Evaluation Manual for the Authorisation of plant protection products and biocides according to Regulation (EC) No 1107/2009

EU part

Plant protection products

Chapter 7 Ecotoxicology: terrestrial; soil organisms

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Board for the Authorisation of plant protection products and biocides

Chapter 7 Ecotoxicology; terrestrial; soil organisms

Category: Plant Protection Products

GENERAL INTRODUCTION

This chapter describes the data requirements for estimation of the effects on soil organisms of a plant protection product and its active substance and how reference values are derived in the EU framework (§1 - §1.5) under Regulation (EC) No 1107/2009 [1].The described risk assessment in this chapter can be used for both the approval procedure for active substances as well as for zonal applications for the authorization of plant protection products (i.e. core registration reports).

Substances that are approved under Regulation (EC) No 1107/2009 and were approved under Directive 91/414/EEC [2] are included in Commission Implementing Regulation (EU) No 540/2011 [3].

This chapter consists of two parts: a part about earthworms (I) and a part about soil micro-organisms (II).

The chapter describes the procedures following the data requirements as laid down in Commission Regulation (EU) No 283/2013 for active substances and in Commission Regulation (EU) No 284/2013 for plant protection products. These data requirements apply for active substances submitted after 31 December 2013 and for plant protection products submitted after 31 December 2015.

A concept guidance is available on the interpretation of the transitional measures for the data requirements for chemical active substances according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 (SANCO/11509/2013 – rev. 0.1).

For further information on the former data requirement as laid down in Commission Regulation (EU) No 544/2011 for active substances and in Commission Regulation (EU) No 545/2011 we refer to the Evaluation Manual for Authorisation of plant protection products according to Regulation (EC) No 1107/2009 version 1.0

I EARTHWORMS AND OTHER NON-TARGET SOIL MESO- AND MACROFAUNA

1. EU FRAMEWORK

In this document, the procedures for the evaluation and re-evaluation of active substances as laid down in the EU are described; the NL procedure for evaluation of a substance is reverted to when no EU procedure has been laid down. The NL-procedure for the evaluation of a substance is described in §2 - §2.5 of part 2 of the Evaluation Manual (plant protection products). This document aims to give procedures for the approval of active substances and inclusion in Commission Implementing Regulation (EU) No 540/2011 [3].

1.1. Introduction

Effects of plant protection products on earthworms are included in the assessment where it cannot be ruled out that the substance or the product reach the soil (see Appendix 1). This chapter describes the procedure for this evaluation.

Guidelines for the risk assessment for earthworms are described in the Guidance Document on Terrestrial Ecotoxicology [4].

Earthworms play a vital role in the ecosystem. For this reason plant protection products should cause no unacceptable and prolonged effects on earthworm populations, not in the

treated part and not beyond.
The risk assessment of the use of pesticides for earthworms serves to prevent that products that present an unacceptable risk to the environment will reach the market. The risk to earthworms must be evaluated in case there is a chance of exposure of these organisms.

The sublethal data on earthworms are used in the higher tier risk assessment for persistence (see Chapter 6 Behaviour and fate in the environment; Behaviour in soil; Persistence). The calculated concentration in soil (PEC_{soil}) is also used for the risk assessment for earthworms. The PEC_{soil} calculation method is presented in Chapter 6 Fate and behaviour in the environment; Behaviour in soil; Persistence.

A decision tree with corresponding explanatory notes is presented in Appendix 2. This decision tree presents the decision scheme for earthworms.

Data requirements, evaluation methodologies, criteria and trigger values that deviate from, or further elaborate, the provisions under EU framework (§1), are described in the NL part (§2 - §2.5). The national further provisions can also be used for inclusion of an active substance in Commission Implementing Regulation (EU) No 541/2011.

1.2. Data requirements

In order to qualify for inclusion of an active substance in Commission Implementing Regulation (EU) No 540/2011 [3] a dossier that meets the provisions laid down in Commission Regulation (EU) No 283/2013 [5] and Commission Regulation (EU) No 284/2013 of Regulation (EC) No 1107/2009 [6] must be submitted for the active substance as well as for the product.

Generally, EU and OECD guidelines for the protocol of experiments are mentioned in Commission Communication 2013/C 95/01 [7].

When according to the applicant a certain study is not necessary, a relevant scientific justification can be provided for the non-submission of the particular study.

The data requirements, and the fact whether or not they are required for certain fields of use, and the corresponding guidelines are summarised in the overview table; see Appendix A to Chapter 7.

1.2.1 Data requirements for the active substance

The text below in grey frames has been taken from Commission Regulation (EU) No 283/2013. The numbering in these grey frames follows the section numbering in this Commission Regulation. Any necessary additions to the text have been added below the grey frames. Question numbers (NL as well as EU) are given below the headings.
The endpoints of the study are given as well, if relevant.

The data requirements regarding the risk of the active substance for earthworms are described in Commission Regulation (EU) No 283/2013, point 8.4 (effects on earthworms).

Introduction

1. All available biological data and information which is relevant to the assessment of the ecotoxicological profile of the active substance shall be reported. This shall include all potentially adverse effects found during routine ecotoxicological investigations. Where

required by the national competent authorities, additional studies, necessary to investigate the probable mechanisms involved and to assess the significance of these effects, shall be carried out and reported on.

2. The ecotoxicological assessment shall be based on the risk that the proposed active substance used in a plant protection product poses to non-target organisms. In carrying out a risk assessment, toxicity shall be compared with exposure. The general term for the output from such a comparison is 'risk quotient' or RQ. It shall be noted that RQ can be expressed in several ways, for example, toxicity:exposure ratio (TER) and as a hazard quotient (HQ). The applicant shall take into account the information from Sections 2, 5, 6, 7 and 8.

3. It may be necessary to conduct separate studies for metabolites, breakdown or reaction products derived from the active substance where non-target organisms may be exposed and where their effects cannot be evaluated by the available results relating to the active substance. Before such studies are performed, the applicant shall take into account the information from Sections 5, 6 and 7.

   Studies undertaken shall permit characterisation of metabolites, breakdown or reaction products as being significant or not, and reflect the nature and extent of the effects judged likely to arise.

4. In the case of certain study types, the use of a representative plant protection product instead of the active substance as manufactured may be more appropriate, for example testing of non-target arthropods, bees, earthworm reproduction, soil micro-flora and non-target terrestrial plants. In the case of certain plant protection product types (for example encapsulated suspension) testing with the plant protection product is more appropriate to testing with active substance when these organisms will be exposed to the plant protection product itself. For plant protection products where the active substance is always intended to be used together with a safener and/or synergist and/or in conjunction with other active substances, plant protection products containing these additional substances shall be used.

5. 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.

6. For those guidelines which allow for the study to be designed to determine an effective concentration (EC x), the study shall be conducted to determine an EC 10 , EC 20 and EC 50 , when required, along with corresponding 95 % confidence intervals. If an EC x approach is used, a no observed effect concentration (NOEC) shall still be determined.

