chemicals for which Enchytraeids were >5 times more sensitive than earthworms.

The Panel concludes that it is likely that the response of *E. fetida* will be representative for Enchytraeids and that remaining uncertainties can be addressed by an appropriate assessment factor. Therefore it is proposed to use *E. fetida* as surrogate test species for Enchytraeids taking into account an appropriate assessment factor.

***Eisenia fetida* compared with gastropods**

Limited data are available for terrestrial gastropods, that might allow us to evaluate whether they can be considered covered by the current battery of toxicity tests for PPPs. Terrestrial gastropods are considered to be suitable bioindicators for metals (e.g. De Vaufleury et al., 2006; Viard et al., 2004; Cortet et al., 1999) and have been used frequently for *in situ* biomonitoring of sites contaminated by heavy metals (see e.g. Cortet et al., 2000). Various studies have been performed assessing the effects of different heavy metals on growth, food consumption, fecundity, mortality and molecular stress responses in terrestrial molluscs, mainly snails (Cortet et al., 2000; de Vaufleury 2015; Triebkorn, 2009). For the retrospective risk assessment of metals, a standard test is requested to assess the soil quality using the vineyard snail *Helix aspersa* Müller (ISO, 2006). In the guideline, a test procedure to assess the effect of exposure to contaminated soil and a procedure to assess the effect of contaminated food mainly on growth (but also lethality) of juveniles of *H. aspersa* are described. This test is well qualified to assess the effect of metal contamination (Triebkorn, 2009) but as emphasised in the guideline itself, the test is not adequate to test volatile compounds. (Sverdrup et al., 2006) showed that it is not very suitable to assess the effects of PAHs.

There is less literature available on the effects of organic chemicals on terrestrial gastropods. Some studies investigate the side-effects of non-molluscicidal organic pesticides on snail and slug individuals; these studies consider contact exposure on contaminated surfaces, exposure *via* oral uptake of contaminated food or contact exposure in contaminated soil (e.g. according to ISO 15952) and record mortality, (shell) growth, sublethal signs of effect (e.g. body swelling, lethargy), histological changes or effects on enzyme activity (Rorke et al., 1974; Schuytema et al., 1994; Coeurdassier et al., 2001; 2002; Triebkorn et al., 2005; Druart et al., 2011; Hartnik et al., 2008).

There are also a few experiments available studying the effects of pesticides on terrestrial gastropod egg development (Iglesias et al., 2003; Druart et al., 2010; Druart et al., 2012). The test protocols allow studying effects on the embryonic development of land-snail eggs and the hatching success.

4504 Bioassays can be performed testing exposure via solid phase (contaminated soil) as well as via
4505 liquid/gaseous phase. The egg stage of terrestrial molluscs usually takes place in soil and therefore
4506 solid phase bioassays have a high ecological relevance for terrestrial gastropods.

4507 Table 33 summarizes studies from public literature testing the effects of pesticides on terrestrial
4508 gastropods exposed via spiked soil that allowed direct comparison with standard toxicity data. An
4509 overview of most available literature is provided by De Vaufleury (2015).

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4510 **Table 33:** Overview of tests with terrestrial gastropods compared with standard toxicity data.

Reference	Test design	Results for tested organic pesticides	Comparable chronic regulatory standard toxicity data
Coeurdassier et al. (2002)	Juveniles of <i>H. aspersa</i> exposed via ISO substrate (spray application) to dimethoate for 7 days.	Dimethoate (technical a.s.) EC50 growth = 150 mg/kg soil EC10 growth = 9.7 mg/kg soil	NOEC <i>E. fetida</i> = 2.87 mg a.s./kg soil dw
Druart et al. (2012)	Eggs of <i>H. aspersa</i> exposed via natural soil and ISO soil (2.9% and 10% OM content, respectively) to tebuconazole (formulation Corail) and glyphosate (formulation Bypass) for ca. 20 days	Tebuconazole (formulation Corail) tested: EC50 = 0.8(nat-soil) / 7.8(OECD sub) mg a.i./kg soil NOEC = 0.9 / 5 mg a.i./kg soil Glyphosate (formulation Bypass) tested: EC50 = 219(nat-soil) / >300(OECD sub) mg a.i./kg soil NOEC = 178 / >300 mg a.i./kg soil	Tebuconazole (formulation Folicur EW 250 – equivalent to Corail): NOEC <i>E. fetida</i> < 1.5 mg a.s./kg d.w. soil (10% OM, spray application). NOEC <i>Hypoaspis aculeifer</i> = 56.2 mg a.s./kg dw soil* No chronic studies testing the effects of the formulation Bypass (a.i. Glyphosate) towards in-soil organisms were available (please refer to EFSA conclusion Tebuconazole, EFSA Journal 2014;12(1):3485 [98 pp.])
Hartnik et al. (2007)	Juveniles of <i>H. aspersa</i> exposed to Alpha cypermethrin, technical via natural Norwegian soil according to ISO guideline. Effects towards <i>E. cypticus</i> , <i>E. fetida</i> and <i>F. candida</i> tested according to standard guidelines.	Sensitivity <i>E. cypticus</i> > <i>E. fetida</i> > <i>F. candida</i> > <i>H. aspersa</i> , based on EC ₁₀ (growth for <i>H. Aspersa</i> and reproduction for all other species). Sensitivity differed less than by a factor of 5.	

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The amount of data available for comparing sensitivity of terrestrial gastropods and earthworms is too limited to draw a conclusion. In two of the three available studies for which a comparison of the sensitivity between gastropods and *E. fetida* could be made, *E. fetida* was more sensitive; in the third study, the gastropods were more sensitive. It should be noted that, for adult gastropods, exposure to pesticides *via* foliage and litter (oral and contact exposure) could present major pathways of exposure. There are several publications available testing also oral/contact exposure and the possibility to test oral exposure is also included in ISO 15952. However, since the exposure *via* foliage and litter (oral and contact exposure) is not covered by current standard testing requirements, it cannot be assumed that the toxicity towards terrestrial gastropods *via* these pathways is covered by current standard toxicity tests.

It is considered a critical issue that standardized test systems recommend using relatively large snail species, since the vast majority of land snail species are tiny (< 1cm diameter in greatest dimension) and some even have maximum diameters of < 1mm (see e.g. Sturm et al., 2006). Due to their smaller ratio of surface area to volume, these smaller species may be more susceptible to pesticide exposure than the current, standard test species.

The panel concludes, that based on the limited amount of data available, no conclusion can be drawn about whether the standard test with *E. fetida* could be protective for gastropods, taking into account an appropriate assessment factor. It is also noted that potentially major exposure pathways, i.e. contact and oral exposure *via* foliage and litter, are not covered by standard testing requirements. At this stage, however, a suitable test system or a suitable testing strategy for gastropods cannot be recommended. For the test systems presented, it is as yet unclear whether the responses of the tested species/life stages are representative of the majority of terrestrial gastropods. Further research is required on this issue with the aim of developing suitable test systems to be used in regulatory practice.

***E. fetida* compared with nematodes**

The bacteria-feeding nematode *Caenorhabditis elegans* Maupas, (Rhabditida: Rhabditidae) has almost ideal properties (e.g. easy to culture, simple body plan, and short generation and life cycle) to be used as a model organism in genetics, developmental biology, and medical biochemistry. The effects of chemicals on *C. elegans* at various organizational levels (molecular, organs, whole organism, and population) have been described. Several types of toxicity assays are available, including whole-organism endpoints such as growth, reproduction and mortality, feeding inhibition, as well as molecular assays measuring the induction of stress-response genes. LC₅₀ and LD₅₀ in *C. elegans* were considered as predictive values of rat toxicity, highlighting the sensitivity of this nematode. *C. elegans* has been utilised widely for toxicity tests, including pesticides such as the fungicide captan and a number of organophosphates (Rajini et al., 2008). *Caenorhabditis elegans* is, however, considerably less sensitive to many common environmental toxicants than other soil invertebrates (Sochova et al., 2007). This may be due in part to the opportunistic lifestyle of *C. elegans*.

Apart from *C. elegans*, various other soil and marine nematode species have been utilised to assess the toxicity of xenobiotic compounds, such as pesticides. The nematode *Panagrellus redivivus* detects toxin concentrations that affect moulting and nematode size through stimulation, inhibition or lethality, enabling it to be used as a bio-monitor (Neher, 2001). It has also been used to ascertain toxic effects of several hundred single chemicals (Neher, 2001). The most sensitive species are classified as cp-4 or cp-5 in the coloniser-persister classification of Bongers (1990).

The most appropriate species would probably be predatory nematodes with K-strategies e.g. *Mononchus* spp., but at the moment no standardized laboratory test is available. Predatory nematodes, such as Mononchidae, feed on other nematodes or on invertebrates in the soil. They are a very sensitive group of nematodes and are not able to adapt quickly to environmental disturbance. They were assigned a cp-4 value in the coloniser-persister classification by Bongers (1990) and to feeding group 5 by Yeates et al. (1993). Nematodes such as Longidoridae, Discolaimidae and Thorneinematidae that belong to cp-5 can also be potential bioindicators. These nematodes have the

longest life spans of all the groups (Ferris and Bongers, 2009). Nematodes in the group also demonstrate the lowest fecundity, lowest metabolic rates and sluggish movement. They are particularly sensitive to toxins, as well as to other disturbances within their environments, due to their permeable cuticles (Ferris and Bongers, 2009).

Studies have focused on the effects of herbicides on plant-parasitic nematodes, especially root-knot nematodes and cyst nematodes (Browde et al., 1994; Dewar et al., 2000; Gilreath and Santos, 2004). In a recent review, Daam et al. (2011) found that nematodes are more sensitive to fungicides (copper sulphate and cupric chloride) than *E. fetida*. This comparison was based on toxicity values of 14 nematode taxa. However, when the effects of copper compounds were tested by using single nematode species, toxicity values were variable compared to those of *E. fetida*.

Nematodes are often chosen as bioindicators of the health status of all in-soil organisms. Zhao and Neher (2013) indicated that herbicides decreased abundance of both fungivores and predators; however, abundance of bacterivores, plant parasites and omnivores increased. Overall, total nematode abundance tended to increase in response to herbicide application.

Although there have been many studies about the interaction of agrochemicals and entomopathogenic nematodes (EPNs), there are limited toxicity data allowing a direct comparison of the sensitivity of EPNs with other model organisms (Koppenhofer et al., 2003). Several studies with insecticides (Garcia del Pino and Jove, 2005) and herbicides (Garcia del Pino and Morton, 2010) have reported absence of sublethal effects of agrochemicals on EPN biology. A direct comparison of the sensitivity of *E. fetida* with nematodes could not be made, since the endpoints in the available tests with nematodes (liquid medium) cannot be compared with the endpoints for earthworm laboratory studies (exposure in soil). Thus it is not possible to draw conclusions about the representativeness of *E. fetida* responses for nematodes.

In conclusion, as stated in chapter 3, nematodes are an important and diverse group of in-soil organisms. Standard laboratory test methods are available but endpoints are not directly comparable to other standard laboratory methods. The most commonly used test species *C. elegans* is probably not the most sensitive species. Other species, as mentioned above, could be useful model species, however no standardized test are available at the moment. Therefore, with the available knowledge, it is not possible to propose a standardize laboratory method that would be representative for effects of PPPs on nematodes.

Conclusion on the representativeness of *E. fetida* for non-arthropod soil invertebrates

The comparison of the sensitivity of *E. fetida* to other non arthropod invertebrates key-drivers shows that limited data are available to compare directly the chronic toxicity of pesticides to different organisms. Available evidence indicates that the response of *E. fetida* might not always be representative nor cover the toxicity of pesticides to other key drivers. It should be noted that, for nematodes, a comparison of sensitivity with *E. fetida* was not possible. With respect to representativeness of *E. fetida* in terms of lifestyle, exposure routes and life cycle it should be noted that *E. fetida* is an epigeic species and does not reflect the traits of e.g. endogeic species. However, currently available data, do not allow us to propose an alternative species as a sensitive indicator.

9.2.2. Representativeness of *Folsomia candida*, *Folsomia fimetaria* and *Hypoaspis aculeifer* for in-soil arthropod invertebrates

For in-soil arthropod invertebrates it was evaluated whether *F. candida*/*F. fimetaria* and *H. aculeifer* are suitable species to represent in-soil arthropod invertebrates (i.e. other springtails and mites other groups that are not tested, e.g. macroarthropods) in the first-tier risk assessment.

***F. candida* and *F. fimetaria* compared with other springtails**

No systematic studies have been published on the sensitivity of *F. candida* and/or *F. fimetaria* compared with other collembolans. Table 34 shows three studies from the public literature. From these, with the exception of the the experiments with picoxystrobin in Schnug et al. (2014), it seems that tests on *F. candida* or *F. fimetaria* with an appropriate assessment factor could be representative of other Collembola. According to the Schnug et al. (2014), the absence of effects on *F. fimetaria* might be due to food and habitat preferences, causing a much lower exposure. However, it is uncertain whether these effects would be also absent in a standard laboratory toxicity test.

Table 34: Overview of tests with springtails, comparing toxicity between species.

Reference	Test design	Results for tested organic pesticides
Wiles and Frampton, 1996	Bioassays, field contaminated soil was studied in the lab. Test was conducted on four species (<i>Isotoma viridis</i> , <i>Isotomurus palustris</i> , <i>Folsomia candida</i> (Collembola: Isotomidae) and <i>Sminthurus viridis</i> (Collembola: Sminthuridae) and three substances: chlorpyrifos, cypermethrin and pirimicarb.	Residues of cypermethrin and pirimicarb were of low toxicity, causing less than 10% mortality; residues of chlorpyrifos were toxic to all four species of Collembolan (from most to least susceptible) <i>S. viridis</i> > <i>F. candida</i> > <i>Isotomurus palustris</i> > <i>Isotoma viridis</i> .
Schnug et al., 2014	Spiked soil, four species <i>Proisotoma minuta</i> , <i>Heteromurus nitidus</i> , <i>Folsomia fimetaria</i> and <i>Protaphorura fimata</i> tested with esfenvalerate, picoxystrobin and triclosan in a soil multispecies test system.	For esfenvalerate differences in LC ₅₀ between species were within factor of 2. All species were not sensitive for triclosan, for picoxystrobin large differences in sensitivity (5 orders of magnitude between LC ₅₀ of most (<i>P. firmata</i>) and less sensitive species (<i>F. fimetaria</i>)). This difference might be due to food and habitat preferences.
Lokke and Van Gestel, 1998	Standard test, three species <i>F. fimetaria</i> , <i>F. candida</i> and <i>Isotoma viridis</i> with copperchloride, LAS and dimethoate.	Direct comparison was difficult because endpoints and soils are different between tested in-soil organisms. Generally, no systematic differences were found between the three species.

In conclusion, very little data are available to compare the sensitivity of *F. candida* and *F. fimetaria* with other springtails. The few available data indicate that *F. candida* or *F. fimetaria* could be representative of other springtails with regard to their toxicological sensitivity and it is therefore recommended to use it as a surrogate test species for Collembola considering an appropriate assessment factor. In one case, *F. fimetaria* appeared to be insensitive compared with other species, but this might be a consequence of food and habitat preferences combined with the test conditions.

***H. aculeifer* compared with other mites**

Lokke and Van Gestel (1998) compared the sensitivity of the herbivorous, oribatid mite *Platynothrus peltifer* with the predatory mite *H. aculeifer* for copper chloride, LAS and dimethoate. The results show that EC₅₀ for reproduction does not differ by more than a factor of 2. For *Oppia nitens*, test methods were also available (Princz et al., 2010), however no data were found that allow comparison of the sensitivity of this species with other mite species.

Since hardly any data were found comparing the sensitivity of *Hypoaspis aculeifer* with other soil mites, no conclusion can be drawn concerning the representativeness and further research is required on this issue.

F. candida compared with *Hypoaspis aculeifer*

From the EFSA conclusions on active substances, chronic toxicity data on *Folsomia candida* and *Hypoaspis aculeifer* were extracted. For completeness, sub-lethal toxicity data on *Eisenia* sp. were also extracted.

For 51 cases and 30 PPPs (10 herbicides, 11 fungicides, 8 insecticides, one acaricide), long-term toxicity data both on *Folsomia candida* and *Hypoaspis aculeifer* were available (see Figure 24). *Hypoaspis* was more sensitive than *Folsomia* for 8/51 compounds (15.7%). However, for three cases out of those eight, *Eisenia* was the most sensitive in-soil organism among the species tested. *Hypoaspis* showed a similar sensitivity than *Folsomia* for 22 compounds out of 51 (41%). These results were confirmed by Owjori et al. (2014), who reported lower to intermediate sensitivity of *H. aculeifer* in comparison with other in-soil organisms for which standardized tests are available (*E. fetida*, *F. candida*, and *Enchytraeus albidus*). Huguier et al. (2015) also concluded that, compared with other in-soil meso-fauna invertebrates, mites were in general as sensitive or less sensitive than other test species, depending on the studied endpoints and chemicals. The status of *H. aculeifer* as the only predator among organisms for which a standardized protocol is available, however, highlights its usefulness in ecotoxicological laboratory studies. Please see Appendix H. for more information about the data used in the analysis. In the standard laboratory test, however, *H. aculeifer* is not exposed via the route food uptake since it is fed with clean prey organisms. For exposure via soil, *H. aculeifer* does not always add information concerning the toxicity of in-soil organisms to tested chemicals compared with the test with *F. candida*. It is therefore recommended to study the possibility of adapting the test guideline in order to take the food-uptake route into account, which would be relevant for a predator such as *H. aculeifer*. Further research is needed in order to assess the sensitivity of *H. aculeifer* since it is not clear whether *H. aculeifer* is representative of other soil mites. Since *F. candida* is the most sensitive species in most of the cases, the mode of action of the substance does not seem to be determinant of the response when comparing *F. candida*, *H. aculeifer* and *E. fetida*.

In conclusion, it was found that *H. aculeifer* is the most sensitive test species for only 3/51 cases. This result indicates that it might be sufficient to test *E. fetida* and *F. candida* with the present test design. *H. aculeifer* represents another trophic level (predator species) in the standard test battery. Since it remains unclear if *H. aculeifer* itself is a good representative for soil mites and the feed-uptake route is not included, it is recommended to adapt the test protocol accordingly.

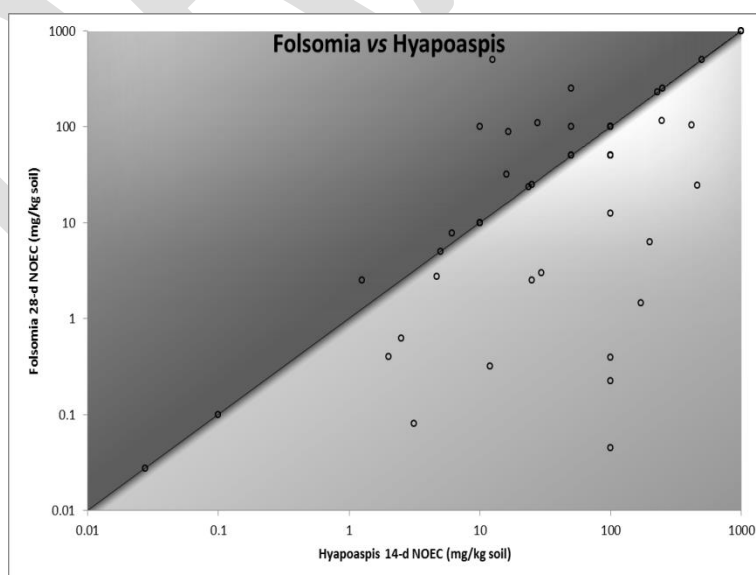


Figure 24: Comparison of sensitivity to a set of PPPs (active substances, metabolites or formulations) of *Folsomia candida* and *Hypoaspis aculeifer*. The solid line indicates a 1:1 relationship between *Folsomia candida* and *Hypoaspis aculeifer*. The light grey area in the figure indicates where *Folsomia* is more sensitive to pesticides.

4667 ***Folsomia candida* compared with Isopods**

4668 For the comparison of the sensitivity of *F. candida* with Isopods, a review study was done based on
4669 literature (ISI WoK) and database search (US-EPA, PPDB database, PAN pesticide database).

4670 Toxicity data on Isopods and Collembola were found for 13 compounds (see Table 35). Most of the
4671 toxicity data reported for isopods were based on mortality or parameters related to individual growth
4672 and feeding performance. Data reporting effects on reproduction and avoidance behavior are scarce.
4673 The best represented isopod species is *Porcellionides pruinosus*, a species with a widespread
4674 distribution, being very abundant in southern European and tropical countries. This is considered an
4675 “in-soil” species, since it can be found in the soil and not predominantly in litter, unlike most isopod
4676 species. It is also abundant near agricultural areas. For Collembola, toxicity data found were mostly on
4677 mortality and reproduction, with few references on avoidance behavior. As expected, *Folsomia*
4678 *candida* was the best represented species.

