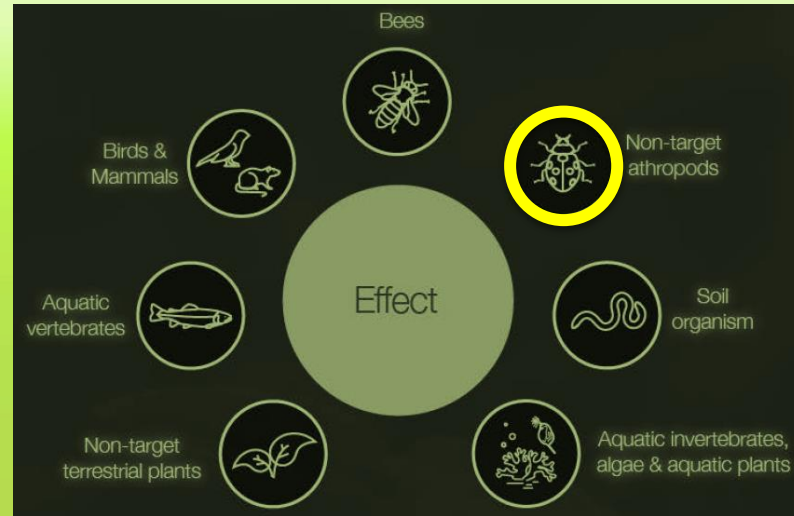


Risk assessment on non-target arthropods in the EU



Edition date:
June 2018

Realisation:
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Overview



- Data requirements (European Union)
- Guidance documents
- Basic study types & related endpoints
- Higher tier studies
- 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 / Scientific Opinion

- Guidance Document on Terrestrial Ecotoxicology, SANCO/10329/2002, rev. 2 final, 17.10.2002
- Guideline to evaluate side-effects of plant protection products to non-target arthropods (Candolfi *et al.*, 2000)
- Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi *et al.*, 2001)
- ESCORT 3: Linking Non-Target Arthropod Testing and Risk Assessment with Protection Goals (CRC SETAC Press, 1–151, 2010)
- Scientific opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods (EFSA Journal 2015;13(2):3996)

Terms & Abbreviations

Term	Explanation
a.s.	Active substance (synonymous to active ingredient)
Effect measurements	In the context of NTA studies , effects are commonly measured for the following endpoints: mortality, reproduction (e.g. number of eggs), repellency etc.
Effect value	Dependent from study design & underlying guideline, effect values (often also referred to as 'endpoints') have different names (abbreviations) as they signify different effect levels that have been measured or calculated. Examples: ER ₅₀ , LR ₅₀ etc.
ER ₅₀	Effect rate at which the tested species show an effect at the 50% level
HQ	Hazard quotient
LR ₅₀	Lethal rate at which 50% of tested species are dead
MAF	Multiple Application Factor (assuming degradation of the substance between the applications)
NOEAER	No Observed Ecologically Adverse Effect Rate
NTA	Non-target arthropods
PPP	Plant Protection Product
prod.	product

Data Requirements - active substances

8.3. Effect on arthropods

8.3.2. *Effects on non-target arthropods other than bees*

Circumstances in which required

Effects on non-target terrestrial arthropods shall be investigated for all active substances except where plant protection products containing the active substance are for exclusive use in situations where non-target arthropods are not exposed such as:

- food storage in enclosed spaces that preclude exposure,
- wound sealing and healing treatments,
- enclosed spaces with rodenticidal baits.

Two indicator species, the cereal aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) and the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) shall always be tested. Initial testing shall be performed using glass plates and mortality (and reproduction effects if assessed) shall be reported. Testing shall determine a rate-response relationship and LR₅₀ ⁽¹⁾, ER₅₀ ⁽²⁾ and NOEC endpoints shall be reported for assessment of the risk to these species in accordance with the relevant risk quotient analysis. If adverse effects can be clearly predicted from these studies then testing using higher tier studies may be required (see point 10.3 of Part A of the Annex to the Regulation (EU) No 284/2013 for further details).

With active substances suspected of having a special mode of action (such as insect growth regulators, insect feeding inhibitors) additional tests involving sensitive life stages, special routes of uptake or other modifications, may be required by the national competent authorities. The rationale for the choice of test species used shall be provided.