   Existing acceptable studies that have been designed to generate a NOEC shall not be repeated. An assessment of the statistical power of the NOEC derived from those studies shall be carried out.

7. All of the aquatic toxicity data shall be used when developing a proposal for environmental quality standards (Annual Average EQS, AA-EQS; Maximum Acceptable Concentration EQS, MAC-EQS). The methodology for derivation of these endpoints is outlined in the 'Technical Guidance for Deriving Environmental Quality Standards([1])' for the Water Framework Directive 2000/60/EC of the European Parliament and of the Council ([2]).

8. In order to facilitate the assessment of the significance of test results obtained, including

the estimation of intrinsic toxicity and the factors affecting toxicity, the same strain (or recorded origin) of each relevant species shall, where possible, be used in the various toxicity tests specified.

9. Higher tier studies shall be designed and data analysed using suitable statistical methods. Full details of the statistical methods shall be reported. Where appropriate and necessary, higher tier studies shall be supported by chemical analysis to verify exposure has occurred at an appropriate level.

10. Pending the validation and adoption of new studies and of a new risk assessment scheme, existing protocols shall be used to address the acute and chronic risk to bees, including those on colony survival and development, and the identification and measurement of relevant sub-lethal effects in the risk assessment.

Effects on non-target soil meso- and macrofauna
(283/2013; 8.4)

Earthworm – sub-lethal effects
(283/2013; 8.4.1)

8.4. Effects on non-target soil meso- and macrofauna

8.4.1. Earthworm – sub-lethal effects
A test shall provide information on the effects on growth, reproduction and behaviour of the earthworm.

Circumstances in which required
Sub-lethal effects on earthworms shall be investigated where the active substance can contaminate soil.
Test conditions
Testing shall determine a dose-response relationship and the EC 10 , EC 20 and NOEC shall enable the risk assessment to be conducted in accordance with the appropriate risk quotient analysis, taking into account likely exposure, the organic carbon content (f oc) of the test medium and the lipophilic properties (K ow) of the test substance. The test substance shall be incorporated into the soil to obtain a homogenous soil concentration. Testing with soil metabolites may be avoided if there is analytical evidence to indicate that the metabolite is present at an adequate concentration and duration in the study conducted with the parent active substance.

Result:
→ 28 d NOEC

Effects on non-target soil meso- and macrofauna (other than earthworms)
(283/2013; 8.4.2)

8.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms

Circumstances in which required
Effects on soil organisms, other than earthworms, shall be investigated for all test

substances, except in situations where soil organisms are not exposed such as:
(a) food storage in enclosed spaces that preclude exposure;
(b) wound sealing and healing treatments;
(c) enclosed spaces with rodenticidal baits.

For plant protection products applied as a foliar spray data on *Folsomia candida* and *Hypoaspis aculeifer* may be required by the national competent authorities. If data are available on both *Aphidius rhopalosiphi* and *Typhlodromus pyri* these may be used in an initial risk assessment. If concern is raised with either species tested under point 8.3.2, data on both *Folsomia candida* and *Hypoaspis aculeifer* shall be provided.

If data on *Aphidius rhopalosiphi* and *Typhlodromus pyri* are not available, then the data set out in point 8.4.2.1 shall be provided.

For plant protection products applied directly to soil as soil treatments either as a spray or as a solid formulation, testing shall be carried out on both on *Folsomia candida* and *Hypoaspis aculeifer* (see point 8.4.2.1).

Note on 8.4.2 (and 10.4.2):
The text of point 8.4.2 (and 10.4.2) leaves open for the national competent authorities a choice on how to require the fulfillment of this data requirement in case of foliar applications. (i.e. due to the (multiple) use of the word 'may' in the second alinea). The text therefore leaves room for two options in case of foliar applications:

A) Studies with *Folsomia candida* and *Hypoaspis aculeifer* are always required

B) Studies with *Folsomia candida* and *Hypoaspis aculeifer* are only required when:
- no data is available for *Aphidius rhopalosiphi* and *Typhlodromus pyri,* or:
- a risk is identified for *Aphidius rhopalosiphi* or *Typhlodromus pyri.*

The Ctgb working approach will be option A. The reason for this choice is as follows:

- The tests and risk assessment for *Typhlodromus* and *Aphidius* are considered not a good indicator for the risk to in-soil species, due to the different exposure route (in soil versus residues on plant leaves) and due to the different triggers that are used for risk assessment (HQ based on ER50 with trigger value 2 versus TER based on NOEC with trigger 5).
- The risk assessment for soil organisms based solely on earthworms and soil micro-organisms is, from a scientific point of view, considered as limited.

The above will be used by Ctgb in the role of EU Rapporteur Member State for Annex I listing of an active substance, or as Zonal Rapporteur Member State for authorisation of a plant protection product.

Species level testing
(283/2013; 8.4.2.1)

8.4.2.1 Species level testing

A test shall provide sufficient information to perform an assessment of the toxicity of the active substance to the soil invertebrate indicator species *Folsomia candida* and *Hypoaspis aculeifer*.

Test conditions
Testing shall determine a dose-response relationship and the EC 10 , EC 20 and NOEC shall enable the risk assessment to be conducted in accordance with the appropriate risk quotient analysis, taking into account likely exposure, the organic carbon content (f oc) of the test medium and the lipophilic properties (K ow) of the test substance. The test substance shall be incorporated into the soil to obtain a homogenous soil concentration. Testing with soil metabolites may be avoided if there is analytical evidence to indicate that the metabolite is present at an adequate concentration and duration in the study conducted with the parent active substance.

1.2.2 Data requirements for the product

The text below in grey frames has been taken from Commission Regulation (EU) No 284/2013. The numbering in these grey frames follows the section numbering in this Commission Regulation. Any necessary additions to the text have been added below the grey frames. Question numbers (NL as well as EU) are given below the headings.
The endpoints of the study are given as well, if relevant.

The data requirements regarding the risk of the plant protection product for earthworms are described in Commission Regulation (EU) No 284/2013, point 10.6 (effects on earthworms and other soil non-target macro-organisms, believed to be at risk).

Generally, EU and OECD guidelines for the protocol of experiments are mentioned in Commission Communication 2013/C 95/02 [8].

Introduction

1. Testing of the plant protection product shall be necessary where its toxicity cannot be predicted on the basis of data on the active substance. Where testing is necessary, the aim shall be to demonstrate whether the plant protection product, taking account of content of active substance, is more toxic than the active substance. Thus bridging studies or a limit test may be sufficient. However, where a plant protection product is more toxic than the active substance (expressed in comparable units), definitive testing shall be required. Possible effects on organisms/ecosystems shall be investigated, unless the applicant shows that exposure of the organisms or ecosystems does not occur.

Tests and studies conducted using the plant protection product as test material necessary to assess the toxicity of the active substance shall be reported in the context of the relevant data requirement concerning the active substance.

