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Guideline on the environmental risk assessment of medicinal products for human use

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This guideline replaces 'Guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 corr 2)'.

Keywords	***Environmental risk assessment, ERA, Human medicinal products, PBT/vPvB***

* Page 7: The wording in Section 3.1 has been amended to clarify requirements for excipients. Editorial corrections have been made throughout the guideline text.

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Guideline on the environmental risk assessment of medicinal products for human use

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Definitions

AF	Assessment factor
Antibacterial	Effective against bacteria
Antimicrobial	Effective against bacteria and fungi
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BCF_{FISH}	Bioconcentration factor in fish
BMF	Biomagnification factor
BW	Body weight
CHMP	Committee for Medicinal Products for Human Use
COMP	Committee for Orphan Medicinal Products
CLP	Classification, Labelling and Packaging
CMR	Carcinogen, Mutagen or Reprotoxic (when chronic exposure) classification
D_{ow}	Octanol/water distribution coefficient for ionisable compounds
DART	Developmental and reproductive toxicity studies
DEE	Daily energy expenditure
DT_{50}	Degradation half-life of substance (in a given compartment)
EAS	Endocrine active substance
EC_{10}	Effect concentration at which 10% effect (mortality, inhibition of growth, reproduction, etc) is observed compared to the control group
EC_{50}	Effect concentration at which 50% effect (mortality, inhibition of growth, reproduction, etc) is observed compared to the control group
ErC_{10}	Effect concentration at which 10% effect for inhibition of growth rate is observed compared to the control group
ECHA	European Chemicals Agency
EPAR	European public assessment report
ERA	Environmental risk assessment
FELS	Fish early life stage (toxicity test)
EQS	Environmental quality standard according to the Water Framework Directive
FOCUS	FOrum for the Co-ordination of pesticide fate models and their USe
F_{PEN}	Market penetration factor
GLP	Good Laboratory Practice
GMO	Genetically modified organism
HMP	Human medicinal product

IARC	International Agency for Research on Cancer
IC_{50}	Inhibitory concentration at which 50% effect is observed compared to the control group
K_D	Adsorption distribution coefficient
K_F	Freundlich adsorption coefficient
K_{FOC}	Organic carbon normalised adsorption partition coefficient
K_{ow}	Octanol/water partition coefficient
LOAEL	Lowest observed adverse effect level
Log D_{ow}	Logarithm of octanol/water distribution coefficient for ionisable compounds
Log K_{ow}	Logarithm of octanol/water partition coefficient
MoA	Mode of action ((eco)toxicological)
MAA	Marketing authorisation application
MAH	Marketing authorisation holder
NER	Non-extractable residues
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOE_rC	No observed effect concentration for growth rate
OC	Organic carbon
OECD	Organisation for Economic Co-operation and Development
PAR	Public assessment report
PEC	Predicted environmental concentration (in a given compartment)
pK_a	Dissociation constant
PL	Package leaflet
PNEC	Predicted no effect concentration
PBT	Persistent, Bioaccumulative and Toxic (substance classification)
QSAR	Quantitative structure–activity relationship
3Rs	3Rs principle for animal testing (Replacement, Reduction and Refinement)
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ	Risk quotient (for a given compartment)
SimBaFi	Simulation model bank filtration
SmPC	Summary of product characteristics
STP	Sewage treatment plant
vPvB	very Persistent and very Bioaccumulative (substance classification)

Executive summary

It is mandatory for a marketing authorisation application (MAA) for a medicinal product for human use (HMP) to include an environmental risk assessment (ERA). This ERA is based on the use of the product and the physico-chemical, ecotoxicological, and fate properties of its active substance(s). This guideline describes how to perform this ERA and how to evaluate potential risks to the environment arising from the use of the medicinal product, with the aim of protecting aquatic and terrestrial ecosystems including surface water, groundwater, soil, species at risk of secondary poisoning and the risk for the microbial processes in sewage treatment plants (STPs). Furthermore, the identification of potential hazards of the active substance of a medicinal product is described. The guideline also includes consideration of potential precautionary and risk mitigation measures and provides guidance on how to report the findings in an Environmental Risk Assessment Report.

1. Introduction (background)

The purpose of this guideline is to describe the assessment of the potential environmental risks and hazards of HMP. It outlines general considerations and the recommended stepwise procedure of assessment.

2. Scope and legal basis

An ERA is required for all new MAAs for a medicinal product submitted through a centralised, mutual recognition, decentralised or national procedure.

For type II variations, the ERA dossier should be updated if there is an anticipated increase in the environmental exposure, e.g. a new indication which results in an increase in the extent of the use. For extension applications according to Annex II of Commission Regulation (EC) No 1085/2003, an ERA is also required if there is an anticipated increase in the environmental exposure, e.g. an extension application of an oral medicinal product to include a dermal patch. The environmental data previously submitted in the original dossier of the same marketing authorisation holder (MAH)[1] may serve as a basis for the revised ERA for the variation or extension application, or for a new MAA with the same active substance.

An ERA is not required for renewals of marketing authorisations. If new data emerge in the post-authorisation phase that require an update to the ERA, the updated ERA shall be submitted as a type IB C.I.z variation. For further details, please refer to the EMA pre-authorisation guidance, Q&A No 3.4.2 (EMA, 2023).

Directive 2001/83/EC, as amended, relates to those risks to the environment arising from the use, storage and disposal of medicinal products and not to risks arising from the synthesis or manufacture of medicinal products. This guideline is focused on environmental risks associated with the use of HMPs.

This guideline does not apply to medicinal products consisting of genetically modified organisms (GMOs). For further details on those, please refer to the Agency's pre-authorisation guidance Q&A 3.4.3.

[1] Same Marketing Authorisation Holder (MAH) is defined in section 2.8 of the Notice to Applicants, volume 2A, Chapter 1

For MAAs for radio-pharmaceutical precursors for radiolabelling, and for radiopharmaceuticals, additional requirements on emission standards for radiation set by Council Directives 2013/59/Euratom should be taken into account.

In accordance with Article 8(3) of Directive 2001/83/EC, as amended, the potential environmental risks posed by the use of medicinal products shall be evaluated and, on a case-by-case basis, specific arrangements to limit this risk shall be considered. Under the current requirements, the outcome of the ERA should not constitute a criterion for refusal of a marketing authorisation.

According to Directive 2001/83/EC, applicants are required to submit an ERA irrespective of the legal basis (see Figure 2 Q2a-d and Figure 3 Q2a-b).

The environmental risk assessment should be provided in Module 1.6 of the MAA (see section 9). Any missing data or studies should be justified by the applicant.

In the interest of animal welfare, the principles of 3Rs (Replacement, Reduction and Refinement) in accordance with Directive 2010/63/EU should be implemented whenever possible.

3. General Principles

3.1. The Environmental Risk Assessment

The ERA is performed for a product containing one or more active substances. All pharmacologically active substances in the product need to undergo an ERA. For fixed combination products, the ERA is performed separately for each active substance within the product. Excipients are out of scope of the guideline.

3.1.1. Identification of the substance for which the ERA is performed

The ERA should be performed for the pharmacologically active substance, which in most cases is the parent compound. The ERA dossier should contain information on the identification of this substance or these substances, which should be at a minimum:

- CAS number
- Molecular formula, molecular weight
- Structural formula
- Physico-chemical information on the substance that could influence test protocols used, e.g. highly lipophilic substances.
- Information (one sentence) on the pharmacological profile, including whether the substance is an antibiotic, antiparasitic or endocrine active substance (EAS) (a tailored testing or specific assessment strategy will be needed, see section 4.3)

3.1.2. Total residue approach

The 'total residue approach' assumes that the active substance is completely excreted as parent compound without metabolism or assuming that metabolites have similar or lower toxicity than that of the parent compound.

Metabolism of the active substance may be taken into account in Phase II; see section 4.2.3.2.

In most cases, the ERA is conducted on the parent compound but for a prodrug, the pharmacologically relevant substance will generally be the active metabolite. However, there may be instances where a prodrug is incompletely converted to the active metabolite (i.e., <50% conversion) and excreted largely (>50%) intact or via a metabolic pathway that does not generate the active moiety. In these rare cases, the selection of the substance or substances for which the ERA is conducted should be justified, and scientific advice should be sought from regulatory agencies (section 8).

3.1.3. Test guidelines

Data generated by or on behalf of the applicant in order to meet the ERA data requirements specified in this guideline should be compliant with Good Laboratory Practice (GLP) where applicable and preferably follow the most recent test guidelines issued by the Organisation for Economic Co-operation and Development (OECD) or comparable international validated test guidelines. For substances that are difficult to test e.g. very lipophilic substances, the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, 2019) should be applied. Quantitative structure-activity relationships (QSARs) and read-across currently cannot replace the studies requested in this guideline. However, in light of the 3Rs principles, increasing knowledge of QSARs and the development of validated alternative assays is encouraged, to potentially replace *in vivo* assays.

A number of methods used in this guideline are based on methods described in the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (e.g. ECHA, 2016; ECHA, 2017; ECHA 2023 a-c), the Water Framework Directive environmental quality standards (EQS) (European Commission, 2018) guidelines, as well as OECD guidance documents and technical guidelines. In case of future revisions of these guidelines, or regulatory uptake of new tests, the revised version of the relevant method or test guideline should be used.

3.1.4. Publicly available data

For active substances that are already marketed, information may be available in the public domain. To prevent repetition of (animal) studies and to use all relevant data available, the Applicant should provide a complete literature review (see section 6.1 on data search). When other MAHs have already performed relevant studies, they are encouraged to share data with the Applicant, in order to minimise the number of tests having to be re-performed and also in accordance with 3Rs principles. (European) Public Assessment Reports (PARs and EPARs) and reviews or summary data from other regulatory frameworks cannot be used as data in the ERA dossier. Endpoints are owned by the company who submitted them in the original procedure and cannot be used by other applicants without a letter of access. If the applicant has a letter of access, the applicant also should have the study reports available and submit those. Of note, (1) endpoints may have been evaluated using older standards or in different frameworks and not meet current standards, (2) EPARs may not have been updated with new data or changed assessments during former procedures.

All data submitted (whether study reports or peer reviewed literature) should contain enough information to permit assessment of the reliability of the study performed (see section 6.2 on evaluation of studies).