Data Requirements - active substances

8.3.2.1. Effects on *Aphidius rhopalosiphi*

A test shall provide sufficient information to evaluate the toxicity in terms of LR₅₀ and NOEC of the active substance to *Aphidius rhopalosiphi*.

Test conditions

Initial testing shall be performed using glass plates.

8.3.2.2. Effects on *Typhlodromus pyri*

A test shall provide sufficient information to evaluate the toxicity in terms of LR₅₀ and NOEC of the active substance to *Typhlodromus pyri*.

Test conditions

Initial testing shall be performed using glass plates.

Source: Regulation (EU) No. 283/2013

According to Regulation (EU) No. 283/2013, testing on NTA should be conducted with the formulated plant protection product.

Data Requirements - plant protection products

10.3. Effects on arthropods

10.3.2. Effects on non-target arthropods other than bees

Circumstances in which required

Effects on non-target terrestrial arthropods shall be investigated for all plant protection products except where plant protection products containing the active substance are for exclusive use in situations where non-target arthropods 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 the requirements set out in point 8.3.2 of Part A of the Annex to Regulation (EU) No 283/2013.

For plant protection products, two indicator species, the cereal aphid parasitoid *Aphidius rhopalosiphii* (Hymenoptera: Braconidae) and the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) shall be tested. Initial testing shall be performed using glass plates, and both mortality and effects on reproduction (if assessed) shall be reported. Testing shall determine a rate-response relationship and LR₅₀⁽¹⁾, ER₅₀⁽²⁾ and NOEC endpoints shall be reported for assessment of the risk to these species in accordance with the relevant risk quotient analysis.

For a plant protection product containing an active substance suspected of having a special mode of action (for example insect growth regulators, insect feeding inhibitors) additional tests involving sensitive life stages, special routes of uptake or other modifications, may be required. The rationale for the choice of test species used shall be provided.

⁽¹⁾ LR₅₀: abbreviation for 'Lethal Rate, 50 %', that is to say the application rate required to kill half the members of a tested population after a specified test duration.

⁽²⁾ ER₅₀: abbreviation for 'Effect Rate, 50 %', that is to say the application rate required to cause an effect on half the members of a tested population after a specified test duration.

Data Requirements - plant protection products

10.3.2.1. Standard laboratory testing for non-target arthropods

The test shall provide sufficient information to evaluate the toxicity of the plant protection product to the two indicator species (*Aphidius rhopalosiphi* (Hymenoptera: Braconidae) and *Typhlodromus pyri*) (Acari: Phytoseiidae) in accordance with the relevant risk quotient analysis.

Where adverse effects are indicated, testing using higher tier studies shall be required (see points 10.3.2.2 to 10.3.2.5) for further details. In higher tier assessment the risk quotient analysis used for standard laboratory non-target arthropod testing is not appropriate.

For test methods, see ESCORT 2 and Candolfi et al., 2000 (IOBC methods 2000)

10.3.2.2. Extended laboratory testing, aged residue studies with non-target arthropods

The tests shall provide sufficient information to evaluate the risk of the plant protection product for arthropods using a more realistic test substrate or exposure regime.

Circumstances in which required

Further testing shall be required where effects are seen following laboratory testing in accordance with the requirements set out in point 10.3.2.1 and where the relevant risk quotient analysis indicates a risk to the standard indicator non-target arthropod species.

Firstly, the indicator species affected in standard Tier 1 laboratory testing (point 10.3.2.1) shall be tested. In addition, where an in-field risk is indicated to one or both standard indicator species, testing of one additional species shall be required. Where an off-field risk to the standard indicator species is indicated, testing of one further additional species shall be required.

An aged residue study shall be conducted with the most sensitive species to give information on the time scale needed for potential re-colonisation of treated in-field areas.

Test conditions

(a) Extended laboratory studies

Extended laboratory studies shall be carried out under controlled environmental conditions, by exposing laboratory-reared test organisms, or field collected specimens, to fresh and dried pesticide deposits applied to natural substrates, for example leaves, plants or natural soil under laboratory or field conditions.