2. All potentially adverse effects found during routine ecotoxicological investigations shall be reported and such additional studies, which may be necessary to investigate the mechanisms involved and assess the significance of these effects, shall be undertaken and reported.

3. Whenever a study implies the use of different doses, the relationship between dose and adverse effect shall be reported.

4. Where exposure data are necessary to decide whether a study has to be performed, the data obtained in accordance with Section 9 shall be used.

For the estimation of exposure of organisms, all information on the plant protection product and on the active substance shall be taken into account. A tiered approach shall start with

default worst-case parameters for exposure and be followed by a parameter refinement based on the identification of representative organisms. Where relevant, the parameters set out in this Section shall be used. Where it appears from available data that the plant protection product is more toxic than the active substance, the toxicity data for the plant protection product shall be used for the calculation of appropriate risk quotients (see point 8 of this introduction).

5. The requirements laid down in this Section shall include certain study types that are set out in Section 8 of Part A of the Annex to Regulation (EU) No 283/2013 (such as standard laboratory tests with birds, aquatic organisms, bees, arthropods, earthworms, soil micro-organisms, soil meso-fauna and non-target plants). While each point shall be addressed, experimental data with a plant protection product shall be generated only if its toxicity cannot be predicted on the basis of data on the active substance. It may be sufficient to test the plant protection product with that species of a group that was most sensitive with the active substance.

6. A detailed description (specification) of the material used as provided for in accordance with point 1.4 shall be provided.

7. In order to facilitate the assessment of the significance of test results obtained, the same strain of each species shall, where possible, be used in the various toxicity tests specified.

8. The ecotoxicological assessment shall be based on the risk that the proposed plant protection product poses to non-target organisms. In carrying out a risk assessment, toxicity shall be compared with exposure. The general term for the output from such a comparison is ‘risk quotient’ (RQ). RQ may be expressed in several ways, for example, toxicity:exposure ratio (TER) and as a hazard quotient (HQ).

9. For those guidelines which allow for study to be designed to determine an effective concentration (EC x), the study shall be conducted to determine an EC 10 and EC 20 along with corresponding 95 % confidence intervals. If an EC x approach is used, a NOEC shall still be determined.

Existing acceptable studies that have been designed to generate a NOEC shall not be repeated. An assessment of the statistical power of the NOEC derived from those studies shall be carried out.

10. For solid formulations an assessment of the risk from dust drift on to non-target arthropods and plants shall be required. Details on the likely exposure levels shall be presented in accordance with Section 9 of this Annex. For aquatic life, the risk of movement of the whole particle as well as dust particles shall be considered. Until agreed dust dissipation rate assessments are available likely exposure levels shall be used in the risk assessment.

11. Higher tier studies using a plant protection product shall be designed and data analysed using suitable statistical methods. Full details of the statistical methods shall be reported. Where appropriate, higher tier studies shall be supported by chemical analysis to verify exposure has occurred at an appropriate level.

12. Pending the validation and adoption of new studies and of a new risk assessment scheme, existing protocols shall be used to address the acute and chronic risk to bees, including those on colony survival and development, and the identification and measurement of sub-lethal effects in the risk assessment.

Effects on non-target soil meso- and macrofauna
(284/2013; 10.4.)

Earthworms
(284/2013; 10.4.1)

10.4. Effects on non-target soil meso- and macrofauna
10.4.1. Earthworms
The possible impact on earthworms shall be reported unless the applicant shows that it is not likely that earthworms are exposed, directly or indirectly.

A risk assessment for earthworm shall be conducted in accordance with the relevant risk quotient analysis.

Earthworms - sublethal effects
(284/2013 ; 10.4.1.1)

10.4.1.1. Earthworms – sub-lethal effects
The test shall provide information on the effects on growth and reproduction of the earthworm.
Circumstances in which required
The sub-lethal toxicity of a plant protection product to earthworms shall be investigated if the relevant criteria as defined in point 8.4.1 of Part A of the Annex to Regulation (EU) No 283/2013 are met, and the toxicity of the plant protection product cannot be predicted on the basis of the data for the active substance, unless the applicant shows that no exposure occurs.
Test conditions
Testing shall determine a dose-response relationship and the EC 10 , EC 20 and NOEC shall enable the risk assessment to be conducted in accordance with the appropriate risk quotient analysis, taking into account likely exposure, the organic carbon content (f_{oc}) of the test medium and the lipophilic properties (K_{ow}) of the test substance. The test substance shall be incorporated into the soil to obtain a homogenous soil concentration. Testing with soil metabolites may be avoided if there is analytical evidence to indicate that the metabolite is present at an adequate concentration and duration in the study conducted with the parent active substance.

Result:
→ 28-d NOEC

Eartworms - field studies
(284/2013; 10.4.1.2)

10.2.1.2. Earthworms - field studies
The test shall provide sufficient data to evaluate effects on earthworms under field conditions.

Circumstances in which required
Where the relevant risk quotient analysis indicates a chronic risk to earthworms a field study to determine effects under practical field conditions shall be conducted and reported as an option for refined risk assessment.
Test conditions
The study design shall reflect the proposed use of the plant protection product, the

environmental conditions likely to arise and species that will be exposed.

If a study is to be used for risk assessment in relation to metabolites, their concentrations occurring shall be confirmed analytically.

Effects on non-target soil meso- and macrofauna (other than eartworms)
(284/2013; 10.4.2)

10.4.2. Effects on other soil non-target macro-organisms

Circumstances in which required
Effects on soil organisms (other than earthworms) shall be investigated for all plant protection products, except in situations where soil organisms are not exposed such as:
(a) food storage in enclosed spaces that preclude exposure;
(b) wound sealing and healing treatments;
(c) enclosed spaces with rodenticidal baits.

Testing shall be required if:
— the plant protection product contains more than one active substance,
— the toxicity of a plant protection product cannot be reliably predicted to be either the same or lower than the active substance tested in accordance with point 8.4.2 of Part A of the Annex to Regulation (EU) No 283/2013.

For plant protection products applied as a foliar spray, data on the relevant two non target arthropod species might be taken into account for a preliminary risk assessment. If effects do occur on either species, testing on *Folsomia candida* and *Hypoaspis aculeifer* shall be required (see point 10.4.2.1).

If data on *Aphidius rhopalosiphi* and *Typhlodromus pyri* are not available then the data outlined in point 10.4.2.1 shall be required.

For plant protection products applied as soil treatments directly to soil either as a spray or as a solid formulation, then testing shall be required on both *Folsomia candida* and *Hypoaspis aculeifer* (see point 10.4.2.1).

Note on 10.4.2:
See note on 8.4.2.

Species level testing
(284/2013; 10.4.2.1)

10.4.2.1 Species level testing
The test shall provide sufficient information to perform an assessment of the toxicity of the plant protection product to the soil invertebrate indicator species *Folsomia candida* and *Hypoaspis aculeifer*.

