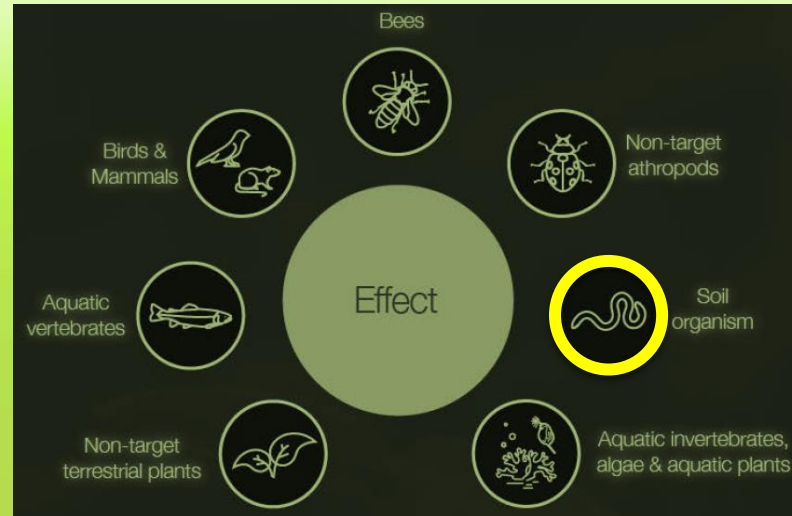


Risk assessment on soil organisms in the EU



Edition date:
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Realisation:
tier3 solutions GmbH
Leverkusen

Overview



- Regulations & data requirements (EU)
- Guidance documents & guidelines
- Basic study types & related endpoints
- Correction of the endpoint
- Virtual Standard Risk Assessment Example
- Potential refinement options

Regulations (European Union)

Regulation (EC) No. 1107/2009 concerning the placing of plant protection products on the market

& corresponding regulations:

- Regulation (EU) No. 283/2013
= data requirements for active substances
- Regulation (EU) No. 284/2013
= data requirements for plant protection products
- Regulation (EU) No. 546/2011
= Uniform Principles

Guidance documents / guidelines

- Guidance Document on Terrestrial Ecotoxicology, SANCO/10329/2002, rev. 2 final, 17.10.2002
- Test guidelines & guidances (OECD, ISO):
 - OECD TG 207 or ISO 11268-1 (Earthworm, acute)
 - OECD TG 222 or ISO 11268-2 (Earthworm, chronic)
 - ISO 11268-3 (Earthworm field test)
 - OECD TG 226 (*Hypoaspis aculeifer*, chronic)
 - OECD TG 232 or ISO 11267 (*Folsomia candida*, chronic)
 - OECD TG 216 (Nitrogen transformation)
 - OECD TG 217 (Carbon transformation)
 - OECD No. 56 (Litter bag)
- Semi-Field Methods for the Environmental Risk assessment of Pesticides in Soil (Schaeffer et al., 2011)
- Kula et al. (2006), Technical recommendations for the update of the ISO Earthworm field test guideline (ISO 11268-3)
- Environmental risk assessment scheme for plant protection products. Normes OEPP/EPPO Standards. PP 3/7 (revised)
- Generic Guidance for Tier 1 FOCUS Groundwater Assessments, V2.2 (May 2014)

Future guidance/ scientific opinions

OUTLOOK...

- EFSA Scientific Opinion on soil risk assessment:

Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms (EFSA Journal 2017;15(2):4690)

- New PEC_{soil} guidance:

EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil (EFSA Journal 2017;15(10):4982)