(b) Aged residue studies

Aged residue studies shall assess the duration of effects on in-field non-target arthropods. They shall involve ageing of plant protection product deposits under field conditions (use of rain protection may be advisable), with exposure of the test organisms on treated leaves or plants either in the laboratory, under semi-field conditions or a combination of both (such as mortality assessment under semi-field conditions and reproduction assessment under laboratory conditions).

Data Requirements - plant protection products

10.3.2.3. Semi-field studies with non-target arthropods

The tests shall provide sufficient information to evaluate the risk of the plant protection product for arthropods taking field conditions into account.

Circumstances in which required

Where effects are seen following laboratory testing in accordance with the requirements set out in point 8.3.2 of Part A of the Annex to Regulation (EU) No 283/2013 or point 10.3.2 of this Annex (for example relevant trigger values are breached), semi-field testing shall be required.

Test conditions

The tests shall be conducted under representative agricultural conditions and in accordance with the proposed recommendations for use, resulting in a realistic worst case study.

In semi-field testing the results from lower tier testing as well as the specific questions to be addressed shall be taken into account. In the selection of species for semi-field testing, the results from lower tier testing as well as the specific questions to be addressed shall be taken into account.

Testing shall include lethal and sub-lethal endpoints (for example integrated parameters in field studies), but such endpoints shall be interpreted with care since they are subject to high variability.

Source: Regulation (EU) No. 284/2013

Data Requirements - plant protection products

10.3.2.4. Field studies with non-target arthropods

The tests shall provide sufficient information to evaluate the risk of the plant protection product for arthropods taking field conditions into account.

Circumstances in which required

Where effects are seen following testing in accordance with the requirements set out in point 8.3.2 of Part A of the Annex to Regulation (EU) No 283/2013 or in accordance with points 10.3.2.2 or 10.3.2.3 of this Annex, and where the relevant risk quotient analysis indicates a risk to non-target arthropods, field testing shall be required.

Test conditions

The tests shall be conducted under representative agricultural conditions and in accordance with the proposed recommendations for use, resulting in a realistic worst case study.

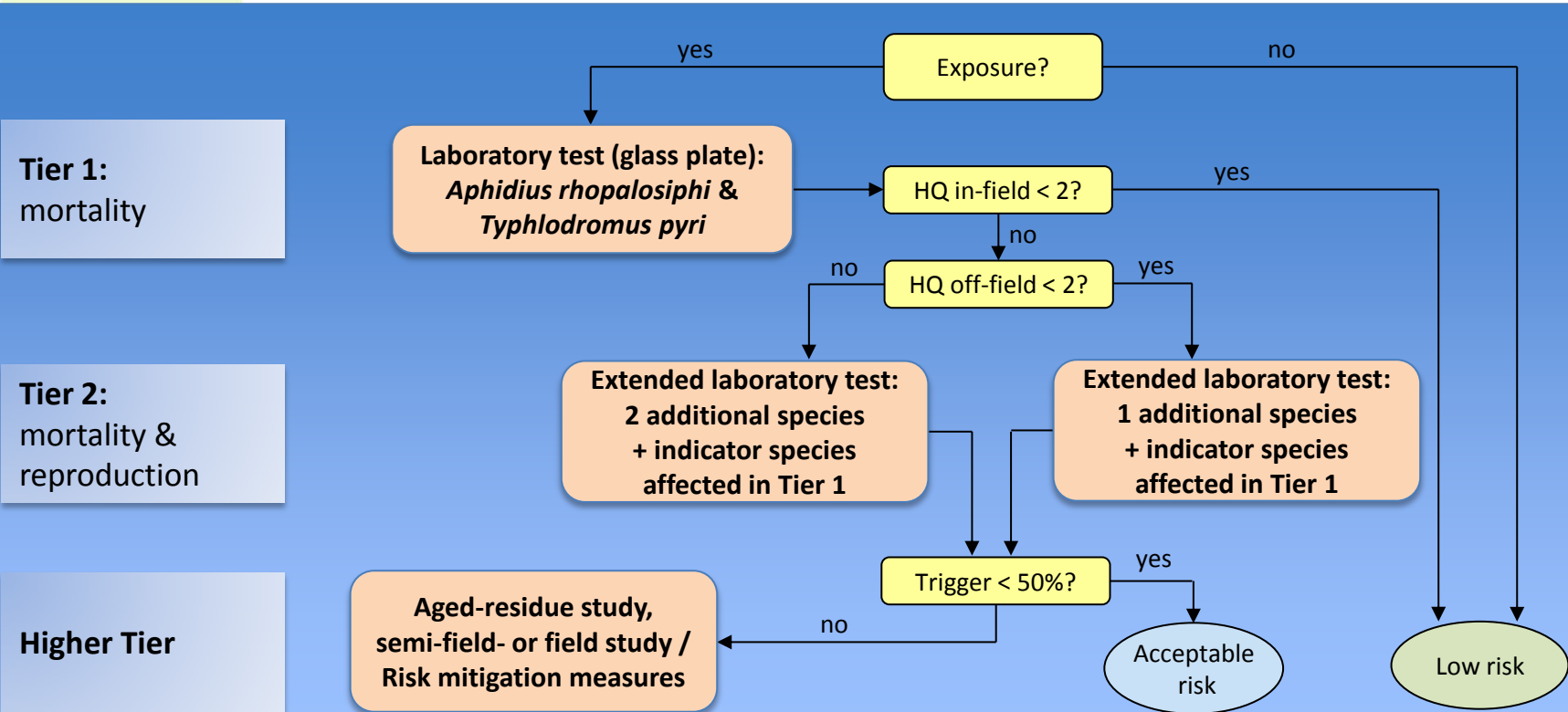
Field trials shall allow the determination of short- and long-term effects on naturally occurring arthropod populations of a plant protection product following application in accordance with the proposed use pattern for the plant protection product under normal agricultural conditions.