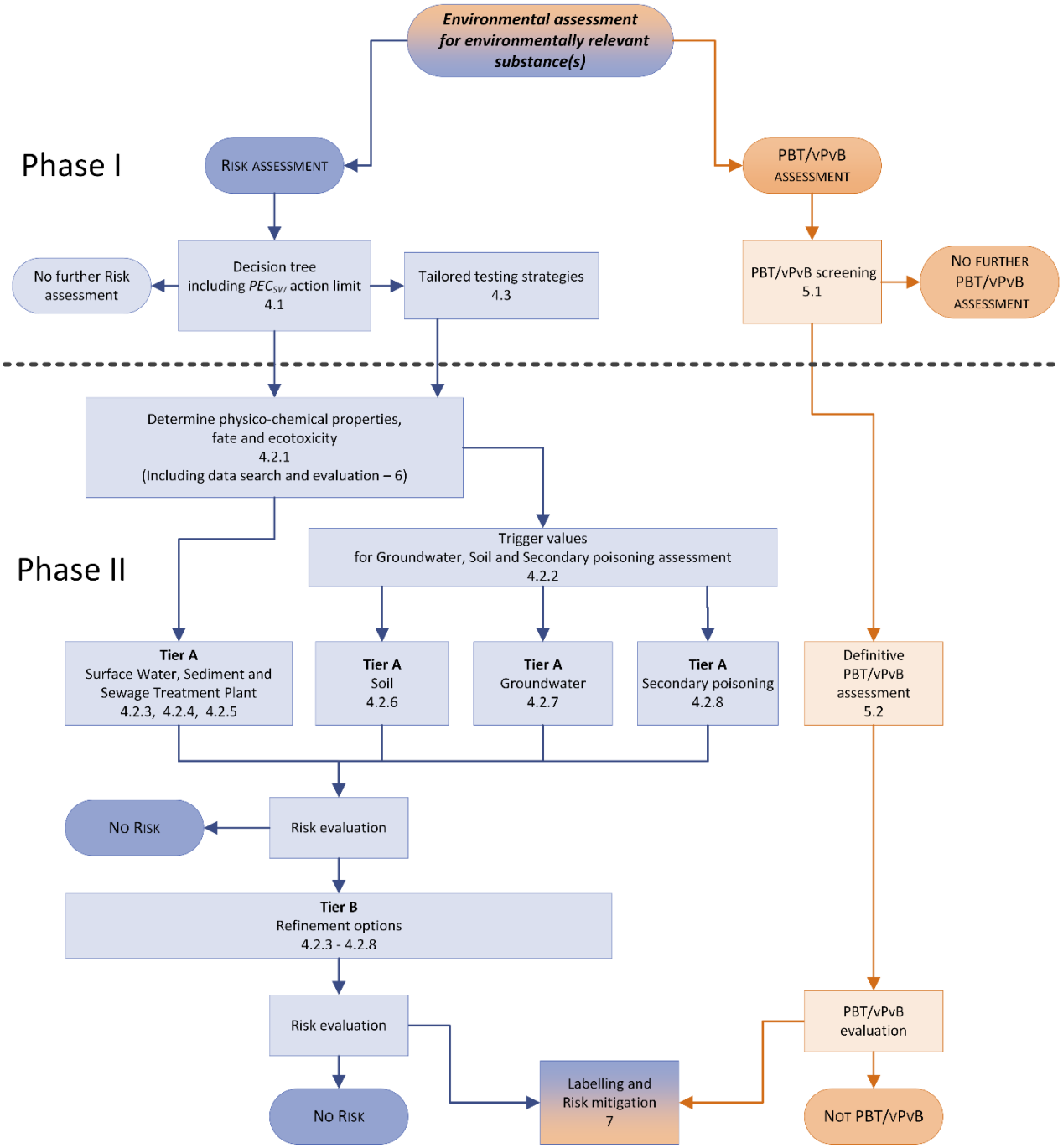
3.2. Overview of the risk assessment and hazard assessment

For each ERA, both a risk assessment and a hazard assessment are required (see Figure 1). The risk assessment reflects the possibility of an effect occurring and is an evaluation of both exposure of organisms in the environment to the active substance, and ecotoxicity. The hazard assessment concerns the identification of intrinsic properties of an active substance that could render it harmful to

the environment regardless of the levels of exposure. Active substances poorly degraded in the environment (persistent; P), that accumulate in organisms (bioaccumulative; B) and are toxic (T) or very persistent and very bioaccumulative (vPvB), are identified in the PBT/vPvB assessment.

The ERA may consist of a justification for not submitting some or all ERA studies. However, this only applies to certain cases, which are specified in section 4.1 and 5.1. For some substances with a specific toxicity profile or mechanism of action (e.g. EAS, antibacterials and antiparasitics), a specific assessment strategy is necessary (see Section 3.2.1).

Figure 1: Overview of the risk assessment and PBT/vPvB assessment including references to section numbers in the main text.



In **Figure 1**, NO RISK is defined as PEC/PNEC < 1.

3.2.1. Risk assessment

In Phase I, a decision tree (Figure 2, section 4.1) is followed to identify the products that require a Phase II assessment. The Phase I decision tree concludes with the calculation of a Predicted Environmental Concentration in surface water (PEC_{SW}), based on the predicted use of the product. When this PEC_{SW} is ≥ the action limit of 0.01 µg L^{-1}, a Phase II assessment (section 4.2) should be performed. As outlined in Figure 2, a specific assessment strategy is necessary for some groups of substances due to either of the following reasons:

(1) They may affect population-relevant endpoints in organisms in the environment (see section 4.3.2) at concentrations < 0.01 µg L^{-1} and should enter Phase II regardless of their PEC value (Figure 2, Q4).
(2) A specific mechanism of action requires a tailored testing strategy (Figure 2, Q7). Currently, specific guidance is given for antibacterials and EAS in section 4.3.

Table 1: Examples of groups of substances that require specific assessment strategies

Substance group	Enter Phase II regardless of PEC	Tailored testing
Endocrine active substances	Yes	Yes
Antibacterials	No	Yes
Antiparasitics*	Yes	No

* Antiprotozoals are exempted.

This guideline provides guidance on specific assessment strategies only for EAS antibacterials and antiparasitics (See Table 1 above). However, if the applicant considers there might be other substances for which a specific assessment strategy is relevant due to their specific toxicity profile or mode of action (MoA), they are encouraged to seek scientific advice from the relevant Competent Authority.

In Phase II Tier A, the PEC_{SW} is compared to an acceptable environmental concentration, the Predicted No Effect Concentration (PNEC). When a risk is identified in Tier A, a Tier B assessment with PEC_{SW} refinement and, if warranted, further effect studies should be performed.

The studies that should be performed in Phase II Tier A on physico-chemical characteristics, fate and ecotoxicity are described in section 4.2.1. The requirement for a risk assessment for certain environmental compartments (soil and groundwater) depends on whether trigger values are met by the outcome of these studies.

The Phase II risk assessment for the surface water compartment, including options for risk refinement, is described in section 4.2.3. Sections 4.2.4. - 4.2.6. give guidance on Phase II risk assessment and risk refinement for sediment, functioning of STPs, soil and groundwater, respectively. The marine environment is not assessed separately; the freshwater assessment is considered sufficiently conservative to also address risk to the marine environment due to the additional dilution in open marine waters. The assessment of risk to predators consuming contaminated prey (secondary poisoning) is described in section 4.2.7.

The Applicant should also perform a targeted data search to identify endpoints of significance to the ERA from scientific literature. Information on data search and evaluation is provided in section 6.

3.2.2. PBT/vPvB assessment

The PBT and vPvB assessment concerns the identification of certain intrinsic properties of the active substance, for which the long-term risks to the environment are unpredictable. Hence, PBT/vPvB assessment is performed independently of exposure calculations and environmental exposure to PBT/

vPvB substances should be minimised. The assessment of PBT and vPvB properties is described in section 5, starting with a PBT/vPvB screening decision tree for all active substances (Figure 3, section 5.1). Depending on the outcome of the PBT/vPvB screening, a definitive PBT/vPvB assessment may be required (section 5.2).

For substances which do not meet the trigger for definitive PBT/vPvB assessment (logarithmic octanol/water partitioning coefficient (log K_{ow}) > 4.5), an assessment of PBT/vPvB properties may still be required. This will be the case if the outcomes obtained in Phase II of the risk assessment demonstrate that the B- and T-criterion are met, or if the vB-criterion is met (see Table 18).

However, the outcome of the PBT/vPvB assessment does not influence the risk assessment. Phase I of the risk assessment is always performed, including for active substances that are not PBT/vPvB. Also, if an active substance is identified as PBT/vPvB but it does not meet the action limit for Phase II risk assessment, a Phase II risk assessment is not necessary.

3.2.3. Finalization of risk and PBT/vPvB assessment

When a risk is identified and/or a substance is classified as PBT/vPvB, this information should be included in the summary of product characteristics (SmPC), and risk mitigation measures should be discussed. These are described in section 7.

The structure of the ERA report is described in section 9.