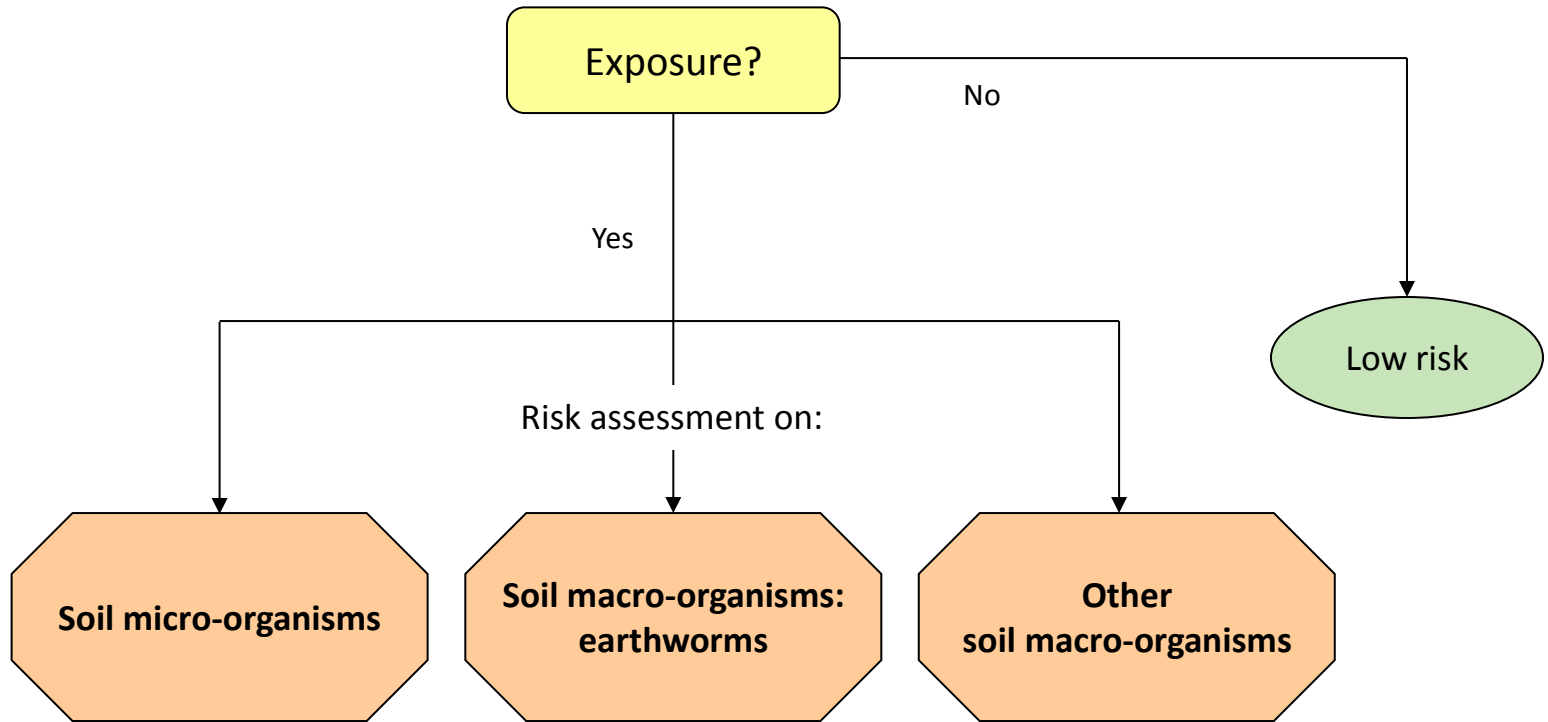
- Guidance to develop specific protection goals:

Guidance to develop specific protection goals options for ERA at EFSA, in relation to biodiversity and ecosystem services (EFSA Journal 2016;14(6):4499)

