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

EU part

Plant protection products

Chapter 7 Ecotoxicology; aquatic

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Board for the Authorisation of plant protection products and biocides

Chapter 7 Ecotoxicology; aquatic

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Changes in the Evaluation Manual

Evaluation manual PPP EU part Chapter 7 Aquatic			
Version	**Date**	**Paragraph**	**Changes**
2.1	October 2016	1.2	Text from data requirements deleted from the Manual, replaced with reference/links to Regulations (EU) No 283/2013 and 284/2013. Short list of data requirements included in the text.
		1.2.3	Criteria for relevant metabolites are adjusted
		1.3	Further elaboration or clarification on risk assessment issues that are used by Ctgb included in the text of 1.3: - Points of attention regarding the use of NOEC or NOEAEC from micro-/mesocosmstudies - Expression of the endpoints from aquatic studies - Algae (Methodology for calculating the section-by-section coefficient of variation in algal studies (OECD 201) - PECsw-twa - Further elaborations of the criteria reported in the EFSA guidance document on aquatic risk assessment - With respect to SSD and micro-/mesocosm studies reference is made now to EFSA aquatic GD
2.2	January 2020	1.3	Conclusions regarding the aquatic risk assessment of the EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, July 2019, are included in the text: - Additional information on relevant endpoint for algae and macrophytes (A.1); - Additional information regarding the geomean approach (A.2); - General recommendations on mesocosm experiments (representativeness and vulnerability of the communities tested, experimental design of mesocosm experiments, effect classes, consideration of indirect effects, representative ness of mesocosm studies when the risk assessment at lower tiers is triggered by a non-freshwater species) (B.1); - Extrapolation of studies between different agroclimatic conditions (B.2) - Use of refined exposure studies as Tier

			2C (B.4.3) - Alternative test design in Myriophyllum studies (B.4.4) - Minimum detectable difference (B.4.5) - How to express the endpoint for sediment-dwelling organisms when tested in the presence of sediment (B.4.6)
		I.1 and II.1	Sentence included on the administrative EFSA guidance
2.3	July 2020	Chapter 1.3	Bullet points from the final agreements from the 4th CZHW in Ecotoxicology, Dessau, Sept 20-21 2018 on 'Risk mitigation measures', 'Refined exposure studies' and 'Derivation of endpoints for aquatic tests with instable substances' included.
2.4	February 2022	Chapter 1.3	Bullet points from the final agreements from the 5th CZHW in Ecotoxicology, Brno, November 2019 on 'PECsw_TWA"and 'Mixture risk assessment calculator tool' included.
2.5	October 2022	Chapter 1.3	Bullet point from the final agreements from the 5th CZHW in Ecotoxicology, Brno, November 2019 on the 'Geomean acute' included.
2.6	July 2023	Chapter 1.3	Bullet points from the final agreements from the 6th CZHW in Ecotoxicology, Ede (NL), June 2022 on aquatic issues are included. Furthermore the information regarding the ERO-RAC or ETO-RAC (point B.2.3) has been updated according to the results of the CZHW in Liverpool, 2017.

GENERAL INTRODUCTION

This chapter describes the data requirements for estimation of the effects of a plant protection product and its active substance on the aquatic environment and STP, and how reference values are derived in the EU framework (§1 - §1.5) under Regulation (EC) No 1107/2009.

This chapter consists of two parts: a part about effects on aquatic and sediment dwelling organisms (I), and a part about effects on sewage treatment plants (STPs) (II),

I AQUATIC AND SEDIMENT DWELLING 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.

Notifiers preparing an assessment report for active substances need to comply with the relevant guidance, instructions and format laid down in the EFSA Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances.

1.1. Introduction

This chapter describes the risk assessment of plant protection products for aquatic and sediment dwelling organisms.

This chapter is related to Chapter 6 Fate and behaviour in the environment; behaviour in surface water, sediment and sewage treatment plant (STP). That chapter describes the determination of estimated or measured concentrations in the sediment.

Guidelines for the risk assessment for aquatic organisms are described in the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290).

For sediment organisms these guidelines can be found in Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001).

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 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 and Commission Regulation (EU) No 284/2013 of Regulation (EC) No 1107/2009 must be submitted for the active substance as well as for the product,.

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

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.

1.2.1. Data requirements for the active substance

The date requirements regarding the risk of the active substance for aquatic organisms are described in part A of Commission Regulation (EU) No 283/2013, point 8.2 (effects on aquatic organisms).

Point 8.2 consists of the following data requirements:

8.2.1:	Acute toxicity to fish
8.2.2:	Long-term and chronic toxicity to fish
8.2.2.1:	Fish early life stage test
8.2.2.2:	Fish full life cycle test
8.2.2.3:	Bioconcentration in fish
8.2.3:	Endocrine disrupting properties
8.2.4:	Acute toxicity to aquatic invertebrates
8.2.4.1:	Acute toxicity to *Daphnia magna*
8.2.4.2:	Acute toxicity to additional aquatic invertebrate species
8.2.5:	Long-term and chronic toxicity to aquatic invertebrates
8.2.5.1:	Reproductive and developmental toxicity to *Daphnia magna*
8.2.5.2:	Reproductive and developmental toxicity to an additional aquatic invertebrate species
8.2.5.3:	Development and emergence in *Chironomus riparius*
8.2.5.4:	Sediment dwelling organisms
8.2.6:	Effects on algal growth
8.2.6.1:	Effects on growth of green algae
8.2.6.2:	Effects on growth of an additional algal species
8.2.7:	Effects on aquatic macrophytes
8.2.8:	Further testing on aquatic organisms

1.2.2. Data requirements for the product

The date requirements regarding the risk of the plant protection product for aquatic and sediment dwelling organisms are described in Commission Regulation (EU) No 283/2013, point 10.2 (effects on aquatic organisms).

Point 10.2 consists of the following data requirements:

10.2.1:	Acute toxicity to fish, aquatic invertebrates or effects on algal growth and macrophytes
10.2.2:	Additional long-term and chronic toxicity on fish, aquatic invertebrates and sediment dwelling organisms
10.2.3:	Further testing on aquatic organisms

1.2.3. Data requirements for metabolites

Metabolites in the water phase

For metabolites that are formed at more than 10 % at any timepoint or between 5 and 10 % at two or more occasions or at more than 5 % at the end of the study, a risk assessment (RA) is needed. In general, RA for metabolites formed below 5 % or below 10 % (observed at a single occasion) is not considered necessary. However, if there is reason to believe that a metabolite formed at < 5 % has intrinsic properties comparable to the parent substance in terms of its biological target activity, or that it has certain structural properties indicating high reactivity (i.e. mutagenicity) or endocrine disrupting properties or that it has unacceptable

toxicological properties, then that metabolite may be ecotoxicologically relevant and a RA is needed. Data on transformation rate, bioconcentration and acute toxicity to algae, invertebrates and fish are required for such metabolites.

Metabolites in the sediment phase
Major metabolites in the sediment phase are metabolites of which in the laboratory study into the transformation in a water/sediment system the concentration in the sediment phase after 14 days is higher than or equal to 10% of the added amount of active substance.

Data on the toxicity to sediment dwelling organisms are required for such metabolites.
Minor metabolites (formed in a concentration lower than 10% of the amount of added active substance) should be taken into consideration as well, because they may well be ecotoxicologically relevant. Hence, all available information and expert judgement should be used to assess if metabolites <10% give rise to particular concern..

The data requirements mentioned in these sections do not always need to be met by means of experimental studies. Applicants may also answer the open questions by means of other available information in support of a scientific and rational risk assessment.

Valuable sources of information are e.g.:

- consideration of molecular structure of the metabolite (active part intact?);
- the occurrence of metabolites in the medium in existing tests with the active substance or major metabolites;
- general knowledge on the relationship between the toxicity of the metabolite and its parent substance (e.g. from the aquatic base set (fish, daphnia, algae);
- information on pesticidal activity from biological screening data;
- available knowledge on related compounds;

Further information is given in the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290) with respect to the water phase and in the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001) regarding the sediment phase.

1.3. Risk assessment

Aquatic organisms
The risk assessment methodology for aquatic organisms has in EU context been elaborated in the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290). Each study is analysed and evaluated separately. The final conclusion and the endpoint per aspect (such as LC_{50} fish and NOECecosystem) are presented in a list of endpoints.

Risk assessment is based on comparison with endpoints. The risk evaluation for aquatic organisms follows a tiered approach. The first tier is based on model data as regards exposure and on laboratory data as regards toxicity. This is a general conservative evaluation of the behaviour and toxicity of the substance in the environment.
Where the criteria of the first tier of the evaluation are not met, there is the possibility to submit supplementary data for conducting a refined risk evaluation (higher tier).

Further information about the method to determine the exposure concentration is given in Chapter 6 Fate and behaviour in the environment; Behaviour in surface water, sediment and sewage treatment plant (STP), §1.3. The estimated exposure concentration is then compared with the toxicity data for the different aquatic organisms.

Sediment dwelling organisms
The risk assessment methodology for sediment dwelling organisms has in EU context been elaborated in the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001).

What is written above for aquatic organisms about endpoints, risk assessment, higher tier and exposure concentrations also applies to sediment dwelling organisms.

In addition, further elaboration or clarification on risk assessment issues that are used by Ctgb are included in the text below:

A. Issues EFSA aquatic guidance document
Certain parts of the aquatic guidance document Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290) are still under discussion, e.g. the relevant endpoints for algae and aquatic plants and the geomean approach. Many Member States commented on these parts and expressed their concerns. The actual situation is that there is no agreement between the Member States about the approach to follow on these points. Member States asked for an update of the Guidance Document to deal with the concerns. It is decided by EFSA that a corrigendum of the aquatic GD is necessary on these issues; as long as such a corrigendum is not performed, Member States follow their own approach.

A.1 Relevant endpoints for algae and macrophytes
In the EFSA aquatic guidance document (Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290)) it is strongly recommended to use the ErC50 value as the endpoint for algae/macrophytes in risk assessment. In the former guidance (SANCO) the lowest endpoint (EbC50, EyC50, ErC50) had to be selected for the risk assessment. Because the ErC50 value is in most cases higher than the EC50 based on biomass or yield the protection level for algae and macrophytes will be lower when following the recommendation of the new guidance document.
In the peer review meeting on recurring issues on ecotoxicology of October 2018 (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019) Germany presented a meta-analysis of Tier 1 and higher tier data. It was shown that Tier 1 endpoints expressed in terms of growth rate (i.e. ErC50 values) for algae and *Lemna* are respectively 6.9- and 3.5-fold higher than the Eb/yC50 values. Furthermore, comparison of Tier 1 data with endpoints from mesocosm studies indicated that the Tier 1 RAC calculated using ErC50 values is only protective in 42% of cases; while the same comparison based on EbR50 indicated a sufficient level of protection in 75% of the cases.
The experts acknowledged this concern. However, considering the available scientific knowledge, it was suggested that EFSA further considers this issue in the context of the revision of the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290) by taking into consideration all the available scientific knowledge on this aspect (e.g. van Wijngaarden and Arts, 2018).

For EU-dossiers it was decided to use the ErC50 in the risk assessment and to mention all endpoints (ErC50, EbC50 and EyC50) in the LoEP (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, December 2015), so that for the product assessment MSs can choose the endpoints they consider most appropriate. For the zonal assessments there is no decision yet taken by the Central Zone Steering Committee (CZSC).

The standard test duration of algae tests is 72 hours, according to the relevant OECD guideline. However, also tests with a duration of 96 hours and 120 hours are available. According to the new aquatic GD of EFSA (2013), algae tests with a test duration of 72-h and 96-h are

acceptable. If endpoints are available at 72-h as well as 96-h the lowest of the two should be used for risk assessment.

With respect to the endpoints from 120-h tests the endpoints at 72-h and 96-h should be determined, if possible. The lowest of the two should be used for risk assessment. If it is not possible to determine the endpoints at 72-h and/or 96-h, the 120-h endpoint is used for risk assessment.

The standard test duration of *Lemna* tests is 7 days, according to the relevant guideline. However, also 14-day endpoints are sometimes available. If the last endpoint is lower than the 7-d endpoint, the 14-d endpoint should be used for risk assessment, because there is no reason to assume that the endpoint at 14 days is less reliable (in consultation with Gertie Arts from WUR Environmental Research).

A.2 Geomean approach

For using the geometric mean in risk assessment additional data than the ones defined in the data requirements are needed. However, in some cases, two endpoints are sufficient for carrying out the geomean approach.

For using the geomean approach, the endpoints should be derived by highly comparable tests (including duration of the tests and how these tests cover the life cycle of the tested species).

At the zonal harmonisation workshop in Vienna (2015) it was decided that the geomean is only accepted for the acute risk assessment. The geomean is accepted for the chronic risk assessment of algae and *Lemna* (not *Myriophyllum*) but not for fish and invertebrates. However, there is a concern that the level of protection is not sufficient for each single active substance and PPP. Germany has made a proposal for a decision scheme in which it is decided whether the lowest endpoint or a geomean should be used.
In the peer review meeting on recurring issues on ecotoxicology of October 2018 (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019) the following was decided for the assessment of active substances at EU level: in cases where the RACgeomean is greater than the lowest endpoint, the lowest endpoint should be used to calculate the RAClowest. The minimum modified AF for deriving the RAClowest should be 20 for invertebrates and 30 for fish. The experts suggested that the approach should be further considered with the revision of the EFSA PPR Panel (2013).
There was no agreement for using a geometric mean for chronic data. This should be further considered together with the entire approach when the aquatic guidance (Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290)) is revised.

In the Central Zone Harmonisation Workshop in Brno, 12-14 November 2019, the following was agreed (bullet point):
The majority of MS agreed with the proposed Tier 2A scheme for acute risk assessment (steps 1 and 2):

Step 1 - Is lowest EP < $RAC_{geomean}$?

- Yes: use **RAC_{lowest}** (EFSA, 2013)

  Note: RAC_{lowest} = lowest EP / AF ≥ 20 for invertebrates and ≥ 30 for vertebrates (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019)
- No: Go to 2

Step 2 - $RAC_{geomean}$ and RAC_{lowest}
Compare $RAC_{geomean}$ and RAC_{lowest} (lowest EP /AF 60), report both, use the **lowest RAC**.
If using RAC_{lowest}, add **blocktext**:
"The RAC_{lowest} (i.e. endpoint of the most sensitive species tested divided by an AF of ≥ 60) is considered as a "safety net" to the $RAC_{geomean}$, especially relevant when the lowest available endpoint of the dataset is in a range close to the $RAC_{geomean}$. In the current situation, the use of the RAC_{lowest} instead of $RAC_{geomean}$ helps to reduce the shift in the protection level that will be achieved for species situated close to this trigger."
Note:
Step 1 is in line with the EFSA Technical Report (2019)
Step 2 is advanced to the approach agreed in the EFSA Technical Report (2019).