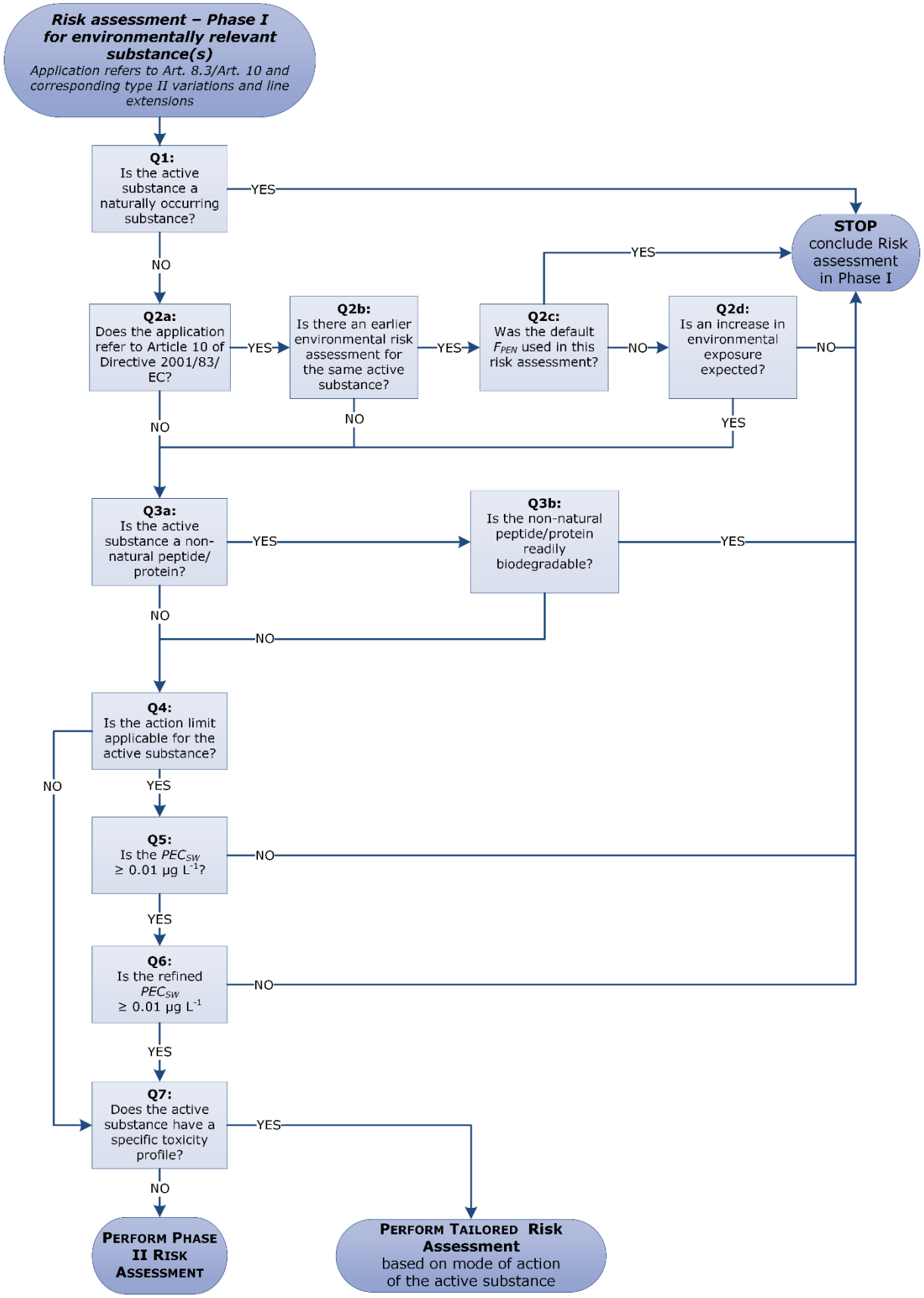
4. Risk Assessment

4.1. Phase I Risk Assessment

This section presents guidance on how to conduct the Phase I risk assessment. The potential for environmental exposure is assessed based on the nature of the active substance and the intended use. In Phase I, products that require a more extensive Phase II risk assessment – either standard or tailored - are identified based on their use and/ or environmental exposure.

The Phase I risk assessment is presented as a decision tree (Figure 2). The questions in the decision tree are described in detail below Figure 2. The outcome of Phase I may be that the risk assessment stops, or that a Phase II risk assessment is required. The basis for a decision not to proceed to a Phase II risk assessment needs to be provided in the ERA report.

Figure 2: Phase I Decision tree (Q: question)



Questions in Phase I Risk Assessment Decision tree (Figure 2):

Q1: Is the active substance a naturally occurring substance?

In the case of medicinal products comprised of naturally occurring substances (e.g. vitamins, electrolytes, amino acids, peptides, proteins, nucleotides, carbohydrates and lipids) as active substance, the ERA may consist of a justification for not submitting ERA studies. Such a justification could be, for example, that due to the physico-chemical nature of the active substance these products are unlikely to pose a risk to the environment. Alternatively, based on the environmental fate and/or common presence in the environment, these products are unlikely to alter the concentration or distribution of the substance in the environment. As defined in Directive 2004/24/EC, the same criteria apply to herbal medicinal products.

However, there may be exceptional cases where further justification for the absence of studies might be necessary, e.g. when an active substance is classified as being a carcinogen, mutagen, or toxic for reproduction (CMR) or PBT/vPvB (see section 5).

Vaccines without adjuvants are unlikely to result in a risk to the environment and the ERA may consist of a justification for not submitting ERA studies on this basis. Adjuvants contained in vaccines may however require additional justification for the absence of ERA studies according to the principles outlined above.

Q2a: Does the application refer to Article 10 of Directive 2001/83/EC?

According to Directive 2001/83/EC, applicants are required to submit an ERA for all initial MAAs and certain post-authorisation applications (e.g. extension applications) regardless of the legal basis. Applications under Art 10(1)-generic medicinal products, Art 10(3)-hybrid and Art 10(4)-similar biological applications[2] are not exempt from submitting an ERA. Likewise, an ERA is required for Art 10a-well established use/bibliographical, Art 10b-fixed combinations and Art 10c-informed consent applications.

Q2b: Is there an earlier environmental risk assessment for the same active substance?

The ERA remains specific to the applied medicinal product and it remains under the responsibility of the Applicant. However, in order to avoid unnecessary repetition of studies, and in particular animal studies in line with 3Rs principles, applicants are encouraged to share their data. If the current applicant has access to an ERA that was performed earlier by another MAH, the study reports may be submitted together with the written consent from the owner of the data, as part of the ERA of the new application.

Where relevant ERA studies were performed earlier by another MAH but data sharing is not agreed with this MAH, the Applicant is responsible for providing a complete and satisfactory ERA as part of their application. In the specific instance of generics where data sharing is not agreed, if a relevant ERA[3] was considered satisfactory by an EU National Competent Authority and the applicant is able to justify that the scientific conclusions reached for the relevant ERA remain applicable to their generic product, repetition of ERA studies will generally not be required. In such cases, it is expected that appropriate information and/or risk mitigation measures should be included in the product information of the generics (SmPC, PL) and aligned with those reflected in the relevant ERA.

[2] The ERA may already have stopped at Q1 for these products, as due to the physico-chemical nature of the active substance, they are unlikely to pose a risk to the environment.
[3] This should be understood as the ERA of the reference medicinal product or of any other relevant medicinal product containing the same active substance.

If the relevant ERA is not considered complete in accordance with the current guideline (e.g. studies are missing, or increased environmental exposure may be anticipated [see Q2d]), the applicant should conduct the missing studies and/or update their ERA accordingly.

Q2c: Was the default market penetration factor (F_{PEN}) used in this risk assessment?

The market penetration factor (F_{PEN}) is defined as the fraction of the population receiving the active substance daily. If the default F_{PEN} (0.01) was used in this earlier risk assessment - and provided that the dosage of the new indication is the same - the outcome of the risk assessment will not change and the risk assessment stops. However, if a refined F_{PEN} was used, the applicant should verify whether the respective F_{PEN} is still appropriate, and if not, update the F_{PEN} accordingly (see Q6). The outcome of the risk assessment may change.

Q2d: Is an increase in environmental exposure expected?

An increase in environmental exposure may be expected when e.g. a new indication or a new patient population is added, the maximum daily dose is increased, a new route of administration or a new pharmaceutical form is added, or a marketing authorisation is applied for in a member state with a higher prevalence of the disease. If a refined F_{PEN} was used in the previous ERA, an applicant applying for a marketing authorisation in a new member state should compare the prevalence in this new member state to the prevalence used to refine F_{PEN} in the previous ERA. If the environmental exposure for any reason is increased compared to the environmental exposure used in the previous ERA, the ERA should be updated accordingly. It is the responsibility of the Applicant/MAH to determine whether the environmental exposure is expected to increase and to provide the appropriate updated ERA/justifications.

Q3a: Is the active substance a non-natural peptide/protein?

Peptides and proteins that have been structurally modified using non-natural amino acids to increase biostability are considered non-natural.

Protein-drug conjugates including natural proteins do not belong to this group and require standard assessment of the non-protein-moiety.

Q3b: Is the non-natural peptide/protein readily biodegradable?

For non-natural peptides/proteins, an additional screening step should be performed to demonstrate that they will be quickly degraded in the STP and will not enter the environment.

When the non-natural peptide/protein is demonstrated to be excreted in amounts < 10% of the dose or shown to be readily biodegradable in an OECD 301 test, the ERA stops.

Q4: Is the PEC_{SW} action limit of 0.01 µg L^{-1} applicable to the active substance?

For active substance that may pose a risk to organisms in the environment at concentrations < 0.01 µg L^{-1}, the action limit may not be applicable. Examples include EAS and antiparasitics (antiprotozoals exempted). For EAS, a tailored testing strategy is also required (see Q7).

Q5: Is the $PEC_{SW} \geq$ 0.01 µg L^{-1}?

In Phase I, the PEC calculation is restricted to the surface water compartment. The PEC_{SW} is calculated using default values and the following assumptions:

- 1% of a population receives the active substance daily.
- The sewage system is the main route of entry of the active substance into the surface water. The calculation is based on an averaged wastewater flow of 200 L per inhabitant per day for a population of 10,000 inhabitants.

- There is no biodegradation or retention of the active substance in STP.
- There is no metabolism in the patient.

The PEC_{SW} concentration can be calculated using the following formula in Equation 1:

$$PEC_{SW} = \frac{DOSE_{AS} \cdot F_{PEN}}{WASTEW_{INHAB} \cdot DILUTION} \qquad \textbf{Eq. 1}$$

Parameters used in Equation 1:

Parameter	Description	Unit	Default value
PEC_{SW}	Predicted environmental concentration for surface water calculated in Phase I	[mg L^{-1}]	-
$DOSE_{AS}$	Maximum daily dose of the active substance consumed per patient	[mg patient^{-1} d^{-1}]	-
F_{PEN}	Fraction of a population receiving the active substance	[patients inh^{-1}]*	0.01
$WASTEW_{INHAB}$	Amount of wastewater per inhabitant per day	[L inh^{-1} d^{-1}]	200
DILUTION	Dilution factor	[-]	10

* Note that the unit of F_{PEN} is defined as [patients inh^{-1}] for reasons of clarity. Since $DOSE_{AS}$ is usually represented in [mg patient^{-1} d^{-1}], redundant units like 'patients' and 'inh', were introduced to provide insights in deriving the PEC_{SW}. Mathematically, the parameter F_{PEN} is a fraction and is thus unitless.

If the PEC_{SW} value is < 0.01 µg L^{-1} and no other environmental concerns are apparent, it is assumed that the medicinal product is unlikely to represent a risk to the environment following its recommended usage in patients and no further risk assessment is required.

Q6: Is the refined PEC_{SW} ≥ 0.01 µg L^{-1}?

PEC_{SW} may be refined by refining the F_{PEN} value based on prevalence data and/or based on the treatment regimen. For medicinal products, which can be used for more than one indication, the calculation of refined PEC_{SW} should consider all designated indications for the product. The total PEC_{SW} is the sum of the PEC_{SW} for each indication, which should be calculated using the maximum prescribed dose for each indication. The other default values representing a realistic worst case environmental exposure scenario should not be replaced by other data. If the refined PEC_{SW} value is < 0.01 µg L^{-1}, and no other environmental concerns are apparent (e.g. the active substance is a potential EAS or antiparasitic), it is assumed that the medicinal product is unlikely to represent a risk for the environment following its recommended usage in patients; in that case, no further risk assessment is required.