Test conditions
Testing shall determine a dose-response relationship and the EC 10 , EC 20 and NOEC shall enable the risk assessment to be conducted in accordance with the appropriate risk quotient analysis, taking into account likely exposure, the organic carbon content (f oc) of the test medium and the lipophilic properties (Kow) of the active substance in the plant protection product. The plant protection product shall be incorporated into the soil to obtain

a homogenous soil concentration.

Higher tier testing
(284/2013; 10.4.2.2)

10.4.2.1 Species level testing
The tests shall provide sufficient information to evaluate the risk of the plant protection product for soil organisms (other than earthworms) using a more realistic test substrate or exposure regime.

Circumstances in which required
Further testing shall be required where significant effects are seen following laboratory testing in accordance with the requirements set out in point 8.4.2.1 of Part A of the Annex to Regulation (EU) No 283/2013 or in accordance with point 10.4.2.1 of this Annex and where risk is indicated following the relevant risk quotient analysis.

The need to perform such studies and the type and conditions of the studies to be performed shall be discussed with the national competent authorities.

Test conditions
Higher-tier tests may take the form of community/population studies (for example. terrestrial model ecosystems, soil mesocosms) or field studies. Timing, levels and routes of exposure shall reflect those of the proposed use of the plant protection product. Key effect end-points include: changes in community and population structure of both micro and macro-organisms; species diversity; number and biomass of key species/groups.

1.2.3 Data requirements for metabolites

Data about the effects on earthworms are required for metabolites formed in the laboratory study into the (an)aerobic transformation route in soil.
For a general discussion about metabolites see §1.2.3 in Chapter 7 Ecotoxicology; Terrestrial; Birds and mammals.

1.3. Risk assessment

The risk assessment methodology for earthworms has in EU context been elaborated in the Guidance Document on Risk Assessment for Terrestrial Ecotoxicology [4].

Each study is summarised and analysed separately. The final conclusion and the endpoint per aspect (such as 14-d LC_{50}) are presented in a list of endpoints.
The risk is assessed against the endpoints and a relevant trigger value.

Further elaborations of the EU evaluation methodology:

Combination toxicity
Combination toxicity must be determined when plant protection products contain several active substances. Combinations of plant protection products of which the combination (tank mix) is recommended in the directions for use are also considered as combination products.

When evaluating the side effects of combination products on non-target organisms, the question arises whether the risk estimate must be based on a toxicity test with the combination product or whether a reasonable risk estimate can be made on the basis of the toxicity data of the separate active substances. There is no European Guidance in the field of combination toxicology.

It should be noted that for non-target soil meso- and macrofauna other than eartwhorms, testing with the formulation is required when the product contains more than one active substance (see data requirement 10.4.2).

It is possible to base the risk assessment of a combination product on toxicity tests with the formulation.
The *acute* toxicity test can lead to varying results because the quantity and the quality of the co-formulants may not be constant and the formulation may change the availability of the active substances. For the acute risk assessment, the combination toxicity on the basis of the tests with the product is compared with the combination toxicity based on the toxicity research with the separate active substances.

In the assessment the risk of the combination products is determined on the basis of the lowest TER value, as calculated by the toxicity of the separate active substances or the toxicity of the product.

The fact that the ratio between the active substances changes by differences in sorption and degradation rate plays a role in establishing *chronic* toxicity. This means that the concentration of the combination product in the environment (the PEC) cannot be predicted because the separate active substances may behave differently after application.
For chronic risk assessment it is therefore preferred to determine the toxicity of the combination product on the basis of toxicity research with the separate active substances.

Combination toxicity is determined on the basis of concentration addition.
In theory, three different effects are to be expected when two or more substances are used in a mixture:
- the substances may weaken each others' toxic effects (antagonism)
- the effects of the substances may be additive
- the substances may potentiate each others' toxic effects (synergism).
  Although the effects of mixtures of active substances in plant protection products have only been studied to a very limited extent and not for all relevant species and toxicological endpoints it is expected that active substances in a combination product or tank mix together contribute to the toxicity of that product or that tank mix. The extent to which the active substances are contributing is poorly known. The available data indicate that also in case of partial addition the extent of combination toxicity does not deviate strongly from concentration addition. In view of these considerations the evaluation of the toxicity data of combination products or tank mixes is based on concentration addition. In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration.

  The combination TER can be calculated according to the following formula:
  - $TER_{combi} = 1/((1/TER_{substance\ 1})+(1/TER_{substance\ 2})+(1/TER_{substance\ 3}))$

An acceptable risk is expected when TER_{combi} > trigger.

1.4. Approval

This section describes the approval criteria for active substances (section 1.4.1) and plant protection products (section 1.4.2 and 1.4.3). For the EU approval procedure of active substances a representative formulation has to be included in the dossier. Therefore section 1.4.1 to 1.4.3 apply. For the zonal applications of plant protection products only section 1.4.2 and 1.4.3 apply.

1.4.1 Approval of the active substance

Regulation (EC) No 1107/2009 Annex II Directive 91/414/EEC provides the procedure and criteria for the approval of an active substances, safeners and synergists pursuant to Chapter II of Regulation (EC) No 1107/2009.

Point 3 of Annex II of Regulation (EC) No 1107/2009 gives the criteria for the approval of an active substance. The texts specifically applicable to the aspect earthworms are presented below.

3. Criteria for the approval of an active substance

3.1. Dossier

The dossier submitted pursuant to Article 7(1) shall be sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.

3.3. Relevance of metabolites
Where applicable the documentation submitted shall be sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.

3.8. Ecotoxicology
3.8.1. An active substance, safener or synergist shall only be approved if the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The assessment must take into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.

1.4.2 Evaluation of plant protection products

The principles for the evaluation regarding the effects on the environment are presented in the Commission Regulation (EU) No 546/2011 [9]. These are the relevant sections of the introductory principles, the general principles and the specific principles Environmental effects.
The specific principles Environmental effects, part Effect on species that are no target species are as regards earthworms and other soil macro-arthropods in the text below printed in a grey frame. This text, including numbering, is the literal text from Commission Regulation (EU) No 546/2011.

2.5.2.5. Member States shall evaluate the possibility of exposure of earthworms and other non-target soil macro-organisms to the plant protection product under the proposed conditions of use; if this possibility exists they shall evaluate the degree of short-term and long-term risk to be expected to these organisms after use of the plant protection product according to the proposed conditions of use.
(a) This evaluation will take into consideration the following information:
(i) the specific information relating to the toxicity of the active substance to earthworms and to other non- target soil macro-organisms as provided for in the Annex to Regulation (EU) No 544/2011 and the results of the evaluation thereof;

(ii) other relevant information on the active substance such as:
- solubility in water,
- octanol/water partition coefficient,
- Kd for adsorption,
- vapour pressure,
- hydrolysis rate in relation to pH and identity of breakdown products,
- photodegradation rate and identity of breakdown products,
- DT50 and DT90 for degradation in the soil;

(iii) all relevant information on the plant protection product as provided for in the Annex to Regulation (EU) No 545/2011, including the effects on earthworms and other non-target soil macro-organisms;

(iv) where relevant, other authorized uses of plant protection products in the area of envisaged use, containing the same active substance or which give rise to the same residues;

(b) This evaluation will include:

(i) the lethal and sublethal effects,

(ii) the predicted initial and long-term environmental concentration,

(iii) a calculation of the acute toxicity/exposure ratio (defined as the quotient of LC50 and predicted initial environmental concentration) and of the long-term toxicity/exposure ratio (defined as the quotient of the NOEC and predicted long-term environmental concentration),

(iv) where relevant, the bioconcentration and persistence of residues in earthworms.