4679 **Table 35:** Number of bibliographic records found per isopods and collembolan species and
4680 different PPPs

	<i>Circonis cus ornatus</i>	<i>Porcel lio dilatatus</i>	<i>Porcel lio scaber</i>	<i>Porcellion ides pruinosus</i>	<i>Folso mia candida</i>	<i>Folso mia fimeta ria</i>	<i>Orches ella cincta</i>	<i>Sinell a curvis eta</i>
Abamectin			5		4	2		
Atrazine				1	2		2	
Benomyl				8	10			
Carbofuran				3	4			
Copper				2	1	1		
Diazinon			14	6	4			
Dimethoate		1	8	6	14			
Endosulfan		1			3			
Glyphosate				1	3			
Imidacloprid			9		4			
L-cyhalothrin	5			18	3			1
Lindane				5	6			
Spirodiclofen				1	1			

4681

4682 The analysis of the toxicity data for Isopoda was done first by comparing the toxicity considering
4683 exposure *via* food and soil. Data for the different isopod species were pooled and differences between
4684 soil types were taken into account when possible. When more than one value exists for the same
4685 parameter and same exposure pathway, the geometric mean was calculated.

4686

Table 36: Acute and chronic endpoints measured in Isopoda and *Folsomia candida* for several PPPs

	Isopods (Food)		Isopods (Soil)		Folsomia spp	
Abamectin			LC50 (Nat soil)	71	LC50 (Nat soil)	5.1
			NOEC_growth (Nat soil)	3	EC50 (Nat soil)	1.4
Atrazine			AC50* (Nat soil)	153.1	NOEC_repro	40
					LOEC_repro	80
					EC50 (Nat soil)	43.2
Benomyl	LC50	34679.7	LC50 (Sandy soil)	1068.8	NOEC_mort (Nat soil)	1
	NOEC_growth	1			LC50 (OECD)	13-18
					EC50 (OECD)	8-11
					EC50 (Nat soils)	2-10
					AC50 (OECD)	4-15
Carbofuran	LC50	486	LC50 (Sandy soil)	31.02	NOEC_repro (OECD)	0.01
					LC50 (Nat soils)	0,06-0,09
					EC50 (Nat soils)	0,06-0,12
Copper			AC50 (Nat soil)	922.1	AC50 (OECD)	18
					EC50 (Nat soil)	1200
Diazinon	LC50	182.6	LC50 (Sandy soil)	3.34	LC50 (OECD)	0.1
	NOEC_growth	>100			NOEC_repro (OECD)	0.01
	NOEC_feeding	>100			EC50 (Nat soil)	0.288
Dimethoate	LC50	>75	LC50 (Nat soil)	34	LC50 (Nat soil)	1.5
			LC50 (OECD)	44	LC50 (OECD)	0.6
			EC50_growth (Nat soil)	17.5	NOEC_repro (Nat soil)	2.7
			EC50_growth (OECD)	41.2	EC50_repro (Nat soil)	0.77
			AC50 (Nat soil)	33.2	NOEC_repro (OECD)	2.7
Endosulfan	NOEC_feeding	50			LC50 (Nat soil)	0.08
					AC50 (Nat soil)	0.5
					EC50 (Nat soil)	0.05
Glyphosate			AC50 (Nat soil)	39.7	EC50_repro (Nat soil)	0.42
					NOEC_avoid (Nat soil)	1.2
Imidacloprid	NOEC_mortality	>25			LC50 (TAS)	20.96
	NOEC_growth	>25			NOEC_mort (TAS)	10
	NOEC_feeding	10			EC50 (TAS)	0.06
					NOEC (TAS)	0.01
L-cyhalothrin			NOEC_mort (TAS**&OECD)	0.16	NOEC_repro (OECD)	7.6
			NOEC_mort (Nat soils)	0.17	EC50 (OECD 5%)	5.64
			LC50 (TAS&OECD)	0.61		
			LC50 (Nat soils)	0.31		
			NOEC_repro (OECD)	0.1		
			NOEC_repro (Nat soil)	0.1		
			EC50_repro (OECD)	0.4		
			EC50_repro (Nat soil)	0.13		
Lindane			LC50 (OECD)	80	LC50	1
			AC50 (Nat soil)	35.3	EC50 (OECD)	0.13
					NOEC (OECD)	0.03
					EC50 (Nat soil)	0.8
Spirodiclofen			AC50 (Nat soil)	0.9	EC50_repro (Nat soil)	0.65

*AC50: concentration inducing avoidance in 50% of the tested animals

**TAS: Tropical artificial soil

For the four compounds where comparable data are available (mortality data only), contact exposure to soil was revealed to lead to more adverse effects for isopods than food exposure. Although data are scarce, toxicokinetic studies performed by Sousa et al. (2000) with lindane revealed that isopods were able to accumulate more when exposed *via* soil than when exposed *via* food (the reason was mainly the high excretion rates observed and the slower degradation of the compound in the soil matrix). Despite this trend, this comparison between exposure routes should be interpreted carefully since none of these studies considered another important exposure route for these animals, contact in litter.

The sensitivity of Isopoda and Collembola was compared for soil exposure only using the same parameters and measurement endpoints, directly for eight compounds (mainly acute data) and indirectly for five of the compounds. In this last comparison the parameters assessed were not the same, but it is possible to infer some trend in sensitivity by looking at the differences between values obtained.

For seven out of eight compounds (abamectin, benomyl, carbofuran, copper, diazinon, dimethoate and lindane), Collembola showed a higher acute and chronic sensitivity than Isopoda (several orders of magnitude higher). Indirect comparisons (looking also at chronic parameters) revealed a similar trend. The exception was L-cyhalothrin, where *P. pruinus* was more sensitive than *F. candida* with effects on reproduction occurring at lower concentrations,

As indicated in section 6.5, isopods are key drivers for several ecosystem processes, especially those linked to organic matter decomposition and nutrient cycling. Although they can take up chemicals by being in contact with contaminated soil (*via* the cuticle), the main route of exposure for most isopod species is in litter. Isopods can take up chemicals *via* contact with moist litter surfaces, but their exposure is higher when they feed on contaminated litter material (Peijnenburg et al., 2012).

When considering effects of PPPs on isopods exposed *via* soil, existing data show that their sensitivity is covered by other test species, e.g., the collembolan *Folsomia candida* (see above). However, the assessment of effects *via* exposure to contaminated litter, especially *via* food consumption, should be considered when designing a more appropriate hazard assessment of these compounds.

Despite the absence of a fully standardized ecotoxicity test (i.e., ISO or OECD guideline) on isopods, there is a vast experience in the literature on using them as model organisms for ecotoxicological evaluations of PPPs, ranging from assessing individual and population-level parameters (e.g. Vink et al., 1995; Jansch et al., 2005; Zidar et al., 2012; Morgado et al., 2016) to ecotoxicogenomic studies (e.g., Costa et al., 2013a; 2013b). Most published studies with PPPs focus on three species (*Porcellio scaber*, *Porcellio dilatatus* and *Porcellionides pruinosus*) and address lethal effects and effects linked to food consumption (measuring consumption, assimilation and assimilation efficiency) and their direct effects on energy allocation (measuring energy reserves) and, ultimately on growth (measuring biomass changes) at an individual level (Ribeiro et al., 2001; Stanek et al., 2006; Drobne et al., 2008; Zidar et al., 2012; Ferreira et al., 2015). Some studies also address behavioural parameters (Engenheiro et al., 2005; Loureiro et al., 2005; 2009; Santos et al., 2010). Although all these parameters can influence the onset of reproduction, number of offspring and, ultimately, population growth, it would be important to have more information on the direct effects of chemicals on reproductive parameters. In fact, the number of studies addressing direct effects on isopod reproduction are quite rare (e.g. Jansch et al., 2005).

All these aspects, allied to the need to assess effects to key in-soil organisms having a relevant exposure to PPPs *via* consumption of litter debris, prompt the need to develop further a standardized test addressing both feeding and reproduction parameters. Despite the few papers on the optimization of culture and test conditions (e.g., Caseiro et al., 2000), further research on the optimal isopod species, test design (e.g., test media, test duration, type of parameters), and litter material to use is needed. The extensive information already existing in literature could be a good starting point for a proposal for an ISO or OECD guideline needed to cover this group of key drivers *via* this particular and relevant exposure route to PPPs.

In conclusion, based on the available literature, *F. candida* may be protective for chronic effects on isopods when exposed *via* soil in the laboratory, taking into account an appropriate assessment factor. However, Isopoda are key drivers for several ecosystem processes and the preferential route of exposure for most isopod species is litter on the soil surface. Therefore, the panel recommends development of a standardized test addressing both feeding and reproduction parameters, in order to assess effects to key in-soil organisms having a relevant exposure to PPPs *via* consumption of litter debris. The available information on isopods renders this a good candidate for developing an ISO or an OECD guideline.

Conclusion on the representativeness of *Folsomia candida*/*Folsomia fimetaria* and *Hypoaspis aculeifer* for non-arthropod soil invertebrates

The few available data, indicate that *F. candida* and/or *F. fimetaria* could be representative of other springtails with regard to their toxicological sensitivity. Since hardly any data were found comparing the sensitivity of *H. aculeifer* with other soil mites, no conclusion can be drawn concerning its representativeness for other mite species. Even though with regard to the representativeness for the ecosystem, *H. aculeifer* seems important since it is the only predator in the test battery for which a standardized test protocol is available, an analysis of its toxicological sensitivity indicated that it showed a relatively low sensitivity compared with *F. candida*. Based on the available literature, *F. candida* may be protective for chronic effects on isopods when exposed *via* soil in the laboratory

However, none of these studies considered an important exposure route for this animals, i.e., contact in the litter on the soil. Therefore, no conclusion can be drawn on that respect. In order to take this important exposure route into account, the panel recommends development of a standardized test addressing both feeding and reproduction parameters, to assess effects to key in-soil organisms having a relevant exposure to PPPs *via* consumption of litter debris.

9.2.3. Conclusion and recommendations for the choice of the standard laboratory test system for invertebrates

A lot of data is available concerning the toxicity of PPPs to individual species of soil invertebrate. Only limited data are available, however, that allow a sound scientific comparison, due to differences in substance, test conditions, duration, exposure route and/or endpoint.

Making an overall comparison of the sensitivity of current standard test species, Frampton (2006) and Daam et al. (2011) show that *E. fetida* is often not the most sensitive species when compared with soil arthropods, such as springtails, arachnids and isopods. Daam et al. (2011), compared the sensitivity of *E. fetida* in the standard laboratory test to the sensitivity of other standard laboratory test species. The authors concluded that testing only *E. fetida* was not predictive for a number of other species, and that the inclusion of *F. candida* in the test battery significantly lowered uncertainty due to inter-species variability, which has been implemented in the most recent version of the data requirements (Reg 283/2013 and 284/2013). No conclusion could be drawn, however, on the representativeness of the current test battery for many of the other relevant invertebrate key drivers also due to limited availability of toxicity data.

With respect to representativeness of *E. fetida*, in terms of lifestyle, exposure routes and life cycle, it should be noted that *E. fetida* is an epigeic species and does not reflect the traits of e.g. endogeic species. A robust comparison of sensitivity could rarely be performed. No comparison with *E. fetida* was possible for nematodes. Since currently available data do not allow us to propose alternative species, it is concluded, that for the time being *E. fetida* should be used as a representative species for non-arthropod invertebrates in first-tier risk assessment with an appropriate assessment factor. Research is required with the aim of developing suitable tier 1 test systems to complement current testing of non-arthropod soil invertebrates with regard to the sensitivity of test species and tested exposure routes.

The few data found, indicate that *F. candida* and/or *F. fimetaria* could be representative of other springtails with regard to their toxicological sensitivity. For the time being, *F. candida* and *F. fimetaria* are therefore considered suitable model species for springtails. Since hardly any data were found comparing the sensitivity of *Hypoaspis aculeifer* with other soil mites, no conclusion can be drawn concerning their representativeness for mites, and further research is required on this issue. Even though with regard to the representativeness for the ecosystem, *H. aculeifer* seems important since it is the only predator in the test battery for which a standardized test protocol is available, an analysis of its toxicological sensitivity indicated that due to their comparably low sensitivity it might be omitted from the tier 1 test battery. Based on the available literature *F. candida* may be protective for chronic effects on isopods when exposed via soil in the laboratory and may be an appropriate representative species for isopods considering an appropriate safety factor. However, none of these studies considered an important exposure route for these animals, i.e. for *H. aculeifer* contact via food, and for isopods contact in the litter layer.

Therefore, it is recommended to adapt the test with *H. aculeifer* to take food uptake into account.

Isopods are key drivers for several ecosystem processes and the preferential route of exposure for most isopod species is the litter layer. Therefore, the panel recommends to develop a standardized test addressing both feeding and reproduction parameters, to assess effects to key in-soil organisms having a relevant exposure to PPPs via consumption of litter debris. The available information on isopods renders this a good candidate for developing an ISO or an OECD guideline.

Overall, considering the available data, the Panel considers that the current test battery with the use of an appropriate (calibrated) assessment factor might cover the intra and inter species variability in soil, with the exception of the toxicity of in-soil organisms when exposed via food and via the litter layer. Therefore, the Panel recommends further research aiming at investigating the toxicity of PPPs on in-soil organisms other than the standard ones (*E.fetida*, *F.candida* and *H.aculeifer*) to allow a better estimation of their representativeness in terms of sensitivity compared to the other key drivers as identified in chapter 5 (i.e. Enchytraeids, Isopods, Nematodes, terrestrial molluscs). In addition, further research is needed on the toxicity of in-soil organisms when exposed via food uptake and via the litter layer. It is recommended to adapt the test with *H.aculeifer* to take food uptake into account, and to develop a standardized test with isopods, to take exposure via the litter into account.

9.3. Choice of standard laboratory test methods for microorganisms

9.3.1. General considerations for microbes

A description of the available test methods for microorganisms is reported in Appendix I.

While general toxicity-test systems using single microbial species or strains (e.g. Johnson et al., 2009; Dijksterhuis et al., 2011) can give information both on direct effects on the test organism and on specific toxicity mechanisms or degradation pathways, they are of limited use in prospective risk assessment for microbes in general. Their main disadvantage is the quite low representativeness of one species to the vast microbial genotypic and phenotypic diversity of soils. Also, a big majority of microorganisms currently cannot be cultured as pure isolates, but can only be studied in the context of more or less complex natural communities (e.g. (Wagner, 2004)). Hence, using field samples in mesocosm or microcosm experiments with entire communities has substantially higher environmental relevance and has recently been advocated (Puglisi, 2012); (ECHA, 2014; Karpouzias et al. 2014).

In community-level tests using (semi-)field studies with natural soil, longer term chronic effects induced by pesticides can be determined, taking taxonomic and functional shifts in microbial communities into account. Endpoints can relate to function (activities or processes), biomass (total biomass, or biomass for taxonomic or functional sub-groups) or structural properties (community structure or diversity) (see Appendix I.). Interpretation of the outcomes of microbial community-based tests is generally more straightforward for functional than for structural endpoints. While for the former, a rate decrease (in e.g. nitrogen transformation or a specific enzymatic reaction) can be

considered as an adverse effect on certain populations, for the latter, a changed structure pattern (e.g. disappearance of some operational taxonomic units but appearance of others) is more difficult to translate into potential environmental risk.

Chemical pesticides (and other organic pollutants) can influence microorganisms in two opposite ways: i) the compound is toxic to the microbe and has a negative impact on its metabolic activity and growth; and ii) for a microbe exhibiting tolerance to the compound, it may act as a carbon source and growth substrate and, hence, stimulate growth of those organisms. A consequence of the possible dual effects of pesticides on different metabolic and functional groups of microbes is that spiking soil with pesticides commonly results in increases in microbiological parameters. Accordingly, in the terrestrial dataset of the systematic literature review of responses of microorganisms to pesticides (Puglisi, 2012; external procurement funded by EFSA), a substantial part of the entries in the database represented significant increases. The summary evaluation of the data was reported separately for type of substance (fungicides, herbicides and insecticides) and type of assay (based on activity, biomass or community structure). Regarding effects on microbial activities, 39-47% of entries showed no significant effects, 21-32% significant decrease and 11-22% significant increase. For assays based on biomass, corresponding numbers were 26-55% (no effect), 11-23% (decrease) and 14-44% (increase). Interestingly, it was noted (Puglisi 2012) that for both the activity- and biomass-based endpoints, increases in response were more common for insecticides than for either fungicides or herbicides. This could perhaps imply that insecticides generally have less adverse effects on soil microorganisms than do fungicides and herbicides. In the analysis of effects on endpoints based on community structure (using PCR-DGGE, PLFA, BIOLOG etc.), a different and qualitative approach was taken using three classes: significant changes, transient significant changes and no significant changes. The majority (80-95%) of the entries showed significant changes in community structure, while the remainder showed either no or transient effects.

It is important to keep in mind that, in community-based tests, a no-effect outcome in a functional endpoint after pesticide spiking to soil is a net response, and not proof that no populations have been affected in either a negative or a positive way. In community-based measurements of chronic effects, functional redundancy – i.e. if organisms performing a certain function are inhibited or eliminated, other organisms take over and fill the niche – can also hide effects on specific populations within functional groups, thereby complicating the interpretation of test outcomes.

9.3.2. Prospects for improving test strategies for microorganisms

Phylogenetic or functional groups as well as individual species of organisms contribute to several of the different ESs, perhaps most evidently within the microorganisms. To begin with, all soil microorganisms principally contribute to the ESs genetic diversity, cultural services and food-web support. Apart from this, taking denitrifying bacterial taxa as an example, these bacteria will often also contribute to the ESs nutrient cycling, natural attenuation and possibly pest and disease control. Additionally, although knowledge regarding both the structural and functional properties of soil microbes is growing rapidly, for many functions and activities performed by soil microbes (especially common functions performed by many taxonomical groups), confining the functions to certain phylogenetic units is still a challenge.

Based on the above it is concluded that, for selection of the most relevant community-based microbial test systems, the best strategy seems to be to consider assays that cover as wide a range as possible of processes and key drivers, while it is hardly realistic to design a set-up containing specific assays for specific key drivers. The currently required community-based functional tests of effects on N transformation (EU and North America) and on general microbial activity (CO₂ evolution; North America) take the activity of a wide selection of microbes into account, but are considered comparatively coarse and insensitive. MicroResp is a recent development of the BIOLOG method that can give more detailed information regarding the capacity of a sample to degrade different types of compounds, and the effect of pollutants on degradation (Campbell et al. 2007). It describes the functional/physiological capacity for degradation and measures microbial activity as CO₂ evolution

directly from e.g. soil separately for each substrate. So far, MicroResp has not been used to study effects of pesticides on soil microbial processes but it is recommended that its capacity in that respect is determined in future research.

The aim of finding broad microbial tests that cover as much as possible of phylogenetic and functional variation may need to be balanced against the aim of sensitivity. For illustration, functional redundancy can be expected to contribute less to insensitivity when studying more specialised functions than those functions that are widely distributed among many microbial groups. One example is that potential nitrification has been found to be relatively sensitive to pollutants (Pell et al., 1998), since the ammonia-oxidising bacteria mediating the first step represent a small group of fastidious, slow-growing lithotrophic bacteria. In recent years, however, it has become evident that some archaeans can also oxidise ammonia in soil (Prosser and Nicol, 2012).

Currently, there is no data requirement related to mycorrhizal fungi. Their importance in providing several important ecosystem services is, however, well known and reported in the literature. In addition, mycorrhizae provide specific functions (e.g soil formation) that represents an exclusive trait of mycorrhizae in general and arbuscular mycorrhizal fungi in particular.

A standardized test with arbuscular mycorrhizal fungi was proposed by ISO (ISO/TS 10832:2009) where the acute effect of chemicals (or contaminated matrices) on spore germination of the arbuscular mycorrhizal fungal species *Glomus mosseae* (strain BEG12) is assessed. The test is conducted over 14 days at 24 °C on sand or OECD artificial soil, with the percentage of germinated spores, in relation to the number of recovered spores, being the measured parameter.

The test species (*G. mosseae*) has been proposed as a model species to assess the toxicity of different chemicals due to its widespread distribution around the world and its high abundance to crop systems (Smith & Read, 2002). When testing different PPPs, however, Giovannetti et al. (2006) found that spore germination was only a sensitive parameter towards some of the fungicides tested but not to the tested herbicides. On the other hand, mycelium growth (also at the asymbiotic phase) proved to be a much more sensitive parameter, being inhibited by all fungicides and herbicides tested.