In the same Central Zone Harmonization Workshop in Brno also the following was agreed (bullet point):
All MSs agreed that the status quo on the use of Tier 2A approach for the chronic risk assessment as in EFSA Technical Report (2019) does not apply to primary producers, i.e. the chronic geomean can be used in the risk assessment for primary producers while it cannot be used in the risk assessment for vertebrates and invertebrates.

The majority of MSs agreed with the proposed Tier 2A scheme for chronic risk assessment of primary producers (steps 1 and 2):

Step 1- Is lowest EP < $RAC_{geomean}$?

- Yes: use **RAC_{lowest}** (EFSA, 2013)

  Note: RAC_{lowest} = lowest EP / $AF_{overall}$ ≥ 6
- No: Go to 2

Step 2- $RAC_{geomean}$ and RAC_{lowest}
Compare $RAC_{geomean}$ and RAC_{lowest} (lowest EP /AF 8), report both, use the **lowest RAC**.
If using RAC_{lowest} , add **blocktext**:
"The RAC_{lowest} (i.e. endpoint of the most sensitive species tested divided by an $AF_{overall}$ of ≥ 8) is considered as a "safety net" to the $RAC_{geomean}$, especially relevant when the lowest available endpoint of the dataset is in a range close to the $RAC_{geomean}$. In the current situation, the use of the RAC_{lowest} instead of $RAC_{geomean}$ helps to reduce the shift in the protection level that will be achieved for species situated close to this trigger. "

B. Other issues

B.1 General recommendations on mesocosm experiments

In the peer review meeting on recurring issues on ecotoxicology of October 2018 (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019) several general recommendations on mesocosm experiments were expressed (see below).

B.1.1 Representativeness and vulnerability of the communities tested.

The AF applied to the NOEC or NOAEC (for deriving the ETO- or ERO-RAC) is used for spatio-temporal extrapolations (for values of the AF, see Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290) p. 127; tables 34 and 35); it does not cover other elements (e.g. low representation of some vulnerable taxa).
It should be considered that the community represented is usually dominated by R-

strategists, with high reproductive potential, and which are therefore of low vulnerability. This concern is particularly relevant for ERO derivation.

For invertebrates, this concern can be addressed by ensuring a sufficient number of EPT (Ephemeroptera, Plecoptera, Trichoptera) species. These taxa are generally quite vulnerable due to their reproductive cycles and to their high sensitivity to some substances. It is noted that EPT are also an important component of a functioning ecosystem. It was, however, noted that these taxa are generally not particularly abundant in mesocosms, and that most of them prefer cold fast-running water, while most mesocosm experiments are carried out in pond-like structures. Some experts also suggested that it may be appropriate to build up a list of the species/taxa which should be present in the mesocosms.

It was agreed that the absence or low abundance of vulnerable groups, i.e. EPT, should not necessarily result in the invalidation of the experiment. However, their absence should trigger the need for further considerations, e.g. the selection of a higher AF and/or request for further testing to confirm that EPT are not among the most sensitive species. In such assessment, particular consideration should be paid to the mode of action of the active substance.

B.1.2 Experimental design of mesocosm experiments

Recommendations were made on establishment time, recolonization, emergence, insect instars, replicates, number of samples and sampling times. For these issues reference is made to the report of the meeting (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019), section 4.3.

B.1.3 Effect classes

The terminology for effect classes currently included in the (Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290)) is based on the definitions by Brock et al. (2006) and De Jong et al. (2008) and modified to add the information about the minimum detectable difference (MDD).

Effect class 2 (slight effects) is defined as ‘Effects concern short-term and quantitatively restricted responses usually observed at individual samplings only’.

MDD classes do not propose a quantification for ‘slight effects’, but they do set to 50 % the limit for MDD able to detect ‘small effects’ (MDD class IV).

Brock et al. (2015) suggested that a class 2 effect can be set if the MDD is < 70 % on the sampling after the effect, or < 90 % on the two samplings after the effect. The paper also added that class 2 effects can be set when, on the sampling after the effect, the percentage deviation from controls is less than 20 %.

It must be noted that the decision scheme in the (Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290)) for the setting of the NOEC on the basis of effect class 2 concentration does not specify an MDD trigger nor a proper percentage effect for the sampling times following the one indicating an effect. This indeed opens up possible interpretation on the criteria to be used for setting class 2 effect concentrations. This should be further clarified in the revision of the guidance document.

B.1.4 Consideration of indirect effects

Community interactions (indirect effects; food chain effects) are to be appropriately considered when assessing effects of PPPs. For example, if the recovery option is selected for algae in a study with a herbicidal mode of action, the study should be critically evaluated for potential effects on higher trophic levels (e.g. zooplankton).

B.1.5 Representativeness of mesocosm studies when the risk assessment at lower tiers is triggered by a non-freshwater species

The current aquatic guidance (Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290)) was developed to perform risk assessments for freshwater environments, in accordance with the data requirements specified in Commission Regulation (EU) No 283/2013 and Commission Regulation (EU) No 284/2013. The same AGD, however, does not exclude the opportunity of using data from non-freshwater (marine or brackish) species in the risk assessment scheme. On the contrary, endpoints for these species are regularly used in the evaluations of active substances and PPPs.

Data from ecotoxicological tests on non-freshwater species can refer to species at all trophic levels (e.g. *Skeletonema costatum* for primary producers, *Americamysis bahia* for aquatic invertebrates and *Cyprinodon variegatus* for fish). It is not unusual that the lower tier risk assessment is driven by non-freshwater species. When the evaluation at these lower tiers highlights a potentially high risk, an option to refine the assessment is to conduct mesocosm studies on freshwater communities. Non-freshwater species are hardly represented in such mesocosms, and therefore it is questionable whether these studies are adequate to derive an endpoint able to cover the organisms represented at lower tiers by non-freshwater species.

Usually, the presence of other organisms considered taxonomically similar to the most sensitive non-freshwater species is taken into account to solve the issue. However, the concept of 'taxonomically similar' is open to many interpretations: the term 'taxon' indicates a group of organisms with similar characteristics that can be applied to all the hierarchical levels of biological classification.

The role of phylogeny was discussed at the meeting and some experts disagreed about the use of this approach. It was highlighted that phylogeny is very fluid and hence difficult to be relied upon.

The proposal of setting a 'fixed' taxonomic hierarchical limit is problematic, as for some groups it is possible to get a better picture (more sub-group represented) than for others. However, a minimum level to be addressed was proposed on the basis of the comparison between *A. bahia* and the more closely related taxa that are often tested in mesocosms (Gammarids and Isopods). On this basis the minimum level to be matched should be the superorder. However, a general rule should be to consider which is the closest taxon that can reasonably be tested in a mesocosm, considering its autecology.

Overall, a stepwise procedure was proposed and agreed upon:

Step 1: check whether in the mesocosm the taxa closely related to *A. bahia* are included as the minimum representativeness requirement.

- If the mesocosm does not meet the minimum representativeness requirement, it cannot be considered to cover the risk for the most sensitive taxonomic group.
- If the mesocosm covers the minimum representativeness requirement, go to step 2.

Step 2: check that the 'representative surrogate taxa' (those taxonomically similar to the marine species driving the risk assessment at Tier 1) respond to the treatment, showing clear effects.

- If the 'representative surrogate taxa' respond to the treatment, the mesocosm is considered representative and can be used to address the risk assessment.
- If the 'representative surrogate taxa' do not respond to the treatment, go to step 3.

Step 3: perform further analysis and additional laboratory experiments might be requested with the 'representative surrogate taxa'. This would allow a better interpretation of the mesocosm by verifying whether the sensitivity of the 'representative surrogate taxa' is similar to that of the marine species untested in the mesocosm.

B. 2 Points of attention regarding the use of NOEC or NOEAEC from micro-/mesocosmstudies

B.2.1 Total period of effects

When extrapolating the results from a mesocosm study to a proposed application regime for a product, it has to be kept in mind that the total period of effects in the whole season may not be longer than 8 weeks, if the NOEAEC (based on recovery) is used for risk assessment. It must also be kept in mind that for certain compounds like Insect Growth Regulators the effects can appear later in the study. The period before the appearance of the effects is in that case not taken into account.

In certain cases it is not clear from the GAP how many crop-cycles are possible in a growing season (GAP only presents the uses for one crop-cycle). It is important to have the right information in order to be able to apply the right endpoint from the micro-/mesocosm study. In cases that the NOEAEC value cannot be used because the total period of effects is greater than 8 weeks, the NOEC (based on class 1 effects) from the micro-/mesocosm study may be used for risk assessment, if there is no accumulation of the substance in the water-phase. If there is a build-up of the active substance in the water, the mesocosm study is in principle not appropriate to use in the risk assessment, because the number of applications and therefore the maximum concentration in practice is higher than in the mesocosm study.

B.2.2 Product with two or more active substances

Another issue is the question which endpoint to use from a micro-/mesocosm study if it concerns a product with two or more active substances and a mesocosm study is only available for one or more of the active substances separately, but not for the product. In that case the recovery endpoint (NOEAEC) cannot be used for risk assessment, because the presence of the other active substance(s) in the product can hamper the recovery of the affected species. Hence, in these cases the NOEC (based on class 1 effects) should be used for risk assessment.

B.2.3 ERO-RAC or ETO-RAC

With regard to core assessments, it was agreed during the harmonization meeting in Vienna (2015) to use the ETO-RAC, if available. The Central Zone Steering Committee (CZSC) decided that the ERO-option should be applied in case no ETO (NOEC) is reported in the LoEP (Warsaw, May 2015). However, meanwhile DE started a discussion on a third option on CIRCABC.

In the Central Zone harmonisation workshop (CZHW) in Liverpool in February 2017 and the CZSC of May 2017 the following has been decided:
'The Central Zone (CZ) Ecotoxicology Harmonisation Group are of the view that an "ecological threshold option" (ETO) should be determined when assessing a mesocosm study. Furthermore, the CZ Ecotoxicology Harmonisation Group considers that an ETO should be used to set the regulatory acceptable concentration (RAC). In light of this, when a new mesocosm study is considered as part of an application for product approval, an ETO should be determined and used, along with an appropriate assessment factor of 2-3 (see Table 8 and 9 of EFSA (2013)) to generate the RAC.
(According to EFSA (2013), the Applicant *may* be able to demonstrate that "all relevant processes that determine population viability and the propagation of effects to the community-, ecosystem- and landscape-level" (Section 5.5 of EFSA (2013)) have been considered. If the Applicant has addressed all the issues regarding recovery, then it may be feasible to determine an "ecological recovery option" (ERO) and along with an appropriate assessment factor of 3-4. Both endpoints – ETO and ERO – as well as the corresponding

RAC should be quoted in the core. MS may wish to use the ERO as the justification of using this endpoint may be MS specific (e.g. minor use in a specific area etc.). However, the over-riding view of the CZ Ecotoxicology Harmonisation Group is to use the ETO approach.
As regards what endpoint to use "if the ETO is not report", it is assumed that this is related to where an active substance has been reviewed and as part of that assessment a mesocosm study has been considered and an endpoint agreed. The terms ERO and ETO are new terms and will only be relevant to those active substances considered after EFSA (2013) was noted (i.e. for those dossiers received after 1st January 2015 – see SANCO/10605/2014 – rev. 0 (11 July 2014) Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters) and it is assumed that both endpoints – ERO and ETO will be presented in the LoEP of the EFSA conclusion. The terms ERO and ETO are unlikely to appear in EFSA conclusions prior to this date. As for those active substances that were considered prior to the implementation of EFSA (2013), it is likely that there will be endpoints based either on recovery, minimal or "no effects" as well as a range of associated assessment factors. It is important to have a consistent way in which these previously agreed endpoints are interpreted and used, especially when it is considered that EFSA (2013) should be used for the assessment of products considered after 1st January 2015 – see SANCO/10605/2014. With this in mind, outlined below is a proposal in which the variety of endpoints and assessment factors could be dealt with:
Where an ERO or ETO has not been defined in the list of endpoints, it is assumed that an ETO is broadly equivalent to a NOEC whilst an ERO is equivalent to a recovery based endpoint. It should be noted that in previous assessments the period for recovery may have been longer than specified in EFSA (2013).

1. If the endpoint presented is a NOEC, then this could be assumed to be equivalent to an ETO, hence an assessment factor of 2 could be applied to this endpoint to derive an ETO RAC.
2. If the endpoint presented in the LoEP is based on a recovery endpoint and hence may be quoted as a NOAEAC, then the original assessment in the DAR should be considered, and if a NOEC has been determined as part of the study evaluation[1], then this, along with an assessment factor of 2 should be used.
3. If the endpoint presented is a RAC or some other endpoint where the effects endpoint and the assessment endpoint have been combined and it is unclear from the LoEP what the exact effects endpoint is, then the original assessment in the DAR should be considered and if a NOEC has been determined, then this, along with an assessment factor of 2 should be used. If a NOEC has not been determined, then one should be determined from the study summary in the original DAR if possible.

For points (2) and (3) above, if a NOEC has not been determined, the following course of action is proposed:

1. Can one be determined on the basis of the evaluation in the DAR? If so, then use that along with an assessment factor of 2
2. If a NOEC was not quoted in the DAR and cannot be determined on the basis of the study evaluation, revisit the original study; it is not proposed to re-evaluate it, but to see if a NOEC was determined. If it was, then it is proposed to use that, providing that it is lower than the NOAEAC quoted in the LoEP.
3. If a NOEC cannot be determined due to effects at the lowest concentration then there needs to be a consideration of how many species and what the level of effects were. If there was an impact on two species and the effect deemed to be a Class 2[2] effect[3], then it may be feasible to use this endpoint

[1] It is not proposed to revisit the study but to work from the original assessment. Whilst the NOEC may not have been subject to detailed discussion during the peer review stage it is assumed that the study will have been and hence endpoints other than the previously agreed endpoint can be considered reliable.

[2] See for example Section 2.1.6 of EFSA (2013).

[3] There needs to be a consideration of any additional information in the DAR that could put the effects in to

along with an assessment factor of 3. If there is uncertainty regarding the relevance of the effects at the lowest concentration then it is proposed to go back to the Applicant and for further information[4].