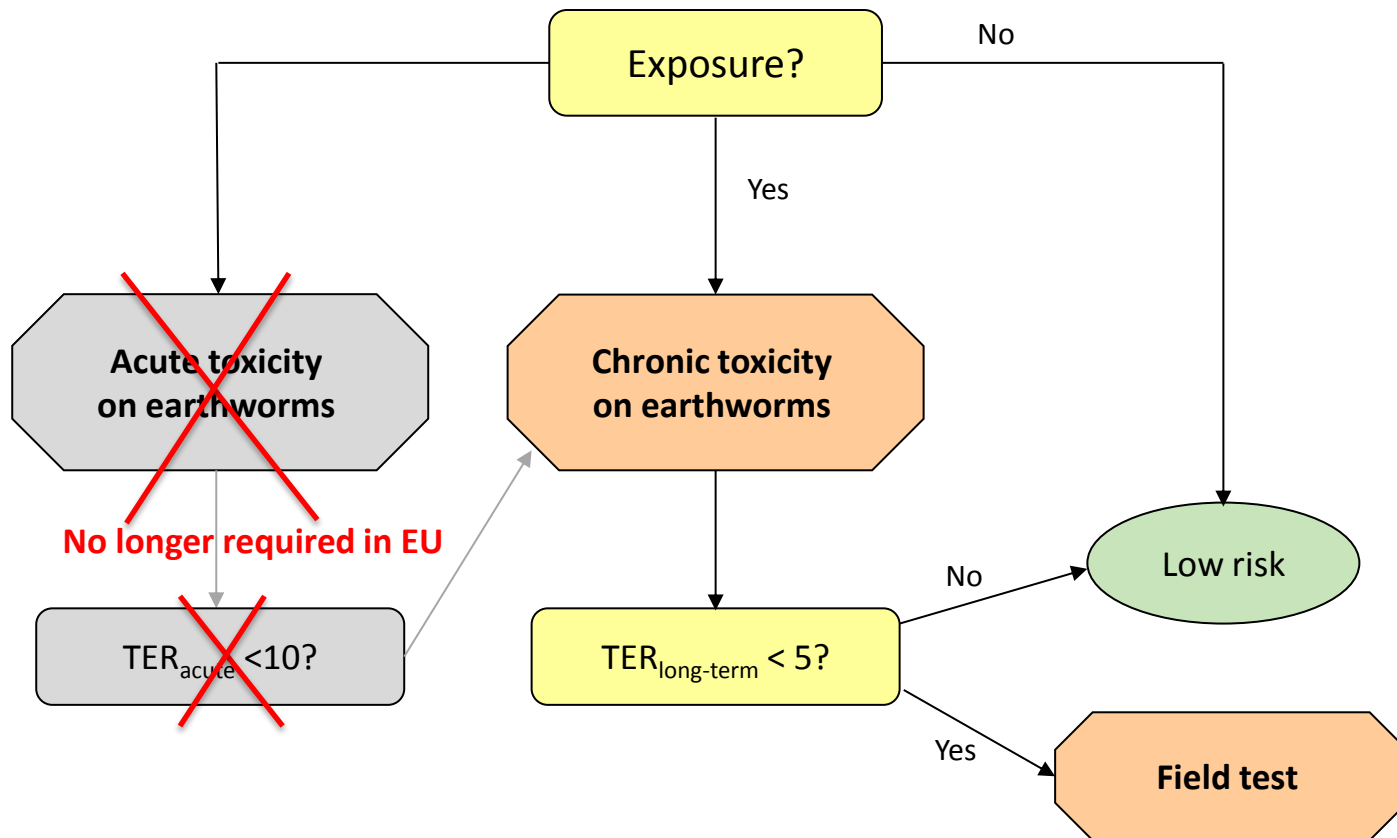
Terms & Abbreviations

Term	Explanation
a.s.	Active substance (synonymous to active ingredient (a.i.))
dws	Dry weight soil
Effect measurements	In the context of studies on soil organisms, effects are commonly measured for the following endpoints: survival, growth, reproduction, feeding activity
Effect value	Dependent from study design & underlying guideline, effect values (often referred to as 'endpoints' have different names (abbreviations) as they signify different effect levels that have been measured or calculated. Examples: EC ₁₀ , NOEC etc.
EC ₁₀	10% effect concentration; concentration causing 10% effect compared to control
LC ₅₀	Lethal concentration; concentration causing 50% mortality compared to control
log P _{ow}	Octanol-Water Partition Coefficient (synonymous to log K _{ow})
NOEC	No Observed Effect Concentration
OECD	Organisation for Economic Co-operation and Development
PECsoil	Predicted Environmental Concentration in soil (calculated by environmental modeling)
PPP	Plant protection product
prod.	product
RA	Risk Assessment
TER _{lt}	TER long-term; Toxicity to Exposure Ratio (long-term)