These results could indicate that, when using arbuscular mycorrhizal fungi as a test species, parameters other than spore germination could be assessed, increasing the informative value of the assessment. This could indicate some improvements in the existing protocol to accommodate the measurement of extra parameters. In a recent study, Mallmann (2016) demonstrated the practicability and reproducibility of the ISO protocol, together with its ability to be used with other arbuscular mycorrhizal fungi species (five species were tested) and to measure other parameters, including the growth of the asymbiotic mycelium. Moreover, since the sensitivity to some chemicals can vary between strains of the same species, studies like this one that focus also on evaluating the inter- and intra-species variation in sensitivity should be performed and the more appropriate test species and strain should be selected.

Conclusion

From the above (and from Appendix I.) it can be concluded that a number of methods exist to measure soil microbial properties of soil in terms of abundance, activities/functions as well as community structure. A number of interpretation problems are identified, e.g. effects on functional endpoints might be diluted by functional redundancy; regarding structural endpoints, the interpretation in the sense of their connection with certain functions is hard to define.

Novel methods are continuously developed that are able to record a broad spectrum of functional and structural endpoints. These methods need further adjustment and standardisation for use in risk assessment of pesticides, but may become useful tools in the future.

Since the present test aimed at N transformation covers a number of processes, it is considered a relevant indicator, most obviously for the functions nutrient cycling and food-web support. In the present test design for agrochemicals, it is prescribed to test two dosages and to determine at different points in time whether effects are >25%. When after 28 days effects are >25%, the test can be prolonged to 100 days. For non-agrochemical substances, however, a dose-response design is prescribed. In order to assess whether the effects fit with the magnitude and the temporal scale mentioned in the SPG chapter, it is recommended to use a dose-effect design for agro-chemicals as well.

Activities of protozoa and many fungi are, to a substantial extent, covered by the N transformation test, whereas, mycorrhizae are not incorporated. It is therefore recommended to add a test with mycorrhizal fungi. The ISO test with *Glomus mosseae* allows for adaption of the endpoints measured or to test other species/strains. Further research and development is needed to improve the test design.

9.4. Conclusions and recommendations concerning the choice of standard test species

In summary, based on the analyses presented above it is recommended to use as standard test species in the first tier: *E. fetida*, *F. candida* and/or *F. fimetaria*, *H. aculeifer* and N-transformation.

Exposure *via* food uptake is only partly included in the standard laboratory tests, since normally uncontaminated food is often provided after exposure. When food is an important exposure route, the test protocols might be adapted better to include this route. Therefore it is recommended to adapt the test with *H. aculeifer* in order to take exposure *via* food into account. This can be accomplished by giving live food (e.g. young specimens of *Folsomia candida*) that have been feeding on yeast spiked with the PPP to be tested. Research is needed to infer properly the spiking doses and the exposure time of *F. candida* to the contaminated yeast, taking into account the toxicokinetics of the PPP in the collembola, in order to obtain the desired body residues in the food to be offered to the mites.

Isopods are key drivers for several ecosystem processes and the preferential route of exposure for most isopod species is the litter layer or litter debris on the soil surface. Therefore, the panel recommends development of a standardized test addressing both feeding and reproduction parameters, in order to assess effects on key in-soil organisms having a relevant exposure to PPPs *via* consumption of litter debris. The available information on isopods renders this a good candidate for developing an ISO or an OECD guideline.

The present standard test species do not cover mycorrhizal fungi. Mycorrhizal fungi are considered very important for many ecosystem service. Due to their peculiarity, they are not considered covered by the N transformation test. It is therefore recommended to add an additional standard test with mycorrhizal fungi. The ISO test with *Glomus mosseae* allows for adaption of the endpoints measured or to test other species/strains. Further research and development is needed to improve the test design.

Most tests are designed for the most common modes of action. In general, for specific or new modes of action, it should be carefully checked whether the current standard test methods are sufficiently protective.

Most test methods are designed for spray applications, but can be adapted in order to test effects of soil fumigants, treated seeds and granules. Methods need to be developed for testing effects of other application methods. For soil fumigants, experience is available for nematodes (see e.g. Zasada et al, 2007, Ma et al., 2001). For the off-field, an option for granules would be simulating exposure with dust particles.

9.5. Additional laboratory testing

When assessing active substances and PPPs, additional laboratory-toxicity data exceeding the regulatory requirements (Commission Regulation (EU) No 283/2013 and No 284/2013) might be

available, due to the legal obligations to submit scientific peer-reviewed open literature data. This open literature data may be used in identifying additional toxicity data for relevant species, which are not captured in the standard test package. Additional data on relevant species can be used to address intra and inter species variability. From the analyses of the sensitivity of test organisms (see section 9.2), used in the standard laboratory tests, it is clear that species may vary markedly in their sensitivity to PPPs and this difference may have a large contribution to the uncertainty of the assessment based on current standard test species.

This variation in direct toxicity can be described by constructing an SSD (species sensitivity distribution). The SSD is a statistical distribution estimated from a sample of laboratory-toxicity data and visualised as a cumulative distribution function. SSDs are used to calculate the concentration at which a specified proportion of species is expected to suffer direct toxic effects. These concentrations, the hazardous concentrations, are expressed as HC_x values and represent the value that affects a specific proportion (x %) of species. For regulatory purposes, usually the HC₅ is used, the hazardous concentration to 5 % of the species tested. When compared with the first-tier effect assessment on the basis of standard test species, SSDs have the advantage of making more use of the available laboratory-toxicity data for a larger array of species. They describe the range of sensitivity rather than focusing on a single value, they enable estimates to be made of the proportion of the species affected at different concentrations, and they can be shown together with confidence limits showing the sampling uncertainty due to the limited number of species tested (EFSA PPR Panel, 2013). The concept of SSDs is applied in e.g. aquatic risk assessment or terrestrial non-target-plant risk assessment. In the guidance document for aquatic risk assessment (EFSA PPR Panel, 2013) other options are given as well, such as the geomean-assessment factor. The latter means that, when not enough data are available to apply a reliable SSD, the geomean of comparable toxicity data for species of the same taxonomic group could be used for further risk assessment (instead of the lowest, most sensitive endpoint). Further research is needed in order to draw conclusions about the use of this type of alternative for the in-soil risk assessment.

Frampton et al. (2006) described the sensitivity range of in-soil organism by using the SSD approach for acute toxicity data. It is clear from table 31 and Appendix C that test methods addressing chronic toxicity are available for a number of additional species. It is, however, also clear that the number of test protocols is limited, especially within certain groups.

For additional information on the minimum number of toxicity data and other practical points (e.g. use of unbound values, etc) for constructing a SSD, please refer to the EFSA PPR Panel (2013).

SSD might be a powerful tool to reduce uncertainty in the risk assessment linked to intra- and inter-species variability in sensitivity to PPPs. Contrary to aquatic organisms, however, there is only limited experience in combining toxicity data of in-soil organisms in SSD, especially for PPPs. Further research is needed with regard, in particular to combining toxicity data from different groups of in-soil organisms. According to the EFSA PPR Panel Aquatic Guidance (EFSA PPR Panel, 2013), the toxic mode of action of a PPP should be taken into account when constructing SSDs. Chronic toxicity data on different groups of in-soil organisms are, however, rarely available.

9.6. Additional test methods addressing specific questions and issues

9.6.1. Addressing transgenerational effects

Campiche et al. (2007) proposed a multigeneration test with *F. candida* to complement the risk assessment of insect growth regulators. Their results indicated that some endocrine disruptors had effects on several generations even though only the F₀ generation was exposed. The potential of transgenerational effects may present an uncertainty that could be addressed by additional testing. It should be investigated during the development of the guidance document how relevant transgenerational effects are for the response of field communities and how transgenerational effects

differ between species. Ernst et al. (2015) proposed a multigeneration test with Collembola to address recovery in a laboratory study. This proposal is discussed in Appendix G.

9.6.2. Bioaccumulation

The assessment of potential effects of bioaccumulation or biomagnification on in-soil organisms is not included in the procedure for risk assessment. The guideline for bioaccumulation in terrestrial oligochaetes (OECD, 2010) is aimed at calculating the bioaccumulation factor (BAF) in order to assess the potential for secondary poisoning in birds and mammals (EFSA, 2009a). The literature concerning bioaccumulation in in-soil organisms is aimed at concentrations that might affect predators, not at effects on in-soil organisms themselves (e.g. Gobas et al., (2015)). Effects of bioaccumulation are partly inherently included in the tests, since the test substance can accumulate in the test organism during the test. No information is available on whether e.g. substances with a relative low bioaccumulation potential might have effects on in-soil organisms in the long term.

9.6.3. Avoidance

For a number of test organisms, avoidance tests are available, standardized or from literature (Table 37).

Table 37: Overview of avoidance test methods.

Taxonomic group	Test species	Reference
Earthworms	<i>Eisenia fetida</i> / <i>Eisenia andrei</i>	(ISO, 2008)
Enchytraeids	<i>Enchytraeus albidus</i>	(Amorim et al., 2008a,b)
Mites	<i>Oppia nitens</i>	(Owojori et al., 2011)
Woodlice	<i>Porcellionides pruinosus</i>	(Loureiro et al., 2005)
Springtails	<i>Folsomia candida</i> / <i>Folsomia fimetaria</i>	(ISO, 2011)

Avoidance tests are mainly used for retrospective risk assessment, e.g. the assessment of contaminated soils. Avoidance test are of relatively short duration (48 h), and thus give quick results. In a number of cases, the sensitivity of the avoidance test is compared with the acute toxicity data for a specific substance, and appears to be representative and thus suitable for assessing the contamination (e.g. Rombke (2008); De Silva and Amarasinghe (2008)).

Avoidance tests are not part of the current risk assessment for PPPs. Some authors suggest that the avoidance test is sensitive and could be used as an alternative to an acute toxicity test (e.g. Garcia et al. (2008) for benomyl, carbendazim and lambda-cyhalothrin). Other authors, however, (e.g. Novais et al. (2010), based on results for phenmedipham, atrazine, carbendazim, pentachlorophenol, dimethoate and lindane) show that avoidance behaviour for *E. albidus* was not clearly correlated with survival and reproduction and do not recommend use of the avoidance test as an alternative to toxicity tests.

Results of avoidance tests can yield valuable information interpreting e.g. effects found in long-term laboratory studies as compared to field studies. Moreover, assuming that avoidance response to a certain dose of a PPP is inversely related to the potential (re)colonization of an area contaminated with that PPP dose, avoidance results can help to predict recolonization of in-field areas (Renaud, 2012). If coupled with population models, avoidance tests can help in predicting spatially based recovery of in-field populations (Meli et al., 2014). Avoidance tests might well play a role as an alternative for range-finding tests for chronic toxicity studies.

9.6.4. Biomarkers

Numerous studies with biomarkers are available. For an overview see e.g. Kammenga et al. (2000) and Fontanetti et al. (2011). Biomarkers can be used as a sensitive indicator for exposure, but also to predict ecological effects. Information from biomarker assays could provide supporting information for risk assessment. In particular, they might be especially useful in the case that the standard tests do

not show effects within the duration of the test but, due to the mode of action of the substance, delayed or long-term effects can be expected.

In an external EFSA report (Duncan et al., 2009), a review was made of long-term effects on invertebrates after short-term pulsed exposure. In that review, concerning in-soil organisms, only an example related to effects of diazinon on *Porcellionides pruinosus* is mentioned. Five weeks after application an effect on protein content occurred, that might result in effects on reproduction. It is not clear whether these effects would have been detected in the standard laboratory tests for prospective risk assessment.

9.7. (Semi)-field methods for higher tier testing

9.7.1. Available (semi)-field methods

Appendix D gives an overview of (semi)-field studies evaluated by Brown et al. (2009) and Schaeffer et al. (2008) with some additions from literature, but has no claim of being exhaustive.

As is evident from Appendix D., a variety of methods is available. In current regulatory field-test protocols, tests are performed in-field on replicated plots and study effects on ‘natural’ in-soil populations present at the time of the experiment. In other field-study methods, specimens were added to the system. In some cases, test systems are chosen on an uncontaminated soil (e.g. grassland), and the test substance is applied to the test soil or system. In the current procedure, laboratory tests are performed with arthropods, non-arthropods and microorganisms. Some field-study methods (e.g. the earthworm field study) are aimed at refining the particular first-tier test. In chapter 4, it is proposed to study the natural assemblages of the soil community. It is clear from Appendix D that most field studies are aimed at effects on natural assemblages of the soil community.

Semi-field studies are defined as controlled, reproducible systems that attempt to simulate the processes of and interactions between components in a portion of the terrestrial environment, either in the laboratory (small scale) or in the field, or somewhere in between. Single-species field studies with earthworms are submitted for the peer-review process in a standardised way.

The mobility of the test species in semi-field studies is artificially limited, using e.g. enclosures or TMEs. TMEs can be conducted in the field, but it is also possible to move the TMEs to the laboratory and to study effects under controlled conditions.

Schaeffer et al. (2007) classify the different types of semi-field studies according to a number of ecological and performance criteria (Table 38). They distinguish three main types of semi-field studies: the assembled system, the terrestrial models ecosystem, and field enclosures.

Table 38: Rough classification of three semi-field methods according to eight ecological and performance criteria. The grey shading indicates whether the criteria are fulfilled or not (from Schaeffer et al., 2008).

Ecological criteria	Assembled System	TME (Terrestrial Model Ecosystem)	Field enclosures	
	Relevance	Artificial food chain, but no real competitors/prey-predators	Natural community	Increased density of predators
	Endpoints	No community measures	All ‘known’ parameters can be measured	All ‘known’ parameters can be measured

Performance criteria	Flexibility	No crop simulation simulation possible	Most soils, except very sandy/very dense soils	All soils
	Sensitivity			
	Practicability			
	Reproducibility/repeatibility		Exact results not reproducible because of natural plasticity	Several studies, but few publications
	Experience	If similar approaches are combined	EU ringtest	
	Standardization	None	ASTM guideline and UBA draft available	IOBC guideline available

5097

High/good/many	
Medium/fair/numerous	
Low/poor/few	

5098

5099 From the table above, it is clear that assembled systems have a low score for a number of criteria.
5100 Studies with assembled systems can be very useful to answer specific scientific questions. Their role
5101 for risk assessment, however, is limited because *inter alia* it is hard to predict what happens when an
5102 additional species is added to the system. As a result of the classification, Shaeffer et al. (2007)
5103 conclude that TMEs are the most promising method for assessing effects of pesticides.

5104 In the intermediate tier 2 of the proposed risk-assessment flow chart (see section 7.8 and Figure 20),
5105 an option is given to the applicant to provide (or to the risk assessor to require) additional information
5106 on effects to particular soil-organism groups of interest, gaining relevance by assessing mainly indirect
5107 effects of the substance of concern towards these groups.

5108
5109 The proposed test for this tier is a microcosm set up using natural, defaunated soil (sieved and
5110 submitted to several freezing/thawing cycles) to which fragments of the natural community of the
5111 group of in-soil organisms of interest is added (e.g., microarthropods, nematodes, mesofauna including
5112 microarthropods and enchytraeids). These systems resemble the existing gnotobiotic tests (Schäffer et
5113 al., 2011), but have a major difference; instead of assembling the tested community with a limited
5114 number of fully known species, usually those existing in laboratory cultures, these microcosms contain
5115 a true fraction of the natural community collected in the field, thus increasing the number of species in
5116 the system and therefore the possible interactions between them (e.g. either *via* competition or
5117 predation).

5118
5119 In brief, samples are collected in the field, the natural community is extracted (by adopting standard
5120 methods for each organism group – see Römbke et al. (2006) for the sampling and extraction methods
5121 for different organism groups) and then it is added to the spiked natural soil on the microcosms.
5122 Depending on the group of interest, different methods can be adopted to decrease the variation on the
5123 number and composition of the community in each replicate microcosm.

5124
5125 Although the experiment is set up to focus on a specific group of in-soil organisms (e.g.,
5126 microarthropods), species from other groups could be added to the system to increase the realism (e.g.,

nematodes or enchytraeids). Microorganisms are always included by re-inoculating the soil with a microbial suspension extracted from the same natural soil.

This means that the microcosm setup can be tailored according to data needs and to the type of substance. For instance, if focusing on an insecticide, the group of interest could be the microarthropods. This means that, although other species from other groups (e.g., enchytraeids) could be added to the microcosm to increase realism, only the microarthropods could be assessed at the end. Organisms can be identified at species or at life-form group level, or using any other trait-based typology of interest.

Besides having the advantage of working with a relevant fraction (in terms of number of species) of the natural community of interest, assessing not only direct effects to several species but also indirect effects *via* interactions among them, these systems have the advantage to ally the reproducibility of laboratory single species tests (mainly regarding the possible number of replicates) with a gain in ecological realism.

There is limited experience of assessing effects of chemicals, especially PPPs, using this approach. Effects of carbofuran have been assessed on nematode and microarthropod communities from temperate and sub-tropical soils by Chelinho et al. (2011, 2014). Data on nematodes showed not only the decrease in abundance and richness, but a shift in community composition of these organisms (although no shifts were detected on feeding groups). Data on microarthropods clearly showed that the direct effect on Collembola (an overall reduction in abundance and species richness together with shifts in community composition favouring epigeic over euedaphic species) clearly affected the abundance of competitor-mite species (an increase in oribatids was observed) and originated a decrease in the abundance of predatory mites.

Whilst not aiming to replace a TME or a field study, where a higher level of ecological realism can be obtained, these test setups could help to obtain more realistic information on both direct and indirect effects on specific communities of interest with much less workload and a higher level of statistical power. Nevertheless, due to the limited experience available, further research is needed to refine some methodological aspects, namely on the size of the test vessels according to the community of interest, number of replicates, and the duration of the experiment to accommodate the variation in the life cycles between species.

The experience with TMEs is increasing. Protocols are available from ASTM (1993), UBA (1994) and an updated protocol was presented at the PERAS workshop (see section 2.3). Knacker et al. (2004) describe a number of TMEs exposed to carbendazim in different regions, comparing the results from the TMEs with those in the field. In his thesis, Scholz-Starke (2013), describes the results of TME studies with lindane. The pros and cons of four types of TME (defined by Knacker et al. (2004)) are discussed: closed, homogenous TMEs; closed, intact TMEs; open, homogenous TMEs; and open, intact TMEs. TMEs are suited to studying effects on a number of invertebrate species, including some earthworm species. It is also possible to include effects on micro-organisms, including functional endpoints, by e.g. including litterbags or bait lamina tests in the TME. Different aspects of TMEs correlated with their use as higher tier studies related to the risk-assessment flowchart presented in section 7.8 (see also Figure 20) are discussed below.

9.7.2. Addressing specific protection goal in (semi-) field studies

Specific protection goals for in-soil fauna

Specific protection goals are proposed for in-soil organisms as drivers of particular ecosystem services, both in-field and off-field. When interpreting field studies, it needs to be made sure that they are able to address the SPGs and to detect relevant effects on the key drivers. The currently proposed specific protection goals for in-soil animals are (these may be adapted following the risk manager consultation):

5178 In-field: For earthworms, enchytraeids, microarthropods, macro-detritivores (e.g. isopods), nematodes,
 5179 molluscs (slugs and snails), small effects (10%<35%) up to months on abundance/biomass of
 5180 populations are tolerable.
 5181 For enchytraeids, microarthropods, macro-detritivores (e.g. isopods), nematodes optionally also
 5182 medium effects (35% <65%) up to weeks on abundance/biomass of populations are tolerable.
 5183 Off-field: For all organisms only negligible effects ($\leq 10\%$ or NEL).

5184 **Statistical power to detect relevant magnitudes of effects in field studies**

5185 It is important to understand the power of various field study designs to detect effects at magnitudes
 5186 relevant to the specific protection goals. For earthworms, in chapter 3 it is indicated that in-field small
 5187 effects on abundance and biomass (10-35%) for months are acceptable, off-field no effects ($\leq 10\%$ or
 5188 NEL) are acceptable. The present earthworm field study according to ISO 11268-3 (ISO, 1999), with
 5189 improvements of Kula et al. (2006), however, is aimed at recovery after one year. Often, only a limited
 5190 number of dosages is used and, in the test design used, it is not possible in practice to detect effects of
 5191 less than 50% on overall abundance and/or biomass with sufficient statistical significance. For
 5192 individual species, which have lower abundances, the ability to detect effects will be lower. The
 5193 standard endpoints of the test thus do not fit with the required level of protection. In the test protocol,
 5194 it is also prescribed to measure effects after three and six months, indicating that potentially the
 5195 duration of effects is in line with the data requirements. In the present design, however the field test
 5196 with earthworms will not be able to show whether effects of 10-35% occur, or to derive a NEL or an
 5197 EC₁₀. An example how to calculate the power of the test is given in De Jong et al. (2006). This implies
 5198 that it might be needed to adapt the study protocol, in order to obtain results that can be used to
 5199 address the acceptability of effects identified for the SPGs.