Prevalence: F_{PEN} can be refined by submitting European disease prevalence data for the sought indication(s). Such data should be published by a reliable and independent source, e.g. a peer-reviewed scientific journal or the World Health Organization (WHO) (e.g. the International Agency for Research on Cancer (IARC)). It is assumed that 100% of the patient population is taking the medicinal product for the relevant disease(s) daily and thus the F_{PEN} reflects the prevalence of the disease. If regional differences exist, F_{PEN} should be calculated for the member state or region with the highest prevalence of the disease. This member state should be one of the member states included in the authorisation procedure. Prevalence data at subnational level (i.e., for regions smaller than a country) can also be used in the risk assessment, provided they are of good quality as described above and justification for use in the risk assessment is provided. Prevalence data should be as recent as

possible, preferably not older than 5 years. If older data is used, a justification should be provided. For orphan drug submissions, F_{PEN} can be refined based on the prevalence for which the medicinal orphan drug designation was based, as adopted by the Committee for Orphan Medicinal Products (COMP; www.ema.europa.eu/en/committees/committee-orphan-medicinal-products-comp). One year prevalence data should be used unless use of other prevalence data (e.g. multiple year prevalence, lifetime prevalence or incidence if appropriate) can be justified considering epidemiologic and posology data available for the supported indication.

Treatment regimen: F_{PEN} may be refined taking the worst-case treatment period ($t_{TREATMENT}$) and worst-case number of treatment repetitions per year ($n_{TREATMENT}$) into consideration. This is easily done for products intended for single use (e.g. during surgery, diagnostics, etc.) or other products with a well-defined treatment regimen. For example, for an anti-cancer drug administered for five days in four-week cycles, $t_{TREATMENT}$ equals 5 days and $n_{TREATMENT}$ would be 13 per year. The posology should be clearly reflected in the SmPC. For other treatment patterns, F_{PEN} refinement based on an intermittent treatment regimen should be based on clinical considerations and justified by a reliable and independent source. In exceptional cases, refinement based on clinical considerations is possible without the presence of public literature. This is only acceptable if these clinical considerations are well-described and based on clinical data in the dossier; for instance, in the case of anti-cancer treatment with a maximum number of treatments per year (e.g. once every 3 weeks) where severe adverse effects prevent an increase in treatment regimen. Refinement based on treatment regimen is not justified for pharmaceuticals dosed 'as needed' unless this is based on published scientific literature.

The following approach may be used for the refinement of F_{PEN} by prevalence data and/or by treatment regimen using Equation 2:

$$F_{\mathrm{PEN-REFINED}} = \frac{P_{\mathrm{REGION}} \cdot t_{\mathrm{TREATMENT}} \cdot n_{\mathrm{TREATMENT}}}{N_{\mathrm{D}}} \qquad \textbf{Eq. 2}$$

The $F_{PEN\text{-}REFINED}$ should be used for the calculation of refined PEC_{SW} using Equation 3:

$$PEC_{SW} = \frac{DOSE_{AS} \cdot F_{PEN-REFINED}}{WASTEW_{INHAB} \cdot DILUTION} \qquad \textbf{Eq. 3}$$

Parameters used in Equations 2 and 3:

Parameter	Description	Unit	Default value / reference
$F_{PEN\text{-}REFINED}$	Refined fraction of a population receiving the active substance during a given time	[patients inh^{-1}]	See Equation. 1
P_{REGION}	Prevalence for the region with the highest prevalence, as described above	[patients inh^{-1}]	
$t_{TREATMENT}$	Duration of one treatment period	[d]	
$n_{TREATMENT}$	Number of treatments per year	[yr^{-1}]	
N_D	Number of days per year	[d yr^{-1}]	365

Parameter	Description	Unit	Default value / reference
PEC_{SW}	Predicted environmental concentration in surface water based on $F_{PEN\text{-}REFINED}$	[mg L^{-1}]	
$DOSE_{AS}$	Maximum daily dose of the active substance consumed per patient	[mg $patient^{-1}$ d^{-1}]	
$WASTEW_{INHAB}$	Amount of wastewater per inhabitant per day	[L $inh^{-1}d^{-1}$]	200
DILUTION	Dilution factor	[-]	10

If the PEC_{SW} value based on a refined F_{PEN} is < 0.01 µg L^{-1} and no other environmental concerns are apparent, it is assumed that the medicinal product is unlikely to represent a risk for the environment following its recommended usage in patients and no further risk assessment is required.

Q7: Does the active substance have a specific toxicity profile?

A tailored testing strategy is needed for compounds with a specific MoA (e.g. EAS, antibacterials and antiparasitics). More information on identification and tailoring of studies for EAS and other specific active substances can be found in section 4.3.

4.2. Phase II Risk Assessment

4.2.1. Determination of physico-chemical properties, fate and ecotoxicity

Physico-chemical properties of active substances are important drivers for environmental fate and toxicity. The determination of some of these properties is therefore mandatory for the assessment. Table 2 gives an overview of the studies to be performed on physico-chemical properties, fate and ecotoxicity in Tier A risk assessment. The base set of data cannot be omitted even if studies such as OECD 303A and OECD 314B show degradation in STPs, because the availability of STPs varies across Europe and removal efficiencies for pharmaceuticals vary considerably. A description of the studies is provided below.

Experimental studies should preferably follow the test guidelines issued by the OECD or the European Commission. It is recognised that there are other test guidelines, approaches and methods that are capable of providing an equivalent environmental risk assessment. If methods other than those described in this section are used, a justification should be included in the Environmental Risk Assessment Report. All tests, including non-standard tests, should undergo a reliability evaluation and can only be used if deemed 'reliable' or 'reliable with restrictions' (as described in section 6.2).

Table 2: Studies to be performed for Phase II Tier A risk assessment

Study	Guideline
Physico-chemical properties (4.2.1.1)	
Water solubility	OECD 105
Octanol/Water Partitioning[#]	OECD 107 or 123
Dissociation in Water	OECD 112

Study	Guideline
UV-Visible Absorption Spectrum*	OECD 101
Melting Point/Melting Range*	OECD 102
Vapour Pressure*	OECD 104
Fate properties (4.2.1.2)	
Adsorption - Using a Batch Equilibrium Method with 3 soils and 2 sludges	OECD 106
Activated Sludge Sorption Isotherm*	OPPTS 835.1110 (EPA)
Ready Biodegradability Test	OECD 301
Aquatic toxicity (4.2.1.3)	
Algae, growth inhibition	OECD 201
Daphnia sp., reproduction	OECD 211
Fish, Early life stage toxicity	OECD 210
Functioning of STP (4.2.1.3)	
Activated sludge, respiration inhibition	OECD 209
Sediment toxicity (choose one or more of the tests below) (4.2.1.3)	
Lumbriculus sp., spiked sediment	OECD 225
Chironomus, sediment-water toxicity	OECD 218/219
Chironomus, sediment-water life-cycle toxicity	OECD 233

* Not mandatory.
# Study also requested for Phase I PBT/vPvB screening.

4.2.1.1. Physico-chemical characteristics

Water solubility

The water solubility of the active substance should be determined experimentally, using the most appropriate method according to the OECD 105 test guideline. For dissociating compounds, the test should be performed at pH 5, 7 and 9. Aqueous solubility should be determined before determining the octanol water partitioning coefficient (K_{ow}) (see below). Both values should be compared to evaluate the plausibility of their respective results. Additionally, information on solubility and partitioning should be taken into account when designing and/or evaluating fate and ecotoxicity tests.

Octanol/water partition coefficient (K_{ow})

The log K_{ow} needs to be determined experimentally for all active substances, as it is used for secondary poisoning-screening and PBT/vPvB-screening. The K_{ow} should be determined using the shake-flask method (OECD 107) or the slow-stirring method (OECD 123). A calculated value is only acceptable in certain cases, for example when a reliable K_{ow} measurement is technically not feasible.

For compounds with log K_{ow} > 4, the shake-flask method cannot be used and only the slow - stirring method is acceptable. This range of applicability is based on OECD guidelines 123 and 107.

For dissociating compounds, Log D_{ow} values should be determined as a function of pH covering an environmentally relevant pH-range (at least 3 pH values ranging from pH 5 to 9), e.g. by measuring the pH-lipophilicity profile (log D as function of pH). In addition, ion-corrected log D_{ow} for the neutral

molecule should be reported together with the respective dissociation constant (pK_a) value(s). The ion-corrected D_{ow} is equal to K_{ow} and can be calculated with Equation 4.[4] For neutral molecules, D_{ow} will approximate K_{ow}.

$$K_{ow} = D_{ow} \cdot (1 + 10^{(pH - pK_a)}) \quad \textbf{Eq. 4}$$

Parameters used in Equation 4:

Parameter	Description	Unit	Default value / reference
K_{ow}	Octanol/water partition coefficient for neutral compounds	[-]	-
D_{ow}	Octanol/water distribution coefficient for ionisable compounds	[-]	-
pH	Acidity or basicity of an aqueous solution	[-]	-
pK_a	Acid dissociation constant	[-]	-

If the D_{ow} value (for dissociating substances) at any pH value between pH 5 and pH 9 meets the trigger value for assessment of secondary poisoning (log K_{ow} ≥ 3) and/or if any ion-corrected D_{ow} value meets the trigger for PBT/vPvB assessment (log K_{ow} > 4.5), further assessment is required (see sections 4.2.8 and 5). In case of a neutral compound the log K_{ow} value can be used to compare to the triggers.

Dissociation constant (pK_a)

The pK_a should be determined for dissociating compounds. The results of this study are used to verify exposure concentrations in fate and ecotoxicity tests. Additionally, the information is required to determine the octanol/water partition coefficient.

4.2.1.2. Fate studies

Along with mandatory studies on physico-chemical properties, mandatory fate studies should be included in the ERA in order to evaluate the fate and predict the environmental exposure of the medicinal product. These mandatory studies are listed in Table 2.

Sorption to soil and sludge

Adsorption/desorption studies generate essential information on the mobility of the active substance and its distribution in the soil and water compartments. This is a complex process that depends on many factors including chemical properties, characteristics of the soil and climatic factors. Therefore, different sludge and soil types should be used in order to cover as widely as possible the interactions of the active substance with sludge and soils.

A study according to OECD 106 using 2 types of sludge and 3 soil types, which differ in organic carbon (OC) content and soil texture, is preferred. The results are used to evaluate the requirement for soil and groundwater assessment (section 4.2.2) and to perform PEC calculations for soil and sediment in Phase II Tier A. In Phase II Tier B, adsorption data for at least 2 types of sludge, preferably from two

[4] This equation is applicable to monoprotic acids and bases for the proton releasing (acidic) reaction. In more complicated situations, assistance may be sought by QSAR software or data published in scientific literature.

different STPs are necessary for PEC_{SW} refinement (SimpleTreat modelling, section 4.2.3.2). Adsorption data for at least 3 soils are needed for equilibrium partitioning calculations in the sediment risk assessment (Section 4.2.4) and refinement of PEC_{GW} in Tier B (section 4.2.6.2). An overview of Phase II risk assessment steps where adsorption data are needed is listed in Table 3 below.