1.4.3 Decision making for plant protection products

The principles for decision making as regards the effects on the environment are presented in Commission Regulation (EU) No 546/2011. These are the relevant sections of the introductory principles, the general principles and the specific principles Environmental effects.

The specific principles Environmental effects, part Effect on species that are no target species are as regards earthworms and other soil macro-organisms in the text below printed in a grey frame. This text, including numbering, is the literal text from Commission Regulation (EU) No 546/2011.

2.5.2.5. Where there is a possibility of earthworms being exposed, no authorization shall be granted if the acute toxicity/exposure ratio for earthworms is less than 10 or the long-term toxicity/exposure ratio is less than 5, unless it is clearly established through an appropriate risk assessment that under field conditions earthworm populations are not at risk after use of the plant protection product according to the proposed conditions of use.

Note on 2.5.2.5:

Soil meso- and macro organisms other than earthworms are not explicitly mentioned under 2.5.2.5, however the common approach in EU-risk assessment for these organisms is to use the same triggers for the toxicity/exposure ratio as for earthworms (i.e. 10 for acute and 5 for chronic effects).

1.5. Developments

New guidance is in development at EFSA with the revisions of the Guidance documents on Persistence (9188/VI/97 rev.8) and Terrestrial Ecotoxicology (SANCO/10329/2002). Until the revision of these guidance documents is finished, the methods as described in 1.4.2 are used for risk assessment.

II SOIL MICRO-ORGANISMS

1. EU FRAMEWORK

In this document, the procedures for the evaluation and re-evaluation of active substances as laid down in the EU are described; the NL procedure for evaluation of a substance is reverted to when no EU procedure has been laid down. The NL-procedure for the evaluation of a substance is described in §2 - §2.5 of part 2 of the Evaluation Manual (plant protection products). This document aims to give procedures for the approval of active substances and inclusion in Commission Implementing Regulation (EU) No 540/2011 [3].

1.1 Introduction

Effects of plant protection products on soil micro-organisms are included in the assessment if the substance or product reaching the soil cannot be ruled out (see Appendix 1, Chapter 7 Ecotoxicology; Terrestrial; Earthworms).

Guidelines for the risk assessment for soil micro-organisms are described in the Guidance Document on Terrestrial Ecotoxicology [4].

Soil micro-organisms play a vital role in the ecosystem. For this reason plant protection products should cause no unacceptable and prolonged effects on soil micro-organism populations, not in the treated part and not beyond.
The risk assessment of the use of pesticides for soil micro-organisms serves to prevent that products which present an unacceptable risk to the environment will reach the market. The risk to soil micro-organisms must be evaluated in case there is a chance of exposure of these organisms.

The data on soil micro-organisms are used in the higher tier risk assessment for persistence (see Chapter 6, part Persistence).

The decision tree with corresponding explanatory notes is presented in Appendix 3.
This decision tree summarises the decision scheme for soil micro-organisms.

Data requirements, evaluation methodologies, criteria and trigger values that deviate from, or further elaborate, the provisions under EU framework (§1), are described under NL framework (§2 - §2.5). The national further provisions can also be used for inclusion of an active substance in 540/2011.

1.2 Data requirements

In order to qualify for inclusion in Commission Implementing Regulation (EU) No 540/2011 a dossier that meets the provisions laid down in Commission Regulation (EU) No 283/2013 [5] and Commission Regulation (EU) No 284/2013 of Regulation (EC) No 1107/2009 [6] must be submitted for the active substance as well as for the product.

Generally, EU and OECD guidelines for the protocol of experiments are mentioned in Commission Communication 2013/C 95/01 [7].

When according to the applicant a certain study is not necessary, a relevant scientific justification can be provided for the non-submission of the particular study.

The data requirements, and the fact whether or not they are required for certain fields of use, and the corresponding guidelines are summarised in the overview table; see Appendix

A to Chapter 7.

1.2.1 Data requirements for the active substance

The text below in grey frames has been taken from Commission Regulation (EU) No 283/2103. The numbering in these grey frames follows the section numbering in this Commission Regulation. Any necessary additions to the text have been added below the grey frames. Question numbers (NL as well as EU) are given below the headings.
The endpoints of the study are given as well, if relevant..

The data requirements regarding the risk of the active substance for soil micro-organisms are described in Commission Regulation (EU) No 283/2013, point 8.5 (effects on soil non-target micro-organisms).

Introduction

1. All available biological data and information which is relevant to the assessment of the ecotoxicological profile of the active substance shall be reported. This shall include all potentially adverse effects found during routine ecotoxicological investigations. Where required by the national competent authorities, additional studies, necessary to investigate the probable mechanisms involved and to assess the significance of these effects, shall be carried out and reported on.

2. The ecotoxicological assessment shall be based on the risk that the proposed active substance used in a plant protection product poses to non-target organisms. In carrying out a risk assessment, toxicity shall be compared with exposure. The general term for the output from such a comparison is ‘risk quotient’ or RQ. It shall be noted that RQ can be expressed in several ways, for example, toxicity:exposure ratio (TER) and as a hazard quotient (HQ). The applicant shall take into account the information from Sections 2, 5, 6, 7 and 8.

3. It may be necessary to conduct separate studies for metabolites, breakdown or reaction products derived from the active substance where non-target organisms may be exposed and where their effects cannot be evaluated by the available results relating to the active substance. Before such studies are performed, the applicant shall take into account the information from Sections 5, 6 and 7.

   Studies undertaken shall permit characterisation of metabolites, breakdown or reaction products as being significant or not, and reflect the nature and extent of the effects judged likely to arise.

4. In the case of certain study types, the use of a representative plant protection product instead of the active substance as manufactured may be more appropriate, for example testing of non-target arthropods, bees, earthworm reproduction, soil micro-flora and non-target terrestrial plants. In the case of certain plant protection product types (for example encapsulated suspension) testing with the plant protection product is more appropriate to testing with active substance when these organisms will be exposed to the plant protection product itself. For plant protection products where the active substance is always intended to be used together with a safener and/or synergist and/or in conjunction with other active substances, plant protection products containing these additional substances shall be used.

5. 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.