5200 In TME studies, the number of replicates can be higher; a dose response design can be followed,
 5201 which means that it is more realistic to derive EC_x values from the results, with a higher statistical
 5202 reliability than in the case of field studies. Since the distribution of in-soil organisms in agricultural
 5203 fields might be highly variable, variation in TME studies can also be high and the statistical power
 5204 should be checked. Earthworms can be tested in TME studies as well (Rombke et al., 2004). However,
 5205 the variation appeared to be relatively high, so it is also questionable in this case whether TME studies
 5206 with earthworms would be able to yield a reliable EC₁₀ or EC₃₅ value.

5207 Scholz-Starke (2013) conducted TME studies that on average were able to detect between approx. 5-
 5208 10% (nematodes) and 40-50% (collembolans and enchytraeids) deviation from the control level. The
 5209 MDD (minimal detectable difference) can be decreased by increasing the number of replicates. This
 5210 can be the number of TME cores, but in the case of low numbers an option can be to increase the size
 5211 of the cores so that the number of samples within the core (and thus the number of individuals) can be
 5212 increased. MDDs can also be decreased by improving the sampling techniques (Brock et al. 2015).

5213 In the case of experiments using natural communities of in-soil organisms (suggested as a surrogate
 5214 reference tier), control and treatment communities will often have a different composition. If the
 5215 differences in composition are potentially large, a large number of replicates will be needed to achieve
 5216 a MDD less than 100%. Such differences can partially be accounted for by the Henderson-Tilton
 5217 (1955) calculation or related methods. However, with such a method, it is not possible to obtain a
 5218 statistical quantity like the MDD.

5219
 5220 Until now, a power calculation has to our knowledge not been performed for any of the field protocols
 5221 available for in-soil organisms. To be able to use the field protocols in regulatory practice, sufficient
 5222 replication and abundance of relevant species should be ensured to be able to detect the above-
 5223 mentioned magnitudes of effect.

Assessing long-term effects and studying recovery

At present the only field study that is widely used to study effects on in-soil organisms is the field study with earthworms. At present, recovery one year after application is taken as an endpoint; for further details see chapter 2.

It is therefore recommended, for the time being, to study community recovery only experimentally in field studies. As noted earlier (see section 7.4), recovery over long time periods may be best addressed by population modelling, but impacts and recovery at community levels cannot be assessed using population models and therefore need to be assessed using field studies. As stated in section 7.3, population modeling can address the impact and recovery from year on year application of PPP on in-soil organisms species in a system approach. Effects of PPP use and recovery at community levels cannot be assessed using population models for the time being and therefore need to be investigated in (semi) field studies with intact communities of in-soil organisms.

Depending on the fate and behavior of the substance in soil, the application timing, the lifestage of the exposed organism as well as toxicokinetics and toxicodynamics, effects on the community might be detectable at later stages. This might be the case even if there are no effects visible in the short term or if recovery of short-term effects is observed. Long term impacts may be related e.g. to disrupted trophic interactions or to reproductive effects. Several species of in-soil organisms are univoltine, some may only be able to complete one generation after several years (please refer to section 3.2.1 and 7.9). Therefore, the timeframes for assessing impacts and recovery at community levels in (semi) field studies with in-soil organisms need to be appropriate to detect delayed effects of the application of a test substance.

As can be seen in the figure below, field sizes in Europe are mostly above 10 ha/field, in intensively managed areas up to 60 ha per field. It is likely that, considering the relatively low dispersal ability of in-soil organisms (see section 3.2.2) and the average field sizes in Europe, recolonization of fields by in-soil organisms from off-field within a year will be very limited. Experiments studying external and internal recovery in the same plots will yield only a very limited informative value as to whether SPGs are fulfilled.

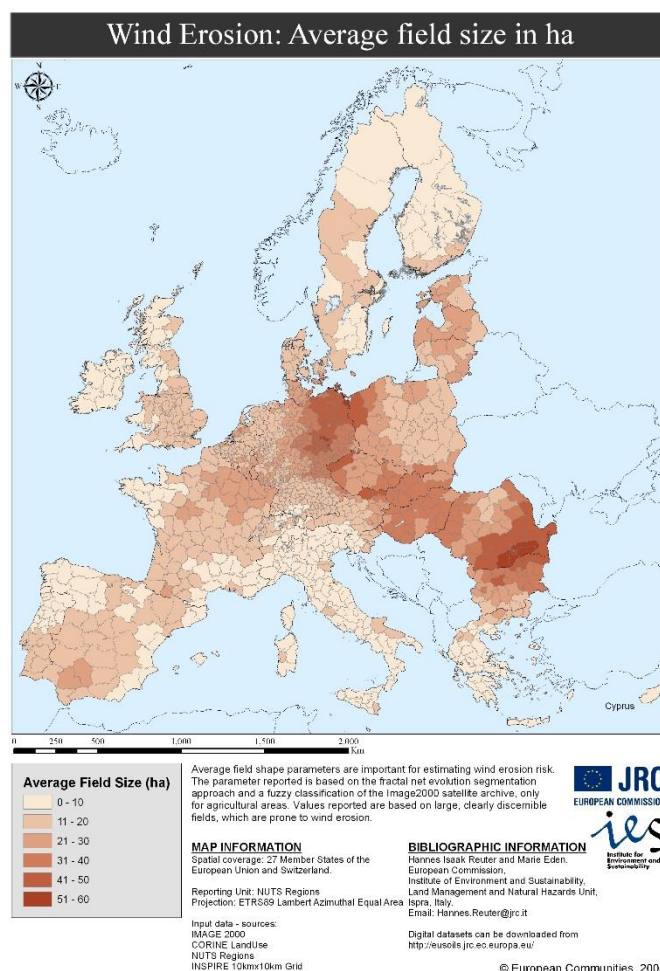


Figure 25: Average field sizes in Europe (see Reuter, H.I. and Eden, M. (2008))

In the example of earthworm field studies performed according to ISO 11268-3 (ISO, 1999), plot sizes are 8x8-9x9m and plots lie 2-4m apart in a randomized block design. Considering the mean dispersal rates for different earthworm species (up to 10m per year, see section 3.2.2), it is very likely that significant migration between plots and the surrounding area will occur within a year, which will be the case to a much lower extent for the majority of European fields being mostly >1000 times larger than study plots in earthworm field studies. So external recovery is likely to be severely overestimated in such studies.

A recent study by Ernst et al., 2015 aims to address recovery of Collembola in a multigeneration study testing *F. candida*. This approach does not, however, sufficiently address the properties of the types of potential stressors and the specific features of the landscape, i.e. variations in land use, and the types, spatial distribution and connectivity of habitats, as reported in section 7.4 and is therefore, for itself, not considered suitable for assessing recovery (please refer to Appendix G.).

In order to incorporate recovery in the risk assessment, it is important to keep in line with the specific protection goals. Protection goals are defined in chapter 6, indicating an acceptable magnitude and duration of effects as well as the relevant spatial scale. On the basis of protection goals, focal taxa, focal communities and/or focal landscapes should be identified, based on relevant traits. For in-soil organisms, it is suggested that risk assessment is performed at the in-field scale (i.e. encompassing variation of factors that determine local differentiation of populations) and at field-boundary level

(encompassing variation between in-field and off-field habitats representing); please refer to section 6.1.

Scholz-Starke (2013) conducted TME studies with lindane. Stability of the TMEs could be influenced by e.g. effects of isolation, effects on diversity by predators, or removing of soil for sampling purposes. Scholz-Starke defined four criteria that should be met in order to ensure stability of the system: abundance, diversity, similarity and soil removal. The results show that TMEs are relatively stable, and therefore suited to detect the possibility for recovery, even for a relatively persistent substance as lindane, and for a recovery period of one year. This implies that the duration of a TME study is such that the majority of the taxa present will be able to show reproduction.

For field experiments to assess the risk at the field scale, it is suggested to choose a test design that excludes unrealistic external recovery (e.g. by enclosing study plots as in TMEs or surrounding the plot by a large enough strip of PPP treated area). This will allow a realistic worst-case prediction of long-term effects at the local scale.

It is possible to study the interaction at the field-boundary scale to determine the contribution of migration to recovery under realistic conditions. In order to be able to make generalized predictions, however, the information from field-boundary experiments needs to be integrated in the risk assessment.

9.7.3. Exposure in field studies

In both current regulatory field studies and proposed new methods like TMEs, the test substance is applied as described in the GAP and the concentration after exposure is measured in the soil. In earthworm field studies, analysis of initial soil concentrations is regularly performed following the recommendations of Kula (2006). In TME studies, exposure is in general measured, often in separate cores (e.g. Knacker et al. (2004), Scholz-Starke (2013)). In contrast with laboratory studies, the substance is not mixed through the soil in order not to disturb the soil structure. Increasingly, a dose-response design is followed. Measurement of concentrations in combination with a dose-response design is needed to determine the consequences of risk-mitigation measures on direct, local PPP effects in off-field habitats or to help extrapolating to different in-field situations. In most standard lab studies, food is supplied after mixing the substance through the soil. Therefore the uptake of the test substance *via* food cannot be quantified easily. In a field experiment, exposure *via* food is included by definition, since the in-soil organisms eat the food from the exposed environment.

9.7.4. Extrapolation/validation

In a large scale study at four locations in Europe, TME were conducted and results were compared with effects in the field (Knacker et al., 2004). Studies were conducted using carbendazim as the test substance. Functional endpoints were studied: nutrient cycling, soil-enzyme activity, microbial SIR and bacterial growth, feeding activity of in-soil organisms and organic matter decomposition. Although carbendazim did not result in clear effects for all parameters, and sometimes the variation between plots and location was rather large, the effects found were similar for the TME and the field situation. As structural endpoints, nematodes, microarthropods, enchytraeids and lumbricids were studied. Although variability was again sometimes high, no differences were found between effects in TMEs and the field situation.

When a valid field study is conducted, the uncertainty of its outcome with regard to the potential outcome under actual field situation should be assessed. Uncertainties that should be checked could be connected to, for example:

- product
- dosage
- method of application
- time, frequency and interval of application

- 5322• type of ecosystem (depends on abiotic factors as soil, climate and on composition of non-target
- 5323 groups)
- 5324• location and isolation of the test system
- 5325• region
- 5326• history of the test system
- 5327• crop and crop-stage
- 5328• in-field and off-field.

5329 These aspects are discussed in detail in De Jong et al. (2005).

5330 The assessment of the reliability of field studies and the interpretation of the effects is complex. It is
 5331 important that field studies are reported in detail in a uniform way, to enhance a uniform assessment of
 5332 the field studies. De Jong et al. (2010) and De Jong et al. (2006) describe in detail the assessment of
 5333 higher tier studies for non-target arthropods and earthworms. These guidance documents distinguish
 5334 between the assessment of the reliability of the field study as such, but also give guidance for the
 5335 interpretation of the results. Such guidance does not exist for the other higher tier studies with in-soil
 5336 organisms, but would be helpful for the interpretation of field-study results.

5337 **9.7.5. General recommendations for further development of existing field-study methods**

- 5338 • To characterise uncertainties with regard to exposure in field studies and to determine the
 5339 worst-case character of exposure would require the measurement of the concentrations in the
 5340 field study.
- 5341 • Measurement of relevant concentrations in combination with a dose–response design could
 5342 prove helpful to determine the consequences of risk-mitigation measures on direct, local PPP
 5343 effects in off-field habitats or help extrapolating to different in-field situations.
- 5344 • It is important to understand the power of various field-study designs to detect effects and to
 5345 evaluate recovery at magnitudes and time scales relevant to the specific protection goals. For
 5346 interpretation of field experiments, not only representativeness but also vulnerability of the
 5347 species present is important.
- 5348 • Long-term field studies, over years, would be an important research priority. These would
 5349 provide both validation for long-term impacts as assessed by population modelling, and
 5350 indicate whether the population effects can be used as a surrogate for community effects. In
 5351 addition, detailed data developed from long-term studies could be used to feed into food-web
 5352 models to improve understanding of community interactions and energy-flows between
 5353 functional groups (Ruiter et al, 1998). In this way food-web models may become tractable for
 5354 predictive in-soil ERA.

5355

5356 Apart from field studies, there are other methods for refinement of lower tier uncertainties. One useful
 5357 possibility is monitoring of actual in-soil populations in the field. Monitoring of the actual in soil
 5358 population can be extremely helpful, especially considering the baseline level of population as
 5359 reference control in the same field and areas where the substance should be applied. For new
 5360 substances, however, it is not really possible to monitor the long-term effects of the use of the
 5361 substance in the field situation. For re-registration of existing products, where concern exists for in-in-
 5362 soil organisms, monitoring data can provide useful information about the effects on the actual
 5363 community in the field situation.

9.7.6. Recovery related to the specific protection goals

In all cases, whether an effect or the duration on an effect is acceptable requires consideration of the specific protection goal. It is important to differentiate whether the aim is to maintain biodiversity, or if the aim is to ensure the provision of a certain level of the ecosystem service (e.g. pest control, food-web support) during a certain period of time. For example, for maintenance of biodiversity at field-boundary level the assessment could focus on whether external recovery from off-field habitats to in-field takes place. However, if the assessment aims at the protection of a certain level of ecosystem function (e.g. food web support, pest control), then impacts may be unacceptable even if the in-field community returns to its pre-disturbed state at a later time.

9.8. Testing metabolites

In line with the PPR Opinion on sediment organisms (EFSA PPR Panel, 2015b) and with the Aquatic Guidance Document (EFSA PPR Panel, 2013), the risk assessment to in-soil organisms of the relevant metabolites should be performed when they are formed or accumulated in the soil compartment. As reported in the Aquatic Guidance Document (EFSA PPR Panel, 2013), in order to minimise the testing, it may be possible to estimate metabolite toxicity by using non-testing methods i.e. by identifying the presence of the toxophore (the active part of the molecule). It is noted, that the non-testing approach for deriving toxicity data would require valid QSAR models for the test species. If the potential effects of the metabolite are not sufficiently addressed by the effect assessment of the parent compound and the toxophore is still present in the metabolite, or it is unclear if the toxophore is present in the metabolite, then specific toxicity tests with the metabolite on the relevant test species (most sensitive with the parent) should be performed. If an assessment indicates that the toxophore is no longer present, in the first instance, it may be assumed that the toxicity of the metabolite is equal to the toxicity of the parent compound for all first-tier test species.

9.9. Modelling approaches to Surrogate Reference Tier and Recovery

Currently, the biological level at which the model should be targeted is the population. As can be seen from the general framework (see Figure 20 and section 7.3), population modelling is considered to be an evaluation that integrates the effects of multiple exposures over time into the lower-tier tests. Since recovery is an integral part of the population response, once we apply the systems approach we no longer need to consider long-term recovery explicitly, since if this does not occur, the population will decline. However, for some SPGs it might be necessary to consider short-term recovery e.g. to maintain a ecosystem service needed at shorter time-scales.

Which type of model is appropriate for the systems approach to population modelling will depend on what data are available, and the modelling question. For in-soil organisms, spatial dynamics are not considered as being important, hence a greater range of model types can be considered than would be the case should action at a distance be important in the assessment.

Depending on model formulation, the model can be generic or specific to e.g. a given species type, chemical type, landscape type. The domain of applicability of the model, including the extent of acceptable extrapolations, should be described by the modeller (EFSA PPR Panel, 2014b).

Since population modelling is considered to be an integration of multiple exposures over time into the lower-tier tests, refinement of the models is permissible only by refinement of the exposure. This strategy is designed to prevent the population modelling becoming highly complex from a regulatory perspective by limiting its use to the pre-selected species and scenarios used for tier 1. This means that the models will need to be designed to work with the standard data from the submission dossier as the inputs, and only these values will be modified by the user, i.e. users will not be able to modify the ecological processes or parameter values for behaviour and ecology of the chosen species.

9.9.1. Overview of different types of effect models for populations

The types of model used in population-level risk assessment have been classified and reviewed, e.g., by Munns et al. (2008) and briefly by EFSA PPR Panel, (2014b). Here we give only an outline summary. There are three main types of model in use; these are: scalar (unstructured), structured (e.g. matrix) and individual-based modelling (IBM, also known as agent-based modelling (ABM)). The model types differ in the degree of detail in the representation of the population and processes that can be represented.

Scalar models – These are the simplest form in which all individuals are treated as identical. They do not differ in age or in any other characteristic and so the population can be fully represented by a single scalar variable representing population size. Density dependence can be incorporated in scalar models, but in general they lack the necessary descriptive power to cope with multiple applications that might affect different stages of an animal's life-cycle differently.

Structured-population models – these are often implemented as matrix models. Distinction can be made between individuals in different categories e.g. ages, sizes. The population is divided into age or size classes – e.g. juveniles and adults – and this gives the population structure, its structure being described by the numbers in each class. All individuals within an age or size class are treated as identical, but there may be variation between the classes in their survival or reproductive rates per unit time. The characteristics of the individuals in each class are entered into the cells of a matrix, and this allows computation of how population structure changes as time progresses. Models of this form can be quite complex.

Individual-based models - In individual-based models (IBMs or ABMs), each individual animal in a population is modelled separately. Individuals may interact with their environment (e.g., depleting food resources by feeding) and with each other (e.g. predation, reproductive behaviour). Individuals should differ in their characteristics, such as their age and size and energy reserves, and each acts according to its modelled needs, e.g., for food, or a mate, or to care for its offspring.

9.9.2. Considerations of models used for in-soil populations

Scalar models are probably too simple to be employed to model a system with complicated, temporally-variable inputs and differential effects on life-stages, but structured population models will often be suitable for this purpose. If complex, these models do suffer from issues of mathematical tractability, but require fewer parameters and therefore fewer explicit assumptions compared with IBMs. Model-development time is also shorter. These models would therefore be most suitable in cases where general assumptions need to be made in the face of uncertain data. A further advantage is that, since these models are mathematical rather than logical, uncertainty is also easier to quantify.

IBMs provide the most detailed description of the effects of chemicals on populations, and generally use more parameters than other model types. The advantages of IBMs are that they can represent the effects of chemicals applied in environments that may change seasonally and can incorporate feedback loops and therefore include more complex ecological interactions than is possible with other model types. IBMs therefore provide the richest potential for prediction if the relevant mechanisms are included (Topping et al., 2015). IBMs do not suffer from mathematical tractability issues, but they do require greater input of resources to development and testing than the other model types. IBMs have a further distinct advantage in that they can incorporate TK/TD models directly.

Toxicokinetic-toxicodynamic models (TK/TD models) - these are not population models but work at the individual level. In these models, the uptake of a chemical and its distribution between the organs of the body and its subsequent biotransformation and elimination processes, are collectively referred to as toxicokinetics. Also modelled are the effects of the chemical where it causes harm within the body, with consequences on individual performances and or life-cycle trait values, referred to as toxicodynamics. These models can be used to refine an exotoxicological assessment when exposure over time is considered variable and important (e.g. in the case of vertical movement in earthworms).

9.9.3. Issues arising in selection of modelling focus

Selection of species – there is no *a priori* reason for selection of one species over another in terms of population modelling. Therefore species selection should be justified in terms of representativity and expected vulnerability based on demographic and ecotoxicological traits. The following demographic traits are relevant to the assessment of population recovery (Rubach et al., 2011):

- Life span;
- Survival to reproduction;
- Generation time (i.e. the interval between reproductive events);
- Voltinism (i.e. the number of reproductive events per year);
- Number of offspring (i.e. clutch size per reproductive event).

In addition, the ecotoxicological components of life-stage sensitivity and methods of incorporating multiple and long-term exposure need to be considered.

Spatial and temporal variation – for both the surrogate reference tier models and recovery, it is necessary to have a realistic, worst-case scenario of exposure during time and space. Since we do not consider action at a distance to have an appreciable influence on the assessment, spatial dynamics may not be necessary in most cases. In the case of vertical movement, however, the same argument applies to in-soil organisms as to NTAs in space (see EFSA PPR Panel, 2015a), and integration of exposure and effects may need to be a dynamic modelling process rather than a combination of statistical distributions. In this case TK/TD modelling will be needed. In order to maximise usefulness by integration with long-term factors and to prevent creation of further parallel tests, however, the TK/TD modelling should be integrated into population models.