If the Applicant has represented the mesocosm study for product registration purposes, possibly along with a consideration of minimum detectable difference (MDD), then this should be considered along with any previous comments made during the peer review process regarding the robustness of the mesocosm study(ies) and a ETO (and possibly an ERO) derived.
Whilst the above outlines a proposal regarding the use mesocosm studies, it is proposed that EFSA (2013) should be used to derive other higher tier endpoints, for example those associated with the use of multispecies data (e.g. SSD).

See for extrapolation of studies between different agroclimatic conditions point B.2.4.

B.2.4 Extrapolation of studies between different agroclimatic conditions
In the peer review meeting on recurring issues on ecotoxicology of October 2018 ((EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019), the issue about extrapolation between different agroclimatic conditions was discussed. In the case of mesocosms, the majority of the experts at the meeting agreed that the no observable effect concentration (NOEC) and the ecological threshold option (ETO) regulatory acceptable concentration (RAC) can be used in the risk assessment with the assessment factor (AF) recommended by aquatic guidance (EFSA PPR Panel, 2013), and this can be considered as independent of the experimental conditions (e.g. the climatic zone). However, when an ecological recovery option (ERO) RAC is derived, the extrapolation between zones should be considered carefully taking into account the fact that the ability for recovery may vary pending on the agroclimatic conditions. A case-by-case evaluation should be carried out, based on the information available.

B.3 Expression of the endpoints from Tier 1 test and formulation tests (with one or more active substances) for unstable substances

At Tier 1, laboratory standard tests must be performed under standard (i.e. mostly worst case) exposure. Therefore, OECD guidelines recommend that the concentrations should be maintained and must be > 80 % and < 120 % of nominal at the end of the exposure period (or at the end of the renewal period for semi-static design).
If the concentration cannot be maintained (i.e. if the substance is dissipating ‘fast’), the validity of the study should be questioned and the test may be rejected as highlighted during the EFSA peer review meeting on general recurring issues in ecotoxicology (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, December 2015).

During this EFSA peer review meeting, Member States agreed that in principle:
1) **Nominal concentrations** can be used to express the toxicity from any kind of test if the test concentrations were maintained at ± 20 % of the nominal at all times throughout the test including the study end sampling. Mean measured is also an option for this situation.

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perspective. There also needs to a consideration of whether the impacted species are key and the only relevant ones. For example, if the compound effects moulting and there are only two species that go through a moult in the mesocosm study, then this is of greater concern, compared to say a broad spectrum toxicant where there is an impact on one species in the lowest concentration.

[4] Further information could be in the form of a minimum detectable difference analysis to try to provide some indication as to the robustness of the effects observed on key species.

2) **Initial measured concentrations** can be used to express the toxicity from any kind of test if the initial test concentrations were below 80 % of the nominal and this concentration was maintained throughout the test (within ± 20 % of the initial) including the final sampling. Mean measured is also an option for this situation.
3) **Mean measured concentrations** must be used to express the toxicity from any kind of test when the test concentrations were not maintained within the range of ± 20 % of the nominal or initial measured, but significant concentrations of the test item were still present at the end of the exposure period (or at the end of the renewal period for semi-static design).
4) When the test concentrations were not maintained and significant residues were not present at the end of the exposure period (or at the end of the renewal period for semi-static design), the **validity of the study should be questioned**.

It was also pointed out that further clarifications should be provided in the AGD.
In practice (and not due to a causal relation), however, semi-static and/or flow-through design is rarely used for tests with:
- algae for which semi-static tests are very uncommon and flow-through tests not established in the regulatory context, due to the technical complexity when conducting the test;
- formulated products with one or more active substance, especially for tests with algae.
This proposal addresses these issues. It especially considers the cases where the recovery of an active substance at the end of a test is < 80 % (i.e. the test substance is dissipating fast) and where requesting a new semi-static or flow-through test (as required by EFSA, 2015) may not be feasible or desirable (i.e. algae tests and vertebrate tests).

An adequate expression of the endpoint from formulated product tests is needed:
- for the purposes of classification and labelling, and
- as the basis for mixture toxicity assessment since it should enable an assessment of potential synergism or additive toxicity due to one or more co-formulants or additional active substances.

The described approach aims to serve both purposes.
Until a revision of the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290) , this position paper is intended to fill the gap as an interim solution, i.e. for such cases where above-cited requirements 3 and 4 cannot be easily fulfilled and performing tests under semi-static or flow-through conditions are an issue.

A paper regarding this issue has been discussed during the Central Zone Harmonisation Workshop in Dessau, 20-21 September 2018 and later agreed on by the MS of the Central Zone (“Expressing endpoints from Tier 1 tests and formulation tests (with one or more active substances) for unstable substances”).The approach is included as Appendix J in the EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019. Reference is made to this document.

B.4 Other issues discussed between Member States

The following issues from the EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, December 2015 and EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019 are also relevant:

B.4.1 Algae (Methodology for calculating the section-by-section coefficient of variation in algal studies (OECD 201) (from EFSA, 2015).

Based on the clarification provided at the meeting, it was clear that the methodology to be used for calculating the CV for section-by-section specific growth rates is the following: calculate specific growth rates for first control replicate for day 0-1, 1-2 and 2-3 and then calculate CV for first control replicate. Use the same approach to calculate CV values also for

2nd and 3rd control replicates. Then calculate the mean CV.

B.4.2 PECsw-twa – Further elaborations of the criteria reported in the EFSA guidance document on aquatic risk assessment (from EFSA, 2015)

The experts at the meeting considered there is a need to have further clarifications and corrections on the EFSA aquatic guidance document regarding the application of the PECsw;twa. The main issues identified were 1) identification of organisms for which the reciprocity appraoch is applicable (e.g. fish, *Lemna, Daphnia,* all); 2) indication of the duration over which linear reciprocity needs to be determined (e.g. entire study, part of the study); 3) recommendation on how to express the endpoint (all study or just the linear part?) in case reciprocity is only determined for a part of the study; 4) clarification regarding the criteria to assess linearity (e.g. R^2 value, p-value of the regression, etc.); 5) clarification on the assessment of the latency.

It was agreed that until further guidance on reciprocity and latency of effects is available, then the use of TWA approaches are unlikely to be sufficiently robust to be used in regulatory risk assessment.

B.4.3 Use of refined exposure studies as Tier 2C (from EFSA, 2019)

At the meeting, Germany presented an update on the Central Zone Harmonisation Meeting in Dessau, 20-21 September 2018, regarding the use of refined exposure studies. A position paper was also made available before the meeting. Nevertheless, it was pointed out that a complete agreement could not be reached at the central zone level regarding these kinds of experiments. The MSs of the central zone agreed on the following two pre-requisites:
- the GAP must be covered in terms of exposure pattern, and
- if a refined exposure toxicity is delivered by the applicant, all information must be provided in order to facilitate its evaluation and potential implementation in the RA.

Although no final agreement was reached, most MS consider:
 that the Tier 2C approach should generally not be supported at zonal level, considering that implementation in ERA is complex and linked to high uncertainties
 if a conclusion of low risk based on a lower tier approach with RMM is possible this should be favoured over a conclusion based on a Tier 2C approach, considering the uncertainties related to such a Tier 2C approach
 if applicants still decide to deliver a refined exposure toxicity test (Tier2C option), a lower tier (e.g. Tier 1) risk assessment should always be also presented up to FOCUS step 4 with an agreed level of Risk Mitigation Measures (RMM).

Representatives from the northern zone reported that this kind of refinement is not considered acceptable for their zonal assessments. It was explained that this is mainly due to doubts that the FOCUS profiles can accurately reflect exposure in the field (particularly as they are currently based on limited time simulations). It was, however, noted that the same doubt should also apply to the use of mesocosms, for which exposure profiles are also compared to the FOCUS predictions. Other concerns were related to the uncertainties in the extrapolation of the results to the field, e.g. the uncertainties on the life stage of the tested species which are exposed in this kind of test. It was indeed highlighted that it is very difficult to have a match of the pulsed exposure with the most sensitive life stage, particularly when knowledge is lacking about which is the most sensitive stage.
It was also noted that the use of the Tier 2C refinement may be problematic for populations of short-lived species (e.g. algae, aquatic plants, daphnids). Indeed, some potential recovery may take place in these tests, while ERO is not an option at Tier 2, as recovery in the field would be influenced by the relationship with other species. For primary producers, it was suggested that an EC10 be used instead of an EC50, in order to reduce the possibility of an effect that it is ‘absorbed’ by a subsequent recovery (it should be noted that this approach is already included in the position paper presented by Germany). In addition, repeated measurements over time of the relevant endpoint(s) help to detect whether a possible recovery takes place. For daphnids and other short-lived invertebrates, testing at the

individual level (i.e. not using populations) should exclude any concern about recovery at the population level, since only repair mechanisms at the level of the individual occur.
In the approach (still not agreed) initially suggested for the central zone, a prerequisite for carrying out refined exposure tests is to provide a risk assessment using endpoint(s) from experiments carried out under constant exposure and that includes mitigation measures. Everyone agreed that providing a lower tier risk assessment with mitigation measures is a reasonable approach for all kinds of refinement. However, it was also highlighted that this does not relate specifically to Tier 2C in any way. It was also agreed that showing a low risk with mitigation measures at lower tiers should not be considered as a reason to avoid an assessment of the available higher tier studies.
It was agreed that the scheme for assessing Tier 2C should be reconsidered and possibly further developed in the revision of the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290).

B.4.4 Alternative test design in Myriophyllum studies (from EFSA, 2019)
It was agreed that Myriophyllum studies performed to OECD TG 239 but with an alternative test design (i.e. one shoot per pot per test vessel) should be considered acceptable.

B.4.5 Minimum detectable difference (from EFSA, 2019)
The MDD, presented in the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290). and the paper by Brock et al. (2015), is considered to be a valid tool to help with the evaluation of the biological results to assess the statistical power – or the absence of power – of a study to detect treatment-related direct effects. It should preferably be reported on non-aggregated data for the relevant taxon and time points. An issue linked to the unclear beta-error associated with the MDD in the available documents mentioned above was raised by Germany.
It was concluded that the use of the MDD is supported and that further considerations and clarifications will be addressed in the revision of the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290).

B.4.6 How to express the endpoint for sediment-dwelling organisms when tested in the presence of sediment (from EFSA, 2019)
During the Pesticide Peer Review Meeting 133 (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, December 2015) it was discussed how the endpoints for aquatic Tier 1 studies should be expressed. It was agreed that 'the toxicity endpoint for Tier 1 studies (i.e. mean measured, nominal or initial measured), should not depend on the study design, on the physical chemical or environmental fate parameters, on technical difficulties when testing, or on how the endpoint would be used in the first-tier risk assessment. The choice must depend on the actual exposure throughout the whole exposure period of that particular test. Where a suitable exposure throughout the whole period was not demonstrated, none of the endpoints should be used in first-tier risk assessments.' This discussion did not specifically cover the case of the toxicity tests on sediment-dwellers when tested in the presence of sediment.
The studies more frequently available for addressing the effects on sediment dwellers are performed on *Chironomus riparius* (OECD 218 and OECD 219).
In the context of the peer review of the active substance risk assessment, the issue of how the concentrations should be expressed in the case of sediment-dweller toxicity testing was often raised. In particular, there have been instances in which it was questionable to express the endpoints as measured concentrations at the beginning of the test, i.e. in the cases where the concentrations were not maintained in the whole system.
EFSA recommended that the decision on how to express the endpoint for the sediment-dwellers is based on the assessment of the mass balance calculation in order to determine

the repartition of the substance in the various compartments. In this view the submission of mass balance calculations as part of the dataset for the sediment-dwellers is highly recommended, particularly in the case of the substances that are difficult to test (concentrations poorly maintained in the test system). In the latter cases, it is also relevant that intermediate measurements in the various compartments are performed (see also Commission Regulation (EU) No 283/2013, Section 8.2.5.3). When a mass balance is available, it is possible to consider the recommendations of the Pesticide Peer Review Meeting 133 (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, December 2015). It is additionally recommended that the key endpoints from the sediment-dweller studies are always presented in terms of mg substance/kg dry sediment and mg substance/L water. This would ensure that both exposure via water and sediment are covered for sediment-dwellers.
Where the concentrations in the test system are not maintained, the recommendations of the Pesticide Peer Review Meeting 133 (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, December 2015) should be considered, i.e. express the endpoint as the mean measured concentration using mg substance/kg dry sediment and/or mg substance/L water, accordingly, if significant levels are detected in the sediment or in the water or in both. The calculations should be based on geometric mean concentrations. It is proposed to further discuss whether, in such cases, the use of these studies in a Tier 2C approach, similar to the proposal in the EFSA aquatic guidance document (Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290)) for the refined exposure studies, would be suitable. This means that it should be demonstrated that the exposure in the study simulates a realistic worst-case exposure relative to the predicted exposure. In this view, a comparison between the exposure in the test system and the expected exposure (FOCUS profiles) should be performed. In order to follow this approach, intermediate analytical measurements should be performed in the course of the study.
It is acknowledged that issues similar to those for the sediment-dwellers could also occur for toxicity tests with the rooted macrophyte *Myriophyllum spicatum* (OECD TG 239). In those cases it is suggested that the same approach as above is applied. It is noted that OECD TG 239 already highlights that 'if there is evidence that the concentration has declined (i.e. is not maintained within 20 % of the nominal or measured initial concentration in the treated compartment) throughout the test, then analysis of the results should be based on the geometric mean concentration during exposure or models describing the decline of the concentration of the test chemical in the treated compartment'.
Overall, the experts agreed with the proposal to use the mass balance for checking whether the concentrations were adequately maintained. Practical examples of the needed calculations are included in Appendices G and J of EFSA, 2019 (EFSA technical report: Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, June 2019).

B.4.7 Risk mitigation measures (RMM) (bullet point from CZHW 2018, Dessau)
In the Central Zone Harmonisation Workshop in Dessau, 20-21 September 2018, the following was agreed (bullet point):
- The MS agreed that RMM up to 90% drift reduction and 30 m buffer zone should be presented in the core assessment.

B.4.8 PECsw-TWA approach aquatic organisms (bullet point from CZHW 2019, Brno)

Application of the PECsw-TWA approach for aquatic organisms gives rise to problems that are difficult to overcome, when based on the existing guidance. Therefore:

- The PECsw-TWA approach should currently not be used in zonal assessments due to lacking guidance and harmonisation at EU-level and other concerns (e.g. most sensitive life-stage tested, extrapolation to other species), to avoid inconsistent evaluations;

- To gain knowledge and experience with these assessments the information submitted by applicants might be included in the RRs, but together with a general statement that it has not been considered further.