The targeted endpoint for adsorption studies should be the Freundlich adsorption coefficient (K_F), determined in line with Tier 3 of OECD 106, and defined as the ratio between the content of the substance in the soil/sludge phase and the mass concentration of the substance in the aqueous solution, under the test conditions, when adsorption equilibrium is reached. The OC normalised adsorption partition coefficient (K_{FOC}) relates K_F to the OC content of the soil sample.[5]

Table 3: Use of adsorption data in Phase II risk assessment

Adsorption data needed in Phase II	Tier A	Tier B
Surface water	Not needed	SimpleTreat - Input: • lowest $K_{FOC,SLUDGE}$* for partition coefficient in raw sewage (Kp_S) and activated sludge (Kp_{AS}) Refined PEC_{SW}-calculation: • lowest $K_{FOC,SOIL}$ for FACTOR (sorption on suspended matter in surface water)
Sediment	PEC_{SED}-calculation: • $K_{SUSP\text{-}WATER}$ with highest $K_{FOC,SOIL}$**	Not needed
Groundwater	Trigger: • lowest $K_{FOC,SLUDGE}$* PEC porewater (PEC_{PW})-calculation: • Kp_{SOIL} with lowest $K_{FOC,SOIL}$**	SimBaFi - Input: • lowest $K_{F,SOIL}$ **
Soil	Trigger: • highest $K_{FOC,SLUDGE}$* SimpleTreat - Input: • highest $K_{FOC,SLUDGE}$* for partition coefficient in raw sewage (Kp_S) and activated sludge (Kp_{AS})	Not needed

* $n_{SLUDGE} \geq 3$: geometric mean, $n_{SLUDGE}=2$: worst case
** $n_{SOIL} \geq 4$: geometric mean, $n_{SOIL} = 3$: worst case

In order to extract the active substance from sludge or soil, the best available extraction techniques should be used. This means that various extraction methods should be used with increasing strength,

[5] Only if it is impossible to determine K_F and K_{FOC}, the adsorption distribution coefficient (K_D) and K_{OC} may be used.

e.g. according to the methodology proposed by ECETOC (2013). The evaluation of the feasibility of various extraction techniques should be reported in the final study report. Usually, a direct method with radiolabelling provides the most robust information.

Ready biodegradability

The ready biodegradability of a substance should be determined according to OECD 301 (or 310). The microbial community should not be pre-exposed to the test compound in this test, and addition of more inoculum is not allowed. The results of OECD 301 (or 310) are used to trigger the soil and groundwater assessment, both as PBT screening information and in the SimpleTreat calculation. For PEC refinement in Phase II Tier B OECD 301 as well as OECD 302 or 314 B can be used. If OECD 308 is available for PBT assessment or PEC refinement for groundwater in Tier B, the OECD 301 may not be required. Not readily biodegradable substances are considered potentially persistent in the PBT screening (see section 5.1). For more information, see REACH R.11.

4.2.1.3. Ecotoxicity studies

To determine the aquatic ecotoxicity, chronic ecotoxicity data i.e. No Observed Effect Concentration (NOEC) or 10% effect concentration (EC_{10}) for species from three trophic levels is required (See Table 2). The risk assessment for the aquatic and sediment compartment is based on chronic exposure and effects because the emission of pharmaceutical residues into surface water is assumed to be continuous.

Studies with other aquatic test species and/or studies providing other endpoints than the standard OECD endpoints (growth, mortality, reproduction)[6] may also be used, provided they are relevant for population dynamics according to the description in the Water Framework Directive Environmental Quality Standards (European Commission, 2018).

The ecotoxicity tests should be performed under the conditions as described in their respective test guidelines. For ionisable substances the aquatic ecotoxicity studies should be conducted at a stable pH consistent with the most bio-available form of the test chemical (usually the non-dissociated form or the form with the most neutral molecule species). For more information see OECD Guidance Document No. 23 (OECD, 2019 as amended). Validity criteria as described in the test guidelines should be reported and if these are not met, the test should be repeated.

Concentrations should be measured analytically. Results should preferably be based on measured concentrations, but nominal concentrations can be used if measured concentrations are within 80-120% of nominal concentrations. When a reliable concentration-response curve is observed (see e.g. recommendations in OECD technical test guidelines), the NOEC as well as the EC_{10} should be reported. Unless the quality of the data does not allow the determination of EC_{10}, the EC_{10} is preferred over the NOEC for PNEC derivation, even if the former is higher than the latter.

A limit or preliminary test, as defined in the respective OECD ecotoxicity guidelines, is sometimes used to determine the correct exposure concentrations. Such tests can only replace a definitive test when no effects are observed at the limit concentration and no risk is identified. If a risk is identified, a concentration-response relationship should always be established using an appropriate concentration range. Similarly, when no EC_{10} or NOEC can be determined because there is a significant effect at the lowest test concentration, the test should be repeated with lower test concentrations to establish a

[6] Many ecotoxicological behavioural endpoints have not yet been established and standardised to indicate changes relevant at a population level. Such endpoints may however be very relevant for neuro-active substances and when standardised guidelines become available, be taken up in a tailored testing strategy for neuro-active substances.

correct concentration-response relationship. Dispensation from conducting studies requires evidence that they are technically not feasible.

Regarding the algal test, the use of a green alga is generally recommended for OECD 201. For some compounds, such as antibacterials, the use of cyanobacteria is more appropriate (see section 4.3.1). In both situations, specific growth rate is the preferred endpoint. The OECD 201 endpoint 'yield' (biomass) is not used in the ERA, even if the endpoint yield (biomass) results in a lower (no-)effect concentration (see also section R.7.8.4.1. in ECHA, 2023a). The high growth rate of algal cells under optimal laboratory testing conditions makes it possible for algal population to recover within the 72h test duration as a result of a decline in exposure concentration (e.g. through hydrolysis and photolysis). However, recovery aspects should be disregarded, as algae act as a model organism for all aquatic photoautotrophic organisms, including aquatic macrophytes with a much longer generation time.

For EAS, the fish early life stage (FELS) toxicity test should be replaced by other, more sensitive test(s), see section 4.3.2.

4.2.2. Trigger values for soil, groundwater, and secondary poisoning

For substances entering Phase II risk assessment, the surface water, sediment and STP compartments always require assessment. If the active substance meets certain trigger values, the risk assessment should also be performed for soil, groundwater and/or secondary poisoning. These trigger values are outlined below.

Soil

Human pharmaceuticals enter wastewater after use and excretion. In STP, they can be partially or completely transformed into transformation products. The parent compound and transformation products are either bound to sewage sludge or emitted with the STP effluent.

Active substances with high affinity for OC or solids have a greater likelihood of accumulating in sludge and ending up in the soil. In cases where the active substance is readily biodegradable, a soil assessment is not required. However, substances with lower adsorption affinity may also be present in sludge at high concentrations, when the release to STPs is high. Hence, the final exposure of soil organisms depends on both main parameters, i.e., the properties of the pharmaceutical (K_{FOC} value) and the total release to the wastewater flow, which again depends on the dose and the fraction of a population receiving the active substance during a given time. The PEC_{SW} calculated in Phase I, directly reflects these parameters, as it disregards processes such as biodegradation or retention of the active substance in the STP. Hence, the PEC_{SW} is used in combination with K_{FOC} to trigger assessment of the soil compartment, see Table 4 and section 4.2.6. These active substances may also contaminate the porewater of agricultural soils after sludge application, which needs consideration by an additional groundwater assessment.

Groundwater

Active substances can enter groundwater by at least two routes; from freshwater streams receiving wastewater via bank infiltration (section 4.2.7.1), and/or through leaching from sludge-amended soils via porewater (section 4.2.7.3). The relative importance of the two routes depends on the chemical-physical properties of the active substance, as these determine the relative distribution between sludge and wastewater. In both scenarios conservative approaches are implied. For example, the predicted groundwater concentration is based upon a premise that porewater concentrations leach to groundwater without dilution.

A risk assessment for groundwater via bank filtration is required when the $K_{FOC,SLUDGE}$ is ≤ 10,000 L kg^{-1}, unless the substance is readily biodegradable (see Table 4 and section 4.2.7.1).

A risk assessment for groundwater via porewater is required when the soil assessment is triggered and the $K_{FOC,SLUDGE}$ is between 1,000 and 10,000 L kg^{-1}, unless the substance is readily biodegradable (see Table 4 and section 4.2.7.2).

The highest of both PEC_{GW} (bank filtration or porewater) should be used for the risk assessment.

Table 4: Combined trigger values for substances entering a Phase II risk assessment for soil compartment and/or groundwater

Input Values		Compartments Triggered		
		Soil assessment	**Groundwater assessment**	
$K_{FOC,SLUDGE}$* [L kg^{-1}]	**PEC_{SW} [µg L^{-1}]**		via bank filtration	via porewater
$K_{FOC,SLUDGE}$ > 10,000	Not relevant	Yes (see 4.2.6)	No	No
5,000 ≤ $K_{FOC,SLUDGE}$ ≤ 10,000	≥ 1	Yes (see 4.2.6)	Yes (see 4.2.7.1)	Yes (see 4.2.7.3)
2,500 ≤ $K_{FOC,SLUDGE}$< 5,000	≥ 2	Yes (see 4.2.6)	Yes (see 4.2.7.1)	Yes (see 4.2.7.3)
1,000 ≤ $K_{FOC,SLUDGE}$ < 2,500	≥ 3	Yes (see 4.2.6)	Yes (see 4.2.7.1)	Yes (see 4.2.7.3)
$K_{FOC,SLUDGE}$ < 1,000	Not relevant	No	Yes (see 4.2.7.1)	No

* n_{SLUDGE} ≥ 3: geometric mean, n_{SLUDGE}=2: worst case

Secondary poisoning

If the log K_{ow} (or log D_{ow} for dissociating compounds) is ≥ 3 (see section 4.2.8), a bioconcentration factor (BCF) needs to be determined experimentally. If this BCF is ≥ 100 L kg^{-1}, a secondary poisoning assessment is requested.

4.2.3. Surface water

To determine a potential risk to the surface water compartment, the PEC_{SW} (as calculated in Phase I) is compared to the $PNEC_{SW}$. This PNEC is derived using experimental chronic ecotoxicity data for freshwater species (Table 2) because continuous exposure of the aquatic environment via effluents from STPs is assumed. When the PEC/PNEC ratio is ≥ 1, a risk to the aquatic compartment as a whole (not a particular sensitive group of species) is indicated. If a risk is identified in Phase II Tier A, a refined assessment may be performed in Phase II Tier B.

4.2.3.1. Phase II Tier A assessment for surface water

Exposure assessment for surface water

The final PEC_{SW} as calculated in Phase I should be used (see Equation 1-3).

Effect assessment for surface water

To derive a PNEC, chronic ecotoxicity data for species from at least three trophic levels (algae, daphnia and fish) are required, as described in section 4.2.1.

The $PNEC_{SW}$ is calculated by applying an assessment factor (AF) of 10 to the lowest EC_{10} or to the effect concentration at which 10% effect for inhibition of growth rate is observed compared to the control group (E_rC_{10}) or NOEC value from the aquatic test species. The AF is an expression of the degree of uncertainty in the extrapolation from a limited number of test species to complex ecosystems in the actual environment and accounts for inter-species variations in sensitivity, intra-species variability, and laboratory data-to-field impact extrapolation.