General risk assessment scheme

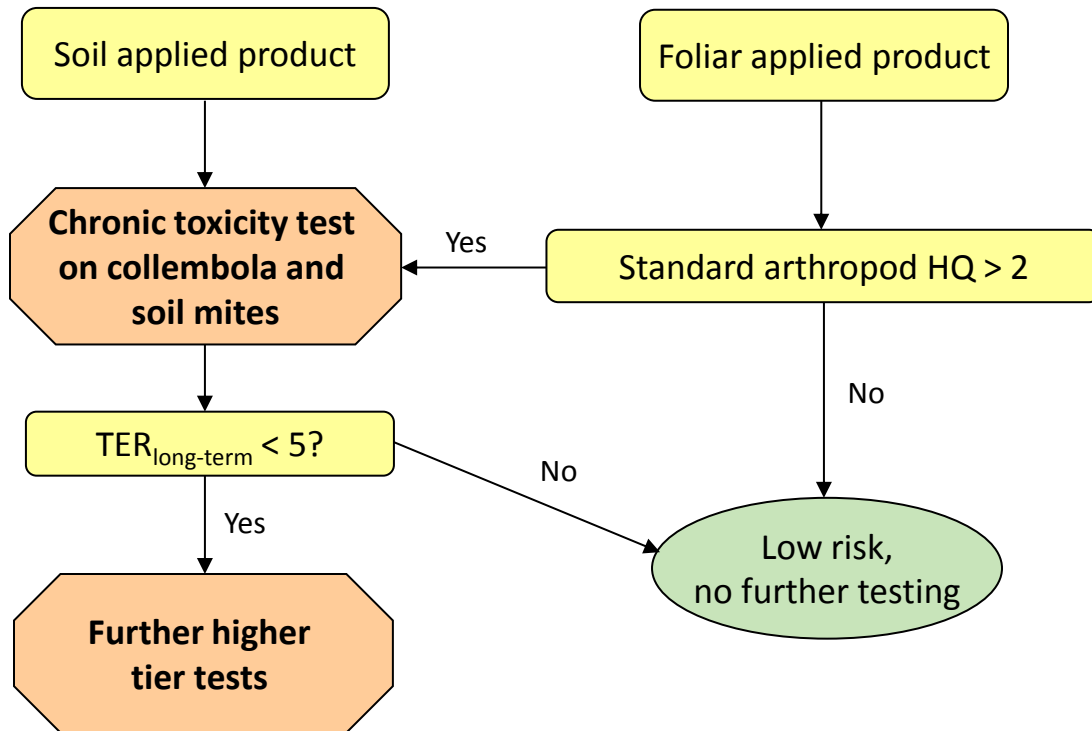


RA scheme - Earthworms

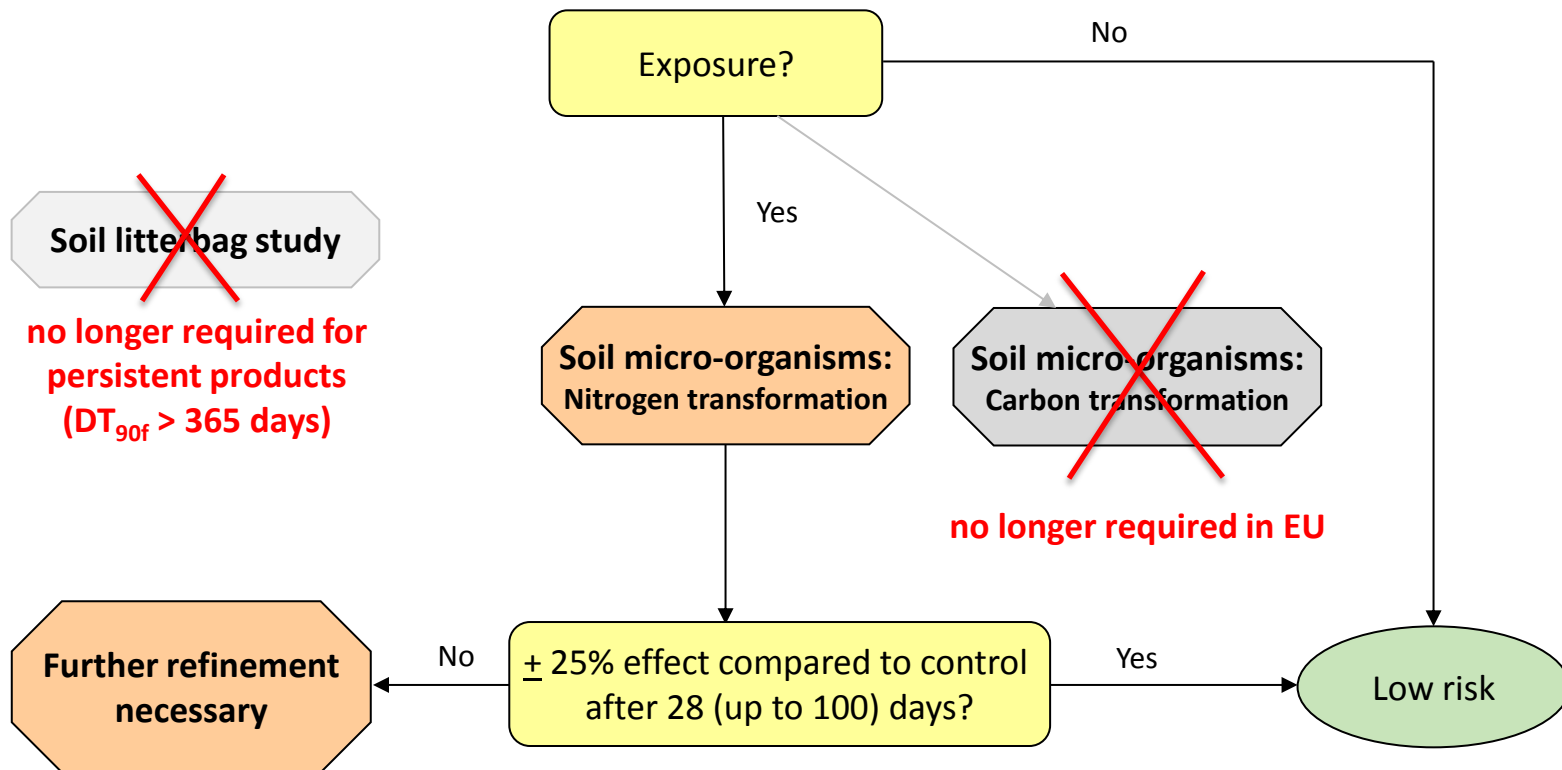


RA scheme – Other soil macro-organisms

Formulations containing two or more active substances also need to be tested



RA scheme – Soil micro-organisms



Test species

Earthworms:

- *Eisenia fetida*

Other soil macro-organisms:

- *Hypoaspis aculeifer* (soil mite)
- *Folsomia candida* (Collembola)

Study types – tier 1

- *Acute toxicity, Earthworms* → No EU data requirement
(OECD TG 207)
- Chronic toxicity, Earthworms (*Eisenia fetida*)
(OECD TG 222, ISO 11268-2)
- Chronic toxicity, Collembola (*Folsomia candida*)
(OECD TG 232, ISO 11267)
- Chronic toxicity, Soil mites (*Hypoaspis aculeifer*)
(OECD TG 226)
- Nitrogen transformation
(OECD TG 216)
- *Carbon transformation* → No EU data requirement
(OECD TG 217)

Study types – higher tier

Laboratory tests:

- More realistic exposure
e.g. natural soil, seed treatment, spray application
- 2-generation, aged residues (Collembola)
(OECD TG 232 modified; Ernst *et al.*, 2016)

Semi-field or field tests:

- Earthworm field study (population level)
(ISO 11268-3, Kula *et al.*, 2006)
- Soil micro-arthropod semi-field (TME) or field study (population & community level)
(Schaeffer *et al.*, 2011; Kula *et al.*, 2006)

Earthworms, chronic

OECD TG 222, ISO 11268-2

- Test species: *Eisenia fetida*
- Test item mixed into artificial soil
- Dose-response test or limit test
 - (max. dose: 1000 mg test item/kg dry weight soil)
- Test duration: 56 days
- Assessments: Mortality, reproduction, growth and other sub-lethal effects (feeding activity)



Endpoint: NOEC / EC₁₀ [mg a.s./kg dws]

Collembola

OECD TG 232, ISO 11267

- Test species: *Folsomia candida*
- Test item mixed into artificial soil