B.4.9 Validity criteria of algae test OECD 201 (bullet point from CZHW 2022, Ede)

The protocol is not developed for non-standard species, also for standard species some criteria may not be appropriate in all cases. A proposal was made for harmonized interpretation of validity criteria for non-standard species. The proposal is acceptable and the CZ MS will use the outlined criteria for non-standard species. The proposal is presented in Appendix 2.

B.4.10 Aquatic organisms – Herbicides with unexpectedly low toxicity to macrophytes: necessity for a different exposure design (overspray) in toxicity tests with emergent and floating macrophytes (bullet point from CZHW 2022, Ede)

For certain herbicides the current testing methodology does not adequately represent the exposure scenario nor the MoA. The majority of MS agreed if the toxicity of an herbicide is below the level of acute toxicity classification in emergent macrophytes, the applicant would be asked to either provide justification that direct exposure is not relevant or provide a test including an overspray exposure scenario. Such a test could be e.g. from the adapted test protocol of existing guideline for terrestrial NTTPs (OECD 227) in which an overspray scenario has been added. The risk assessment for macrophytes would then be performed adding an additional scenario, using the spray drift value for NTTPs, the endpoint from an overspray test (in mg a.s./ha) and the trigger of 10 for aquatic plants. This would be in addition to the “usual” aquatic risk assessment according to FOCUS.

B.4.11 Aquatics and NTTPs – SSD (bullet point from CZHW 2022, Ede)

A proposal was presented for the harmonized evaluation and interpretation of SSD data.

The MS agreed to use the approach when evaluating SSDs (aquatic and NTTP) in future dossiers and to bring the paper forward to the EFSA to be considered in the next general issues meeting. The proposal is presented in Appendix 3.

B.4.12 Acute fish testing with PPPs: Limit vs DR tests (bullet point from CZHW 2022, Ede)

A proposal to minimize vertebrate testing in fish (acute toxicity tests with formulations) was presented. Under certain circumstances only limit tests, or no test, could be accepted rather than a full dose-response test.
The CZ MS agree to follow the proposal when considering whether an acute fish toxicity test is needed with the formulation under consideration for future dossiers. The proposal is presented in Appendix 4.

B.5.0 Tools

B.5.1 Mixture risk assessment calculation tool (from CZHW 2019, Brno)
A tool for the mixture risk assessment calculations (called “AGD_AquaMix_v1.15”) was developed by a group of Member States from the central and northern zone and was published on the 21st of January 2021 in the CIRCABC Expert exchange forum. It can now be downloaded at the EFSA Knowledge Junction (https://zenodo.org/record/4593676).
The tool is intended to be an extension and implementation of the assessment given in the aquatic guidance document (EFSA Journal 2013;11(7):3290) and to facilitate the associated

mixture calculations. Alongside the tool itself an FAQ was developed as separate file, in which proposals are given for the assessment of complex mixture risk assessment topics (e.g. how to handle metabolites).
This tool will be further developed in the future.

Decision-scheme
A decision scheme with corresponding explanatory notes is presented in Appendix 1.
This decision tree summarises the decision scheme for aquatic and sediment dwelling organisms.

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

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

Point 3 of Annex II of Regulation (EC) No 1107/2009 gives the criteria for the approval of an active substance.

1.4.2. Evaluation of plant protection products

The principles for the evaluation regarding the effects on the environment are presented in Commission Regulation (EU) No 546/2011 (i.e. the Uniform Principles).

The specific principles for decision making for aquatic organisms are included in Part B Evaluation, point 2.5.2.2.

1.4.3. Decision making for plant protection products

The principles for the decision-making regarding the effects on the environment are presented in Commission Regulation (EU) No 546/2011 (i.e. the Uniform Principles).

The specific principles for decision making for aquatic organisms are included in Part C Decision making, point 2.5.2.2.

1.5. Developments

Hormone-disturbing substances
It is known that substances may disturb endocrine systems of organisms.
Endocrine substances may in an early life stage cause damage of which the effects only manifest themselves later, possibly only in a next generation. It is recognised that the current available chronic toxicity tests are not adequate to demonstrate potential endocrine effects. This is why in an international programme, organised by OECD, toxicity tests (including fish) are being developed to identify endocrine-disturbing substances. For the time being, data on mammals may give an indication.

In the process of revision of 544/2011 and 545/2011 data requirements regarding endocrine disruption will be taken into account by setting several data requirements.

Organisms in groundwater
Studies of the biological groundwater ecosystem have led to the notion that the groundwater ecosystem is a system as such which needs protection [1,2]. Active substances and/or metabolites should for this reason be evaluated for their effects on the groundwater ecosystem in the future.

In the absence of more specific information and harmonised test guidelines, it may be assumed that groundwater organisms have the same sensitivity as taxonomically and physiologically related organisms in surface water. Crustaceans represent the most important groundwater taxa and – from a provisional scientific point of view – data on crustaceans in surface water are considered as suitable and adequate to cover the risk to groundwater organisms. Recovery observed in higher tier tests, however, is possibly not relevant for organisms in groundwater. Currently, harmonised schemes for exposure and risk assessment are not available. Further research should therefore be carried out in this field.

Ecological modelling
Reference is made to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290).
Section 11.3 of this document gives information about the state-of-the-art of the use of mechanistic effect models in regulatory environmental risk assessment.

In the near future, the PPR Panel will elaborate scientific opinions on good modelling practice and more specifically on modelling within the aquatic RA. Since there is a lack of experience and guidance for these approaches in RA, the use of mechanistic modelling within the authorisation of PPPs has to be evaluated carefully and case-by-case until special guidance becomes available.

Risks of fungicides to aquatic fungi
Almost no information is available concerning the potential risks of fungicides (or PPPs in general) to aquatic fungi. Maltby *et al.* (2009)[3] compiled aquatic ecotoxicity data for a series of fungicides. The dataset included acute single-species data for 42 fungicides, semi-field data for 12 fungicides and covered seven modes of action and different exposure regimes. SSDs were constructed for separate taxonomic groups (*i.e.* fish, invertebrates, and primary producers) and for all groups together. They conclude that there is no evidence to suggest that derived threshold values based on hazardous concentrations (HC_p) from acute aquatic SSDs would pose a risk to aquatic hyphomycetes. However, laboratory toxicity data on fungi were not included in the datasets, since they were not available. In the micro/mesocosm studies reviewed, only functional responses of micro-organisms in the form of litter decomposition received attention. None of the semi-field studies specifically studied structural endpoints of fungi. Maltby *et al* (2009)[3] therefore also concluded that the underlying data is limited in number and that further research on nontarget fungi should be conducted. The relevance of further research into the sensitivity of aquatic fungi was demonstrated recently in screening studies by Dijksterhuis *et al.* (2009, 2011)[4, 5] and CBS (2009)[6]. Their data indicate that HC_5 concentrations derived by Maltby *et al*. (2009)[3] for ergosterol inhibitors may show an effect on aquatic fungi. Further research is needed to address the relevance of aquatic fungi as additional non-target groups in the risk assessment of PPPs. Special attention should be paid to the selection of appropriate test species, given the enormous diversity within the kingdom of fungi. When these data are collated, it will be a risk manager decision to set the specific protection goal for aquatic fungi (e.g. structure and/or function).

Sediment organisms
Regarding sediment organisms the following EFSA Opinion was published:

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015. Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water. EFSA Journal 2015;13(7):4176, 145pp. doi:10.2903/j.efsa.2015.4176. This opinion is assumed to be input for future guidance.

Multiple stress and mixture toxicity
In many crops during the growing season more than one compound will be used. In some crops this can add up to more than 50 applications and some of these compounds will be applied together, e.g. an herbicide together with an insecticide and/or fungicide. Sometimes even two or three herbicides or two or three fungicides or two insecticides may be applied simultaneously, up to 5 or 6 compounds at the same time. When these combinations (e.g. tank mixes) are not sold as a formulation the legislative process does not take account for the potential combined effects of the use of these tank mixes. Neither does the legislative process take into account that different compounds of the same group (e.g. insecticides) or of different groups (e.g. insecticides, herbicides, fungicides) are used over time in the same growing season.

When a compound is allowed on the market this decision is sometimes based on the potential of recovery. Whether under different crop scenarios the recovery option is appropriate to use in the derivation of the RAC needs to be evaluated from an ecological point of view, since during the growing season drainage ditches may be affected multiple times by the use of plant protection products. EFSA is planning to take this topic into account.

II EFFECTS ON A SEWAGE TREATMENT PLANT (STP)

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.

Notifiers preparing an assessment report for active substances need to comply with the relevant guidance, instructions and format laid down in the EFSA Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances.

1.1. Introduction

This chapter serves to estimate the risk to micro-organisms in the STP.

This chapter is related to Chapter 6 Fate and behaviour in the environment; behaviour in surface water, sediment and sewage treatment plant (STP).

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 Commission Implementing Regulation (EU) No 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 283/2013and Commission Regulation (EU) No 284/2013 of Regulation (EC) No 1107/2009 [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 and Commission Communication 2013/C 95/02.

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.

1.2.1. Data requirements for the active substance

The date requirements regarding the effects of the active substance on sewage treatment plants (STPs) are described in Commission Regulation (EU) No 283/2013, point 8.8 (effects on biological methods for sewage treatment).

Point 8.8 consists of the following data requirements:
8.8: Effects on biological methods for sewage treatment

1.2.2. Data requirements for the product

According to Commission Regulation (EU) No 284/2013, no data are required for the risk assessment for an STP.

1.3. Risk assessment

Risk assessment is carried out as described in §1.3 of Chapter 6 Fate and behaviour in the environment; behaviour in surface water, sediment and sewage treatment plant (STP).

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

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

Point 3 of Annex II of Regulation (EC) No 1107/2009 gives the criteria for the approval of an active substance.

1.4.2 Evaluation of plant protection products

Commission Regulation (EU) No 546/2011 (i.e. the Uniform Principles), contains no specific criteria for risk assessment as regards sewage treatment.

1.4.3 Decision making for plant protection products

Commission Regulation (EU) No 546/2011 (i.e. the Uniform Principles), contains no specific criteria for decision making as regards sewage treatment. However, for the national assessment the threshold level used for risk assessment is 0.1 * EC50 STP value.

1.5. Developments

None.

2. APPENDICES

Appendix 1 Explanatory notes decision tree Risk to aquatic and sediment dwelling organisms based on Regulation (EC) 1107/2009

1) For each active substance, information concerning toxicity to aquatic organisms (Commission Regulation (EU) No 283/2013: point 8.2) must be provided, unless it can be demonstrated that it can be ruled out that the substance reaches surface water during good (agricultural) use of the product, in compliance with the WG/GA (Statutory Use Instructions/Directions for Use). For the purposes of labelling in the European framework, data concerning acute toxicity of the active substance to algae, aquatic invertebrates and fish, and the ready biodegradability of the active substance must always be provided. For each product in principle data concerning toxicity to aquatic organisms must be provided if the toxicity of the plant protection product cannot be predicted on the basis of the data for the active substance (Commission Regulation (EU) No 284/2013, point 10.2).

2) The acute toxicity research (283/2103 point 8.2.1/8.2.4/A8.2.6) must be carried out in accordance with standardised methods with representatives of at least 3 different trophic levels, i.e., algae, aquatic invertebrates and fish.
For fish acute toxicity data are always required for rainbow trout (*Oncorhynchus mykiss*). Seven fish should be used, also in a limit test.
For herbicides and growth regulators a standard test with higher aquatic plants must be submitted (283/2013 point 8.2.7) as well as a test with a second algal species from a different taxonomic group.
For pesticides with an insecticidal mode of action data are required for *Daphnia* sp. (*D. magna* preferred) and an additional arthropod (preferably a *Chironomus* test, if data on *Americamysis bahia* are not already available).
If a long-term/chronic study on insects is already available there is no need to require additionally an acute one.
Except for the active substance and the product, data about metabolites formed in the water and sediment phase of water/sediment systems are required as well. For metabolites that are formed at more than 10 % or between 5 and 10 % at two or more occasions or at more than 5 % at the end of the study, data is needed. In general, data for metabolites formed below 5 % or below 10 % (observed at a single occasion) is not considered necessary. However, if there is reason to believe that a metabolite formed at < 5 % has intrinsic properties comparable to the parent substance in terms of its biological target activity, or that it has certain structural properties indicating high reactivity (i.e. mutagenicity) or endocrine disrupting properties or that it has unacceptable toxicological properties, then that metabolite may be ecotoxicologically relevant and data is needed.Data on transformation rate, bioconcentration and acute toxicity to algae, aquatic invertebrates and fish are required for such metabolites.
Metabolites should in general also be tested with *Lemna*, *Chironomus* or other species if these taxa have been the most sensitive with the active substance. If it can be demonstrated that certain taxonomic groups are clearly less sensitive to the active substance (by a factor of 100) than other groups, testing can be limited to those which are the most sensitive ones. If testing reveals that the toxicity of the metabolite to one taxonomic group is similar to the parent or higher then testing may be required on all taxonomic groups.
Major metabolites in the sediment phase are metabolites of which in the laboratory study into the transformation in a water/sediment system the concentration in the sediment phase after 14 days is higher than or equal to 10% of the added amount of active substance. Data on the toxicity to sediment dwelling organisms are required for such metabolites.

Minor metabolites should be taken into consideration as well.
The data requirements mentioned in this section do not always need to be met by means of experimental studies.
Applicants may also answer the open questions by means of other available information in support of a scientific and rational risk assessment. Valuable sources of information are e.g.:

- consideration of molecular structure of the metabolite (active part intact?);
- the occurrence of metabolites in the medium in existing tests with the active substance or major metabolites;
- general knowledge on the relationship between the toxicity of the metabolite and its parent substance (e.g. from the aquatic base set (fish, daphnia, algae);
- information on pesticidal activity from biological screening data;
- available knowledge on related compounds;

Further information is given in the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290).

3) Also chronic toxicity data (283/2013 point 8.2.2/8.2.5) must be submitted , unless there is 90% or more loss of the original substances over 24 hours via hydrolysis.

4) A chronic study with fish and *Daphnia* sp. is required. For fish this should be a Early life-stage test, unless a fish full life-cycle (FFLC) test is provided. An FFLC may be required depending on the persistence and bioaccumulative potential of the substance; the following critera applies: BCF > 1000 ánd the elimination during the 14 day depuration phase in the bioconcentration study <95% ánd the substance is stable in water or sediment ($DegT_{90}$ > 100 days).
For pesticides with an insecticidal mode of action preferably the most sensitive standard test arthropods (*Daphnia, Chironomus, Americamysis*) from the acute Tier 1 data set should be selected as test species in the chronic effect assessment. If in the acute assessment a certain standard test arthropod is a factor of 10 more sensitive a chronic test with this arthropod should be performed.