9.9.4. Model development

Model development should follow the guidelines given by EFSA PPR Panel (2014b). This involves the following steps implemented in a modelling cycle, which is repeated until the model is considered to perform satisfactorily compared to predefined criteria (adapted from EFSA PPR Panel (2014b)):

1. The problem formulation sets the scene for the use of the model within the environmental risk assessment. It therefore needs to explain clearly how the modelling fits into the risk assessment and how it can be used to address protection goals. In all cases, for the evaluation of in-soil population impacts, the critical issue is the development of a baseline model that represents the state of the population under normal, realistic worst-case conditions, but without the stressor to be evaluated. Evaluation of the model will initially be based on the baseline, and only later on the implementation of the regulated stressor.
2. Model formulation. Based on the problem definition, a conceptual model is designed. The conceptual model provides a general and qualitative description of the system to be modelled. It characterises the environmental and biological processes and their interactions and interdependencies.
3. Model formalisation. In this step, model variables and parameters are defined and linked together into mathematical equations or algorithms. The result of this step is called the formal model.
4. Model implementation. In the following step the formal model is transferred into a computer model by implementing the model equations into computer code. The computer code should be verified to check if it correctly represents the conceptual and formal model.
5. Model set up. In this step, model parameter values are estimated and the computer model is combined with one or more environmental scenarios. The result is called the regulatory model. Note that the regulatory model includes both the computer model and the environmental scenarios. Model analysis, including sensitivity analysis, uncertainty analysis

5504 and comparison with observed data, is an essential part of the procedure to set up the
5505 regulatory model.

5506 In all steps, documentation of the procedure should be provided, and both the model and the computer
5507 code or mathematical equations should be documented and explained clearly for risk managers.

DRAFT

10. CONCLUSIONS AND RECOMMENDATIONS

10.1. Conclusions

The Panel has proposed Specific Protection Goal (SPG) options to be considered in the risk assessment of in-soil organisms exposed to Plant Protection Products (PPPs). Key driver organisms in soils are soil bacteria, mycorrhiza and other fungi, nematodes, earthworms and enchytraeids, microarthropods, gastropods and macroarthropods. The Panel has identified those ecosystem services that are important in agricultural landscapes and are provided by key-driver, in-soil organisms. These ecosystem services are the provision of genetic resources and biodiversity, the maintenance of cultural services, nutrient cycling, pest and disease control, natural attenuation of xenobiotics and toxins, the formation of soil structures and water-retention capacity, and the support of food webs in agricultural landscapes.

The Panel proposes different SPG Options for in-soil organisms living in-field and in off-field areas. Options are discussed for the magnitudes of in-field effects that can be suffered by key drivers without severely compromising the ecosystem-service performance. SPG Options are specific for each key driver group. This is because species traits affect the exposure and the sensitivity of the organisms towards the PPP and also the degree of recovery after disturbance.

Exposure assessments for in-soil organisms are performed according to the EFSA Guidance Document for predicting environmental concentrations in soil (EFSA, 2015). Scenarios are available for the three regulatory zones in accordance with Regulation EC No 1107/2009 of the European Parliament and the Council. Exposure assessments consist of five tiers for which user user-friendly software tools have been developed. The purpose of the exposure assessment is to consider the total area of the crop where it is intended that the PPP should be applied. In order to account for differences in the spatial statistical distribution of crops, scenario-adjustment factors were defined to correct the PEC calculations if necessary.

The soil-exposure scenarios are currently limited to annual field crops under conventional and reduced tillage. However, scenarios for the selection and parameterisation of scenarios for permanent crops and row crops on ridges are under development and will come available soon. Data on the litter layer are scarce and it is suggested that no litter layer, in the sense of a conventional soil horizon, is present in the majority of permanent crops. Grassland or bare soil scenarios are appropriate here. Litter layers might, however, become more important in the future; good soil-management practices are promoting an increase of organic matter on the soil, so there may be a shift to a more sustainable management of this litter layer.

The presence of PPPs on off-field non-target surfaces is mainly caused by the emission of the applied product out of the field by spray drift and runoff. Drift is currently considered to be the most important factor for off-field emissions to non-target surfaces. Drift is defined as droplet drift, but vapour drift and dust drift are also considered to be important emissions in particular cases. The basic processes for run-off emissions were described by FOCUS (FOCUS, 2001). The methodology was originally developed for surface waters but it can be transferred to off-crop areas as described by EFSA PPR Panel (2014a). Several datasets exist for deposition of drift and dust and exchange of air-borne substances with receptor surfaces,. These datasets should be combined in order to produce harmonised approaches (e.g. drift curves).

For soil microorganisms, a number of methods exist to measure soil microbial activity as function and structure. A number of interpretation problems are identified i.e. effects on functional endpoints might be diluted by functional redundancy and for effects on structural endpoints the interpretation in the sense of their impact on the functions. Novel methods are under development in order to be able to measure a broad spectrum of functional and structural endpoints. These methods need further

5555 development and adjustment for use in risk assessment of pesticides, but are promising tools for the
5556 near future.

5557 There is no experience in combining toxicity data in SSD for in-soil organisms. Further research is
5558 needed with regard to several issues, in particular:

- 5559 • Combination of toxicity data from different groups of in-soil organisms. According to the
5560 EFSA PPR Panel Aquatic Guidance, the toxic mode of action of a PPP should be taken into
5561 account when constructing SSDs. Chronic toxicity data on different groups of in-soil
5562 organisms are, however, rarely available.

5563 The intermediate tier 2 of the proposed risk-assessment flowchart comprises a microcosm experiment
5564 with a fraction of the natural community. An option is given to the applicant to provide (or for to the
5565 risk assessor to require) additional information on effects of PPPs on particular in-soil organisms of
5566 interest, assessing mainly indirect effects of the substance on these groups. In order to address this
5567 subject, a microcosm setup is proposed, using natural, defaunated soil to which fragments of the
5568 natural community of the group of in-soil organisms of interest is added. Due to the limited experience
5569 available, further research is needed to refine some methodological aspects, and also to the
5570 interpretation of the results for risk assessment.

5571 The use of terrestrial model ecosystems (TMEs) is suggested as a most promising method for the
5572 surrogate reference higher tier, next to field tests. TMEs can be designed such that they can show the
5573 desired level of magnitude and duration of effects. Since TMEs can last and remain stable for up to
5574 one year, they will also be able to detect effects on reproduction of the majority of the species.

5575 Evaluation of recovery of in-soil organisms' species requires a systems approach at the population
5576 level to determine the effect of a PPP on the population. This is because assessment needs to take into
5577 account the state of the population before exposure to the PPP, as well as long-term and multiple
5578 application effects on the population. Spatial dynamics are not thought to be generally important for
5579 recovery of in-soil organisms in-field, although there is evidence of large-scale spatial phenomena
5580 such as travelling waves in soil invertebrates that may affect recovery (Ridsdill-Smith et al, 2013).
5581 Since recovery is an integral part of the population response, the application of the systems approach
5582 to population modelling removes the need to consider long-term recovery explicitly; if recovery does
5583 not occur, the population will decline. For some SPGs, however, it might be necessary to consider
5584 short-term recovery e.g. to maintain an ecosystem service needed at shorter time-scales.

5585 10.2. Recommendations

5586 The Panel has identified Service Providing Units (SPUs) for different ecosystem services. The most
5587 critical SPU-ecosystem service combination has to be selected for the final SPG option. For ecosystem
5588 services that address structural parameters, e.g. biodiversity, the Panel has identified the *populations*
5589 of different species of in-soil organisms as SPUs (invertebrate SPUs). For ecosystem services based on
5590 soil processes, e.g. nutrient cycling, the Panel has identified as SPUs the abundance and biomass of
5591 different *functional groups* of in-soil organisms (microbial SPUs). Defining SPUs merely at the level
5592 of functional groups, however, may lead to a loss of functional performance under unfavourable
5593 conditions. In order to support the long-term functional role of in-soil organisms in agricultural soils, it
5594 is recommended to define the SPU as the abundance/biomass of populations of the species that make
5595 up the different functional groups.

5596 In order to link exposure and effects reliably, the Panel recommends measuring the exposure
5597 concentration in laboratory tests and in field-test systems. Exposure could be measured using the two-
5598 step extraction procedure that is proposed in EFSA (2015). This consists of a 24-hour extraction with a
5599 0.01 M CaCl₂ solution to characterise the pore-water concentration and a solvent extraction to
5600 characterise the total extractable mass. Measurements should be performed so that it is possible to
5601 characterise adequately the maximum concentration in both space and time. The Panel does not

5602 recommend calculating the pore-water concentration from the concentration in total soil using the
5603 partitioning coefficient. The Panel considers the use of calculated pore-water concentrations
5604 acceptable only for legacy studies where only the nominal concentration is available. It is now
5605 common practice to divide the obtained toxicity endpoint of lipophilic substances by a factor of two.
5606 The Panel considers that scaling of the toxicity endpoint is only justified when only the nominal
5607 concentration is available (legacy studies) and when the ERC is expressed in terms of a pore-water
5608 concentration. In all other cases, scaling of the toxicity endpoint is not justified.

5609 A tiered approach to assessing the effects of PPPs on in-soil organisms should include a relatively
5610 simple, robust set of tests as the lowest tier. After reviewing the existing tests available, the Panel
5611 recommends carrying out tier 1 toxicity tests on five species/process.

5612 The Panel recommends that further research should be conducted on the sensitivity of test species and
5613 tested exposure routes with the aim of developing improved test systems for tier 1 assessment. This is
5614 particular relevant for non-arthropod invertebrates where currently no robust conclusion can be drawn
5615 on suitable test systems to close existing gaps and on assessment factors.

5616 Exposure *via* food uptake is only partly included in the standard laboratory tests, since
5617 uncontaminated food is normally provided following exposure. When food is likely to be an important
5618 exposure route, the Panel recommends adaptation of the test protocol for a better estimation of the
5619 toxicity due to oral uptake. In particular, the Panel recommends the design of test protocols for
5620 carnivorous, non-target, in-soil invertebrates to account for possible trophic chain effects, such as
5621 biomagnification. Until new tests are developed, it is recommended to adapt the test with *H. aculeifer*
5622 in order to take exposure *via* food into account, so as to address properly the exposure of predators.

5623 Isopods are key drivers for several ecosystem processes and the most important route of exposure for
5624 most isopod species is feeding on litter debris. Therefore, the panel recommends development of a
5625 standardized test addressing both feeding and reproduction parameters, to assess effects on key in-soil
5626 organisms of exposure to PPPs *via* consumption of litter debris. The available information on isopods
5627 makes an isopod test a good candidate for developing an ISO or an OECD guideline.

5628 Since the present test aimed at N transformation covers a number of processes, it is considered a
5629 relevant indicator, at least for the ecosystem services nutrient cycling and food-web support. In the
5630 present test design for agrochemicals, it is prescribed to test two dosages, and to determine at different
5631 points in time whether effects are > 25%. When effects exceed 25% after 28 days, the test can be
5632 prolonged to 100 d. For non-agrochemical substances, however, a dose-response design is prescribed.
5633 In order to assess whether the effects fit with the magnitude and the temporal scale mentioned in the
5634 SPG chapter, it is recommended to use a dose effect design for agro-chemicals as well.

5635 The present standard test species do not include mycorrhizal fungi. Mycorrhizal fungi are considered
5636 very important for many ecosystem services but they are not covered by the N-transformation test. The
5637 ISO test with *Glomus mosseae* allows for adaption of the endpoints measured or to test other
5638 species/strains. Further research and development is needed to improve the test design. It is therefore
5639 recommended to develop an additional standard test with mycorrhizal fungi.

5640 Considering the possibilities for intermediate tier testing, the panel considers that the SSD conceptual
5641 model is very useful, but that standard SSD methodology cannot yet be applied to in-soil organisms
5642 until further guidance will become available on the combination of data for in-soil organisms in SSD.

5643 In the current risk assessment for in-soil organisms exposed to PPPs, the different groups of in-soil
5644 organisms are assessed separately, both at lower tiers and at higher-tier assessment steps. As a
5645 consequence, the evaluation of indirect effects, e.g. *via* food-web interactions, is not possible. In order
5646 to overcome these limitations and to fulfil the legal requirements, the Panel recommends assessment
5647 of the response of *communities* of in-soil organisms to intended uses of PPPs. The assessment of the
5648 in-soil organisms' community response can be performed at higher tiers by investigating the effects of

PPPs in tests with natural assemblages of in-soil organisms, e.g. in field tests or in terrestrial model ecosystems (TMEs). In the calibration of lower-tier assessment steps, direct and indirect effects of PPPs on the community of in-soil organisms should be taken into account in order to derive appropriate assessment factors. Currently, only the earthworm field study is performed on a regular basis. In the risk-assessment scheme proposed here, it is recommended to test soil communities in the higher tier, therefore the sole performance of an earthworm field-study test may not be appropriate any more. The panel also recommends use of TMEs as a tool to study effects on soil communities in the (semi-)field.

The Panel proposes that population modelling is used to integrate long-term and multiple stressor events into the population-impact evaluation as a lower tier test. The assessment should be based on a number of standard models of species identified as being potentially vulnerable to these effects. Since standard models are proposed, the Panel recommends that modification by users should be limited to PPP properties and GAP scenarios.

The Panel recommends taking into account the recommendations of the draft EFSA guidance on Uncertainty in Scientific Assessment when developing guidance for standardised risk assessment for in-soil organisms. In particular, it is recommended that uncertainties affecting the standardised assessment procedure should be systematically identified and described and that as many sources of uncertainty as possible should be included transparently in proposed assessment factors.

The Panel recommends that assessment factors be derived probabilistically on the basis of statistical modelling of the relationships between effects for different species in the various possible lower tier tests, higher tier studies and the surrogate reference tier. In particular, a Bayesian graphical model can exploit information from both experimental data and expert judgement and provides a relatively transparent method for deriving assessment factors in order to ensure high probability of acceptable effects for uses that pass the risk assessment.

10.3. Specific recommendations for further research

- Most test methods are designed for spray applications, but should be adapted in order to test effects of soil fumigants, treated seeds and granules. Methods need to be developed for testing effects of other application methods.
- In current laboratory test systems, in-soil animals are provided with uncontaminated food. It is recommended to develop or to amend laboratory test systems that integrate both contact and oral exposure routes.
- The MicroResp method measures respiration rate in e.g. soil and can describe the capacity of microbial communities of soil to degrade various organic substrates. It is recommended that the capacity of MicroResp to study effects of pesticides on soil microbial processes is determined in future research.
- The Panel recommends adding a test with mycorrhizal fungi. The ISO test with *Glomus mosseae* allows for adaption of the endpoints measured or to test other species/strains. Further research and development is needed to improve the test design in these respects.
- There is only limited experience of combining toxicity data of in-soil organisms in Species Sensitivity Distributions (SSD), especially for PPPs. Further research is needed with regard to combining toxicity data from different groups of in-soil organisms. This further research is aimed at making (chronic) toxicity data available in general, and the role of the toxic mode of action of a PPP and the identification of sensitive groups in particular.

- Mesocosm data for many more active substances are needed to make possible calibration of lower tiers against the Surrogate Reference Tier; calibration would then be based on data rather than on expert judgment alone.
- Research to develop data to support the use of Toxicokinetic-toxicodynamic (TK/TD) modelling for organisms where vertical movement in soil may require dynamic linking between exposure and effects.
- Exposure profiles vary in both time and space. Toxic effects of PPPs in soil organisms should be best linked to internal body concentrations. For the time being, not enough data are available on toxicokinetics-toxicodynamics (TK/TD) of PPPs in soil organisms. For these reasons, the following research needs are proposed
 - Reliable models of movement for endogeic earthworms, within the soil profile
 - Dynamic models of exposure providing soil and pore-water concentrations at all relevant soil depths and varying with time.
 - TK/TD models capable of linking toxicological effects to internal body concentrations in time.
 - Ideally, these three combined components would be integrated into the system model used to develop the population-modelling 'surrogate reference tier'.
- Long-term field studies, over years, are an important research priority. These would provide both validation for long-term impacts as assessed by population modelling, and indicate whether the population effects can be used as a surrogate for community effects. In addition, detailed data developed from long-term studies could be used to feed into food-web models to improve understanding of community interactions and energy-flows between functional groups (Ruiter et al, 1998). In this way food-web models may become tractable for predictive in-soil ERA.
- There is a need to develop a range of representative scenarios and models of focal species for population modelling. These will need to include a definition of the normal operating range for the focal species, a parameterisation of the models, and incorporation of ecotoxicology in the models, and the range of system drivers needed (e.g. weather, regulated and non-regulated stressors). These models and scenarios will need to cover the range of agricultural systems necessary to provide good coverage for any regulatory ERA that may arise.

5729 **GLOSSARY AND/OR ABBREVIATIONS**

AF	Assessment Factor
ASTM	American Society for Testing and Materials
CRP	Community Recovery Principle
DegT ₅₀	Half-life in a medium due to degradation (transformation) processes
DT ₅₀	Half-life in a medium due to degradation (transformation) and other processes (such as volatilisation and leaching)
EC	European Commission
EC _x	Concentration at which x % effect was observed/calculated European and
EPPO	Mediterranean Plant Protection Organization
ERA	Environmental Risk Assessment
ERC	Ecotoxicologically Relevant Concentration
ES	Ecosystem service
ETP	Ecological Threshold Principle
EU	European Union
FOCUS	FORum for Co-ordination of pesticide fate models and their USE
FRP	Functional Redundancy Principle
ISO	International Organisation for Standardization
MDD	Minimal Detectable Difference
Metabolite	Any metabolite or a degradation product of an active substance, safener or synergist, formed either in organisms or in the environment (thus including also oxidation products which may have a larger molecular mass than the parent substance)
NOEC	No observed Effect Concentration
NTA	Non-Target Arthropod
NTTP	Non-Target Terrestrial Plant
OECD	Organization for Economic Cooperation and Development
PEC	Predicted Environmental Concentration
PPPs	Plant Protection Products
PPR Panel	EFSA's Scientific Panel on Plant Protection Products and their Residues
PBT	Persistent, Bioaccumulating and Toxic
POP	Persistent Organic Pollutant
RA	Risk Assessment
RAC	Regulatory Acceptable Concentration
REG	Regulation
SPGs	Specific Protection Goals
SSD	Species Sensitivity Distribution
TER	Toxicity exposure ratio (i.e. NOEC/PEC or EC ₁₀ /PEC)
TK/TD	Toxicodynamics/toxicokinetic
TME	Terrestrial Model Ecosystem
vPvT	very Persistent, very Toxic

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Appendix A. Time of development of some worms belonging to the family Lumbricidae

Species	No of cocoons per worm	Incubation time of cocoons (weeks)	Period of growth per worm (weeks)	Total time for development (weeks)	Growth to maturity (development of clitellum) (days)	Cocoon viability (%)	Reference
<i>Eisenia fetida</i>	11 (per year)	11	55	66			Edwards and Bohlen, 1996
	5.5 (per week)	-	-	-	-		Lavelle and Spain, 2005
	3.7 (per month)	7.7	-	-	-	81.9	Tripathi and Bhardwaj, 2004
	3.5 (over 10 days)	3.3 at 25 °C	-	-	-		Venter and Reinecke, 1988
<i>Dendrobaena subrubicunda</i>	42	8.5	30	38.5			Edwards and Bohlen, 1996
<i>Lumbriculus rubellus</i>	106	16	37	53			Edwards and Bohlen, 1996
<i>Lumbriculus castaneus</i>	65	14	24	38			Edwards and Bohlen, 1996
<i>Aporrectodea rosea</i>	8	17.5	55	72.5			Edwards and Bohlen, 1996
<i>Aporrectodea caliginosa</i>	27	19	55	74			Edwards and Bohlen, 1996
	31.2 at 15 °C	33.4 at 5°C; 17 at 10°C; 8.8-12 at 15 °C; 5.1 at 20 °C	-	-	-	90 at 20 °C	Lowe and Butt, 2005
<i>Allolobophora chlorotica</i>	27	12.5	36	48.5			Edwards and Bohlen, 1996
	9.9 at 10 °C, 27.3 at 20 °C	~60 at 5°C; 13-25 at 10 °C; 7.3-8.4 at 15°C; 10 at 20°C, 4.9-5.7 at 25 °C			84 at 15 °C; 56 at 20 °C	54 at 10 °C; 62 at 15 °C; 65-90 at 20 °C	Lowe and Butt, 2005
<i>Aporrectodea longa</i>	8	10	50	60			Edwards and Bohlen, 1996
	17 at 20 °C	15 at 9.6 °C; 7.7-8.7 at 15 °C; 5.3-8 at 20 °C; 6 at 26 °C			168 at 15 °C; 120 at 20 °C	70 at 15 °C; 47 at 20 °C	Lowe and Butt, 2005
<i>Octodrilus</i>	52	9.4 (in the range 7-10)		-	150	55	Monroy et al., 2007

Species	No of cocoons per worm	Incubation time of cocoons (weeks)	Period of growth per worm (weeks)	Total time for development (weeks)	Growth to maturity (development of clitellum) (days)	Cocoon viability (%)	Reference
<i>complanatus</i>							
<i>Aporrectodea trapezoides</i>	105	-	-	-	-	87	Fernandez et al., 2010
<i>Lumbriculus terrestris</i>	25.3 at 15 °C; 36.9 at 15 °C (field) 10.1 at 20 °C (lab)	39 at 5 °C; 26 at 10°C; 10 at 20 °C; 11.6 at 25 °C			213 at 7.5 °C; 112 at 15 °C; 90 at 20 °C	70 betw. 5-20 °C; 83 at 15 °C; 41 at 25 °C	Lowe and Butt, 2005
<i>Octolasion cyaneum</i>	32.3 at 20 °C	16.3 at 15°C; 12.3 at 20 °C				79 at 15 °C; 77 at 20 °C	Butt, 1993

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7389 **Appendix B. Overview of recovery potential for soil microorganisms**

7390 Table B-1: List of studies reporting significant increase in the measured endpoint compared to the control followed by no differences (from Puglisi, 2012).