- Dose-response test or limit test
 - (max. dose: 1000 mg test item/kg dry weight soil)
- Test duration: 28 days
- Assessments: Mortality, reproduction



Endpoint: NOEC / EC₁₀ [mg a.s./kg dws]

Soil mite

OECD TG 226

- Test species: *Hypoaspis aculeifer*
- Test item mixed into artificial soil
- Dose-response test or limit test
 - (max. dose: 1000 mg test item/kg dry weight soil)
- Test duration: 14 days
- Assessments: Mortality, reproduction

Endpoint: NOEC / EC₁₀ [mg a.s./kg dws]

Nitrogen transformation

OECD TG 216

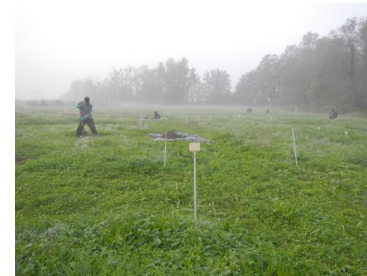
- Tier 1 test (laboratory)
- Natural soil is amended with powdered plant meal and treated with the substance to be tested
- Usually two test concentrations (maximum PEC and 2-10 × maximum PEC)
- Comparison of nitrate formation rate to untreated control after 28 days (prolongation up to 100 days possible)

**Endpoint: Max. concentration with effects ≤ 25 %
[mg a.s./kg dws]**

Higher tier studies

ISO 11268-3, Kula *et al.* (2006), special design

- Earthworm field study
- Application of test substance according to realistic use pattern (i.e. formulated product)
- Monitoring of earthworm population for a period of 1 year
- Population level including single species evaluation



Higher tier studies

Schaeffer *et al* (2011), Kula *et al.* (2006), special design

- Soil micro-arthropod semi-field (TME) or field study
- Application of test substance according to realistic use pattern
- Monitoring of soil micro-arthropod population for a period of 1 year
- Population & community level including single species evaluation



Correction of endpoint

- If $\log P_{ow}^* > 2$,
correct the endpoint by a factor of 2 for
E. fetida, *F. candida* and *H. aculeifer* tested in artificial soil