5) Where in a water/sediment study (283/2013 point 7.2.2.3.) at or after 14 days (283/2013 point 8.2.7) ≥ 10% of the active substance and/or metabolite is found in the sediment or when the substance interferes with moulting hormones (e.g. insect growth regulators), a chronic toxicity test with sediment dwelling organisms (*Chironomus sp.*) (283/2013 point 8.2.7) must be provided unless the EC10/NOEC from the chronic daphnia test (or a comparable study with aquatic insects if this group of organisms is more sensitive) ≥ 0.1 mg a.s./L.

6) Further information on the calculation and determination of the PEC is given in Chapter 6 Behaviour and fate in the environment; behaviour in surface water, sediment and sewage treatment plant (STP).

7) The following criteria must be met:
An active substance and each of its transformation products have in surface water a concentration lower than:

- 0.01 of the LC_{50} for acute toxicity to fish
- 0.01 of the EC_{50} for acute toxicity to aquatic invertebrates
- 0.1 of the EC50 for algae
- 0.1 of the EC50 for aquatic plants

- 0.1 of the NOEC for long-term toxicity to fish and aquatic invertebrates
- 0.1 of the NOEC for long-term toxicity to sediment dwelling organisms

The risk is low if these criteria are met. The product can be authorised in as far as the risk to aquatic and sediment dwelling organisms is concerned.

8&9)A risk is present if the criteria as given under 6) are not met. Such a use is considered as not permissible, unless a further (adequate) risk evaluation shows that there are no unacceptable direct or indirect effects for aquatic and sediment dwelling organisms and organisms that depend on aquatic ecosystems (higher tier). The higher tier risk assessment is performed according to Regulation (EC) 1107/2009 and hence the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290).

10) Research is requested to determine species accumulation and elimination, i.e., the extent to which the substances in question are directly absorbed from the water, retained (bioconcentration factor BCF), and excreted by the organism.
The octanol/water partition coefficient (Kow) (283/2013 point 2.7) of a substance gives information about the bioaccumulating capacity of a substance. Where the logKow of a substance < 3, experimental research is not required. For such organic substances sufficient insight into the bioaccumulating capacity can be obtained from the octanol/water partition coefficient (Kow) (283/2013 point 2.7), for which the following formula (Veith et al., 1979[5]) is used:

logBCF = 0.85*logKow - 0.70 (L/kg)

Experimental research with fish is required for substances with a logKow > 3 (283/2013 point 8.2.2.3), unless the substance is considered not stable, i.e. , more than 90% loss of the original substance over 24 h via hydrolysis. BCF_k (kinetic bioconcentration factor) values should be reported as growth-corrected and as lipid-normalised values (default 5% lipid content).

11) An active substance of a plant protection product and each of its transformation products have a maximum bioconcentration factor lower than:
a. 1000 for readily biodegradable active substances, or
b. 100 for active substances that are not readily biodegradable.

12) Where this is not the case, a risk is present and the use is not permissible, unless a further (adequate) risk evaluation shows that there are no unacceptable direct or indirect effects for aquatic and sediment dwelling organisms and organisms that depend on aquatic ecosystems (higher tier). The higher tier risk assessment is performed according to Regulation (EC) 1107/2009 and hence the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290).

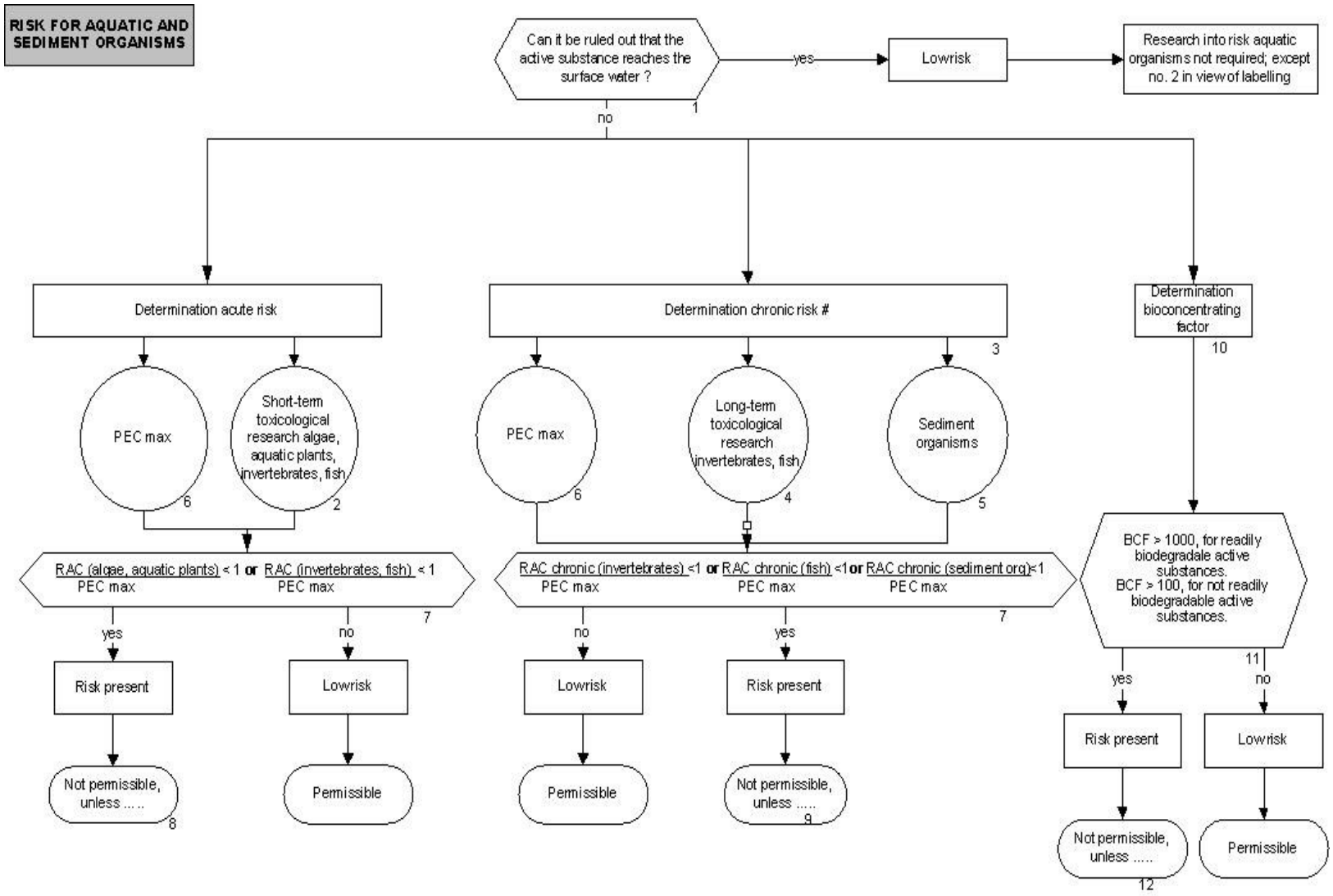
For the higher tier risk assessment triggered by exceeding of the first tier TER values several possibilities exist, e.g.:
- geomean approach;
- SSD approach;
- modified exposure tests;

[5] Veith, G.D., D.L. Defoe and B.V. Bergstedt. 1979. Measuring and estimating the bioconcentration factor of chemicals on fish. J. Fish. Res. Board Can. 36: 1040-1048.

- micro-/mesocosm studies.

For more information about these studies and approaches reference is made to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013; 11(7):3290).

A TER is calculated based on the relevant higher tier Regulation (EC) 1107/2009 toxicity endpoint and the relevant PEC in the edge-of-field ditch. The toxicity endpoint depends on the higher tier approach which is chosen; modified exposure studies are directed on taking into account fate processes under natural conditions; the endpoint will change but in principle the same safety factor will be applied as in the first tier risk assessment. The SSD approach yields an endpoint which is the mean HC5 value with a certain safety factor. More information can be found in the EFSA aquatic guidance.
A micro-/mesocosm study yields a NOEC or NOEAEC. For risk assessment a safety factor is applied (trigger value). The safety factor depends on the endpoint and on the number of studies available. For more information see the EFSA aquatic guidance.
If the TER is lower than the trigger value, a risk is still present; drift reduction measures may be applied. If these are sufficient the risk in the edge-of-field ditch is acceptable.



Unless there is 90% or more loss of the original substance over 24 hours via hydrolysis

Appendix 2: Test Validity of OECD 201 (algae; species other than recommended): stepwise approach when not met

Background information

This working agreement provides guidance in situations where the performance criteria (validity of the test) are not met in tests performed with algal species other than recommended[6] by OECD 201. As result of a species-specific growth pattern the performance criteria might not be met. A stepwise approach was developped to analyse the response of the unexposed control cultures which can help to decide whether or not the test (with species other than recommended) can be considered valid. OECD 201 states the following concerning test validation:

VALIDITY OF THE TEST

11. For the test to be valid, the following performance criteria should be met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day^{-1}. For the most frequently used species the growth rate is usually substantially higher (see Annex 2). This criterion may not be met when species that grow slower than those listed in Annex 2 are used. In this case, the test period should be extended to obtain at least a 16-fold growth in control cultures, while the growth has to be exponential throughout the test period. The test period may be shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached.

- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures (See Annex 1 under "coefficient of variation") must not exceed 35%. See paragraph 49 for the calculation of section-by-section specific growth rate. This criterion applies to the mean value of coefficients of variation calculated for replicate control cultures.

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%.

18. If other species are used, the strain and/or origin should be reported. Confirm that exponential growth of the selected test alga can be maintained throughout the test period under the prevailing conditions.

Please note that *less frequently tested species* (e.g., CV of average specif growth rates) concern the recommended species of diatom and cyanobacteria groups.

Afspraak voor risicobeoordeling

In cases where the validity of the test (OECD 201) is not met the following stepwise approach should be followed:

A1 The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. If not, the test is not valid.

If exponential growth is observed → B1

[6] recommended species: the green algae: *P. subcapitata* and *D. subspicatus*, the diatom: *N. pelliculosa* and the cyanobacteria: *A. flos-aquae* and *S. leopolienesis*). Please note that: *Pseudokirchneriella subcapitata* is called *Raphidocelis subcapitata* now, and was known as *Selenastrum capricornutum. Desmodesmus subspicatus* was formerly known as *Scenedesmus subspicatus*.

B1 Look at the mean CV[7] for section-by-section specific growth rates (e.g., assess effects on the pattern of growth).

The growth factor per day = biomass (number of cells) at 24h ÷ 0h, 48h ÷ 24h and 72h ÷ 48h). Please note that the product of these daily growth factors equals the overall growth factor of A1.

In case growth factors are variable this might indicate that the observed pattern might be species-specific.

Consider the growth factors of each time frame (e.g. 0-24, 24-48 and 48-72 hours) in combination with that of the whole period. Is growth steady over the time course or is it decreasing? In that case have a look at the growth factor in the last 24 hours. Is it still reasonable (growth factor > ~2.5).

A daily growth factor of ~2.5 equals a 72-hours growth factor of 16, when considering constant exponential growth, which is the minimum requirement of OECD 201 (see A1). Therefore, a growth factor of ~2.5 in the last 24 hours is only just acceptable. However, this should not be applied too strict. Always look at the whole picture. A flattening growth is often observed in studies with non-standard species, also in the case of standard test species.

A cut-off value up to 50% can be considered acceptable.

The cut-off value of 35% of OECD 201 is drawn up and validated for the recommended species. t is therefore, considered secondary in case of other species. In OECD 238 and 239 the following is stated: ECx values are only reliable and appropriate in tests where coefficients of variation in the control fall below the effect level being estimated (after OECD 238 and 239). Therefore, coefficients of variation should be < 50% for robust estimation of an EC_{50}.

B2 Look at the CV of average specific growth rates (e.g., variation between replicates).

If the CV of average specific growth rate is acceptable (e.g., < 10%) the variation between replicates is acceptable and the observed variable growth rate is most likely species-specific.In case the CV is much lower than the cut-off value this could support the validity of the endpoints.

Substantial differences between section-by-section specific growth rate and average specific growth rate indicates a deviation from constant exponential growth and close examination of the growth curve is warranted.

B3 Are the confidence intervals of the E_rC_{50} and E_yC_{50} wide or narrow (e.g., degree of precision). In case it is narrow, this could support the validity of the study endpoints. For this the normalised width of the confidence interval approach of

[7] CV = variability of a parameter

EFSA Supporting Publication 2019:EN-1673 (paragraph 2.1 and table E9 of Appendix E) should be used.

NW = *(EC_x, upp – EC_x, low) / EC_x, med*

NW	**Rating**
< 0.2	Excellent
0.2 – 0.5	Good
< 1	Fair
< 2	Poor
≥ 2	Bad

B4 Weigh the overall quality of the study (is the study well performed, e.g. according to the general requirements of OECD 201, was the duration of the study long enough? if prolonged would it have resulted in a lower EC_{50}?). Relevant issues should be discussed.

Use B1 to B4 in a WoE approach to accept or reject the exceedance of the mean CV for section by section specific growth rate and / or the CV of average specific growth rate.

Appendix 3: Proposal for the 6th Central zone harmonization workshop, June 2022. SSD and its exemplary use for aquatic organisms and non-target terrestrial plants- data selection and statistical procedure -

List of abbreviations

AGD	Aquatic Guidance Document
a.s.	Active substance
CI	Confidence Interval
cZone	Central Zone
d.w.	Dry weight
EC	Effect Concentration
ED	Effective Dose
EP	Endpoint
ER	Effect Rate
HC_5	5th percentile of the Hazard Concentration
HR_5	5th percentile of the Hazard Rate
ini	Initial concentration
LC	Lethal Concentration
$LLHC_5$	Lower limit of the confidence interval of the hazardous concentration for 5 % of the species of an SSD
m.m.	mean measured concentration
MoA	Mode of Action
Nom	Nominal concentration
NOEC	No Observed Effect Concentration
NTTP	Non-Target Terrestrial Plants
OECD	Organisation for Economic Co-operation and Development
RA	Risk Assessment
RAR	Regulatory Acceptable Rate
SANCO	Health and Consumer Protection of the European Commission
SE	Seedling Emergence
SSD	Species Sensitivity Distribution
VV	Vegetative Vigour
zRMS	Zonal Rapporteur Member State

Background

This document aims to give detailed guidance for calculating an SSD in ecological risk assessment. Beside some general aspects on the SSD approach, this document deals with the application of the SSD for aquatic organisms and for NTTP. Therefore, it also points out some specific aspects to consider for each of these groups
Recommendations presented in the current document follow those reported in chapter 8. of the Aquatic Guidance Document (AGD) (EFSA Journal 2013;11(7):3290). When judged necessary, further explanations were added based on concrete experiences gained from the

regulatory practice.
The focus is on the selection of data and the statistical procedure.