Table 5: Ecotoxicological studies used in the effect assessment for surface water

Study	Endpoint*	Guideline
Aquatic toxicity (4.2.1.3)		
Algae, growth inhibition	E_rC_{10} or NOE_rC [mg L^{-1}]	OECD 201
Daphnia sp. Reproduction	EC_{10} or NOEC [mg L^{-1}]	OECD 211
Fish, Early life stage toxicity	EC_{10} or NOEC [mg L^{-1}]	OECD 210

* EC_{10} values are preferred over NOECs in the risk assessment.

Risk characterisation

Using the $PNEC_{SW}$, the risk quotient (RQ) for the surface water is determined (Equation 5).

$$RQ_{SW} = \frac{PEC_{SW}}{PNEC_{SW}} \qquad \textbf{Eq. 5}$$

If the surface water RQ is <1, then further testing in surface water is not required and it can be concluded that the active substance is unlikely to represent a risk to surface water.

If the surface water RQ is ≥1, a Phase II Tier B assessment is required, and risk refinement options may be used as described below.

4.2.3.2. Phase II Tier B assessment for surface water

When a risk is established in Tier A, the PEC_{SW} may be refined using one or more of the options below:

- F_{PEN}, if not refined in Phase I Tier A. For more information, see Q6 in section 4.1.
- Metabolism
- Potential removal in the STP.

Refinement of PEC_{SW} using metabolism data

If a potential risk for the medicinal product to the environment has been identified based on the total residue approach, then the total residue approach may be abandoned, and the risk may be refined by subtracting the fractions of metabolites (Equation 6). If the total residue approach is abandoned, a full Phase II risk assessment is required for each excreted metabolite constituting ≥10% of the administered dose. The PEC is then calculated separately for the parent compound and these metabolites and all resulting PEC/PNEC ratios are summed for the evaluation of environmental risk of the product. If it is not possible to perform the ERA for the metabolites excreted in fractions ≥ 10% of the dose, the total residue approach should be used. If a risk is identified and it is not possible to refine the risk by testing the metabolites, the ERA should be concluded with the statement that the use of the product is expected to result in a risk to the environmental compartment(s) concerned.

The following approach may be used for this refinement:

$$PEC_{\mathrm{SW-REFINED}} = \frac{DOSE_{\mathrm{AS}} \cdot F_{\mathrm{PEN}} \cdot F_{\mathrm{EXCRETA}}}{WASTEW_{\mathrm{INHAB}} \cdot DILUTION} \qquad \textbf{Eq. 6}$$

Parameters used in Equation 6:

Parameter	Description	Unit	Default value / reference
$PEC_{\mathrm{SW\text{-}REFINED}}$	Predicted environmental concentration in surface water refined in Phase II Tier B	[mg L^{-1}]	-
F_{PEN}	Fraction of a population receiving the active substance during a given time,	[patients inh^{-1}]	See Equation 1-3
F_{EXCRETA}	Fraction of parent substance excreted	[-]	-
$DOSE_{\mathrm{AS}}$	Maximum daily dose of the active substance consumed per patient	[mg $patient^{-1}$ d^{-1}]	-
$WASTEW_{\mathrm{INHAB}}$	Amount of wastewater per inhabitant per day	[L $inh^{-1}d^{-1}$]	200
DILUTION	Dilution factor	[-]	10

Refinement of PEC_{SW} with STP modelling using the SimpleTreat model

Refinement of PEC_{SW} may also be performed by a model simulation using the latest version of SimpleTreat[7]. The output of SimpleTreat is the fraction of release directed to water by STP ($F_{\mathrm{STP,WATER}}$) and will be derived by incorporating:

- Adsorption of the active substance to sewage sludge in STPs, using the $K_{\mathrm{FOC,SLUDGE}}$ data from the estimation of the adsorption coefficient (OECD 106).
- Test for ready biodegradability in the STP (OECD 301)/measured removal rates using the OECD 314 B study.

[7] (Download: https://www.rivm.nl/en/Topics/S/Soil_and_water/SimpleTreat; instruction: https://www.umweltbundesamt.de/publikationen/application-of-simpletreat-40-in-european-substance).

Table 6: Fate studies used in Phase II Tier B refinement of PEC_{SW}

Study	Endpoint	Guideline
Fate properties (4.2.1.2)		
Adsorption - Using a Batch Equilibrium Method in sludge and soil	$K_{FOC,SLUDGE}$ (L kg_{oc}^{-1}) $K_{FOC,SOIL}$ (L kg_{oc}^{-1}), $K_{F,SOIL}$ (L kg^{-1})	OECD 106
Ready Biodegradability Test	Information if readily/not readily biodegradable	OECD 301*

* OECD 301 can be replaced by OECD 314B or OECD 302.

For local scale assessments it is assumed that one point source is releasing its wastewater to one STP. The local release of the active substance to surface water ($Elocal_{WATER}$), needed as a required input value in Simple Treat, can be estimated as follows:

$$Elocal_{WATER} = \frac{DOSE_{AS} \cdot F_{EXCRETA} \cdot F_{PEN} \cdot CAPACITY_{STP}}{CONV_{mg/kg}} \qquad \textbf{Eq. 7}$$

The concentration in the influent of the STP, i.e. the untreated wastewater, can be calculated from the local release to wastewater and the influent flow to the STP. The influent flow equals the effluent discharge:

$$Clocal_{INF} = \frac{Elocal_{WATER} \cdot CONV_{mg/kg}}{WASTEW_{INHAB} \cdot CAPACITY_{STP}} \qquad \textbf{Eq. 8}$$

The concentration of the effluent of the STP is given by the fraction directed to the effluent and the concentration in untreated wastewater as follows:

$$Clocal_{EFF} = Clocal_{INF} \cdot F_{STP,WATER} \qquad \textbf{Eq. 9}$$

The fraction of the active substance discharged to the water phase in STP ($F_{STP,WATER}$) can be modelled with SimpleTreat (current version 4.1, https://www.rivm.nl/en/soil-and-water/simpletreat). The model is used to estimate chemical emission from STPs and exposure to surface water and sewage sludge. The following input parameters are essential:

- Molecular mass, water solubility, vapour pressure and temperature of determination for the latter two parameters.
- Adsorption of the active substance to sewage sludge in STPs, the K_{FOC} values derived for sludge by the batch equilibrium method (OECD 106) is required. K_{FOC} derived from soil or sediment cannot be considered. The lowest K_{FOC} derived from sludge should be used (n=2). If 3 or more types of sludge are available, the geometric mean can be used.
- Biodegradation in activated sludge as input for Simple Treat can be estimated by three different methods:

- Method 1: estimated from OECD/EU standardised biodegradability tests according to OECD 301 series, 310 or 302 series (recommended). The aquatic first order degradation constant k biodeg [h^{-1}] should be used.
- Method 2: active substance is biodegradable in activated sludge batch test according to OECD 314B. The first order degradation constant k biodeg [h^{-1}] valid for combined aqueous phase/sludge should be used.
- Method 3: active substance is biodegradable in activated sludge simulation test according to OECD 303B. The first order degradation constant k biodeg [h^{-1}] valid for aqueous phase should be used.

No changes of the default values for the operational parameters of the sewage treatment (facility type: municipal) are needed. In the output-sheet the distribution of the mass of active substance over four compartments is given:

- Air [%]
- Water [%] = $F_{STP,WATER}$ [-], needed for refinement of PEC_{SW}
- Primary settler [%]
- Surplus sludge [%]

$F_{STP,SLUDGE}$ is the sum of primary settler and surplus sludge [-]

Calculation of the refined surface water concentration

The starting point for the calculation is the concentration of the active substance in the STP effluent. Dilution in the receiving surface water and adsorption to suspended matter are then considered.

The partition coefficient between suspended matter and water, Kp_{SUSP}, may be estimated from the K_{FOC} of the active substance, determined for soil by taking into account different OC contents of the media. The lowest K_{FOC} derived from soil (n=3) should be used.[8]

$$Kp_{SUSP} = F_{OC,SUSP} \cdot K_{FOC,SOIL} \qquad \textbf{Eq. 10}$$

$$FACTOR = 1 + Kp_{SUSP} \cdot SUSP_{WATER} \cdot CONV_{mg/kg} \qquad \textbf{Eq. 11}$$

$$PEC_{SW-REFINED} = \frac{Clocal_{EFF}}{FACTOR \cdot DILUTION} \qquad \textbf{Eq. 12}$$

[8] If four or more K_{FOC} values derived from soil are available, the geometric mean may be used and the correlation between K_F (or K_D) and organic carbon (OC) may be assessed. If K_F does not correlate with OC, K_F should be used as Kp_{SUSP}. This can be assessed with the Excel-tool Input_Decision freely available: https://www.bvl.bund.de/EN/Tasks/04_Plant_protection_products/03_Applicants/04_AuthorisationProcedure/08_Environment/01_Tool_input_decision/ppp_Tool_Input_Decision_node.html

Parameters used in Equations 7-12:

Parameter	Description	Unit	Default value / reference
$Elocal_{WATER}$	Local release rate to influent wastewater during episode	[kg d^{-1}]	-
$DOSE_{AS}$	Maximum daily dose of the active substance consumed per inhabitant	[mg $patient^{-1}$ d^{-1}]	-
$F_{EXCRETA}$*	Fraction of active substance excreted	[-]	-
F_{PEN}	Fraction of a population receiving the active substance during a given time	[patients inh^{-1}]	See Equation 1-3
$CAPACITY_{STP}$	Capacity of the STP (inhabitants)	[inh]	10,000
$CONV_{mg/kg}$	Conversion factor from kg to mg	[mg kg^{-1}]	1000,000
$Clocal_{INF}$	Concentration in untreated wastewater	[mg L^{-1}]	-
$WASTEW_{INHAB}$	Amount of wastewater per inhabitant per day	[L inh^{-1} d^{-1}]	200
$Clocal_{EFF}$	Concentration of active substance in the STP effluent	[mg L^{-1}]	-
$F_{STP,WATER}$	Fraction of release directed to water by STP	[-]	See SimpleTreat tab**
Kp_{SUSP}	Solids/water partition coefficient for suspended matter	[L kg_{dw}^{-1}]	-
$F_{OC,SUSP}$	Fraction of organic carbon in suspended matter	[kg_{oc} kg_{dw}^{-1}]	0.1
$K_{FOC,SOIL}$	Partition coefficient between organic carbon and water derived from soil	[L kg_{oc}^{-1}]	See Table 3
$FACTOR$	Factor taking the adsorption to suspended matter into account	[-]	-
$SUSP_{WATER}$	Concentration of suspended matter (dry weight)	[mg_{dw} L^{-1}]	15
$PEC_{SW-REFINED}$	Predicted environmental concentration in surface water refined in Phase II Tier B	[mg L^{-1}]	-
$DILUTION$	Dilution factor	[-]	10

* This should include unchanged active substance and the fractions of dose excreted as metabolites unless the total residue approach is abandoned
** See tab 'Distribution' and parameter 'Water'.