NOEC = 1000 mg a.s./kg dws

NOEC_{corr} = 500 mg a.s./kg dws*

* Endpoint corrected by factor of 2 for $\log P_{ow} > 2$

- Using endpoints of studies performed with artificial soil containing 5% peat without correction is no longer accepted by most of the European authorities (PRAPeR** decision, 2012)

* $\log P_{ow}$ = octanol-water Partition Coefficient (Ratio of the solubility of a chemical in octanol to its solubility in water at equilibrium)

** PRAPeR = Pesticide Risk Assessment Peer Review Unit

Metabolites

Major metabolites

- All metabolites formed in amounts of $> 10\%$ of the applied amount of active substance at any time point or two times $> 5\%$ evaluated during the degradation studies, or 5% and increasing.

Minor metabolites

- All metabolites and reaction products that are formed in amounts of $< 5\%$ of the applied amount of active substance at any time during the degradation studies.

→ Major metabolites will be addressed in the risk assessment

Risk Assessment - Example

The following example is based on a virtual product containing two virtual active substances (A and B) and a virtual intended use pattern.

Formulated product	A+B SC 300 (200 g A/L + 100 g B/L)
Intended use pattern	2 x 1.0 L prod./ha, cereals (BBCH 30-69), 14 days interval between the 2 applications
Application rate (active substance A)	2 x 200 g a.s./ha
Application rate (active substance B)	2 x 100 g a.s./ha
Product density	0.980 g/mL

Virtual endpoints (a.s. A)

Endpoints of active substance A and its metabolite(s) for earthworms and other non-target soil macro-organisms

Species	Substance	Exposure System	Ecotoxicological endpoints	Reference
<i>Eisenia fetida</i>	A	Mixed into substrate / 56 d, chronic 10% peat content	NOEC _{repro} = 85.5 mg a.s./kg dws NOEC _{corr} = 42.75 mg a.s./kg dws ^{&}	SANCO/0815/99-Final (2003)
<i>Eisenia fetida</i>	M1-A (metabolite of A)	Mixed into substrate / 56 d, chronic 10% peat content	NOEC _{repro} = 14 mg p.m./kg dws	SANCO/0815/99-Final (2003)
<i>Folsomia candida</i>	A	Mixed into substrate / 28 d, chronic 5% peat content	NOEC = 78 mg a.s./kg dws NOEC _{corr} = 39 mg a.s./kg dws ^{&}	SANCO/0815/99-Final (2003)
<i>Folsomia candida</i>	M1-A (metabolite of A)	Mixed into substrate / 28 d, chronic 5% peat content	NOEC = 120 mg p.m./kg dws	SANCO/0815/99-Final (2003)

a.s. = active substance; p.m. = pure metabolite; dws = dry weight soil

[&] Corrected by factor of 2 for logPow >2

Remark: All values (i.e. endpoints and references) are virtual values

Virtual endpoints (a.s. B)

Endpoints of active substance B and its metabolite(s) for earthworms and other non-target soil macro-organisms

Species	Substance	Exposure System	Ecotoxicological endpoints	Reference
<i>Eisenia fetida</i>	B	Mixed into substrate / 56 d, chronic 10% peat content	NOEC _{repro} = 102 mg a.s./kg dws	EFSA Scientific Report 1001 (2009)
<i>Eisenia fetida</i>	M1-B (metabolite of B)	Mixed into substrate / 56 d, chronic 10% peat content	NOEC _{repro} > 1000 mg p.m./kg dws	EFSA Scientific Report 1001 (2009)
<i>Folsomia candida</i>	B	Mixed into substrate / 28 d, chronic 5% peat content	NOEC = 72 mg a.s./kg dws	EFSA Scientific Report 1001 (2009)

a.s. = active substance; p.m. = pure metabolite; dws = dry weight soil

Remark: All values (i.e. endpoints and references) are virtual

Virtual endpoints (formulated product)

Endpoints of A+B SC 300* for earthworms and other non-target soil macro-organisms

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	A+B SC 300	Mixed into substrate / 56 d, chronic 10% peat content	NOEC _{repro} = 56 mg prod./kg dws NOEC _{corr} = 28 mg prod./kg dws ^{&}	Appendix 2 Testbert, 2008
<i>Folsomia candida</i>	A+B SC 300	Mixed into substrate / 28 d, chronic 5% peat content	NOEC = 171.5 mg prod./kg dws NOEC _{corr} = 85.75 mg prod./kg dws ^{&}	Appendix 2 Testbert, 2007
<i>Hypoaspis aculeifer</i>	A+B SC 300	Mixed into substrate / 14 d, chronic 5% peat content	NOEC = 100 mg prod./kg dws NOEC _{corr} = 50 mg prod./kg dws ^{&}	Appendix 2 Testbert, 2008