The application of the SSD approach for NTTP is described in the Guidance Document on Terrestrial Ecotoxicology (TGD, SANCO/10329/2002 rev 2 final). But this document needs to be urgently revised including the section related to SSD that do not provide much recommendations. Therefore, in this document the recommendations provided for aquatic organisms (EFSA 2013) are analysed in order to assess if they could be applied to NTTP.

To facilitate the reading, specific approaches concerning aquatic organisms and NTTP are presented in separate columns.

Crucial aspects for each section
Data selection:

- For aquatic organisms, follow recommendations of EFSA (2013). Special emphasis regarding insecticides, herbicides and fungicides are given in chapters 8.4.3.1, 8.4.3.2 and 8.4.3.3 of the AGD, respectively.
- For NTTP follow recommendations of SANCO/10329/2002 rev 2 final given in chapter 7.1.
- Be aware of the representativeness of the taxa tested regarding the specific MoA of the a.s.
- Select the same estimates (e.g. EC_{10}; ER_{50}) and preferentially identical variables to calculate an SSD. Note that similar variables as dry weight and fresh weight might be mixed to assess the variable biomass for primary producers (aquatic and NTTP) or for invertebrates.
- EPs should also be expressed with same concentration or rate units.
- Verify that the EPs used are reliable (e.g., calculate the normalised CI around the EP)
- Different test designs – i.e. Tier 1 and tier 2C data (aquatic organisms) and VV and SE data or laboratory and field or semi-field studies (NTTP) cannot be mixed.

Statistical procedure:
- Check detailed procedure regarding censored EP and make sure that the minimum data requirement to conduct an SSD for this organism group is fulfilled.
- Check if the data is unimodal and fits adequately the assumed distribution (e.g. log-normal or log-logistic)
- Check the reliability of the results, with a particular emphasis on the fit and thus choice of the model (log-normal, log-logit, Weibull…)

Special case of primary Producer in aquatic
- If the minimum data requirement is not met because of too many censored E_rC_{50}, instead of going back to lower Tier, we propose the possibility to calculate the SSD with E_yC_{50} values.

Application examples:
- Example on how to report the results as zRMS (approaches 1 and 2)

Selection of Toxicity Data

Effect Side
Selecting toxicity data on the basis of toxic mode of action of the substance

Be aware of the representativeness of the taxa tested regarding the specific MoA of the substance.

Aquatic organisms	NTTP
No deviation to AGD. Follow chapters 8.4.2 and 8.4.3 (p. 92): "*If, for example, the First tier toxicity value for Chironomus is an order of magnitude lower than that of Daphnia and/or Americamysis bahia, it is recommended to construct, in the first instance, a SSD with toxicity data for insects, or to explore which insects and crustaceans (e.g. macro-crustaceans) can be combined in a single SSD on the basis of all relevant information available*." (AGD 2013) As another example for primary producers, in case of auxin herbicides, dicotyledonous species are usually more sensitive. Thus, check that this group is sufficiently represented in the data set and consider constructing an SSD with only dicotyledonous species. In addition, check if rooted macrophytes are sufficiently represented as well.	No deviation to SANCO/10329/2002 rev 2 final (chapter 7.1, Tier2): "*In order to generate data that are useful for probabilistic approaches there should not be a focus exclusively on species assumed to be the most sensitive. If, from the screening data, a specific mode of action is evident, or strong differences in the species sensitivities are identified, this evidence should be used in the selection of the appropriate test species*." E.g., if the First-tier toxicity values are lower for dicotyledonous (which might be the case for auxin herbicides), it might be recommended to construct, in the first instance, an SSD with toxicity data for this group if possible.

Further information regarding the sensitivity of the non-target organisms against the a.s. under evaluation can be found in the respective EU-LoEP(s)/D(R)AR(s) and in addition for NTTP in the efficacy data (c.f., CA B3 or D(R)AR Vol.3 CA/CP -B.3 for zonal and EU applications, respectively). Note that screening data submitted for the evaluation of herbicidal activity of metabolites might also be informative.

Estimates and variables

Terminology:

Endpoint: is the combination of an estimate and a measured variable.
Estimates: is referring to the magnitude of effect described (e.g., ECx, NOEC …)
Variables: is the response variable measured

Aquatic organisms	NTTP
Estimates	
E_rC_{50}: EC_{50} calculated with growth rate EyC_{50}: EC_{50} calculated with yield EbC_{50}: EC_{50} calculated with area under the curve EC_{10}: e.g. reproduction, body weight EC_{50}/LC_{50}	ER_{50}

Variables	
Algae: cell counts (surrogate for biomass and thus most frequently called “biomass”) Macrophytes: frond number, frond area, biomass wet weight, biomass dry weight etc...	Seedling emergence: emergence, mortality, biomass (fresh weight/ dry weight), plant height, visual injury Vegetative vigour: biomass (fresh weight/ dry weight), plant height, mortality, visual injury
Selection of estimates and variables in SSD calculation	
Select identical estimates and preferentially identical measured variables However, for aquatic and terrestrial primary producers, wet weight and dry weight might be pooled to assess the variable biomass (see section 7.1).	
Specific recommendations available for aquatic organisms: **Acute risk assessment:** The AGD sees the possibility to construct an SSD based on NOEC/EC_{10} values. However, no further recommendations are provided regarding the decision making for regulation (*i.e.*, which approach should be then preferred?). In general, LC/EC_{50} values are most robust and reliable and should be used for constructing an SSD. An SSD based on NOEC/EC_{10} values might be suitable in cases when LC/EC_{50} are less reliable (e.g. in case of very steep dose-response curves).	No further specific recommendations available. The SSD is simulated with ER_{50} values as recommended in SANCO (2002)
Chronic risk assessment: Classically, NOEC or EC_{10} values are available for multiple biological variables (e.g., reproduction, body weight, body length...). Select same estimates (e.g. only EC_{10} values) and preferentially identical biological variables as underlying data for an SSD. EC_{10} is the preferred estimate.	

Exposure Side
Test design

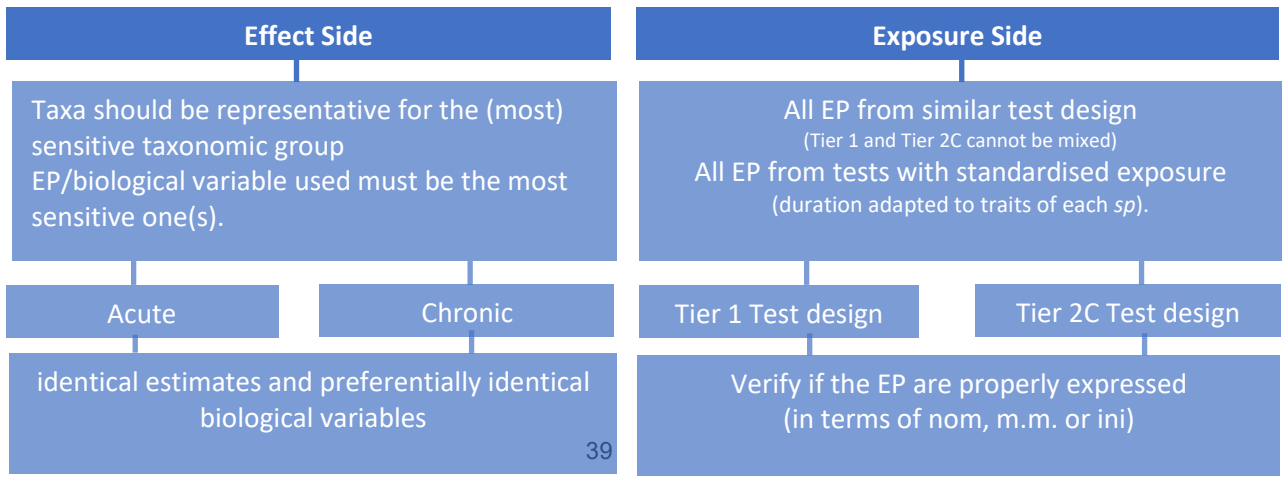
Aquatic organisms	NTTP
Different test designs cannot be mixed	
Note that Tier 1 and Tier 2C data cannot be mixed within an SSD. **SSD based on Tier 1 data:** All endpoints used for the SSD are derived	ER_{50} cannot be mixed within an SSD if they are from - (i) SE and VV tests or - (ii) from tests having different application methods (sprayed *versus* mixed to the

from standard (i.e. OECD) tests; however please note that the duration of the test might differ according to the traits of the tested species (e.g. 48 h for *D. magna* but 96 h for *A. bahia*), as mentioned in the AGD under 8.4.2. Note that for certain insect growth regulators, the standard duration (48–96 hours) of the acute toxicity test may not be sufficient, since latency of effects may occur (refer to AGD 2013, p. 94). **SSD based on Tier 2C data**: In theory, it is possible to calculate an SSD with EPs derived from refined exposure tests (e.g. pulses and/or water-sediments lab tests, i.e. Tier 2 C). In practice, this is problematic since there are a number of critical issues for refined exposure test. In such case, it has to be carefully verified that each single refined exposure test is acceptable for risk assessment.	soil) or - (iii) from tests having different duration or - (iv) from tests with different settings (e.g. from laboratory and semi- or field conditions)

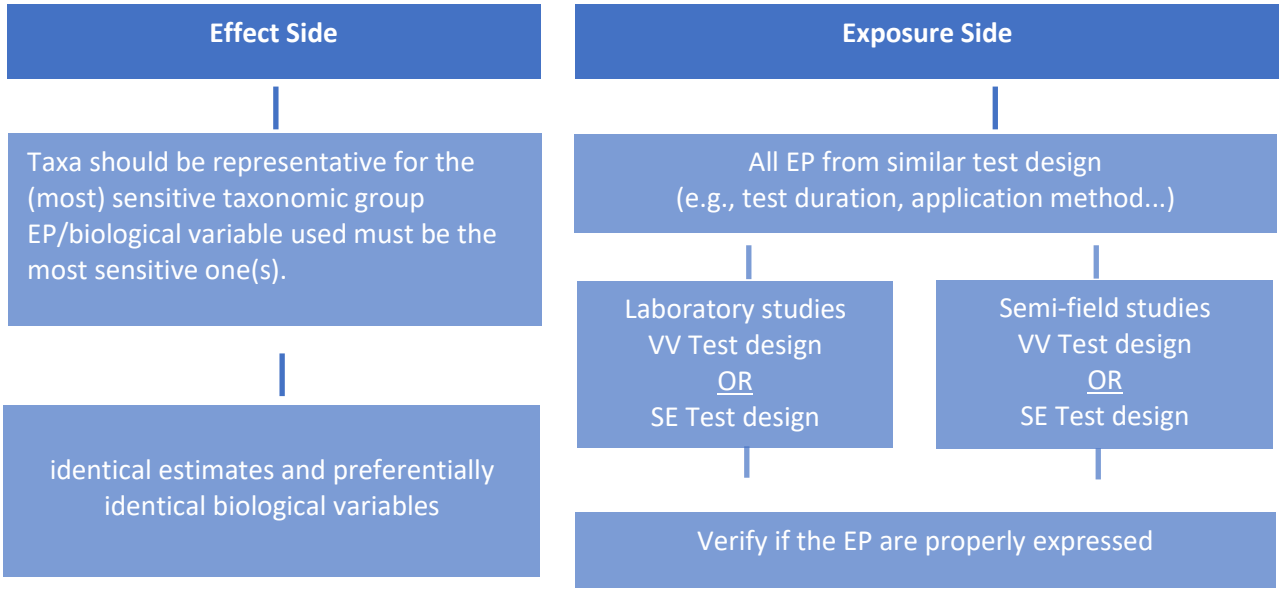
Expression of endpoints

Aquatic organisms	NTTP
For both Tier 1 and Tier 2C tests, carefully verify that the EP is properly expressed in terms of e.g., nom, m.m., or ini. concentrations. . Please refer to section 3.1 in EFSA Supporting publication 2015:EN-924 as well as to Appendix J in EFSA Supporting publication 2019:EN-1673.	All EP should be expressed in the same unit (e.g. in g product / ha).

Summary schemes for data selection
Scheme for data selection for aquatic



Scheme for data selection for NTTP



Statistical procedure

Pooling different types of endpoints

For terminology, please refer to section 5.3.2.

Estimates: Cannot be mixed within an SSD.

Variables: Should in general not be mixed. In case the more sensitive biological variable differs between species (e.g. reproduction for *D. magna versus* body weight for *A. bahia* or plant height *versus* plant biomass for NTTP), different SSD have to be calculated for each variable.
There is an exception for identical variables, such as wet weight and dry weight for aquatic and terrestrial plants (see section 9.1). If available variables differ only slightly, they might be mixed to construct an SSD (e.g. fresh weight and dry weight for primary producers or invertebrates.

For the special case of aquatic primary producers, please refer to section 7.

Censored endpoints

Some endpoints might be expressed as censored values, i.e. less than (<) or greater than (>) values.
Censored EPs are also referred as “unbound values” in AGD.

In principle, censored EP can be dealt as recommended in EFSA (2013),” *i.*e. to include censored EP as “= value” in the SSD data set, only when those EP are out of the range of sensitivity of the species tested. Censored EP within the range of sensitivity of the species tested should be excluded from calculation”. Additionally, EFSA 2013 recommends to conduct an SSD with this potentially restricted data set, only if the minimum number of EP

needed for calculation is still required (*i.e.*, n ≥ 8 and n ≥ 5 for fish). See also section 6.3.1 and 2. below for more details. In case this minimum requirement is not fulfilled, the SSD refinement option should be rejected.

We suggest to enlarge these recommendations to NTTP. This means that in case censored ER_{50} are part of the data set, they should be treated as recommended in EFSA (2013), i.e. > or < ER_{50} should be further considered only when they are out of the range of sensitivity of the species tested. The minimum number of EP available for SSD calculation should be n ≥ 6 as reported in SANCO/10329/2002 rev 2 final.