6. For those guidelines which allow for the study to be designed to determine an effective concentration (EC x), the study shall be conducted to determine an EC 10 , EC 20 and EC 50 , when required, along with corresponding 95 % confidence intervals. If an EC x approach is used, a no observed effect concentration (NOEC) shall still be determined.

   Existing acceptable studies that have been designed to generate a NOEC shall not be repeated. An assessment of the statistical power of the NOEC derived from those studies shall be carried out.

7. All of the aquatic toxicity data shall be used when developing a proposal for environmental quality standards (Annual Average EQS, AA-EQS; Maximum Acceptable Concentration EQS, MAC-EQS). The methodology for derivation of these endpoints is outlined in the 'Technical Guidance for Deriving Environmental Quality Standards([1])' for the Water Framework Directive 2000/60/EC of the European Parliament and of the Council ([2]).

8. In order to facilitate the assessment of the significance of test results obtained, including the estimation of intrinsic toxicity and the factors affecting toxicity, the same strain (or recorded origin) of each relevant species shall, where possible, be used in the various toxicity tests specified.

9. Higher tier studies shall be designed and data analysed using suitable statistical methods. Full details of the statistical methods shall be reported. Where appropriate and necessary, higher tier studies shall be supported by chemical analysis to verify exposure has occurred at an appropriate level.

10. Pending the validation and adoption of new studies and of a new risk assessment scheme, existing protocols shall be used to address the acute and chronic risk to bees, including those on colony survival and development, and the identification and measurement of relevant sub-lethal effects in the risk assessment.

Effects on soil nitrogen transformation
(283/2013; 8.5)

8.5. Effects on soil nitrogen transformation

A test shall provide sufficient data to evaluate the impact of active substances on soil microbial activity, in terms of nitrogen transformation.
Circumstances in which required
The test shall be carried out where plant protection products containing the active substance are applied to soil or can contaminate soil under practical conditions of use. In the case of active substances intended for use in plant protection products for soil sterilisation, the studies shall be designed to measure rates of recovery following treatment.
Test conditions
Soils used shall be freshly sampled agricultural soils. The sites from which soil is taken shall not have been treated during the previous two years with any substance that could substantially alter the diversity and levels of microbial populations present, other than in a transitory manner.

Result:
→ % nitrogen transformation in comparison with the untreated control
→ % carbon mineralisation in comparison with the untreated control

1.2.2 Data requirements for the product

The text below in grey frames has been taken from Commission Regulation (EU) No 284/2103. The numbering in these grey frames follows the section numbering in Commission Regulation. Any necessary additions to the text have been added below the grey frames. Question numbers (NL as well as EU) are given below the headings.
The endpoints of the study are given as well, if relevant..

The data requirements regarding the risk of the plant protection product for soil micro-organisms are described in Commission Regulation (EU) No 284/2013, point 10.7 (effects on soil non-target micro-organisms).

Generally, EU and OECD guidelines for the protocol of experiments are mentioned in Commission Communication 2013/C 95/02 [8].

Introduction

1. Testing of the plant protection product shall be necessary where its toxicity cannot be predicted on the basis of data on the active substance. Where testing is necessary, the aim shall be to demonstrate whether the plant protection product, taking account of content of active substance, is more toxic than the active substance. Thus bridging studies or a limit test may be sufficient. However, where a plant protection product is more toxic than the active substance (expressed in comparable units), definitive testing shall be required. Possible effects on organisms/ecosystems shall be investigated, unless the applicant shows that exposure of the organisms or ecosystems does not occur.

Tests and studies conducted using the plant protection product as test material necessary to assess the toxicity of the active substance shall be reported in the context of the relevant data requirement concerning the active substance.

2. All potentially adverse effects found during routine ecotoxicological investigations shall be reported and such additional studies, which may be necessary to investigate the mechanisms involved and assess the significance of these effects, shall be undertaken and reported.

3. Whenever a study implies the use of different doses, the relationship between dose and adverse effect shall be reported.

4. Where exposure data are necessary to decide whether a study has to be performed, the data obtained in accordance with Section 9 shall be used.

For the estimation of exposure of organisms, all information on the plant protection product and on the active substance shall be taken into account. A tiered approach shall start with default worst-case parameters for exposure and be followed by a parameter refinement based on the identification of representative organisms. Where relevant, the parameters set out in this Section shall be used. Where it appears from available data that the plant protection product is more toxic than the active substance, the toxicity data for the plant protection product shall be used for the calculation of appropriate risk quotients (see point 8 of this introduction).

5. The requirements laid down in this Section shall include certain study types that are set out in Section 8 of Part A of the Annex to Regulation (EU) No 283/2013 (such as standard laboratory tests with birds, aquatic organisms, bees, arthropods, earthworms, soil micro-organisms, soil meso-fauna and non-target plants). While each point shall be addressed, experimental data with a plant protection product shall be generated only if its toxicity cannot be predicted on the basis of data on the active substance. It may be sufficient to test the plant protection product with that species of a group that was most sensitive with the active substance.

6. A detailed description (specification) of the material used as provided for in accordance with point 1.4 shall be provided.

7. In order to facilitate the assessment of the significance of test results obtained, the same strain of each species shall, where possible, be used in the various toxicity tests specified.

8. The ecotoxicological assessment shall be based on the risk that the proposed plant protection product poses to non-target organisms. In carrying out a risk assessment, toxicity shall be compared with exposure. The general term for the output from such a comparison is ‘risk quotient’ (RQ). RQ may be expressed in several ways, for example, toxicity:exposure ratio (TER) and as a hazard quotient (HQ).

9. For those guidelines which allow for study to be designed to determine an effective concentration (EC x), the study shall be conducted to determine an EC 10 and EC 20 along with corresponding 95 % confidence intervals. If an EC x approach is used, a NOEC shall still be determined.

Existing acceptable studies that have been designed to generate a NOEC shall not be repeated. An assessment of the statistical power of the NOEC derived from those studies shall be carried out.

10. For solid formulations an assessment of the risk from dust drift on to non-target arthropods and plants shall be required. Details on the likely exposure levels shall be presented in accordance with Section 9 of this Annex. For aquatic life, the risk of movement of the whole particle as well as dust particles shall be considered. Until agreed dust dissipation rate assessments are available likely exposure levels shall be used in the risk assessment.

11. Higher tier studies using a plant protection product shall be designed and data analysed using suitable statistical methods. Full details of the statistical methods shall be reported. Where appropriate, higher tier studies shall be supported by chemical analysis to verify exposure has occurred at an appropriate level.

12. Pending the validation and adoption of new studies and of a new risk assessment scheme, existing protocols shall be used to address the acute and chronic risk to bees, including those on colony survival and development, and the identification and measurement of sub-lethal effects in the risk assessment.

Effects on soil nitrogen transformation

Laboratory testing
(284/2013 ; 10.5)

10.5. Effects on soil nitrogen transformation

The test shall provide sufficient data to evaluate the impact of the plant protection products on soil microbial activity in terms of nitrogen transformation.