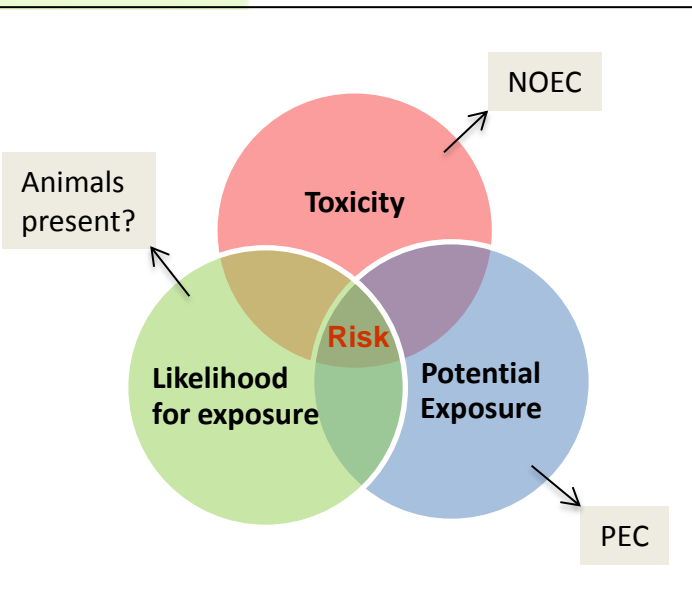
prod. = formulated product; dws = dry weight soil

[&] Corrected by factor of 2 for logPow >2

Remark: All values (i.e. endpoints and references) are virtual

* ,A+B SC 300' = virtual product name

General principles – Toxicity / Exposure



TER = Toxicity to Exposure Ratio

where

Toxicity → Endpoint value from a study (i.e. NOEC, EC₁₀)

Exposure → PEC_{soil} - Predicted Environmental Concentration in soil

$$\text{TER} = \frac{\text{toxicity value (NOEC)}}{\text{exposure (PEC}_{\text{soil}}\text{)}}$$

→ Low risk to soil organisms is indicated if $\text{TER} \geq 5$

(see Uniform Principles as laid down in Reg. (EU) No 546/2011 and verified by Christl *et al.* 2016)

Soil organisms – TER calculation

- TER = Toxicity to Exposure Ratio:

$$\text{TER}_{\text{LT}} = \text{NOEC or EC}_{10} / \text{PEC}_{\text{soil, max}}$$

- PEC_{soil} is calculated & provided by environmental modeling

- *Amount of a.s. reaching the soil (considering interception) is determined*
- *Initial concentration in soil is determined*
- *Degradation is calculated using SFO* kinetics*
- *Accumulation in soil is considered for substances with $\text{DT}_{50} > 90$ days*

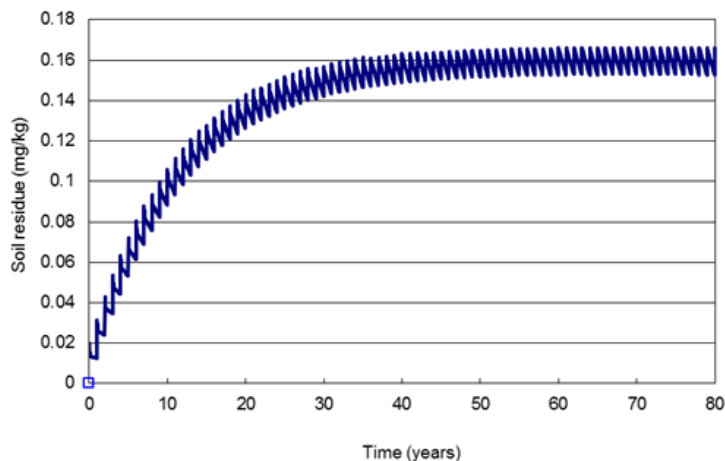
* SFO kinetics = Single First Order kinetics

Soil organisms – PECsoil, accu

If $DT_{50} > 90$ days, accumulation in soil needs to be considered:

Repeated annual applications over a 20 year period are simulated leading to a plateau concentration.

For annual crops, a plateau of the top 20 cm is simulated assuming regular tillage (for perennial crops: top 5 cm, without tillage).