Calculation

Following calculation methods for SSD simulations are possible:

- ETX program: It is the usual approach considering lognormal models and non-censored endpoints.
- R-package fitdistrplus: it is developed by Sandrine Charles from the University of Lyon and implemented in the platform MOSAIC (https://mosaic.univ-lyon1.fr/ssd)[8]. This program has many advantages since:
  - (i) it considers censored values,
  - (ii) it takes confidence interval into account, which is particularly relevant when uncertainties around the EP exist (i.e., large CIs, which often occur in case of NTTP); with this approach, relevant available information regarding the robustness and reliability of the single estimates is included in the SSD, and
  - (iii) it is possible to apply different models (log-normal, log-logistic, Weibull…), whereas in ETX only the log-normal model is used.

UBA developed an Excel Tool connected with R to implement the R-package fitdistrplus. It has been published by UBA on the EFSA Knowledge Junction platform Zenodo on 26 October 2022: https://zenodo.org/record/7249239

Pre-requisite for SSD calculation	
Aquatic organisms	**NTTP**
Sufficient representative toxicity data according to the AGD must be available (see AGD p. 92-93; i.e. n ≥ 5 (only for fish) or n ≥ 8) after that censored EP in the range of species sensitivity have been excluded from data set.	Sufficient representative toxicity data according to SANCO/10329/2002 rev 2 final must be available, the minimum requirement is n ≥ 6 for NTTP. Thus, we suggest a minimum of 6 available ER_{50} after that censored EP in the range of species sensitivity have been excluded from data set.

For calculation, we propose:

- Approach 1: to follow EFSA (2013) that recommends to simulate an SSD only with censored EP that are out of the range of species sensitivity of non-censored EPs (see below)
- Approach 2: additionally, to simulate an SSD with the whole data set (*i.e.*, using all censored and non-censored EP) by using the R-package fitdistrplus (see below). Indeed, in case censored endpoints and/or confidence intervals are available in the

[8] Kon Kam King G. Veber P., Charles S., Delignette-Muller M. L. (2014) MOSAIC_SSD: A new web tool for species sensitivity distribution to include censored data by maximum likelihood. Environmental Toxicology and Chemistry 33(9) 2133-2139

SSD data set, approach 2 (R-package fitdistrplus) might be more appropriate more reliable, as the results of the simulations consider more information than only the EP. See also Green, 2016 and 2018[9,10].However, results of the R-package fitdistrplus simulations might be more complex to evaluate.

Decision on which approach (i.e., 1 or 2) as well as which simulation models is the most appropriate (*i.e.*, log-normal, log-logistic, Weibull…) should be done on a case-by-case basis considering the recommendations provided in section 5.4. In case of the inclusion of "bigger than" censored values (e.g., LC_{50} > 10 mg a.s./L), the approach with *fitdistrplus* provides in our view more reliable results as it considers intervals as such (e.g., LC_{50} > 10 mg/L a.s. belongs to the interval 10; +∞; see below)

Approach 1: Data selection according to EFSA (AGD 2013)

Data are selected excluding censored EPs in the range of species sensitivity and the SSD is performed according to AGD (p. 92-93). Censored EPs out of the range of species sensitivity are considered as non-censored EPs in the SSD (e.g. > 42 mg/L is considered as 42 mg/L).

Although no specific program for SSD calculation is recommended in the AGD and in SANCO (2002), the program ETX is commonly used by MS.
However, we also recommend to use the R-package fitdistrplus as it can consider more than only the lognormal model. Moreover, this approach also takes confidence intervals of single estimates into account, which might be particularly relevant for NTTP (see 5.3 above).
Take decision on which model is the most appropriate according to section 5.4.1.

[9] Green (2016) Species Sensitivity Distribution with censored values. SETAC (Nantes) 2016.

[10] Green, Springer & Holbech (2018) Statistical Analysis of Ecotoxicity Studies ISBN: 978-1-119-48881-1| July 2018| 416 Pages|

Approach 2: Including all censored EP

First, data are selected excluding censored EPs in the range of species sensitivity as in approach 1 (see 5.2). Then, Approach 2 is applied only if sufficient toxicity data according to EFSA (2013) and SANCO (2002) are still available.

In approach 2, data used for the SSD include all censored EP (i.e., within and outside the range of species sensitivity of non-censored EPs) and censored EP are considered as such in the SSD (e.g., LC_{50} > 10 mg a.s. is used as interval : 10; +∞). The SSD is modelled with the R-package fitdistrplus (e.g., available in the platform MOSAIC).

The particularity of the R-package fitdistrplus is that the program can treat "interval values". This means that the package can treat Confidence Intervals (CI) as well as Censored Endpoints.
Indeed, censored values belong to an interval. E.g., LC_{50} > 10 mg a.s./L belongs to the interval [10; +∞[; LC_{50} < 10 mg/L belong to the interval]-∞; 10].

(i) Uncertainty: Perform the SSD analysis with the Confidence Intervals (CI) of EP.
(ii) Censored values: Enter all censored endpoints as an interval as described just above.

When reporting the results with R-package fitdistrplus add the following:
"*SSD calculation is conducted with the R-package fitdistrplus, which allows including censored data and consideration of confidence intervals (for details see https://doi.org/10.1002/etc.2644*)

Note that a detailed example of Approach 2 is given in section 8.

Reliability check
Model selection and model fit

If a calculation method is chosen that enables the application of different models (such as the R-package fitdistrplus), it is advised to fit several models (log-normal, log-logistic, Weibull…) and to compare different criteria to select the model (e.g. Akaike Information Criterion (AIC)). The best fitting model should be selected. Also test statistics from the goodness of fit estimations can be considered for model comparison.

The quality of the model, especially the fit of the underlying distribution, should be checked (i) by visual inspection of the output graph and (ii) if possible the qq-plot (*e.g.* does the model reflect the assumed distribution of the EPs?). If available goodness of fit estimations such as the Cramér–von Mises test can be considered to check if the underlying distribution is significantly deviating from the data set. Note that the check of the model fit and selection might result in the rejection of the SSD simulation.

Furthermore, we highly recommended to check the width of the confidence interval around the median HC_5. Indeed, the model underlying an SSD is always linked with uncertainties expressed in an interval – the confidence interval. Thus, the confidence interval provides the uncertainty of the model and is dependent on the model structure, data structure, and fitting method. Given the uncertainty of the model, the median HC5 (or just HC5) is estimated to be

correct with a probability of 50%, whereas the lower and upper limit HC5 simulate the HC5 with a probability of 95%. It is important to notice that the confidence interval does not provide the confidence existing around the median HC5 but rather provide confidence in the model fit, given that the underlying assumptions of the model are met.
E.g., we advise to compare the position of the $LLHC_5$ to the median HC_5. In case the $LLHC_5$ is less than 1/3 of the median HC_5, reliability and/or protectiveness of the simulated median HC_5 might be questioned (i.*e*., consider rejecting the SSD or eventually select a higher AF or regulate on another Effective Dose proposed below in 6.4.2 below). This is also addressed in the AGD 2013, since it is suggested under section 2.1.4.2 to consider that for "*The lower limit value of the HC5*. If the lower limit HC5 derived from the curve is less than 1/3 of the median HC5, a higher AF in the proposed range may be warranted."

Note also that:

(i) Violation of goodness of fit might be acceptable if the distribution of the data in the lower tail of the SSD is considered as relatively conservative (see AGD 8.4.1).
(ii) In some cases, a split of dataset and conduction of specific SSD might be required (see section 5.3.1 of this position paper or 8.4.1 and 8.4.3 of the AGD).

Choice of the AF (aquatic organisms) or relevant Effective Dose (NTTP)

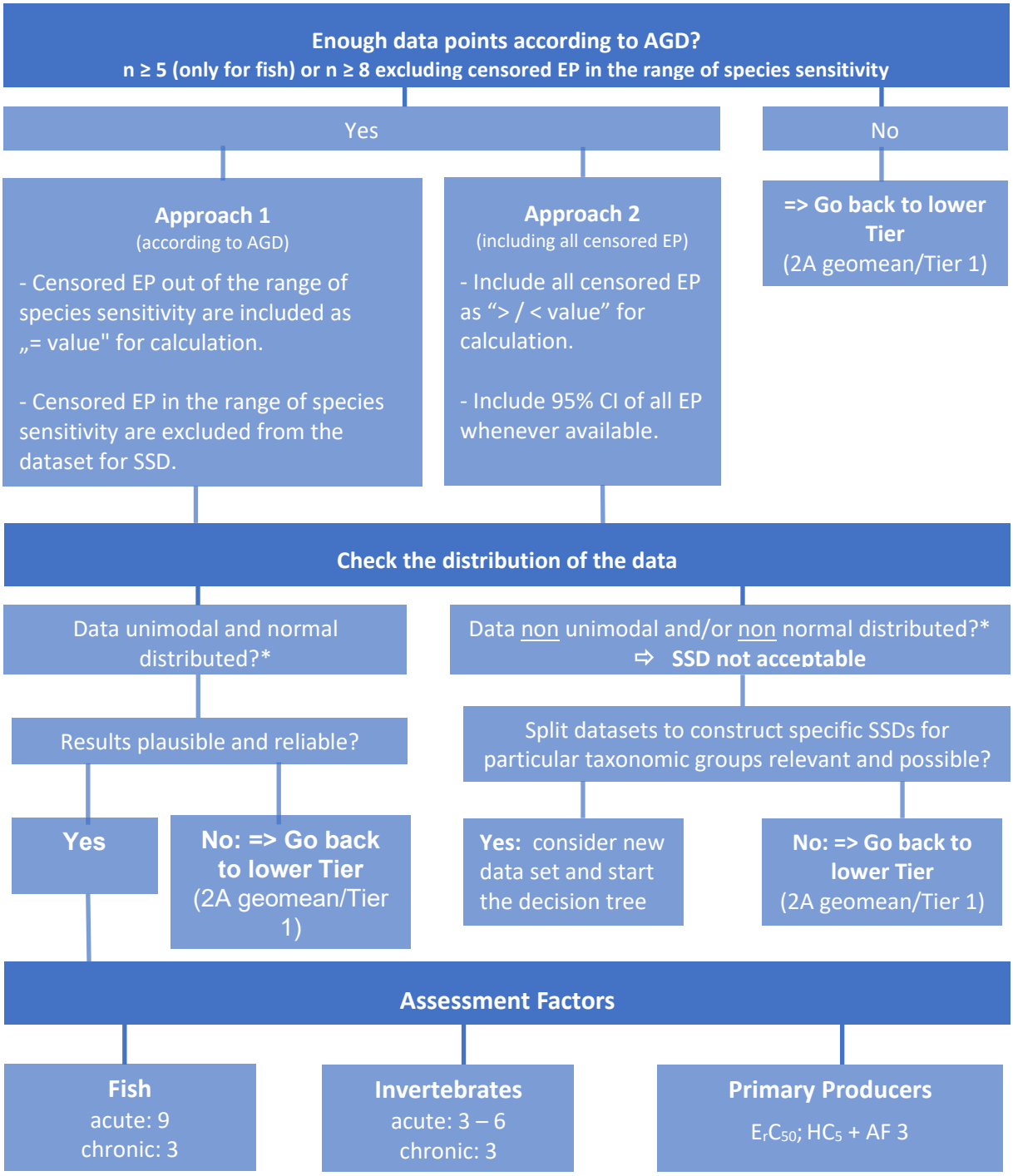
For aquatic organisms, we follow the recommendations provided in EFSA (2013).

For NTTP, SANCO (2002) reports that: "if *the ED50 (Effective dose 50 %) for less than 5 % of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable*", which corresponds to an AF =1. However, SANCO 2002 does not precise whether the Effective Dose should rely on the median or LLHR5. Thus, we suggest to carefully check which ED (median or $LLHR_5$) is the most appropriate according to some recommendations provided in the check list reported in the table below. Note that these recommendations are adapted from those provided in EFSA (2013).

Aquatic organisms	NTTP
Follow recommendations as provided in EFSA (2013) section 2.1.4.2 (p. 20)	We propose to adapt the recommendations provided in EFSA (2013) in section 2.1.4.2, as follow: - If the $LLHR_5$ is less than 1/3 of the median HR_5, then the protectiveness of the median HR_5 should be questioned; the $LLHR_5$ might me better appropriate. - If the median HR_5 is lower than the RAR derived at the lower Tier (i.e., lowest ER_{50}/5), then the relevance of the SSD approach should be questioned. Indeed, in principle following the tiered approach, a RAR higher Tier should be higher than a RAR lower Tier. - Consider the position of the toxicity data in the lower part of the tail of the SSD (around the HR_5). Indeed overall, if they are positioned on the right side of the SSD curve, the derived HR_5 estimate may be considered relatively “conservative” for the most sensitive species. This may indicate that the median HR5 is appropriate. In contrast, if in the lower tail the toxicity data are, overall, positioned on the left side of the SSD curve, this may be a reason to question the protectiveness of the median HR_5. $LLHR_5$ might me better appropriate. - *The steepness of the SSD curve*. In the case of a relatively steep SSD curve (e.g. less than a factor of 100 between lowest and highest ER_{50} value used to construct the SSD curve), the $LLHR_5$ might me better appropriate since exposure concentrations that exceed the RAR may have ecotoxicological consequences for a larger number of taxa. - *Read-across information for compounds with a similar toxic mode of action*. For a PPP with a well-known mode of action, sufficient information on related compounds may be available that allows the evaluation of the predictive value of the median HR_5 and/or lower limit of the HR_5 (e.g. known strong sensitivity of some species but not tested with the PPP under evaluation). This information may be used to decide on the protectiveness of median HR_5 vs $LLHR_5$ or of the whole SSD approach.