Circumstances in which required
The effects of plant protection products on soil microbial function shall be investigated if the toxicity of the plant protection product cannot be predicted on the basis of data for the active substance, unless the applicant shows that no exposure occurs.

Result:
→ % nitrogen transformation in comparison with the untreated control
→ % carbon mineralisation in comparison with the untreated control

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1.2.3 Data requirements for metabolites

Data about the effects on soil micro-organisms are required for metabolites that are formed in the laboratory study into the (an)aerobic transformation route in the soil. For a general discussion about metabolites, see §1.2.3 in Chapter 7 Ecotoxicology; Terrestrial; Birds and mammals.

1.3 Risk assessment

The risk assessment methodology for soil micro-organisms has in EU context been elaborated in the Guidance Document on Terrestrial Ecology [4].

Each study is summarised and analysed separately. The final conclusion and the endpoint per aspect (% nitrogen and carbon mineralisation in comparison with the untreated control) are presented in a list of endpoints.
Risk is assessed against the endpoints.

1.4 Approval

This section describes the approval criteria for active substances (section 1.4.1) and plant protection products (section 1.4.2 and 1.4.3). For the EU approval procedure of active substances a representative formulation has to be included in the dossier. Therefore section 1.4.1 to 1.4.3 apply. For the zonal applications of plant protection products only section 1.4.2 and 1.4.3 apply.

1.4.1 Approval of the active substance

Regulation (EC) No 1107/2009 Annex II provides the procedure and criteria for the approval of an active substances, safeners and synergists pursuant to Chapter II of Regulation (EC) No 1107/2009.

Point 3 of Annex II of Regulation (EC) No 1107/2009 gives the criteria for the approval of an active substance. The texts specifically applicable to the aspect earthworms are presented below.

3. Criteria for the approval of an active substance

3.1. Dossier

The dossier submitted pursuant to Article 7(1) shall be sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.

3.3. Relevance of metabolites
Where applicable the documentation submitted shall be sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.

3.8. Ecotoxicology
3.8.1. An active substance, safener or synergist shall only be approved if the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The assessment must take into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.

1.4.2 Evaluation

The principles for the evaluation regarding the effects on the environment are presented in Commission Regulation (EU) No 546/2011 [[10]]. These are the relevant sections of the introductory principles, the general principles and the specific principles Environmental effects.
The specific principles Environmental effects, part Effect on species that are no target species are as regards microbial activity in the text below printed in a grey frame.
This text, including numbering, is the literal text from Commission Regulation (EU) No 546/2011.

2.5.2.6. Member States shall, where the evaluation carried out under point 2.5.1.1 does not exclude the possibility of the plant protection product reaching the soil under the proposed conditions of use, evaluate the impact on microbial activity such as the impact on nitrogen and carbon mineralisation processes in the soil after use of the plant protection product in accordance with the proposed conditions of use.
This evaluation will take into consideration the following information:
(i) all relevant information on the active substance, including the specific information relating to the effects of non-target soil micro-organisms as provided for in the Annex to Regulation (EU) No 544/2011 and the results of the evaluation thereof;
(ii) all relevant information on the plant protection product as provided for in the Annex to Regulation (EU) No 545/2011, including the effects on non-target soil micro-organisms;
(iii) where relevant, other authorized uses of plant protection products in the area of proposed use, containing the same active substance or which give rise to the same residues;
(iv) all available information from biological primary screening.

1.4.3 Decision making

The principles for decision making as regards the effects on the environment are presented in the Uniform Principles (546/2011). These are the relevant sections of the introductory principles, the general principles and the specific principles Environmental effects.
The specific principles Environmental effects, part Effect on species that are no target

species are as regards soil micro-organisms in the text below printed in a grey frame. This text, including numbering, is the literal text from 546/2011.

2.5.2.6. Where there is a possibility of non-target soil micro-organisms being exposed, no authorisation shall be granted if the nitrogen or carbon mineralisation processes in laboratory studies are affected by more than 25 % after 100 days, unless it is clearly established through an appropriate risk assessment that under field conditions there is no unacceptable impact on microbial activity after use of the plant protection product according to the proposed conditions of use, taking account of the ability of micro-organisms to multiply.

1.5 Developments

New guidance is in development at EFSA with the revisions of the Guidance documents on Persistence (9188/VI/97 rev.8) and Terrestrial Ecotoxicology (SANCO/10329/2002). Until the revision of these guidance documents is finished, the methods as described in 1.4.2 are used for risk assessment.

2 APPENDICES

Appendix 1 Can it be ruled out that the substance reaches the soil?

To answer the above question it is important whether the substance, during or after the application in a not entirely closed system consistent with good agricultural practice, comes into contact with the soil or not.

The first thing that matters is whether the application takes place in the open, or in enclosed spaces (greenhouses (cultures on substrate), barns, bee hives etc.). During applications in enclosed spaces, it is not ruled out *a priori* that the product reaches the soil. This can only be ruled out if the applied water is collected for re-use, or is discharged to a sewage treatment plant in a controlled manner. In the other cases of treatment in enclosed spaces, persistence is relevant.

During outdoor use, the aspect persistence is relevant for nearly all applications. Only for a number of specific application techniques (treatment of wounds by pasting, injection of trees etc.), and applications where the water is collected for re-use or is discharged to a sewer, can it be ruled out that the product reaches the soil.

There are uses where the actual use of the plant protection product takes place at another location, other than the crop cultivation itself (seed treatment, treatment of propagation material, tray treatment etc.). In those cases, the situation of the crop cultivation itself should serve as a basis. This means that, in the case of treated seed or other propagation material, it is not ruled out that the substance reaches the soil.