10.3.2.5. Other routes of exposure for non-target arthropods

Where for particular arthropods (such as pollinators and herbivores) testing conducted in accordance with points 10.3.1 and 10.3.2.1 to 10.3.2.4 is not appropriate, additional specific testing shall be required, where there are indications that exposure by routes other than by contact occur (for example plant protection products containing active substances with systemic activity). Before undertaking such testing, the proposed design to be used shall be discussed with the relevant competent authorities.

Source: Regulation (EU) No. 284/2013

Risk assessment scheme



Study types

Tier 1 (laboratory studies):

- *Aphidius rhopalosiphi* and *Typhlodromus pyri* (indicator species) on glass plates

Tier 2 (extended laboratory studies):

- Extended laboratory studies on natural substrate
- Additional test species on natural substrate

Tier 3 (aged-residue, semi-field or field studies):

- Aged residue study
- Semi-field trial
- Field trial (in-field or off-field full fauna)

Test species



- *Aphidius rhopalosiphi*, parasitic wasp
- *Typhlodromus pyri*, predatory mite
- *Chrysoperla carnea*, lacewing
- *Coccinella septempunctata*, ladybird beetle
- *Orius laevigatus*, flower bug
- *Aleochara bilineata*, rove beetle

Tier 1 studies

Aphidius rhopalosiphi



- Test design: Dose-response or limit test
- Conditions: Worst-case laboratory study on glass plates
- Treatment groups: test item, control, toxic reference
- Replicates: 4 replicates á 10 adults per treatment
- Assessments: mortality

Endpoint: LR_{50} [L product/ha]

Tier 1 studies

Typhlodromus pyri



- Test design: Dose-response or limit test
- Conditions: Worst-case laboratory study on glass plates
- Treatment groups: test item, control, toxic reference
- Replicates: 5 replicates á 20 protonymphs per treatment
- Assessments: mortality after 7 days

Endpoint: LR_{50} [L product/ha]

Tier 2 studies

Extended laboratory study:

- Test design: Dose-response test
- Exposure to pesticide residues applied to natural substrates (e.g. leaves, plants or natural soil)
- Treatment groups: test item, control, toxic reference
- Assessments: mortality and reproduction

Endpoints: LR_{50} , ER_{50} [L product/ha]

Tier 2 studies

Aged residue study:

- The plant protection product is applied to plants and residues are aged for a range of time periods (i.e. 7, 14, 28, ... d) under semi-field conditions (e.g. with rain protection for several weeks)
- A bioassay is started at the end of each aging period
- Each bioassay is equivalent to an extended laboratory study with a single test rate
- Additional bioassays are conducted until 2 subsequent bioassays result in effects (on mortality & reproduction) below 50%
- Endpoint: Required aging period until effects drop below 50%



Higher tier studies

Semi-field study

- Single species test
- Application of the test item to plants or crops under field conditions
- Treated plants (crops) are covered with an enclosure or cage
- Test organism is introduced into the test system
- Rain protection
- Assessment is based on mortality and reproduction (or integrated effect endpoints)

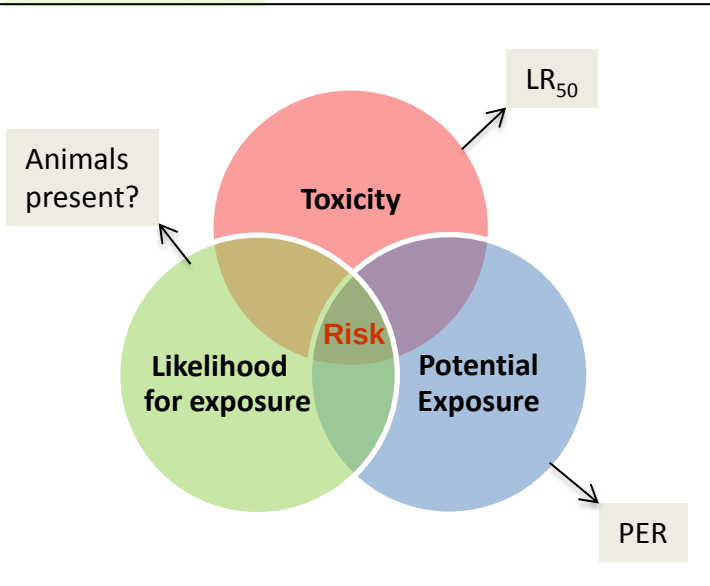
Higher tier studies

Field study

- Application of the test item under realistic agricultural conditions
- In-field or off-field full fauna study
- Naturally occurring non-target arthropod community and populations are assessed
- Duration: up to 1 year with multiple assessment time points
- Endpoint: effects and recovery on community & population level



General principles (Tier 1) - Hazard quotient



HQ - Hazard Quotient

where

Toxicity → Endpoint value from a study (i.e. LR₅₀)

Exposure → PER - Predicted Environmental Rate

$$HQ_{\text{in-field}} = \frac{PER_{\text{in-field}}}{LR_{50}}$$

$$HQ_{\text{off-field}} = \frac{\text{corr. PER}_{\text{off-field}}}{LR_{50}}$$

→ Low risk to non-target arthropods is indicated if $\leq 50\%$ effect

Virtual endpoints

Tier 1 studies

Endpoints of Tier 1 laboratory studies (example)

Species	Test item	Exposure System	Results	Reference
<i>Typhlodromus pyri</i>	A+B SC 300	Laboratory test glass plates (2D)	LR ₅₀ = 3000 mL product/ha	Appendix 2 Testbert, 2016
<i>Aphidius rhopalosiphi</i>	A+B SC 300	Laboratory test glass plates (2D)	LR ₅₀ = 750 mL product/ha	Appendix 2 Testbert, 2016

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

Risk Assessment – Tier 1, in-field

In-field RA

calculate PER (Predicted Environmental Rate)

$$\text{PER}_{\text{in-field}} [\text{mL/ha}] = \text{max. single application rate} [\text{mL/ha}] \times \text{MAF}$$

MAF (→ see Appendix V, ESCORT 2)

Risk Assessment – Tier 1, in-field

In-field RA

calculate $HQ_{in-field}$

$$HQ_{in-field} = \frac{PER_{in-field} [mL/ha]}{LR_{50} [mL/ha]}$$

Risk Assessment – Tier 1, off-field

Off-field RA

calculate $PER_{\text{off-field}}$

$$PER_{\text{off-field}} = \text{max. single application rate} \times \text{MAF} \times (\text{drift factor/VDF})$$