Soil organisms – TER calculation example

First-tier assessment of the chronic risk for earthworms due to the use of A+B SC 300 in cereals

Chronic effects on earthworms			
Product / active substance/ metabolite	NOEC [mg/kg]	PEC _{soil} [mg/kg]	TER _{lt} (criterion TER ≥ 5)
Active substance A	42.75 ^{&}	0.175	244
M1-A	14	0.008	1750
Active substance B	102	0.48	212
M1-B	> 1000	0.24	> 4167
A+B SC 300	28 ^{&}	0.523 [*]	54

 PEC_{soil} values taken from PEC reports

[&] Endpoint corrected for logPow >2

^{*} PEC_{soil formulation} = calculated based on application rate (2 × 1.0 L prod./ha), density = 0.980 g/mL, 80% interception (for BBCH >30), soil bulk density of 1.5 g/mL, and a soil layer of 0-5 cm

TER_{lt} > 5
→ acceptable risk

Soil organisms – TER calculation example

First-tier assessment of the chronic risk for other non-target soil organisms due to the use of A+B 300 SC in cereals

Chronic effects on other soil macro- and mesofauna			
Product / active substance / metabolite	NOEC [mg/kg]	PEC _{soil} [mg/kg]	TER _{lt} (criterion TER ≥ 5)
Active substance A (<i>Folsomia candida</i>)	39 ^{&}	0.96	41
M1-A (<i>Folsomia candida</i>)	120	0.1836	654
Active substance B (<i>Folsomia candida</i>)	72	0.48	150
A+B SC 300 (<i>Folsomia candida</i>)	85.75 ^{&}	0.523 [*]	164
A+B SC 300 (<i>Hypoaspis aculeifer</i>)	50 ^{&}	0.523 [*]	96

PEC_{soil} values taken from PEC report

[&] Endpoint corrected for logPow >2

^{*} PEC_{soil formulation} = calculated based on application rate (2 × 1.0 L prod./ha), density = 0.980 g/mL and 80% interception (for BBCH >30), soil bulk density of 1.5 g/mL, and a soil layer of 0-5 cm

TER_{lt} > 5
→ acceptable risk

Virtual endpoints soil micro-organisms

Endpoints and effect values relevant for the risk assessment for soil micro-organisms

Endpoint	Substance	Exposure System	Ecotoxicological endpoint	Reference
N-mineralisation	A	28 d, aerobic	No unacceptable effects on N-transformation at 4.8 mg a.s./kg dws	SANCO/0815/99-Final (2003)
N-mineralisation	B	28 d, aerobic sandy clay loam	No unacceptable effects on N-transformation at 1.8 mg prod./kg dws	EFSA Scientific Report 1001 (2009)
N-mineralisation	A+B SC 300	28 d, aerobic sandy clay loam	No unacceptable effects on N-transformation at 9.4 mg a.s./kg dws	Appendix 2 Testbert, 2013

a.s. = active substance; prod. = formulated product; dws = dry weight soil

Remark: All values (i.e. endpoints and references) are virtual values

Soil micro-organisms – Risk Assessment example

Max. concentration with effects $\leq 25\%$ is compared to max. PEC_{soil}

Assessment of the risk for effects on soil micro-organisms due to the use of A+B SC 300 in cereals

Intended use	Cereals, BBCH 30-69		
N-mineralisation			
Product / active substance	Max. concentration with effects ≤ 25 % [mg/kg]	PEC _{soil} [mg/kg]	Risk acceptable? (Yes, if PEC _{soil} < max conc. without effect >25%)
Active substance A	4.8	0.96	Yes
Active substance B	1.8	0.48	Yes
A+B SC 300	9.4	0.523*	Yes

PEC_{soil} values taken from PEC report

* PEC_{soil} formulation = calculated separately in Excel

PEC_{soil} < max conc. with effects $\leq 25\%$
→ acceptable risk

Cited publications

Ernst *et al.* (2016), Assessing the potential for intrinsic recovery in a Collembola two-generation study: possible implementation in a tiered soil risk assessment approach for plant protection products. *Ecotoxicology* 25 (1), 1–14.

Christl *et al.* (2016), Recalibration of the earthworm tier 1 risk assessment of plant protection products. *Integrated Environmental Assessment and Management* 12 (4), 643-650.

Conditions for product submission and approval

- The applicant only submits a dossier for registration of a plant protection product, when Environmental Risk Assessment (ERA) showed acceptable risk for all assessment areas
 - Authorities review the submitted dossier (containing study reports, evaluation and risk assessments + any further required data)
 - Authorities grant registration/approval only if they agree on an acceptable risk for all assessment areas
- Special mandatory conditions for use might apply (i.e. risk mitigation measures) which are printed on the label of the plant protection product