Substance	Substance dose		Time to recover (d)	Total experiment time (d)	Method
Iprodione	0.83 AR) ²¹	mg/kg	60	90	FDA
Iprodione	8.3(10X AR) ²²	mg/kg	60	90	FDA
Propargyl Bromide	79 (AR)	mg/kg	28	90	dehydrogenase
Mefenoxam	4 (AR)	mg/kg	60	60	MBC
Copper Oxide	128 (AR)	mg/kg	60	60	MBC
Fenvalerate	1 (NR) ²³	mg/kg	35	35	catalase
Fenvalerate	10 (NR)	mg/kg	35	35	catalase
Fenvalerate	40 (NR)	mg/kg	35	35	catalase
Fenvalerate	80 (NR)	mg/kg	35	35	catalase
Chlorpyrifos	1 (NR)	mg/kg	35	35	catalase
Chlorpyrifos	10 (NR)	mg/kg	35	35	catalase
Chlorpyrifos	40 (NR)	mg/kg	35	35	catalase
Chlorpyrifos	80 (NR)	mg/kg	35	35	catalase
Bromacil	80 (NR)	kg/ha	28	28	phosphatase
Mefenoxam	72 (AR)	µg	120	120	dehydrogenase
Mefenoxam	72 (AR)	a.i./100g	120	120	dehydrogenase
Mefenoxam	72 (AR)	µg	120	120	alkaline phosphatase
Mefenoxam	72 (AR)	a.i./100g	90	120	acid phosphatase
Diazinon	800 (NR)	g a.i./ha	60	150	arginine deaminase
Endosulfan	1 (NR)	mg/kg	98	98	FDA

²¹ AR: application rate according to Good Agricultural Practice

²² 10X AR: 10 times the application rate according to Good Agricultural Practice

²³ NR: not reported. It is not clear from the original study whether the used concentration were chosen according to good agricultural studies

Substance	Substance dose		Time to recover (d)	Total experiment time (d)	Method
Endosulfan	10 (NR)	mg/kg	98	98	FDA
Endosulfan	1 (NR)	mg/kg	98	98	arylsulfatase
Fenpropimorph	1.3 (AR)	mg/kg	30	56	CFU of total bacteria
Diazinon	800 (NR)	g a.i./ha	60	150	dehydrogenase
Hexachlorocyclohexane	7.5 (AR)	kg a.i./ha	45	60	CFU of N-fixing bacteria
Phorate	1.5 (AR)	kg a.i./ha	60	60	CFU of N-fixing bacteria
Carbofuran	1 (AR)	kg a.i./ha	60	60	CFU of N-fixing bacteria
Fenvalerate	0.35 (AR)	kg a.i./ha	60	60	CFU of N-fixing bacteria
Captan	0.125	g a.i./kg	45	65	MBC
		mg	50	56	
Triasulfuron	5 (> AR)	a.i./kg			soil respiration
		mg	50	56	
Triasulfuron	5 (> AR)	a.i./kg			dehydrogenase
		mg	50	56	
Primisulfuron methyl	5 (>AR)	a.i./kg			soil respiration
		mg	50	56	
Primisulfuron methyl	5 (>AR)	a.i./kg			dehydrogenase
		mg	18	56	
Rimsulfuron	5 (>AR)	a.i./kg			soil respiration
		mg	18	56	
Rimsulfuron	5 (>AR)	a.i./kg			dehydrogenase

7391 Table B-2: List of studies reporting significant decrease in the measured endpoint compared to the control followed by no differences (from Puglisi, 2012).

Substance	Substance dose		Time to recover (d)	Total experiment time (d)	Method
Quinalphos	4 (AR)	L/ha	75	105	dehydrogenase
Quinalphos	4 (AR)	L/ha	30	105	phosphatase
Propargyl Bromide	79 (AR)	mg/kg	28	90	acid phosphatase
Chloropicrin	176 (AR)	mg/kg	14	90	beta-glucosidase
		mg/50c	90	90	
Quinalphos	2,5 (NR)	m ²			dehydrogenase
		mg/50c	7	90	
Quinalphos	2,5 (NR)	m ²			alkaline phosphatase
Bensulfuron			1	8	
Methyl	51 (AR)	g/ha			potential nitrification
Bensulfuron	510 (10X		8	8	
Methyl	AR)	g/ha			potential nitrification
		mg	14	56	
Benomyl	51 (AR)	a.i./kg			soil respiration
		mg	28	56	
Captan	125 (AR)	a.i./kg			soil respiration
		mg	28	56	
Chlorothalonil	37 (AR)	a.i./kg			soil respiration
Bensulfuron	0,1 (10X	µg/g	7	45	potential nitrification
Methyl	AR)				
Bensulfuron	1 (100X	µg/g	7	45	potential nitrification
Methyl	AR)				
Nanopropamide	20 (10X	mg	14	56	
	AR)	a.i./kg			soil respiration
2-Phenylethyl			30	60	
Isothiocyanate	10 (NA) ²⁴	mg/kg			microbial respiration
Metam Sodium	300 (AR)	mg/kg	30	60	microbial respiration
Bensulfuron			8	8	
Methyl	51 (AR)	g/ha			CFU of total bacteria
Bensulfuron	510 (10X	g/ha	8	8	CFU of total bacteria

²⁴ NA: not applicable. The substance was applied at different concentrations in order to mimick the field application of metam-sodium

Substance	Substance dose		Time to recover (d)	Total experiment time (d)	Method
Methyl	AR)				
Pentachlorophenol	9,5 (AR)	Kg/ha	8	8	CFU of total bacteria
	95 (10X		8	8	
Pentachlorophenol	AR)	Kg/ha			CFU of total bacteria
		mg	56	56	
Benomyl	51 (AR)	a.i./kg			MBN
		mg	56	56	
Captan	125 (AR)	a.i./kg			MBN
		mg	56	56	
Chlorothalonil	37 (AR)	a.i./kg			MBN
Chlorpyrifos	2 (AR)	mg/kg	3	28	CFU of total bacteria
Chlorpyrifos	2 (AR)	mg/kg	14	28	CFU of total fungi
Bensulfuron			5	45	potential
Methyl	0,01 (AR)	µg/g			nitrification
			3	3	potential
Carbendazim	10 (NR)	mg/kg			nitrification
			3	3	potential
Chloranil	10 (NR)	mg/kg			nitrification
			3	3	potential
Chloroneb	10 (NR)	mg/kg			nitrification
			3	3	potential
Chlorothalonil	10 (NR)	mg/kg			nitrification
			3	3	potential
Dichlone	10 (NR)	mg/kg			nitrification
			3	3	potential
Dodine	10 (NR)	mg/kg			nitrification
			3	3	potential
Fenaminosulf	10 (NR)	mg/kg			nitrification
			3	3	potential
Folpet	10 (NR)	mg/kg			nitrification
			3	3	potential
Maneb	10 (NR)	mg/kg			nitrification
			3	3	potential
Quintozene	10 (NR)	mg/kg			nitrification
Thiram	10 (NR)	mg/kg	3	3	potential

Substance	Substance dose		Time to recover (d)	Total experiment time (d)	Method
Zineb	10 (NR)	mg/kg	3	3	nitrification potential
Chloranil	10 (NR)	mg/kg	1	2	nitrification
Dichlone	10 (NR)	mg/kg	1	2	denitrification
Fenaminosulf	10 (NR)	mg/kg	1	2	denitrification
Thiram	10 (NR)	mg/kg	1	2	denitrification
Zineb	10 (NR)	mg/kg	1	2	denitrification
Chlorothalonil	5.4(100X AR)	g a.i./kg	65	65	MBC
Carbendazim	45(100X AR)	g a.i./kg	65	65	MBC
Forchlorfenuron	0.5 (AR)	mg/kg	15	30	acid phosphatase
Butachlor	100 (NR)	mg/kg	21	21	CFU of total bacteria

7392 Table B-3: Overview of recovery potential for soil microorganisms. List of studies reporting transient effects I the measured endpoint compared to the control
7393 followed by no differences (from Puglisi, 2012).

Substance	Substance dose		Time to recover (d)	Total experiment time (d)	Method
Atrazine	1	mg/kg	30	60	PCR-DGGE of bacterial 16S
Atrazine	2	mg/kg	30	60	PCR-DGGE of bacterial 16S
Atrazine	3	mg/kg	30	60	PCR-DGGE of bacterial 16S
Iprodione	5	mg a.s./g	23	23	PCR-DGGE of bacterial 16S
Iprodione	5	mg a.s./g	23	23	PCR-DGGE of bacterial 16S
Iprodione	50	mg a.s./g	23	23	PCR-DGGE of bacterial 16S
Iprodione	50	mg a.s./g	23	23	PCR-DGGE of bacterial 16S
Carbendazim	0.94	kg a.s./ha	360	360	TGGE of bacterial 16S
Carbendazim	1.88	kg a.s./ha	360	360	TGGE of bacterial 16S
Carbendazim	4.70	kg a.s./ha	360	360	TGGE of bacterial 16S
Acetochlor	50	mg/kg	56	56	PCR-DGGE of fungal communities
			56	56	PCR-DGGE of fungal communities
			56	56	PCR-DGGE of fungal communities
Acetochlor	250	mg/kg			communities
Butachlor	2	mg/kg	21	21	biolog

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Appendix C. Summary of (extended) laboratory test systems with invertebrates identified as potentially relevant by the working group

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Non-arthropod invertebrates					
Earthworms <i>Eisenia fetida fetida</i> / <i>andrei</i>	earthworm reproductive toxicity test 56 d: 28 d exposure of adults; additional 28 d offspring and juveniles artificial soil (10% OM (Sphagnum peat), can according to OECD222 be lowered to 5%. Food (e.g. cow manure) is mixed with the soil source (ISO) or added to soil surface 1 day after application of test substance (OECD).	reproduction NOEC, EC _x mg kg ⁻¹ active substance per dry weight soil (total content, nominal)	verifying amount of test substance applied by a suitable calibration technique contact soil and pore water, oral soil and pore water, oral food substance is mixed with the soil. Note I		ISO, 2012 (11268-2)/ OECD, 2004 (222)
Mollusc <i>Helix aspersa</i>	28 d; semi static (substrate or soil is renewed every 7 days).	Survival, growth	Food and contact		ISO, 2006 (15952)
Earthworms <i>Eisenia fetida</i> / <i>Eisenia andrei</i>	Earthworm acute toxicity test; 14 days Artificial soil (10% OM (Sphagnum peat).	Survival; EC ₅₀ ; mg kg ⁻¹ active substance per dry weight soil (total content, nominal)	Note 1		OECD, 1984 (207)
Nematods <i>Caenorhabditis elegans</i>	Acute toxicity test 24-48 h	Survival; LC ₅₀ , EC ₅₀			ASTM, 2014 (E2172)
Enchytraeids <i>Enchytraeus albidus</i>	Enchytraid reproduction test 21 days (mortality adults) 42 days juvenils artificial soil (10% OM (Sphagnum peat) Test containers 0.02 – 0.25 l, 20 g dry weight soil. Feeding 50 mg of ground rolled oats before introducing the worms. Thereafter, 25 mg food is supplied weekly up to Day 21.	Survival, reproduction NOEC, EC _x mg kg ⁻¹ substance per dry weight soil (total content, nominal)	Note 1		OECD, 2004/ ISO, 2014
Enchytraeid <i>Cognettia sphagnetorum</i>	Enchytraid reproduction test 70 days Modified LUFA soil: 75% sphagnum peat soil : 25 vol% LUFA 2.2 soil (3,9% OM and 5,1% clay). Feeding by adding 1% algae or 0,2% baker's yeast.	Survival, growth, reproduction	Note 1		Rundgren and Augustsson, chapter 6 in Løkke and Van Gestel, 1998.
Arthropods					

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Collembola <i>Folsomia candida</i> / <i>Folsomia fimetaria</i>	Collembolan Reproduction Test in Soil 21 d (F. fimetaria), 28 d (F. candida) artificial soil (5 % Sphagnum peat) Test containers, 30 g soil (ww) Feeding at day 0 and day 14, 2-10 mg of granulated dry yeast is added to each test container	Survival Reproduction relative to control mg kg ⁻¹ as active substance per dry soil (nominal, total content)	Note 1 Guideline gives the option of application of test substance to soil surface.		ISO, 2014 (11267)/ OECD, 2009 (232)
Collembola <i>Folsomia candida</i>	The methodology describes an acute toxicity test for Collembola in an aqueous medium in 100ml sample vials. Effects expected from exposure in pore water can be assessed using this test method. Concentrations were measured several times which allows for the generation of reliable and accurate dose-response curves.	Survival	Test substance in aqueous medium to study exposure in pore water.	Mortality was difficult to determine.	Houx et al., 1996
Collembola <i>Folsomia candida</i>	Collembola multigeneration test. Collembola test is conducted as the standard test, survival and reproduction tested. The second generation is again exposed to the aged residue soil, and effects on the third generation are studied	Survival Reproduction, multi generation			Ernst et al., (2015)
Collembola <i>Sminthurus viridis</i> , <i>Folsomia candida</i> , <i>Isotomurus palustris</i> , <i>Isotoma viridis</i> .	Bioassay: Collembola were contained in the laboratory for 24 hr on pre-sprayed soils aged for varying times in the field. Assessment of the sensitivity of different species. Experiments were conducted on field soil and lufa 2.2 soil.	Survival	Exposure by spraying, deposition measured using water sensitive paper.	The approach could be adapted to for multi-rate dose response testing.	Wiles and Frampton, 1996
Mites <i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i>	<i>Hypoaspis aculeifer</i> Canestrini 14 d artificial soil (5 % Sphagnum peat) As a food source the mite <i>Tyrophagus putrescentiae</i> (Schrank) (Acari: Acaridae) is used.	survival reproduction Both endpoints as % reduction, relative to control, at a certain nominal dose rate (mg kg ⁻¹).	Note 1		OECD, 2008 (226)
Oribatid mite <i>Platynothrus peltifer</i>	14-70 d	Survival Reproduction			Van Gestel and Doornekamp, chapter 8 in Løkke and Van Gestel, 1998.
Oribatid mite <i>Oppia nitens</i>	28 d	Reproduction			Princz et al., 2010

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Insect larvae <i>Oxythyrea funesta</i>	10 d acute toxicity test. artificial soil, 10% OM (Sphagnum peat), Cow dung is added as food for the larvae (3 g at start, 2-3 g after 3 and 7 d.)	Survival; LC50	Note 1		ISO, 2005 (20963)
Centipede <i>Lithobius mutabilis</i>	Acute toxicity test. 28 d for degradable compounds, 84 d for persistent chemicals. Feeding two pupae of housefly a week. Larvae are fed with substrate including the test substance.	Survival; LC50 Growth, respiration rate and locomotive activity (EC20, EC50)	Note 1 Artificial soil (OECD), 10% OM (Sphagnum peat)		Laskowski, Pyza, Maryanski and Niklinska, chapter 11 in Løkke and Van Gestel, 1998.
Millipede <i>Brachydesmus superus</i>	Chronic test 70 d. Food supplied as decomposed leaf litter as polls and baker's yeast.	Survival (EC50)reproduction	Note 1. LUFA 2.2.		Tajovsky, chapter 12 Løkke and Van Gestel, 1998
Isopods <i>Porcellio scaber</i>	28 d	Survival, growth			Hornung, Farkas and Fischer in Løkke and Van Gestel, 1998
Isopods <i>Porcellionides pruinosus</i>	14	Survival, reproduction			Jansch et al., 2005
Carabid beetles <i>Pterostichus oblongopunctatus</i> ; <i>Poecilus cupreus</i>	Different durations; Adult or larval	survival; adult behaviour, respiration			Schrader et al., 1998; Bednarska et al., 2010

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7466 **Appendix D. Semi-field and field test systems**

7467 Potential (Semi-) field approaches to the risk assessment of plant protection products (PPP) towards non-target organisms (partly adapted from Brown et
7468 al., 2008 – EFSA contracted report, Schaeffer et al., 2007 with some additions)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Semi-field studies					
Soil microorganisms and soil mesofauna	TME Species should taxonomic groups that lower-tier risk assessments have identified as being of concern. Preferably a naturally developed soil community with organisms such as arthropods, nematodes and/or oligochaetes. Abundant species like oribatids and predatory mites, as well as collembolans should be present in adequate number. Organisms may be added. TMEs may be considered for studying earthworm populations except for large vertical burrowers (anecics). Natural soil cores including soil organisms	Diversity and abundance NOEC, ECx expressed in mg/kg soil	Plateau concentration and or total annual application is applied on the soil of the TMEs (in order not to disturb the community). One year or more depending on the aim of the study (e.g. recovery of univoltine species) . Concentration in soil is measured.		Schaeffer et al., 2008
Earthworms <i>Dendrobaena octaedra</i> , <i>E. fetida</i>	A forest soil microcosm (forest litter) was used to characterise pesticide toxicity to earthworms. Enables risk in specific circumstances to be evaluated. In this case effects in forest areas with thick litter and high organic matter was evaluated.	burrowing time, weight change and cocoon production survival, reproduction		<i>E. fetida</i> did not thrive in this system, indicating that this methods may not be applicable for all earthworm species	Addison and Holmes, 1995

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Earthworms <i>A. trapezoids</i>	Enclosures were made from PVC pipes. Earthworms were added and then treatments made. 38 days after treatment numbers of earthworms and cocoons assessed.	Survival, number of cocoons		Smaller system that may enable dose rates to be assessed. <i>A. trapezoides</i> is a shallow-burrowing earthworm, and so may not appropriately model the susceptibility or exposure of deeperburrowing worms.	Choo and Baker, 1998
Earthworm <i>Aporrectodea caliginosa</i>	Microcosm system, constructed of using stainless steel enclosures 20 cm deep and 12 cm in diameter (5 replicates per treatment). Natural soil from the study area was used, microcosms were taken to the laboratory. Acetylcholinesterase inhibition in earthworms was shown to strongly correlate with biomass changes.	Biomass, cholinesterase inhibition.	Chlorpyrifos was applied to the soil surface of the microcosm.	Low natural earthworm densities may confound results.	Reinecke and Reinecke, 2007
Soil organisms involved in organic matter breakdown	litter bags containing dried OM (wheat straw) buried in field soil; determination of ash-free dry weight of straw in litter bags after 1, 3 and 6 months after burying bags (or further sampling up to 12 months if 60% mass loss in control is not reached)	mass loss of litter bags in treatment compared to mass loss of litter bags in control endpoint expressed as % effect (mass loss)	plateau concentration + annual cumulative application rate (see dosing method) Concentration in soil measured immediately after application		Römbke et al., 2003