<u>where:</u>	tier 1 (2D*)	Higher tier (3D**)
VDF = Vegetation Distribution Factor	10	-
Drift factor (→ see Appendix VI, ESCORT 2)	dependent from crop type & stage	

* **2D** = 2-dimensional surface (i.e. glass plate or leaf disc)

** **3D** = 3-dimensional structure (i.e potted plant, seedlings)

Risk Assessment – Tier 1, off-field

Off-field RA

include correction factor

$$\text{corrected PER}_{\text{off-field}} = \text{PER}_{\text{off-field}} \times \text{CF}$$

calculate $\text{HQ}_{\text{off-field}}$

$$\text{HQ}_{\text{off-field}} = \frac{\text{corr. PER}_{\text{off-field}} [\text{mL/ha}]}{\text{LR}_{50} [\text{mL/ha}]}$$

<u>where:</u>	tier 1 (lab.)	Higher tier (extended lab.)
CF = Correction factor	10	5

Correction factor: Assessment factor to address uncertainty concerning species sensitivity

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 product/ha in cereals, 14 days interval between the 2 applications
Method	Foliar spraying

Risk assessment - MAF

Multiple Application Factor

Half-life : spray interval	MAF after n applications, where n =							
	1	2	3	4	5	6	7	8
1 : 16	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1 : 8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1 : 4	1.0	1.1	1.1	1.1	1.1	1.1	1.1	1.1
1 : 2	1.0	1.3	1.3	1.3	1.3	1.3	1.3	1.3
1 : 1	1.0	1.5	1.8	1.9	1.9	2.0	2.0	2.0
2 : 1	1.0	1.7	2.2	2.6	2.8	3.0	3.1	3.2
2.3 : 1	1.0	1.7	2.3	2.7	3.0	3.2	3.4	3.5
4 : 1	1.0	1.8	2.5	3.1	3.6	4.1	4.4	4.7
6 : 1	1.0	1.9	2.7	3.4	4.0	4.6	5.1	5.5
8 : 1	1.0	1.9	2.8	3.5	4.2	4.9	5.5	6.0
16 : 1	1.0	2.0	2.9	3.8	4.6	5.4	6.2	6.9

Leaf default

Information in ESCORT 2 (Appendix V p. 45)

(Tier 1 typically starts with default values)

Risk Assessment – Tier 1, in-field

In-field RA

calculate PER (Predicted Environmental Rate)

$$\text{PER}_{\text{in-field}} = \text{max. single application rate [mL/ha]} \times \text{MAF}$$

Example Risk Assessment:

$$\text{PER}_{\text{in-field}} = 1000 \text{ mL/ha} \times 1.7 = 1700 \text{ mL/ha}$$

Risk Assessment – Tier 1, in-field

In-field RA

calculate $HQ_{\text{in-field}}$

$$HQ_{\text{in-field}} = \frac{PER_{\text{in-field}} [\text{mL/ha}]}{LR_{50} [\text{mL/ha}]}$$

Example (*T. pyri*):

$$HQ_{\text{in-field}} = \frac{1700 [\text{mL/ha}]}{3000 [\text{mL/ha}]} = 0.57$$

Risk assessment – Tier 1, in-field

First tier in-field risk assessment for non-target arthropods due to the use of A+B SC 300 in cereals

Intended use		2 x 1000 mL product/ha in cereals (BBCH 30-69)	
Product		A+B SC 300 (virtual product)	
Application rate (mL/ha)		2 × 1000	
MAF		1.7	
Test species	LR ₅₀ (lab.)	PER _{in-field}	HQ _{in-field}
Tier I	(mL/ha)	(mL/ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	3000	1700	0.57
<i>Aphidius rhopalosiphi</i>	750		2.27

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger

HQ > 2 → higher tier testing or higher tier risk assessment is triggered

Risk assessment – off-field

Drift values

Basic drift values for **two applications** (Rautmann *et al.*, 2001) – example „field crops“