Risk characterisation

The RQ for the surface water is determined using the $PNEC_{SW}$ (Equation 13).

$$RQ_{SW} = \frac{PEC_{SW-REFINED}}{PNEC_{SW}} \qquad \textbf{Eq. 13}$$

If the RQ for surface water is < 1, it may be anticipated that the active substance in the medicinal product will not pose a risk to the aquatic environment.

When a risk to the surface water ecosystem cannot be excluded, the applicant should propose adequate precautionary and safety measures to protect surface water ecosystems (see also section 7).

4.2.4. Sediment

For the sediment risk assessment, PEC_{SED} is derived from PEC_{SW} as calculated in phase I (see Equation 1-3) using equilibrium partitioning (EqP) between water and sediment consisting of freshly deposited suspended matter. A $PNEC_{SED}$ is derived using tests with sediment dwelling organisms. Both PEC and PNEC should be based on sediment with equal (normalised) OC content and on a dry weight basis.

4.2.4.1. Phase II Tier A assessment for sediment

Exposure assessment for sediment

K_{FOC} should be determined for a minimum of three soils or sediments (see section 4.2.1.2).[9]

Table 7: Fate study used in Phase II Tier A PEC_{SED} calculation

Study	Endpoint	Guideline
Fate properties (4.2.1.2)		
Adsorption - Using a Batch Equilibrium Method in soil	$K_{FOC,SOIL}$ [L kg_{oc}^{-1}], $K_{F,SOIL}$ [L kg^{-1}]	OECD 106

The concentration of the active substance in wet sediment is calculated according to Equation 14.

$$PEC_{SED,WW} = \frac{K_{SUSP-WATER}}{RHO_{SUSP}} \cdot PEC_{SW} \cdot 1000 \qquad \textbf{Eq. 14}$$

The partition coefficient between suspended matter and water is calculated according to Equation 15.

$$K_{SUSP-WATER} = F_{WATER,SUSP} + \frac{(F_{SOLID,SUSP} \cdot Kp_{SUSP} \cdot RHO_{SOLID})}{CONV_{m3/L}} \qquad \textbf{Eq. 15}$$

The solids/water partition coefficient for suspended matter is calculated according to Equation 16.

$$Kp_{SUSP} = F_{oc,SUSP} \cdot K_{FOC,SOIL} \qquad \textbf{Eq. 16}$$

[9] If four or more K_{FOC} values derived from soil are available, the geometric mean may be used and the correlation between K_F (or K_D) and organic carbon (oc) may be assessed. If K_F does not correlate with OC, K_F should be used as Kp_{SUSP}. This can be assessed with the Excel-tool Input_Decision freely available: https://www.bvl.bund.de/EN/Tasks/04_Plant_protection_products/03_Applicants/04_AuthorisationProcedure/08_Environment/01_Tool_input_decision/ppp_Tool_Input_Decision_node.html.

Parameters used in Equations 14-16:

Parameter	Description	Unit	Default value
$PEC_{SED,WW}$	Predicted environmental concentration in sediment related to wet weight	[mg kg_{ww}^{-1}]	-
$K_{SUSP-WATER}$	Partition coefficient between suspended matter and water	[m^3 m^{-3}]	See Equation 15
RHO_{SUSP}	Wet bulk density of suspended matter	[kg_{ww} m^{-3}]	1,150
PEC_{SW}	Predicted environmental concentration in surface water calculated in Phase I	[mg L^{-1}]	See Equation 1-3
$F_{WATER,SUSP}$	Fraction of water in suspended matter	[m_{water}^3 m_{susp}^{-3}]	0.9
$F_{SOLID,SUSP}$	Fraction of solids in suspended matter	[m_{solid}^3 m_{susp}^{-3}]	0.1
Kp_{SUSP}	Solids/water partition coefficient for suspended matter	[L kg_{dw}^{-1}]	See Equation 16
RHO_{SOLID}	Density of the solid phase	[kg_{solid} m_{solid}^{-3}]	2,500
$F_{OC,SUSP}$	Weight fraction of organic carbon in suspended solids	[kg_{oc} kg_{dw}^{-1}]	0.1
$K_{FOC,SOIL}$	Partition coefficient between organic carbon and water derived from soil	[L kg_{oc}^{-1}]	See Table 3. Determined using OECD 106
$CONV_{m3/L}$	Conversion factor from L to m^3	[L m^{-3}]	1,000

$PEC_{SED,WW}$ is expressed as freshly deposited suspended matter with an OC content of 10%. The PEC_{SED} based on dry weight is obtained by Equation 17.

$$PEC_{SED,DW} = \frac{PEC_{SED,WW} \cdot RHO_{SUSP}}{F_{SOLID,SUSP} \cdot RHO_{SOLID}}$$

$$CONV_{SUSP} = \frac{RHO_{SUSP}}{F_{SOLID,SUSP} \cdot RHO_{SOLID}}$$

$$PEC_{SED,DW} = PEC_{SED,WW} \cdot CONV_{SUSP} \qquad \textbf{Eq. 17}$$

Parameters used in Equation 17:

Parameter	Description	Unit	Default value / reference
$PEC_{SED,DW}$	Predicted environmental concentration in sediment related to dry weight	[mg kg_{dw}^{-1}]	-
$PEC_{SED,WW}$	Predicted environmental concentration in sediment related to wet weight	[mg kg_{ww}^{-1}]	See Equation 14
$CONV_{SUSP}$	Conversion factor	[kg_{ww} kg_{dw}^{-1}]	4.6
RHO_{SUSP}	Bulk density of (wet) suspended matter	[kg_{ww} m^{-3}]	1,150
$F_{SOLID,SUSP}$	Fraction of solids in suspended matter	[m_{solid}^3 m_{susp}^{-3}]	0.1
RHO_{SOLID}	Density of the solid phase	[kg_{solid} m^{-3}]	2,500

The fraction bound residue that may have been determined in fate studies, may not be subtracted from the PEC_{SED}.

Effect assessment for sediment

To determine a $PNEC_{SED}$, a minimum of one toxicity study with sediment dwelling organisms should be performed using a sediment-water test system (Table 8). In general, tests using a spiked sediment procedure are preferred. However, if the characteristics of the test substance make it impossible to spike sediment in a reliable manner (e.g. high water solubility, low binding affinity to sediment) it may be more appropriate to use the spiked water procedure.

For ionisable substances, the sediment ecotoxicity studies should be conducted at a stable pH consistent with the most bio-available form of the test chemical (usually the non-dissociated form or the form with the most neutral molecule species). For more information see OECD Guidance Document No. 23 (OECD, 2019 as amended).

Table 8: Ecotoxicological standard tests with benthic species useful for the effect assessment in sediment

Study	Endpoint[a]	Guideline
Sediment toxicity (choose one or more of the tests below) (4.2.1.3)		
Chironomid, spiked water/sediment	EC_{10} or NOEC [mg kg_{dw}^{-1}]	OECD 218/219
Chironomid, life-cycle study	EC_{10} or NOEC [mg kg_{dw}^{-1}]	OECD 233
Lumbriculus sp., sediment-water toxicity	EC_{10} or NOEC [mg kg_{dw}^{-1}]	OECD 225

[a] EC_{10} values are preferred over NOECs in the risk assessment.

If data from a single chronic sediment test is available, an AF of 100 should be applied to the EC_{10} or NOEC in order to derive the PNEC. If two long-term tests with species representing different living and feeding conditions are available, an AF of 50 may be applied to the lowest EC_{10} or NOEC to obtain the $PNEC_{SED}$.

Results from sediment toxicity tests should be recalculated into a standard sediment with an OC content of 10% (fraction of 0.1) according to Equation 18.[10]

$$EC_{10}\ or\ NOEC_{\mathrm{ST\ SED}} = EC_{10}\ or\ NOEC_{\mathrm{TEST\ SED}} \cdot \frac{F_{\mathrm{OC,ST\ SED}}}{F_{\mathrm{OC,TEST\ SED}}} \qquad \textbf{Eq. 18}$$

Parameters used in Equation 18:

Parameter	Description	Unit	Default value
$F_{OC,ST\ SED}$	Fraction of organic carbon in standard sediment	$kg_{oc}\ kg_{dw}^{-1}$	0.1
$F_{OC,TEST\ SED}$	Fraction of organic carbon in test sediment	$kg_{oc}\ kg_{dw}^{-1}$	-

[10] In case adsorption to soil does not correlate with the organic carbon (see footnote 9) the normalisation of toxicity results to organic carbon should not be applied.

Risk characterisation

Using PEC_{SED} and $PNEC_{SED}$ (both based on dry weight), the RQ for the sediment compartment is determined using Equation 19.

$$RQ_{SED} = \frac{PEC_{SED}}{PNEC_{SED}} \qquad \textbf{Eq. 19.}$$

If the RQ is ≥1, any risk refinement must be performed in Phase II - Tier B.

4.2.4.2. Phase II Tier B assessment for sediment

If a risk is identified in Tier A, refinement of PEC_{SW} (see section 4.2.3.2) may also be used for Tier B sediment assessment. If a risk to sediment organisms still cannot be excluded, the applicant should propose adequate precautionary and safety measures to protect sediment ecosystems (see also section 7).

4.2.5. Sewage Treatment Plant (STP)

The functioning of STPs is essential for good water quality management. Substances with antimicrobial activity may affect microbial processes. The microbial community most likely exposed to the highest concentrations of the substance(s) is the activated sludge community. In order to evaluate the antimicrobial effects of substances, the activated sludge respiration inhibition test (OECD 209) should be used.

4.2.5.1. Phase II Tier A assessment for STP

Exposure assessment for STPs

To determine the risk for STPs, PEC_{SW} as calculated in phase I (see Equation 1-3) should be recalculated into a PEC_{STP}. This is achieved by multiplying the PEC_{SW} by a factor of 10, the PEC_{SW} is derived from the calculated STP effluent concentration by applying a default dilution factor of 10 (see Equation 3).

Effect assessment for STPs

The $PNEC_{MICROORGANISM}$ is based on the respiration inhibition test with activated sludge (OECD 209), by applying an AF of 10 to the EC_{10} or NOEC.

Table 9: Ecotoxicological study used in the effect assessment for STP

Study	Endpoint[a]	Guideline
Functioning of STP (4.2.1.3)		
Activated sludge, respiration inhibition	EC_{10} or NOEC [mg L^{-1}]	OECD 209

[a] EC_{10} values are preferred over NOECs in the risk assessment.