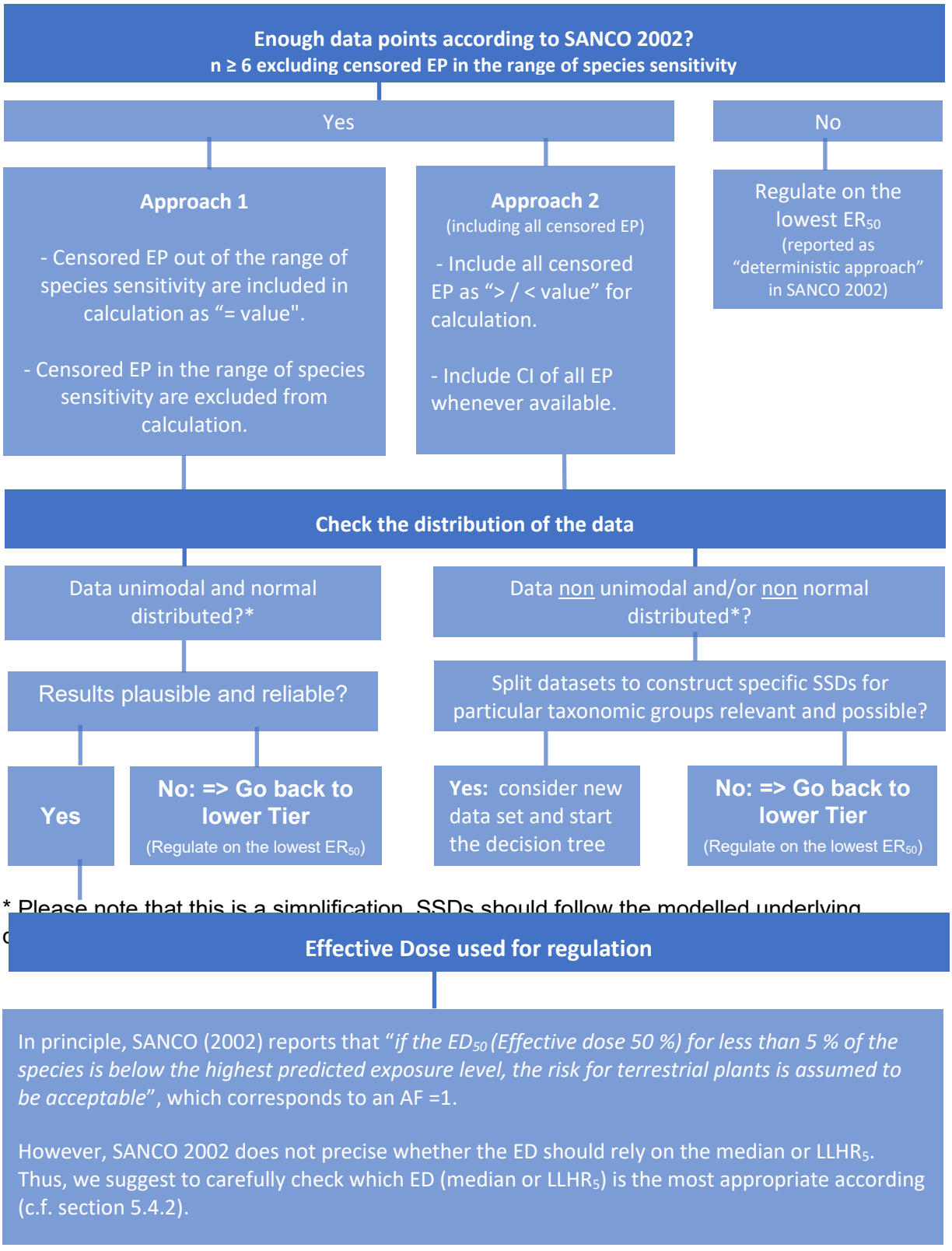
Summary schemes of the SSD procedure

Aquatic organisms: scheme for statistical procedure



* Please note that this is a simplification. SSDs should follow the modelled underlying distribution (usually log-logistic or log-normal, which are similar to the normal distribution).

NTTP: scheme for statistical procedure



Special case of primary producers in aquatic

Pooling endpoints for algae and macrophytes

Variables for aquatic plants do often differ and the AGD is not specific regarding the pooling of such variables. In case several variables are measured, preferably calculate the SSD for each variable independently and regulate on the lowest HC_5. In case only different variables are measured, a pragmatic approach is used to separate the variables for primary producers in two categories:

i) "weight related" (dry weight, wet weight, biomass)
ii) "growth related" (frond number, shoot length, shoot number…)

SSDs can only be conducted for variables from one category (*i.e.*, i or ii).

The AGD recommends to pool algae and macrophytes in a single SSD for primary producers only under the following conditions:

i) In Tier 1 tests, data (EP) on macrophytes and algae differ less than a factor of 10.
ii) No difference in mode of action leading to a sensitivity difference is described or observed (i.e. algae and macrophytes should be randomly distributed along the SSD curve).

Censored endpoints

The occurrence of censored endpoints is usually more common for the E_rC_{50} estimate than for the E_yC_{50} (or E_bC_{50}) estimates. EFSA (2013) is preferably using the E_rC_{50} estimates but at the same time, EFSA is excluding censored EP from the SSD analysis when they are in the range of sensitivity of uncensored endpoints. Therefore, this might lead in some cases to a restricted data set (n <8) and no possibility to apply the SSD

In case the dataset is too small for an E_rC_{50}-SSD analysis (if for E_rC_{50} EP, n < 8 once censored EP in the range of sensitivity have been excluded), alternatively an E_yC_{50}-SSD might be calculated (if for E_yC50 EP, n ≥ 8, as E_yC50 EP are usually not (or less) frequently censored).

Application examples

Higher tier refinement – SSD aquatic invertebrates

The applicant proposed to refine the short-term risk to aquatic invertebrates by conducting an SSD (Tier 2b). Acute data on aquatic invertebrates (either 48 or 96 hours) are shown in the Table below.

Table: Short-term toxicity data to aquatic invertebrates.

Species	EC_{50} in mg/L	95% confidence intervals
Daphnia magna	0.48	0.34 – 0.69
Asellus aquaticus	3.43	2.75 – 4.26
Gammarus pulex	0.23	0.20–0.25
Neocaridina denticulata	>5	Not available
Procambarus sp.	1.2	0.75–1.93

Chironomus riparius	0.44	0.32–0.59
Anax imperator	1.63	Not available
Cloeon dipterum	0.31	0.26–0.38
Notonecta maculata	2.78	Not available
Paraponyx stratiotata	>4	Not available
Plea minutissima	1.29	0.92–1.80
Ranatra linearis	3.33	2.95–3.76
Sialis lutaria	0.96	Not available

Two approaches are used to model the HC_5:

- The inclusion of censored values outside the range of species sensitivity as non-censored values, using software ETX fitting a log-normal distribution to the toxicity data (i.e., equivalent to Approach 1 in 5.3.1) and
- The inclusion of all censored data and the consideration of confidence intervals, using the R-package *fitdistrplus* (for details see http://ubanet/websites/IV1.3/SG1/FG_Aquatik/FGDokumente/Background%20information/documents-%20publications/Kon%20Kam%20King%20et%20al.%20-%202014%20-%20Environmental%20toxicology%20and%20chemistry%20SETAC.pdf)

The available confidence intervals and censored endpoints shown in the Table are taken into account when fitting the SSD model with the R-package *fitdistrplus* (version 1.0.14).

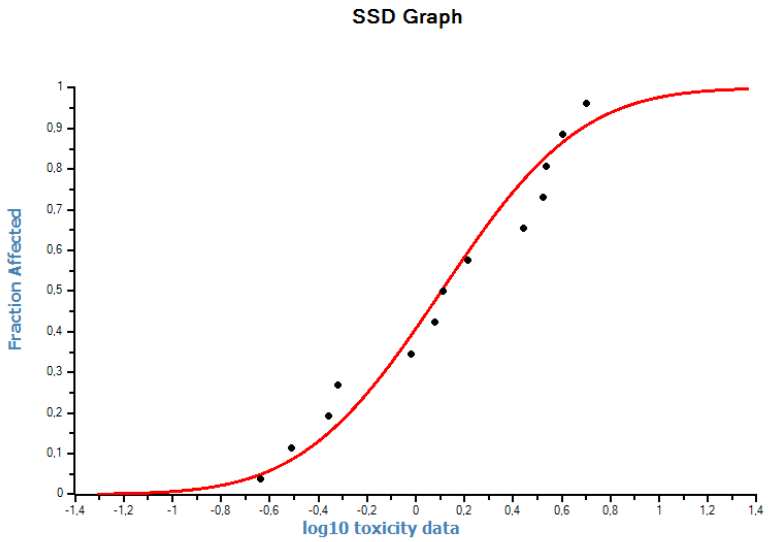
Results ETX:

Test for normality:

Test	**Significance level α = 0.05**
Anderson-Darling	accepted

HC5: 0.223 mg/L (CI: 0.08035 – 0.416)

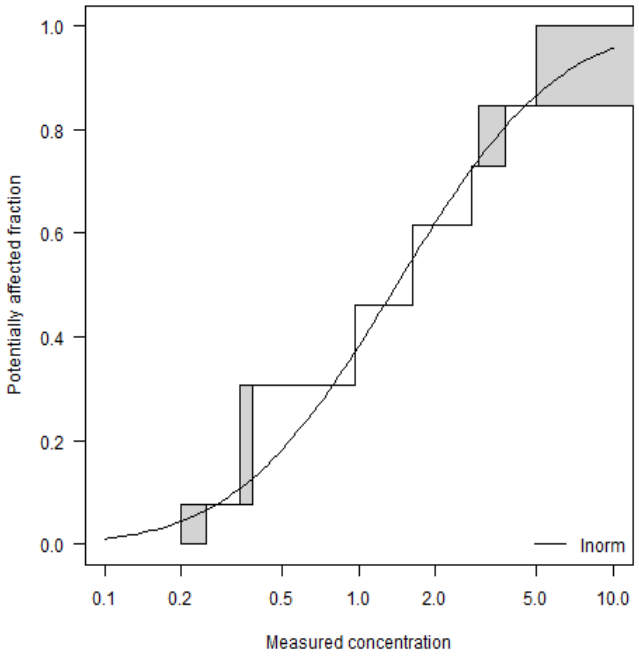
The fitted model by ETX is shown in the following plot.



Results R-package *fitdistrplus* (log-logistic model)*:*
Q-Q plot (not displayed here) indicates that a log-normal distribution of the data can be assumed.

HC5: 0.21345 mg/L (CI: 0.11 – 0.56).

The fitted model derived from the R-package *fitdistrplus* including confidence intervals for single endpoints and censored endpoints is shown in the following plot.



Conclusions on the SSD-HC_5:
The derived HC_5 is in general highly dependent on the fitted model and calculation method. To overcome uncertainties, two statistically sound approaches are used and the more reliable approach is selected. The underlying data in the models can be assumed to follow a log-normal distribution. The calculation with *fitdistrplus* allows to take intervals into account, which in this case due to right censored values and available confidence intervals is relevant. Therefore, the calculations with the R-package *fitdistrplus* is more robust and preferred compared to the calculation with ETX. The HC_5 is 0.21 mg/L.

Notes:
- For determination of the precise AF, WoE shown on page 98 and 99 of the AGD should be taken into account.
- Note that in the plot with *fitdistrplus* displays not all data points, as this would result in an unclear graphic illustration. However, all data points are taken into account for fitting the model and calculation of the HC_5.
-

References:

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EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673 ISSN: 2397-8325.

Appendix 4: Draft proposal for possible use of a limit fish test as alternative to full fish test with formulations

For dossiers without acute fish tox test for the assessed formulation, we follow the requirements/triggers for formulated products as given in Commission Regulation (EU) No 284/2013 and highlighted in the AGD (EFSA 2013), i.e. "*In principle, acute or short-term exposure tests with the formulated products should be carried out on one species from each of the groups of tier 1 aquatic organisms (fish, aquatic invertebrates, algae and/or macrophytes) if the preparation itself may contaminate water. However, where the available information for an a.s. permits the conclusion that one of these groups is clearly more sensitive (factor of 10 difference), only a test using a species of the relevant group needs to be performed*".
Accordingly for acute fish PPP tests, we would consider the following cases (for monoformulation):

1- If the a.s tests show that **fish are at lower risk** (10 fold or more) than other groups of organisms (daphnia/ algae) (i.e. endpoints (EP) deviating of a factor 10 or more): the regulatory praxis is to not follow the data requirement-> no fish PPP test deemed to be necessary.

2- If the a.s. tests indicates that **fish are clearly at risk** (i.e. fish EP is the lowest, deviates of a factor 10 or more): need to perform a PPP fish test -> based on information available, select either a Limit fish test PPP test (may need to be followed by a Dose response-fish test PPP) or directly a Dose response-fish test PPP

3- If the a.s. tests indicates that **fish are potentially at risk** (i.e. fish EP deviating of a factor 10 or less): check if the tests on daphnia and algae indicate a higher tox with the formulation than the a.s. (the tests must be performed with a similar design (e.g. flow-though), and endpoints expressed similarly (e.g. µg a.s./ L)):

- if no higher tox of the formulation is indicated (i.e. deviation of less than a factor 3 between EP of formulation and a.s. tests): it may be assumed that the formulation is also not more acutely toxic to the fish than the a.s. -> no fish PPP test deemed to be necessary
- if a higher tox of the formulation is indicated (i.e. deviation of a factor 3 or more between EP of formulation and a.s. tests): applying the approach proposed ("threshold approach") may be one suitable approach; in such approach, the concentration tested in the fish limit test could be the lowest of the EC50 concentrations available for invertebrate or algae tests performed with the PPP -> Limit fish PPP test requested.
    - If at the concentration tested, the acute toxic effects are lower than 50%, a "> X" LC50 could apply to the fish.
    - If at the concentration tested, the acute toxic effects are higher than 50%, a dose-response test should follow -> Dose response-fish test PPP requested.

Also please note that it is required to conduct chronic studies for formulations where the formulation is more acutely toxic than the a.s. by a factor of 10.

For acute fish test for PPP containing two or more active substances: in principle the same approach as above could apply; but if "the most sensitive taxonomic groups for the individual active substances are not the same, testing on all three/four aquatic groups, that is to say fish, aquatic invertebrates, algae and, where relevant macrophytes, shall be required" (PPP

Regulation 284, section 10.2.1).

3. REFERENCES

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2 HEALTH COUNCIL OF THE NETHERLANDS: COMMITTEE ON PESTICIDES AND GROUNDWATER (1996). Risks of pesticides to groundwater ecosystems. Rijswijk: Health Council of the Netherlands, 1996; publication no. 1996/11E. ISBN:90-5549-135-7.

3 Maltby L, Brock TCM, Van den Brink PJ. 2009. Fungicide risk assessment for aquatic ecosystems: importance of interspecific variation, toxic mode of action and exposure regime. *Environ Sci Technol* 43:7556-7563.

4 Dijksterhuis J, Van Doorn T, Postma J. 2009. De gevoeligheid van oppervlaktewaterschimmels blootgesteld aan azolen en strobilurines die worden toegepast in de landbouw. Centraal Bureau voor Schimmelcultures / Ecofide, Utrecht, Weesp, 32 pp.

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6 CBS 2009. De gevoeligheid van schimmels in het oppervlaktewater voor fungiciden die worden toegepast in de landbouw. Centraal Bureau voor Schimmelcultures, Utrecht, Weesp, 22 pp.