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Soil mesofauna enchytraeids, mites and nematodes	Field study aimed at the structure of the mesofauna community. Plots 3x7 m. Control, treatment and toxic reference (benomyl+chlorpyrifos), 6 replicates. Sampling 2 d before and 1,3,6 and 12 month after application.	Abundance of enchytraeids, mites and nematods. % difference with control	2 applications with test item; concentration in soil was measured	Mites appear to be less sensitive, even to the toxic reference.	Römbke et al., 2009
Soil microorganism communities Invertebrates: <i>Pelodera strongyloides</i> , <i>Enchytraeidae</i>	Microcosm comprised of bean plants <i>P. vulgaris</i> , phytophagous organisms, soil bacteria, fungi, and microinvertebrates.	Assessed endpoints include soil parameters, cellular indicators, indicators at the organism, population and community level		The assessment of multiple taxa at different trophic levels utilizing cellular to community endpoints provides a thorough accounting of potential effects. Methodology requires validation. Ability to use in risk assessment needs to be confirmed.	Burrows and Edwards, 2002 Motheswagner et al., 1992
Earthworm <i>Aporrectodea tuberculata</i>	Microcosms were filled with field collected soil, planted with wheat seedlings, and three earthworms were added to each system. Treatment regime was designed to mimic natural spray events, adding realism to microcosm studies, and endpoints were selected to give insight concerning ecosystem processes.	Microbial biomass, litter decomposition, enzyme activity, bait lamina tests, nutrient leaching measurements were taken periodically, and abundances were quantified at the end.	Pesticide was measured	Collection and containment of natural soil communities may cause problems with non-homogenous replicate communities	Edwards et al., 1998

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Earthworm <i>Aporrectodea trapezoides</i>	Mesocosm study, including the use of mesh bags with organic matter buried in 15 cm deep units with one earthworm. Toxicity thresholds produced using this methodology was very similar to those produced using other methods, indicating that this method has been verified.	Earthworm survival, growth, body accumulation, organic matter decomposition, substrate induced respiration, soil urease activity and total nematode numbers		Use of a single earthworm could limit statistical analysis. Soil chemistry, including pH, may alter sensitivity of soil invertebrate to plant protection products.	Bogomolov et al., 1996
Nematoda Earthworms <i>L. rubellus</i>	Microcosm (using soil cores taken from field), in which three <i>L. rubellus</i> were added to each enclosure. The use of soil cores allows for the collection and analysis of leachate (PPP and nutrients)	Nematode populations, bait lamina test, growth of earthworms, nitraat concentration.	Field soil. Dosages mixed through the soil. Concentration measured after application	Methods for interpretation and extrapolation of microcosm results for use in risk assessments have not yet been developed.	Burrows and Edwards, 2002
Soil community	Reference describes methodology for the automated collection of soil core leachate, irrigation, and analysis of CO ₂ production. Automation streamlines the process of conducting soil microcosm studies.			Unexpected differences detected in CO ₂ production of soil core microcosms were not explained.	Burrows and Edwards, 2004 Hantschel et al., 1994

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Earthworms <i>E. fetida</i> , Enchytraidae <i>Enchytraeus albidus</i>	Both laboratory and field studies were conducted to determine the efficacy of using lab experiments to predict impacts in the field. Soil cores were exposed in greenhouse conditions, while field communities were subjected to overspray. Inhomogeneity of earthworm distribution in field was realistically reflected by the TMEs.			The authors conclude that the abundance and biomass of earthworms are suitable endpoints for assessment of chemicals within TME's but at sites where abundance is low data interpretation may be difficult. Predictability of biomass results derived from TME's is restricted if the number of large earthworms, such as <i>L. terrestris</i> or <i>L. rubellus</i> , is high.	Rombke, et al. 2004
Collembola, Astigmata, Cryptostigmata, Mesostigmatad Prostigmata Microarthropod	TME: Soil cores were collected from multiple fields, irrigated, acclimated for two to four weeks, and treated with compound. Sampling was conducted at weeks 1, 4, 8, and 16 following exposure. Conclusions in TME mirrored those in the field study and thus predictive value of TME is illustrated. Large variations in both Collembola and mite communities.			Differences in the vegetation in the TMEs in the 4 countries possibly caused variation in soil moisture, which may have affected soil microarthropod communities independently of exposure.	Koolhaas et al., 2004

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Nematodes	Methodology was developed concerning the impact of pesticides on soil dwelling nematodes as a part of the ringtesting of Terrestrial Model Validation of TME since field studies showed the same responses to exposure.	Trophic structure, population		High variability of data may conceal effects and increase the likelihood of misinterpretation	Moser et al., 2004
Enchytraeidae, Earthworms Decomposition	TME to measure the breakdown of organic matter. The breakdown of cellulose inserted into a soil column or on the soil surface. Faunal feeding was measured with a bait lamina method. Effects on organic matter decomposition were the same in the TME and the field study and showed a dose response relationship.			The feeding activity of the soil fauna showed a large variability.	Forster et al., 2004
Soil microbial community	A coupled set of experiments (one laboratory and one field) were conducted to describe the impact of a pesticide on soil microorganisms. Various microbial parameters measured. Comparisons on data variability also revealed the absence of significant differences between experiments in all parameters in most cases, indicating that TMEs were able to represent the spatial variability found in the field. Measured responses to the model chemical in TMEs were similar to the field study.			Soil moisture lead to some of the variability in microbial parameters.	Sousa et al., 2004

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Nutrient cycling	TME impact of PPP on soil nutrient cycling. Soil and leachate ammonium and nitrite concentrations were measured following application. Field data showed similar patterns in nutrient levels and thus the TME's predictive value was confirmed.			Variability in moisture or invertebrate activity may confound results. Because soil invertebrates are not homogeneously distributed in field soils, columns may contain significantly different community structures or abundances.	Van Gestel et al., 2004
Field studies					
Earthworm (natural occurring species)	Field study. Increasingly a dose response design is used, and substance is measured in the soil.	Diversity, abundance and biomass. 1, 4 - 6 and 12 months after application. Expressed as kg ha ⁻¹ (active substance), nominal	Duration depends on characteristics of test substance, usually 1 year Field application according to GAP		ISO, 1997 (11268)

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7527 field-validation of a Terrestrial Model Ecosystem (TME) - An instrument for testing
7528 potentially harmful substances: Effects of carbendazim on nutrient cycling. Ecotoxicology, 13,
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7530 **Appendix E. ISO standard in soil microbiology**

Year	Methods	ISO reference
1997	Soil quality -- Determination of soil microbial biomass -- Part 1: Substrate-induced respiration method	ISO 14240-1:1997
1997	Soil quality -- Determination of soil microbial biomass -- Part 2: Fumigation-extraction method	ISO 14240-2:1997
1997	Soil quality -- Biological methods -- Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes	ISO 14238:1997
2002	Soil quality -- Determination of abundance and activity of soil microflora using respiration curves	ISO 17155:2002*
2002	Soil quality -- Laboratory methods for determination of microbial soil respiration	ISO 16072:2002
2004	Soil quality -- Determination of potential nitrification and inhibition of nitrification -- Rapid test by ammonium oxidation	ISO 15685:2004
2005	Soil quality -- Determination of dehydrogenase activity in soils -- Part 1: Method using triphenyltetrazolium chloride (TTC)	ISO 23753-1:2005
2005	Soil quality -- Determination of dehydrogenase activity in soils -- Part 2: Method using iodotetrazolium chloride (INT)	ISO 23753-2:2005
2009	Soil quality -- Effects of pollutants on mycorrhizal fungi -- Spore germination test	ISO 10832:2009**
2010	Soil quality -- Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates	ISO 22939:2010
2010	Soil quality -- Determination of soil microbial diversity -- Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis	ISO 29843-1:2010
2011	Soil quality -- Determination of soil microbial diversity -- Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	ISO 29843-2:2011
2011	Soil quality -- Method to directly extract DNA from soil samples	ISO 11063:2011

Appendix F. Summary of Dutch proposal for risk assessment of persistent substances

Persistence of plant protection products in soil is one of the aspects included in the evaluation of active substances and authorization of PPPs. Under the former regulation at the EU level there was a general agreement on trigger values that indicate the need for further research, but there were different views on the assessment and the interpretation of this additional information at the national level. As a result, member states adopted different evaluation procedures. For example, the Netherlands included a cut-off value of 180 days for the dissipation half-life (DT_{50}) in soil. Since it appeared that this was not in line with the principles of the EU legislation, the Netherlands drafted a proposal for the risk assessment of persistence of PPPs in soil (Van der Linden et al. 2008). Most other countries in the EU did not use a cut-off value.

What are the protection goals?

The Dutch workgroup proposes to consider up to three principles to set protection goals for soil, each having its own timeframe:

Principle to set protection goal	Time scale
Functional Redundancy Principle (FRP)	in year of cropping
Community Recovery Principle (CRP)	2 year post last application
Ecological Threshold Principle (ETP)	7 years post last application

The goals are:

1. Protection of life-support functions of the in-crop soil to allow the growth of the crop and protection of key(stone) species (earthworms) of agricultural soils (FRP). Starting point is that this aspect will be included in the new guidance document anyway.
2. Protection of life-support functions of the soil to allow crop rotation and sustainable agriculture, with overall protection of the structure and functioning of soil communities characteristic for agro-ecosystems (CRP).
3. Protection of life-support functions of the soil to allow changes in land use, with overall protection of the structure and functioning of soil communities characteristic for nature reserves (ETP).

The approach has been developed for the in-crop area.

What are the trigger values?

The half-life for dissipation (DT_{50}) of a chemical from soil acts as a trigger value for evaluation according to one or more of the protection goals. Substances having a DT_{50} above 90 days at 10 °C are evaluated according to the CRP and substances having a DT_{50} above 180 days at 10 °C are additionally evaluated according to the ETP. The values trigger the assessment, but in general additional tests as well.

What is the principle of the risk assessment?

Predicted environmental concentrations (PEC_s) are compared to ecotoxicological relevant concentrations, for instance EC_{50} or NOEC values of indicator species. The assessment evaluates whether, in the realistic worst case exposure, i.e. the 90th percentile, critical values of the exposure / toxicity ratio are exceeded. Substances which exceed the critical value cannot be authorised. The critical values are derived based on EU Technical Guidance Documents; sometimes with a pronounced preference for one of the given options. The assessment can be based both on the total content of the substance as well as on the pore water concentration.

What are the main elements of the assessment?

Both at the exposure side and at the ecotox side, a tiered approach is suggested: ranging from simple and conservative, using higher assessment factors, to more complex and realistic, with lower assessment factors. The first tier of the exposure assessment uses a scenario that is generically vulnerable to persistence. This scenario is run several times, with different input sets in order to

ensure conservative results for both total content and on pore water concentration. The second tier of the exposure assessment uses a spatially distributed model so that the realistic worst case condition is determined during the calculations. At the ecotox side, the CRP and ETP protection goals have separate ecotox assessment schemes, existing of three tiers each.

For the CRP 2 years after the last application *potential* recovery of sensitive soil populations of agro-ecosystems is assured (TER approach based on chronic lab toxicity tests with a basic set of in-soil organisms; SSD approach based on chronic tests and the median HC₅; field experiment approach).

For the ETP 7 years after last application exposure to the PPPs and its metabolites do not affect sensitive populations of in-soil organisms (TER approach based on chronic lab toxicity tests with a larger number of species or the application of a larger AF; SSD approach based on chronic lab toxicity tests and the lower limit of the HC₅ or the use of the median HC₅ value and the application of an AF; model ecosystem approach and extra AF).

Appendix G. Evaluation of the ECPA proposal to evaluate recover in single species multigeneration studies with *F. candida*

ECPA proposed adapting the multigeneration study with *Collembola* (Campiche et al., 2007) to assess the internal recovery of *Collembola* following PPP application (please refer to Ernst et al., 2015).

The study design consists in principle of two subsequent chronic tests with *Folsomia candida*. The reproductive success of the collembolan exposed to PPP is assessed in an artificial soil matrix according to guideline OECD 232 (OECD, 2009). The whole study according to the new design lasts 70 days, the single experiments 28 day each. The artificial soil employed in the tests is contaminated prior to the first test, and is therefore slightly 'aged' at the start of the second chronic tests (42 days). Between the two chronic tests, two weeks are foreseen to rear and synchronize the retrieved organisms from the first test and to select the starting animals for the second tests. The endpoints given at test termination concern survival and reproductive success of *F. candida* in the first chronic test and of *F. candida* F1 generation in the second test with 'aged residues', respectively.

Two main issues need to be discussed with regard to the appropriateness of the study design to assess recovery:

- (i) Test species that has been chosen to address the risk to in-soil organisms in a higher tier approach

The chosen collembolan *Folsomia candida* is a soil dwelling organisms, generally employed in ecotoxicological laboratory testing. It has been shown to be fairly sensitive to toxicants, is easily reared and has a short reproductive cycle (21 days) with a high number of offspring per cycle (e.g. (Krogh, 2008)). In our opinion, the life history traits displayed by *F. candida* might not be representative of ecologically sensitive, vulnerable species of the in-soil organisms' community in the field. Such vulnerable species (e.g. species with long life cycles) are however those that should be particularly considered when addressing long term effects on non-target organisms communities after initial effects due to PPPs use. Please refer for further details on which species' traits should be addressed when considering recovery and/ or recolonization processes to EFSA (2014).

- (ii) Consideration of the reproductive success of adults

The test design does not assess the reproductive success of the first adult generation after 28 days. Since these animals contribute further to the population size development and are also still exposed to the PPPs, it cannot be excluded that there are effects on collembolan populations in the long term solely because the second reproduction test delivers toxicity thresholds above the acceptability criterion.

Moreover, as discussed previously, all (experimental or modelling) approaches for assessing the recovery of in-soil organisms from use of a single PPP need to account for multiple stress caused by normal agricultural practice (e.g. sequential use of different pesticides) that might hinder recovery. This is not considered in the proposed assessment.

Concluding, the outcome of the *F. candida* two generation test (Ernst et al., 2015) may not be fully appropriate to address recovery of collembola.

Appendix H. Comparision of sensitivity between *Folsomia candida* and *Hyapoaspis aculeifer*

Chronic toxicity data on collembolan species (*Folsomia candida*, 28-d NOEC) and mites (*Hyapoaspis aculeifer*, 14-d NOEC) were checked and extracted, when available, from the list of endpoints included in the EFSA conclusions on the risk assessment of active substances published on the EFSA website in the period 2009-2015.

Data were available on both groups of organisms for 51 substances. For completeness, chronic toxicity data on earthworms (*Eisenia fetida*, 56-d NOEC) were also extracted.

The aim of the data collection and extraction was to evaluate the sensitivity of mites, compared to other standard in-soil organisms. The comparison was primarily done with *Folsomia candida*, being mites and collembolan grouped together as micro-arthropods. The toxicity data on mites were, however, also compared to those on *Eisenia fetida* to evaluate the position of mites compared to the other 2 standard species when looking at the sensitivity to PPPs.

The sensitivity ratio (*R*) between the endpoint for *Folsomia candida* and *Hyapoaspis aculeifer* and between *Eisenia fetida* and the mite were calculated. When *R* was higher than 1, the mite species was more sensitive than either *Folsomia* or *Eisenia* spp. The results and discussion are reported in Chapetr 9.

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Table H-1: Chronic toxicity data on in-soil organisms as extracted from the EFSA conclusions on active substances

Test substance	Function of the substance	Endpoint Folsomia (28-d NOEC in mg a.s./kg soil)	Endpoint Hyapoaspis (14-d NOEC in mg a.s./kg soil)	Endpoint Eisenia (56-d NOEC in mg a.s./kg soil)	R (Folsomia/Hyapoaspis)	R (Eisenia/Hyapoaspis)	Reference
Isoproturon	Herbicide	24.3	458.7	14	0.05	0.0305	EFSA, 2015a
Thifensulfuron methyl_metabolite INA4098	Herbicide	0.045	100	0.2	0.0005	0.0020	EFSA, 2015b
Thifensulfuron methyl_metabolite INW8268	Herbicide	100	50	8	2	0.1600	EFSA, 2015b
Famoxadone_m etabolite IN-MN467	Fungicide	25	25	50	1	2.0000	EFSA, 2015c
Famoxadone_m etabolite IN-MN468	Fungicide	50	50	50	1	1.0000	EFSA, 2015c
Iprovalicarb	Fungicide	1000	1000	64	1	0.0640	EFSA, 2015d
Metalaxyl-M (formulation)	Fungicide	89	16.6	35.63	5.36	2.1464	EFSA, 2015e
Tricyclazole (formulation)	Fungicide	32	16	5.2	2	0.3250	EFSA, 2015f
Flupyradifurone (formulation)	Insecticide	1.44	170	1.5	0.01	0.0088	EFSA, 2015g
Florasulam metabolite_5-OH-florasulam	Herbicide	2.5	1.25	0.14	2	0.1120	EFSA, 2015h
Florasulam metabolite_DFP-ASTCA	Herbicide	10	10	0.03	1	0.0030	EFSA, 2015h
Florasulam metabolite_AS	Herbicide	12.5	100	1	0.13	0.0100	EFSA, 2015h

Test substance	Function of the substance	Endpoint Folsomia (28-d NOEC in mg a.s./kg soil)	Endpoint Hyapoaspis (14-d NOEC in mg a.s./kg soil)	Endpoint Eisenia (56-d NOEC in mg a.s./kg soil)	R (Folsomia/Hyapoaspis)	R (Eisenia/Hyapoaspis)	Reference
TCA							
Florasulam metabolite TSA	Herbicide	50	50	10	1	0.2000	EFSA, 2015h
Triasulfuron metabolite CGA 150829	Herbicide	100	100	30	1	0.3000	EFSA, 2015i
Halauixiphen-methyl	Herbicide	500	12.5	5	40	0.4000	EFSA, 2014a
Halauixiphen-methyl metabolite X11449757	Herbicide	2.5	25	10	0.10	0.4000	EFSA, 2014a
Flupyr-sulfuron metabolite IN-J0290	Herbicide	50	100	100	0.50	1.0000	EFSA, 2014b
Flupyr-sulfuron metabolite IN-JV460	Herbicide	50	100	100	0.50	1.0000	EFSA, 2014b
Flupyr-sulfuron metabolite IN-KC576	Herbicide	100	100	25	1	0.2500	EFSA, 2014b
Flupyr-sulfuron metabolite IN-KT982	Herbicide	50	100	100	0.50	1.0000	EFSA, 2014b
Flupyr-sulfuron metabolite IN-KV996	Herbicide	100	100	100	1	1.0000	EFSA, 2014b
Flupyr-sulfuron metabolite IN-KY374	Herbicide	100	10	200	10	20	EFSA, 2014b
Esfenvalerate	Insecticide	0.4	2	0.55	0.20	0.2750	EFSA, 2014c

Test substance	Function of the substance	Endpoint Folsomia (28-d NOEC in mg a.s./kg soil)	Endpoint Hyapoaspis (14-d NOEC in mg a.s./kg soil)	Endpoint Eisenia (56-d NOEC in mg a.s./kg soil)	R (Folsomia/Hyapoaspis)	R (Eisenia/Hyapoaspis)	Reference
2,4-D_metabolite 2,4-DCA	Herbicide	5	5	5	1	1.0000	EFSA, 2014d
2,4-D_metabolite 2,4-DCP	Herbicide	0.625	2.5	5	0.25	2	EFSA, 2014d
Prosulfuron_metabolite CGA 150829	Herbicide	0.225	100	8	0.00225	0.0800	EFSA, 2014e
Pyridate (formulation)	Herbicide	250	250	No data	1	Not applicable	EFSA, 2014f
Ethametsulfuron-methyl	Herbicide	100	100	No data	1	Not applicable	EFSA, 2014g
Sulfosulfuron (formulation)	Herbicide	1000	1000	No data	1	Not applicable	EFSA, 2014h
Sulfosulfuron_metabolite sulfonyl biuret	Herbicide	0.0275	0.0275	0.0275	1	1.0000	EFSA, 2014h
Sulfosulfuron_metabolite sulfosulfuron guanidine	Herbicide	0.1	0.1	0.1	1	1.0000	EFSA, 2014h
Sulfoxaflor (formulation 1)	Insecticide	0.3204	12	0.09	0.03	0.0075	EFSA, 2014i
Sulfoxaflor (formulation 2)	Insecticide	0.08	3.125	0.08	0.03	0.0256	EFSA, 2014i
Sulfoxaflor_metabolite X11519540	Insecticide	10	10	10	1	1.0000	EFSA, 2014i
Sulfoxaflor_metabolite X11579457	Insecticide	10	10	10	1	1.0000	EFSA, 2014i

Test substance	Function of the substance	Endpoint Folsomia (28-d NOEC in mg a.s./kg soil)	Endpoint Hyapoaspis (14-d NOEC in mg a.s./kg soil)	Endpoint Eisenia (56-d NOEC in mg a.s./kg soil)	R (Folsomia/Hyapoaspis)	R (Eisenia/Hyapoaspis)	Reference
Lambda-Cyhalothrin (formulation)	Insecticide	2.73	4.67	No data	0.58	Not applicable	EFSA, 2014j
Metobromuron	Fungicide	23.66	23.66	No data	1	Not applicable	EFSA, 2014k
Tebuconazole	Fungicide	250	50	No data	5	Not applicable	EFSA, 2014l
Chromofenozide metabolite M-010	Insecticide	1000	1000	No data	1	Not applicable	EFSA, 2013a
Metaflumizone (formulation)	Insecticide	110	27.6	119.74	3.99	4.3384	EFSA, 2013b
Chlorantraniliprole	Insecticide	0.39	100	No data	0.0039	Not applicable	EFSA, 2013c
Fluopyram (formulation)	Fungicide	103.8	415	11.42	0.25	0.0275	EFSA, 2013d
Ametoctradin metabolite M650F03	Fungicide	50	100	83.5	0.50	0.8350	EFSA, 2012a
Ametoctradin metabolite M650F04	Fungicide	100	100	100	1	1.0000	EFSA, 2012a
Bixafen (formulation)	Fungicide	7.74	6.15	9.3	1.26	1.5122	EFSA, 2012b
Penflufen (formulation)	Fungicide	115.55	246.5	16.5	0.47	0.0669	EFSA, 2012c
Penflufen metabolite M01	Fungicide	1000	1000	1000	1	1.0000	EFSA, 2012c
Penflufen metabolite M02	Fungicide	500	500	250	1	0.5000	EFSA, 2012c
Sedaxane (formulation)	Fungicide	228	228	2.62	1	0.0115	EFSA, 2012d
Fluxapyroxad (formulation)	Fungicide	2.99	29.64	21.3	0.10	0.7186	EFSA, 2012e

Test substance	Function of the substance	Endpoint Folsomia (28-d NOEC in mg a.s./kg soil)	Endpoint Hyapoaspis (14-d NOEC in mg a.s./kg soil)	Endpoint Eisenia (56-d NOEC in mg a.s./kg soil)	<i>R</i> (Folsomia/Hyapoaspis)	<i>R</i> (Eisenia/Hyapoaspis)	Reference
Tebufenpyrad	Acaricide	6.25	200	0.17	0.03	0.0009	EFSA, 2008

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Appendix I. Advantages and disadvantages of methods to study microbial genetic and functional diversity

(from: Nannipieri et al., 2003; Kirk et al., 2004; Malik et al., 2008; Rastogi and Sani, 2011; Fakruddin and Mannan, 2013; Rincon-Florez et al., 2013 (the list is not considered exhaustive)).