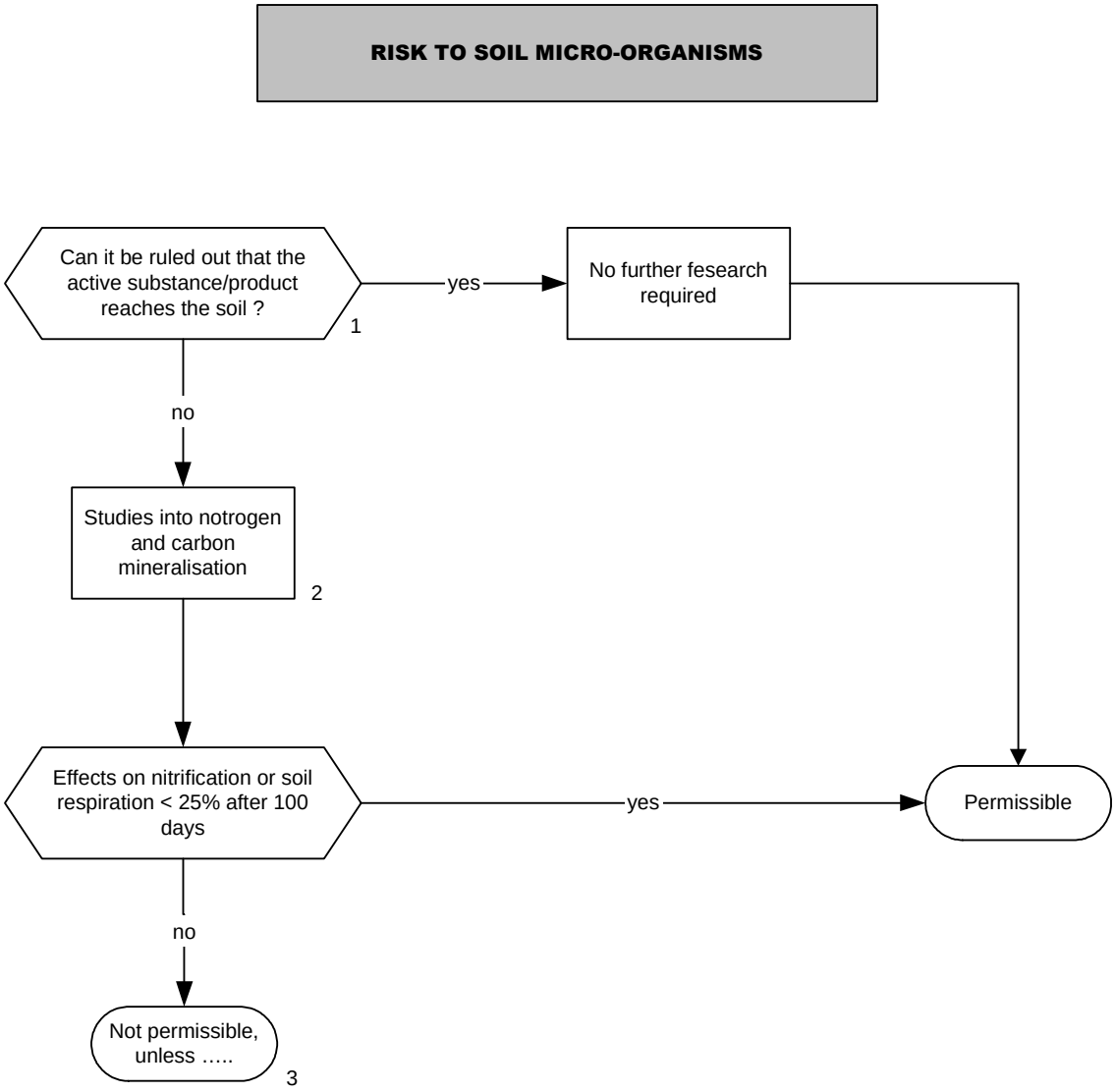
Appendix 2 Explanatory notes decision tree risk to soil micro-organisms

1) Data about the effects on soil micro-organisms are required for each active substance unless it is demonstrated that it can be ruled out that the active substance or product reaches the soil. (see Appendix 1).

   These data are also required for metabolites formed in the laboratory study into the (an)aerobic transformation route in soil. For a general discussion about metabolites, see §1.2.3 in Chapter 7 Ecotoxicology; Terrestrial; Birds and mammals.

2) Research into the effects of the active substance on nitrogen and carbon mineralisation must be carried out in at least 1 soil type. For plant protection products with several active substances it is in principle preferred to test the toxicity of the product/the formulation. The test concentrations must correspond with (or be higher than) the maximum concentrations to be expected in the soil at the field dose. For test guidelines see OECD Guidelines 216 [11] and 217 [12]. Further information about methods is given in a SETAC document: Procedures for assessing the environmental fate and ecotoxicity or pesticides [13].

3) Where the effects on nitrification and soil respiration after 100 days exceed 25%, an adequate risk assessment may demonstrate that there is no risk in the field. There is, however, no protocol for the performance of field studies into the effects on soil micro-organisms. It is recommended to consult the CTGB prior to conducting field studies. Field studies are evaluated on the basis expert judgement. Where effects occur in the field test, recovery can be taken into account.
   The product can be authorised if total recovery occurs before the next season.


RISK TO SOIL MICRO-ORGANISMS
Can it be ruled out that the active substance/product reaches the soil ?
1
yes
No further fesearch required
no
Studies into notrogen and carbon mineralisation
2
Effects on nitrification or soil respiration < 25% after 100 days
yes
Permissible
no
Not permissible, unless …..
3

3 REFERENCES

1 Regulation (EC) No 1107/2009, http://eur-lex.europa.eu/Notice.do?checktexts=checkbox&val=504604%3Acs&pos=1&page=1&lang=en&pgs=10&nbl=1&list=504604%3Acs%2C&hwords=&action=GO&visu=%23texte
2 Directive 91/414/EEC, http://eur-lex.europa.eu/Notice.do?checktexts=checkbox&val=172911%3Acs&pos=3&page=1&lang=en&pgs=10&nbl=3&list=447073%3Acs%2C185439%3Acs%2C172911%3Acs%2C&hwords=&action=GO&visu=%23texte
3 Commission Implementing Regulation (EU) No 540/2011, http://eur-lex.europa.eu/Notice.do?checktexts=checkbox&val=574460%3Acs&pos=6&page=1&lang=en&pgs=10&nbl=6&list=646199%3Acs%2C628324%3Acs%2C615541%3Acs%2C607847%3Acs%2C607130%3Acs%2C574460%3Acs%2C&hwords=&action=GO&visu=%23texte
4 European Commission (2002). Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002 rev. 2 final - noted by the SCFA on 18 October 2002)
5 Commission Regulation (EU) No 283/2013, http://eur-lex.europa.eu/Notice.do?val=724582:cs&lang=en&list=729945:cs,724582:cs,&pos=2&page=1&nbl=2&pgs=10&hwords=
6 Commission Regulation (EU) No 284/2013, http://eur-lex.europa.eu/Notice.do?val=724566:cs&lang=en&list=729902:cs,724566:cs,&pos=2&page=1&nbl=2&pgs=10&hwords=
7 Commission Communication 2013/C 95/01 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2013:095:0001:0020:EN:PDF
8 Commission Communication 2013/C 95/02 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2013:095:0021:0037:EN:PDF
9 Commission Regulation (EU) No 546/2011, http://eur-lex.europa.eu/Notice.do?checktexts=checkbox&val=574598%3Acs&pos=2&page=1&lang=en&pgs=10&nbl=2&list=607713%3Acs%2C574598%3Acs%2C&hwords=&action=GO&visu=%23texte
10 Commission Regulation (EU) No 546/2011, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:155:0127:0175:EN:PDF
11 OECD 216 Soil Microorganisms, Nitrogen Transformation Test *(Original Guideline, adopted 21st January 2000)*
12 OECD 217 Soil Microorganisms, Carbon Transformation Test *(Original Guideline, adopted 21st January 2000)*
13 SETAC (1995) Procedures for assessing the environmental fate and ecotoxicity of pesticides (ISBN 90-5607-002-9).