Basic drift values for two applications Ground sediment in % of the application rate (82 nd percentiles)								
Distance	Field crops	Fruit crops		Grapevine		Hops	Vegetables Ornamentals Small fruits	
[m]		early	late	early	late		Height < 50 cm	Height > 50 cm
1	2.38						2.38	
3		25.53	12.13	2.53	7.23	17.73		7.23
5	0.47	16.87	6.81	1.09	3.22	9.60	0.47	3.22
10	0.24	9.61	3.11	0.35	1.07	4.18	0.24	1.07

Information in ESCORT 2 (Appendix VI pp. 46-50)

Risk assessment – Tier 1, off-field

First tier off-field risk assessment for non-target arthropods due to the use of A+B SC 300 in cereals

Intended use		2 x 1000 mL product/ha in cereals (BBCH 30-69)			
Product		A+B SC 300 (virtual product)			
Application rate (mL/ha)		2 × 1000			
MAF		1.7			
VDF		10 (2D) / - (3D)			
Test species	LR₅₀ (lab.) (mL/ha)	Drift rate (%)	PER_{off-field} (mL/ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
Tier I					
<i>Typhlodromus pyri</i>	3000	2.38	40.46	10	0.013
<i>Aphidius rhopalosiphi</i>	750				0.054

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger

HQ ≤ 2 → acceptable off-field risk

Higher tier Risk Assessment

Virtual endpoints of extended laboratory studies (example)

Species	Test item	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i>	A+B SC 300	Extended lab, potted barley plants (3D)	LR ₅₀ > 4000 mL product/ha ER ₅₀ 2000 mL product/ha	Appendix 2 Testbert, 2017
<i>Coccinella septempunctata</i>	A+B SC 300	Extended lab, detached bean leaves (2D)	ER ₅₀ > 4000 mL product/ha	Appendix 2 Smith, 2016
<i>Chrysoperla carnea</i>	A+B SC 300	Extended lab, detached maize leaves (2D)	LR ₅₀ > 4000 mL product/ha No effect on reproduction	Appendix 2 Smith, 2016

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

Risk assessment – Higher tier in-field

Higher tier in-field risk assessment for non-target arthropods due to the use of A+B SC 300 in cereals

Intended use		2 x 1000 mL prod./ha in cereals (BBCH 30-69)	
Product		A+B SC 300 (virtual product)	
Application rate (mL/ha)		2 × 1000	
MAF		1.7	
Test species Higher tier (ext.lab.)	Rate with ≤ 50 % effect (LR ₅₀ or ER ₅₀) (mL/ha)	PER _{in-field} (mL/ha)	PER _{in-field} below rate with ≤ 50 % effect?
<i>Aphidius rhopalosiphi</i>	2000	1700	Yes
<i>Coccinella septempunctata</i>	> 4000		Yes
<i>Chrysoperla carnea</i>	> 4000		Yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger

PER < LR₅₀ and ER₅₀ → acceptable risk

Risk assessment – Higher Tier off-field

Higher tier off-field risk assessment for non-target arthropods due to the use of A+B SC 300 in cereals

Intended use		2 × 1000 mL prod./ha in cereals (BBCH 30-60)				
Product		A+B SC 300 (virtual product)				
Application rate (mL/ha)		2 × 1000				
MAF		1.7				
VDF		10 (2D) / - (3D)				
Test species Higher tier	Rate with ≤ 50 % effect (LR ₅₀ or ER ₅₀) (mL/ha)	Drift rate	PER _{off-field} (mL/ha)	CF	PER _{off-field} below rate with ≤ 50 % effect?	
<i>Aphidius rhopalosiphi</i>	2000	0.0238	202.3*	5	yes	
<i>Coccinella septempunctata</i>	> 4000		20.23		yes	
<i>Chrysoperla carnea</i>	> 4000		20.23		yes	

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger

*3-dimensional test design (exposure on barley plants); therefore, Vegetation Distribution Factor (VDF) is set to 1

PER < LR₅₀ and ER₅₀ → acceptable risk

Risk mitigation options

In-field risk:

- Reduce application rate or frequency
- Increase application interval

Off-field risk:

- In-field no-spray buffer zones
- Drift reducing application techniques



Air injector nozzles
(http://www.topps-life.org/uploads/8/0/0/3/8003583/drift_short.pdf)

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