	Methods	Brief description	Advantages	Disadvantages
Abundance	Plate counting	Estimation the number of viable cells present on a diluted soil suspension based on their ability to give rise to colonies under specific conditions of nutrient medium, temperature and time	<ul style="list-style-type: none"> Easy Fast Cost effective 	<ul style="list-style-type: none"> 0.1% to 1% of the soil bacterial population can be cultured Interdependency of different organisms upon each other Growth conditions such as temperature, pH, and light and to the fact that some microbial species are cultivable only under certain physiological conditions impact of the growing medium on the colony formed.
	Most Probable Number (MPN)	Estimation of population size without counting cells or colonies. The technique uses a statistical approach in which successive dilutions are made to reach an extinction point. Replicates of each dilution are inoculated into a liquid growth medium and the pattern of positive or negative scores recorded. A statistical table is then used to determine the MPN of viable organisms in the original sample	<ul style="list-style-type: none"> Easy Fast Cost effective 	<ul style="list-style-type: none"> 0.1% to 1% of the soil bacterial population can be cultured
	Fatty acid methyl ester analysis	This method provides information on the microbial community composition based on groupings of fatty acids. Fatty acids make up a relatively constant proportion of the cell biomass and signature fatty acids exist that can differentiate major	<ul style="list-style-type: none"> Culturing of microorganisms is not required Direct extraction from soil Follow specific organisms or communities Useful information on the dynamics of viable bacteria Reproducible 	<ul style="list-style-type: none"> If fungal spores are used, more material is needed Can be influenced by external factors Many fatty acids are common To different microorganisms Time consuming Low number of samples can be treated at the same time

		taxonomic groups within a community. Therefore, a change in the fatty acid profile would represent a change in the microbial population.		
Abundance	Phospholipid Fatty Acid Analysis (PLFA)	The technique is based on the premise that phospholipids are rapidly degraded, therefore phospholipids remaining should belong to living organisms. Phospholipids derived from microbial cell membranes can be used to distinguish specific microbial taxa. These phospholipids contain unique fatty acids composed of different acyl chains and can be used as biomarkers for microbial groups. Changes in the phospholipid profiles are generally related to variation in the abundance of microbial groups	<ul style="list-style-type: none"> ▪ Sensitive detection and accurate quantification of different microbial groups ▪ Rapid and efficient ▪ Useful information on the dynamics of viable bacteria ▪ Reproducible 	<ul style="list-style-type: none"> • Time consuming • Low number of samples can be treated at the same time
	Quantitative PCR (Q-PCR) or real-time PCR	It is a technique that collects amplification data while the PCR occurs. Q-PCR uses either intercalating fluorescent dyes such as SYBR green or fluorescent probes (TaqMan) to measure the accumulation of amplicons in real time during each cycle of the PCR. When the Q-PCR is coupled with a preceding reverse transcription (RT) reaction, it can be used to quantify gene expression template abundance.	<ul style="list-style-type: none"> ▪ Quick, accurate and highly sensitive method for sequence quantification that can also be used to quantify microbial groups ▪ Relatively cheap and easy to implement ▪ Specific amplification can be confirmed by melting curve analysis 	<ul style="list-style-type: none"> • Can only be used for targeting of known sequences. • DNA impurities and artefacts may create false-positives or inhibit amplification
	Method	Brief description	Advantages	Disadvantages
	Mol% Guanine+Cytosine	Methods for analysis of base distribution of DNA.	<ul style="list-style-type: none"> ▪ Not influenced by PCR biases 	<ul style="list-style-type: none"> • Requires large quantities of DNA • Dependent on lysing and extraction efficiency

		It is based on the knowledge that microorganisms differ in their G+C content and that taxonomically related groups only differ between 3% and 5%. It is considered a low resolution method as different taxonomic groups may share the same G+C range.	<ul style="list-style-type: none"> Includes all DNA extracted Includes rare members of community 	<ul style="list-style-type: none"> Coarse level of resolution Not informative on diversity parameters, which are richness, evenness and composition.
	Nucleic acid reassociation and hybridization	<p>DNA reassociation is a measure of genetic complexity of the microbial community and has been used to estimate diversity. Total DNA is extracted from environmental samples, purified, denatured and allowed to re-anneal.</p> <p>Nucleic acid hybridization uses specific probes. These hybridization techniques can be done on extracted DNA or RNA, or in situ. Oligonucleotide or polynucleotide probes designed from known sequences ranging in specificity from domain to species can be tagged with markers at the 5'-end. The relative abundance may represent changes in the abundance in the population or changes in the activity and hence the amount of rRNA content</p>	<ul style="list-style-type: none"> Total DNA extracted Not influenced by PCR biases Study DNA or RNA Can be studied <i>in situ</i> 	<ul style="list-style-type: none"> Lack of sensitivity Sequences need to be in high copy number to be detected Dependent on lysing and extraction efficiency
	DNA microarrays and DNA hybridization	DNA microarray is a miniaturized array of complementary DNA probes (~500–5000 nucleotides in length) or oligonucleotides (15–70 bp) attached directly to a solid support, which permits simultaneous hybridization of a	<ul style="list-style-type: none"> Powerful for rapid characterization as a single array can contain 500-1000 different DNA array Total DNA extracted Not influenced by PCR biases Thousands of genes can be 	<ul style="list-style-type: none"> Only detect the most abundant species Need to culture organisms Only accurate in low diversity systems

		large set of probes complementary to their corresponding DNA/RNA targets in a sample	<ul style="list-style-type: none"> analysed If using genes or DNA fragments, increased specificity 	
	Clone library	This method consists in cloning PCR products and then sequence the individual gene fragments. The obtained sequences are then compared to known sequences in a database, such as GenBnk, Ribosomal Database Project (RBP), Greengenes, etc. Typically cloned sequences are assigned to phylum, class, order, family, subfamily or species at sequence similarity cut-off value of 80, 82, 90, 92, 94 or 97%, respectively.	<ul style="list-style-type: none"> Current 'gold standard' for obtaining the greatest estimate of diversity 	<ul style="list-style-type: none"> Labor-intensive Time-consuming Cost factor Typical clone libraries of 16S rDNA genes contain fewer than 1000 sequences and therefore reveal only a small portion of the microbial diversity present in a sample.
	Denaturing and Temperature Gradient Gel Electrophoresis (DGGE and TGGE)	These methods are considered intermediate resolution techniques. They are able to separate mixtures of PCR products that are similar in length but differ in base pair composition. The PCR amplified DNA have usually a limited size of 500 bp. Separation of bands in both DGGE and TGGE depends on decreased electrophoretically mobility of partial melted double stranded DNA molecules in a gel of polyacrylamide containing a linear gradient of DNA denaturant (DGGE) or linear temperature gradient (TGGE).	<ul style="list-style-type: none"> Rapid and simple Single band can be extracted from acrylamide gel and sequenced or hybridization 	<ul style="list-style-type: none"> Dependent on lysing and extraction efficiency Different fingerprints can be generated from the same DNA mixture One band can represent more than one species (co-migration) Only detects dominant population representing 1% of the total community
	Single Strand Conformation	The technique relies on electrophoretic separation based	<ul style="list-style-type: none"> Same as DGGE/TGGE No GC clamp 	<ul style="list-style-type: none"> PCR biases Some ssDNA can form more than one stable

	Polymorphism (SSCP)	on differences in DNA sequences is single strand conformation polymorphism (SSCP). Single-stranded DNA is separated on a polyacrylamide gel based on differences in mobility caused by their folded secondary structure. When DNA fragments are of equal size and no denaturant is present, folding and hence mobility will be dependent on the DNA sequences.	<ul style="list-style-type: none"> No gradient 	conformation
	Restriction Fragment Length Polymorphism (RFLP)	This method relies on DNA polymorphisms. With this method electrophoresed digests are blotted from agarose gels onto nitro-cellulose or nylon membranes and hybridized with appropriate probes prepared from cloned DNA segments of related organisms.	<ul style="list-style-type: none"> Detect structural changes in microbial community 	<ul style="list-style-type: none"> PCR biases Some ssDNA can form more than one stable conformation
	Terminal Restriction Fragment Length Polymorphism (T-RFLP)	It follows the same principle as RFLP except that one PCR primer is labelled with a fluorescent dye. Resulting PCR products are digested with restriction enzyme and terminal restriction fragments and then separated on an automated DNA sequencer.	<ul style="list-style-type: none"> Simpler banding patterns than RFLP Can be automated large number of samples Highly reproducible Ability to compare differences between microbial communities 	<ul style="list-style-type: none"> Dependent on extraction and lysing efficiency PCR biases Type of Taq can increase variability Choice of restriction enzymes will influence community fingerprint
	Ribosomal Intergenic Spacer Analysis (RISA)/ Automated Ribosomal Intergenic Spacer Analysis (ARISA)/ Amplified Ribosomal DNA Restriction Analysis (ARDRA)	RISA, ARISA and ARDRA provide ribosomal-based fingerprinting of the microbial community. In RISA and ARISA, the intergenic spacer (IGS) region between the 16S and 23S ribosomal subunits is amplified by PCR, denatured and separated on a polyacrylamide gel under denaturing conditions. This region may encode tRNAs and is useful	<ul style="list-style-type: none"> Highly reproducible community profiles 	<ul style="list-style-type: none"> Requires large quantities of DNA (for RISA) PCR biases

		for differentiating between bacterial strains and closely related species because of heterogeneity of the IGS length and sequence. Sequence polymorphisms are detected by silver staining in RISA. In ARISA, fluorescently labeled forward primer is detected automatically. In ARDRA, PCR amplified 16S rRNA fragments are digested or cut at specific sites with restriction enzymes and the resulting digest separated by gel electrophoresis.		
	Random amplified Polymorphic DNA (RAPD)	The technique uses PCR amplification with short (usually 10 nucleotides) primers, which anneal randomly at multiple sites on the genomic DNA under low annealing temperature	<ul style="list-style-type: none"> ▪ Suitable for unknown genomes ▪ Requires low quantities of DNA. ▪ Efficient, fast and low cost 	<ul style="list-style-type: none"> • Low reproducibility • Sensitive to reaction conditions
	FISH	It is used to enable <i>in situ</i> phylogenetic identification and enumeration of individual microbial cells by whole cell hybridization with oligonucleotide probes. A fluorescent molecule or fluorochrome is conjugated with an oligonucleotide probe. In microbiology, 16S rRNA is generally used as a probe due to its genetic stability and high copy number. The fluorescent probe binds to a complementary sequence that can therefore be detected using fluorescence microscopy.	<ul style="list-style-type: none"> ▪ Allows detection and spatial distribution of more than one samples at the same time 	<ul style="list-style-type: none"> • Autofluorescence of microorganisms • Accuracy and reliability is highly dependent on specificity of probe(s)
	Whole microbial Genome sequencing	Whole microbial genomes are sequenced using a shotgun cloning	<ul style="list-style-type: none"> ▪ Rapid method to assess biodiversity and abundance 	<ul style="list-style-type: none"> • Relatively expensive • Replication and statistical analysis are essential

		method that involves (i) extraction of DNS from pure cultures, (ii) random fragmentation of obtained genomic DNS into small fragments of ~ 2 kb, (iii) ligation and cloning of DNA fragments. Once the sequences are obtained, they are aligned and assembled into finished sequences using specialized computer programs.	of many species/organizational taxonomic units simultaneously and at a considerable depth compared to the methods that have been available so far	<ul style="list-style-type: none"> • Computational intensive • Challenging in terms of data analysis
	Next Generation Sequencing (metagenomics)	Metagenomics is defined as the functional and sequence-based analysis of the collective microbial genomes that are contained in an environmental sample. The most widely-used platforms for massive parallel sequencing for assessing soil microbial diversity are Roche 454 Genome Sequencer, HiSeq 2000 and AB SOLiDTM System	<ul style="list-style-type: none"> ▪ Biodiversity can be studied in more detail ▪ Captures polymorphism in microbial communities ▪ Reveals the presence of thousands of microbial genomes simultaneously - Provides information about the functions of microbial communities in a given environment 	<ul style="list-style-type: none"> • High cost • Data analysis is challenging and time-consuming • Difficult to use for low-abundance communities. • The high biodiversity in soil leads to many incomplete genomes -Current sequencing methods and computing power still in its infancy to the high biodiversity in soil
Activity	Method	Brief description	Advantages	Disadvantages
	Enzymatic activity assays and other biochemical assays (e.g. nitrogen transformation test)	Dehydrogenase activity is one of the enzymatic assays which can be carried out to investigate the effects of pesticides on a specific function of microbial community. It represents the intracellular flux of electrons to O ₂ and is due to the activity of several intracellular enzymes catalysing the transfer of hydrogen and electrons from one compound to another. Nitrification rate is the ultimate degradation by micororganisms of nitrogen-containing organic matter, via the process of	<ul style="list-style-type: none"> ▪ Low-cost ▪ Easy and fast method to measure microbial activity for soil samples 	<ul style="list-style-type: none"> • The enzymatic assay do not distinguish the contribution of intracellular from extracellular and stabilized enzyme activities

		ammonification and nitrification, to the respective inorganic end-product nitrate.		
	Fluorescein diacetate (FDA)	FDA is hydrolyzed by free exoenzymes and membrane-bound enzymes that convert the colourless FDA in a coloured fluorescein. Fluorescein can then be quantified by spectrophotometry at 490 nm wavelength	<ul style="list-style-type: none"> Low-cost Easy and fast method to measure microbial activity for soil samples 	<ul style="list-style-type: none"> The measurement of soil microbes by FDA can be contaminated by external sources, e.g. plant matter It is a measurement of the contribution of several enzymes such as non-specific esterases, proteases and lipases
	Stable Isotope Probing (SIP)	The technique allows the characterization or identification of microbial population actively involved in specific metabolic processes in the environment. It involves the incorporation of stable isotope labelled substrates into cellular biomarkers that can be used to identify organisms assimilating the substrate	<ul style="list-style-type: none"> Provides evidence on the function of microorganisms in a controlled experimental setup Directly link microbial phylogeny with function 	<ul style="list-style-type: none"> Incubation and cycling of the stable isotope might cause biases within the microbial communities Lack sensitivity Enrichment bias may not reflect substrate metabolism in the environment
Activity	Community level physiological profiling (CLPP)/ Sole-Carbon-Source Utilization (SCSU) Pattern (BIOLOG)	SCSU or CLPP, also known as Biolog, examines the functional capabilities of the microbial population, and the resulting data can be analyzed using multivariate techniques to compare metabolic capabilities of communities. It uses a commercially available 96-well microtitre plate to assess the potential functional diversity of the bacterial population through sole source carbon utilization (SSCU) patterns. Gram-negative (GN) and gram-positive (GP) plates are available from Biolog.	<ul style="list-style-type: none"> Fast Highly reproducible Relatively inexpensive Able to differentiate microbial communities Generates large amount of data Option of using bacterial, fungal plates or site specific carbon sources (Biolog) 	<ul style="list-style-type: none"> Only represents culturable fraction of community Favours fast growing organisms (then contribution of fungi could not be measured as they usually grow slowly) Only represents those organisms capable of utilizing available carbon sources Potential metabolic diversity, not in situ diversity Sensitive to inoculum density Reproducible results can only be obtained if replicates contain identical community profiles
	Functional Gene Arrays (FGA)	Functional Gene Array identifies or measure genes encoding key	<ul style="list-style-type: none"> Analyzes a vast amount of genetic information 	<ul style="list-style-type: none"> Requires the construction of an array and access to a scanner

	(RNA-based)	enzymes in a metabolic process by measuring mRNA.	simultaneously	<ul style="list-style-type: none"> • Issues with specificity/cross hybridization • Requires normalization • Insufficient sensitivity and reproducibility can be problematic • Limited by the presence of probes on the array • Issues with RNA extraction from soil • Many issues with isolation of RNA from soil • mRNA isolation and often amplification are required for gene expression analyses • Current sequencing methods, databases and computing power are not sufficient yet to cover the high biodiversity in soil.
	Generation Sequencing (Metatranscriptomics)	<p>In a metatranscriptomic study total RNA is first isolated from the sample and structural RNAs are then removed to enrich for mRNA, which is then reverse transcribed into cDNA subject to DNA sequencing using next generation sequencing (NGS) platforms (See metagenomic description). Metatranscriptomic data indicate which of the genes encoded in a metagenome are actually transcribed, and which of the potential metabolic pathways are active (and the level of their activities) on the basis of their transcriptions within a microbial community under certain environmental condition. Metatranscriptomic sequences can be assembled into transcripts, each encoding one or more genes that are transcribed together (in the same direction). In the latter case (known as operons), the intergenic regions between coding genes are relatively short.</p>	<ul style="list-style-type: none"> ▪ Allows rRNA and/or mRNA ▪ Profiling and quantification without ▪ Prior knowledge of sequence 	

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Appendix J. Uncertainty

1. Background

Uncertainty was identified (EFSA Scientific Committee, 2013) as a priority topic: “guidance should be developed on how to characterise, document and explain uncertainties in all steps for human and environmental risk assessment”. A working group was established and the terms of reference stated that the guidance should develop “a harmonised framework applicable to all relevant working areas of EFSA”. The draft guidance will soon go out for public consultation and states that :

‘The Scientific Committee considers that all EFSA scientific assessments must include consideration of uncertainties. Therefore the application of this guidance document is unconditional for EFSA. For reasons of transparency and in line with EFSA 2006, the assessments must say what uncertainties have been identified and what is their impact on the overall assessment outcome. This must be reported clearly and unambiguously’.

2. Purpose of appendix

What are the implications of the SC guidance for an opinion on science underlying a future guidance document?

- It should consider uncertainties in the underlying science and make an assessment of their potential to influence risk assessments
- It should consider any additional uncertainties which need to be considered when developing the guidance
- It should possibly also make an initial evaluation of uncertainties which are likely to affect use of the guidance, distinguishing between those which are likely to be covered by assessment factors (AFs) and those which are not.

3. Main Uncertainties identified by the Working Group in the risk assessment of in-soil organisms:

- Representativeness of test species
- Temporal dynamics of effects
- Interspecies variability
- Are the AFs right?
- Lab-to-field
- Soil variability
- Field exposure dynamics
- Linking test effects to specific protection goals
- Recovery
- Matching up effects and exposure estimates
- Power of higher tier tests
 - number of replicates required
 - field size/recolonisation issues
- Are metabolites covered?

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