Risk characterisation

Using the $PNEC_{MICROORGANISM}$, the RQ for the STP is determined (Equation 20).

$$RQ_{\text{MICROORGANISM}} = \frac{PEC_{\text{STP}}}{PNEC_{\text{MICROORGANISM}}} \qquad \textbf{Eq. 20}$$

When the RQ is ≥ 1, a Phase II Tier B assessment is required, and risk refinement options as described for surface water (section 4.2.3.2) may be used.

4.2.5.2. Phase II Tier B assessment for STP

The exposure concentration in the aeration tank of the SimpleTreat model ($PEC_{\text{AERATION TANK}}$) should be used to refine the RQ for microorganisms. $PEC_{\text{AERATION TANK}}$ is equal to $Clocal_{EFF}$, see also Equation 9 in section 4.2.3.2.

Parameters from SimpleTreat:

Parameter	Description	Unit	Default value/ Reference
PEC_{STP}	Predicted environmental concentration in the STP effluent	[mg L^{-1}]	-
$PEC_{\text{AERATION TANK}}$	Predicted environmental concentration in the aeration tank of the sewage treatment plant.	[mg L^{-1}]	Equal to $Clocal_{EFF}$ (see Equation 9)

4.2.6. Soil

A soil assessment is needed for substances with high release to the STPs, even in scenarios where sorption to the solid fraction is limited (K_{FOC} value <10,000 L kg^{-1}). A set of trigger values depending on a combination of chemical-physical substance properties (K_{FOC}) and the predicted concentration in surface water (see 4.2.2 and Table 4) aims to ensure a soil assessment for substances with high release to the STPs. To determine a possible risk to the soil compartment, the PEC_{SOIL} is compared to the $PNEC_{\text{SOIL}}$. This $PNEC_{\text{SOIL}}$ is derived using experimental long-term ecotoxicity data for soil microorganisms, soil dwelling invertebrates and plant species (Table 2). Since sludge associated active pharmaceutical residues may be available in soil compartment for a long time, short-term effect tests are inappropriate for risk assessment. When the PEC/PNEC ratio is ≥1, a risk to the soil compartment is indicated. If a risk is identified in Phase II Tier A, a refined assessment may be performed in Phase II Tier B.

4.2.6.1. Phase II Tier A assessment for soil

Tier A Exposure assessment for soil

The Tier A exposure assessment considers sludge application as the major entry path for the active substance to be released to the soil environment. In a first step, the initial concentration in soil after the first application is calculated using the predicted concentration of the active substance in sludge. Substances being persistent according to the PBT criteria, i.e. degradation half-life of substance (DT_{50}) >120 days, may accumulate in the soil environment as they are not rapidly degraded. In these

cases, the concentration in soil after repeated sludge application should also be assessed using the approach presented in Equation 22.
In order to consider the degradation of the active substance in soil in between sludge applications, a study on transformation in soil (OECD 307) is required.

Table 10: Fate studies used in Phase II Tier A exposure assessment for soil

Study	Endpoint	Guideline
Adsorption - Using a Batch Equilibrium Method in sludge	$K_{FOC,SLUDGE}$ [L kg_{oc}^{-1}]	OECD 106
Transformation in soil*	DT_{50} [d]	OECD 307

* In the OECD 307 test, a minimum of four soils are requested and the DT_{50} geometric mean value is used. When valid DT_{50} from only three of the four soil types is available, the geometric mean DT_{50} value can still be used. In case of fewer than three DT_{50} being valid, the highest value should be used as DT_{50} in the calculation. Studies must reflect environmental temperatures in Europe. Therefore, studies should preferably be conducted at 12°C, or extrapolation of transformation half-live values to 12°C should be performed. See section 5.2.2.1 for more information.

Concentration in soil after the first sludge application

The calculation of the initial concentration of the active substance in wet soil ($PEC_{SOIL,WW}$) after the first sludge application (t=0) is shown in Equation 21. The default mixing depth and sludge application rates are in compliance with the procedure in the ECHA Environmental Exposure Assessment (R.16) (EU, 2016). The concentration in sewage sludge (C_{sludge}) is calculated in the SimpleTreat model.

$$PEC_{SOIL,WW} = \frac{C_{SLUDGE} \cdot APPL_{SLUDGE}}{DEPTH \cdot RHO_{SOIL}} \qquad \textbf{Eq. 21}$$

Parameters used in Equation 21:

Parameter	Description	Unit	Default value/Reference
$PEC_{SOIL,WW}$	Predicted environmental concentration in wet soil after the first application	[mg kg_{ww}^{-1}]	-
C_{SLUDGE}	Concentration in sludge	[mg kg_{dw}^{-1}]	See SimpleTreat tab*
$APPL_{SLUDGE}$	Yearly sludge application rate in dry weight	[kg_{dw} m^{-2}]	0.5
$DEPTH$	Mixing depth	[m]	0.2
RHO_{SOIL}	Bulk density of wet soil	[kg_{ww} m^{-3}]	1,700

*See tab 'Concentrations' and parameter 'Combined sludge (C_{sludge})'. The emission rate to influent wastewater ($Elocal_{WATER}$) of the active substance is estimated by Equation 7 using a default value of 1 for $F_{EXCRETA}$.

Long-term accumulation in soil

If the active substance is not easily degraded, it may accumulate in soil over time as a result of repeated sludge application. It will continue to accumulate until a steady state level is reached. The number of years to reach steady state depends on the half-life of the substance. The concentration in the steady-state year can be calculated using Equation 22.

$$PEC_{\mathrm{SOIL,SS,WW}} = \frac{PEC_{\mathrm{SOIL,WW}}}{1 - F\mathrm{acc}} \qquad \textbf{Eq. 22}$$

The fraction accumulating after one year is calculated using Equation 23.

$$F\mathrm{acc} = e^{-365 \cdot k} \qquad \textbf{Eq. 23}$$

The first order removal rate can be calculated if the removal rates for transformation, leaching and volatilisation are known, i.e. k=k_{VOLAT} + k_{LEACH} + $k_{\mathrm{TRANSFORMATION}}$.

However, removal by volatilisation and leaching (k_{VOLAT} + k_{LEACH}) may be disregarded assuming that transformation is the main removal constant. Otherwise, guidance for calculating k_{VOLAT} + k_{LEACH} may be found in R.16 ECHA Exposure Assessment (ECHA, 2016). The removal by biotransformation is calculated using Equation 24.

$$k_{\mathrm{TRANSFORMATION}} = \frac{\ln 2}{DT_{50}} \qquad \textbf{Eq. 24}$$

Parameters used in Equations 22-24:

Parameter	Description	Unit	Default value
$PEC_{\mathrm{SOIL,SS,WW}}$	Predicted environmental concentration in wet weight soil in a steady-state situation	[mg kg_{ww}^{-1}]	-
$PEC_{\mathrm{SOIL,WW}}$	Predicted environmental concentration in wet weight soil after the first application	[mg kg_{ww}^{-1}]	See Equation 21
F_{acc}	Fraction accumulating in soil over one year	[-]	-
k	First order removal (dissipation) rate from soil	[d^{-1}]	-
DT_{50}	Half-life for transformation in soil	[d]	-

The PEC_{SOIL} based on dry weight is obtained by Equation 25.

$$PEC_{\mathrm{SOIL,DW}} = \frac{PEC_{\mathrm{SOIL,SS,WW}} \cdot CONV_{\mathrm{SOIL}}}{F_{\mathrm{SOLID,SOIL}} \cdot RHO_{\mathrm{SOLID}}}$$

$$CONV_{\mathrm{SOIL}} = \frac{RHO_{\mathrm{SOIL}}}{F_{\mathrm{SOLID,SOIL}} \cdot RHO_{\mathrm{SOLID}}}$$

$$PEC_{\mathrm{SOIL,DW}} = PEC_{\mathrm{SOIL,SS,WW}} \cdot CONV_{\mathrm{SOIL}} \qquad \textbf{Eq. 25}$$

Parameters used in Equation 25:

Parameter	Description	Unit	Default value / reference
$PEC_{SOIL,DW}$	Predicted environmental concentration in soil related to dry weight	[mg kg_{dw}^{-1}]	
$PEC_{SOIL,SS,WW}$	Predicted environmental concentration in soil related to wet weight	[mg kg_{ww}^{-1}]	See Equation 21 and 22
$CONV_{SOIL}$	Conversion factor	[kg_{ww} kg_{dw}^{-1}]	1.13
RHO_{SOIL}	Bulk density of wet soil	[kg_{ww} m^{-3}]	1,700
$F_{SOLID,SOIL}$	Fraction of solids in soil	[m_{solid}^{3} m_{soil}^{-3}]	0.6
RHO_{SOLID}	Density of the solid phase	[kg_{solid} m^{-3}]	2,500

Tier A Effect Assessment for soil

Four tests on different trophic levels are required for the soil compartment, including a functional test with soil microorganisms, and ecotoxicological tests with soil-dwelling invertebrates and plant species (Table 11). The long-term toxicity to soil organisms should be assessed because active substances in soils may persist for a long time and accumulation of the substance may occur when sludge is applied over consecutive years. The $PNEC_{soil}$ is calculated by applying an AF of 10 to the lowest EC_{10} or NOEC value from the soil test species. For ionisable substances the soil ecotoxicity studies should be conducted at a stable pH consistent with the most bio-available form of the test chemical (usually the non-dissociated form or the form with the most neutral molecule species). For more information see OECD Guidance Document No. 23 (OECD, 2019 as amended).

Table 11: Ecotoxicological studies used in the risk assessment for soil organisms.

Study	Toxicity endpoint[a]	Guideline
Nitrogen Transformation (28 days)*	≤25% of control**	OECD 216
Terrestrial plants***	EC_{10} or NOEC [mg kg_{dw}^{-1}]	OECD 208
Earthworm / Enchytraeid	EC_{10} or NOEC [mg kg_{dw}^{-1}]	OECD 222/OECD 220
Collembola	EC_{10} or NOEC [mg kg_{dw}^{-1}]	OECD 232

* Studies should be conducted at test concentration equal to and 10 times the maximum PEC.
** An assessment factor (AF) is not relevant to this endpoint – when the difference in rates of nitrate formation between the lower treatment (i.e. the maximum PEC) and control is equal to or less than 25% (negative or positive) at any sampling time before day 28, the active substance can be evaluated as having no long-term influence on nitrogen transformation in soils.
***Six plant species from six different families should be tested. It is highly recommended to use species belonging to six different families of four dicotyledonous (including a Brassica species) and two monocotyledonous species, which represent the types of plants grown on agricultural land, which would receive a sludge application.
[a] EC_{10} values are preferred over NOECs in the risk assessment.

Risk characterisation

Using PEC_{SOIL} and $PNEC_{SOIL}$ (both based on dry weight), the RQ for the soil compartment is determined by Equation 26.

$$RQ_{\mathrm{SOIL}} = \frac{PEC_{\mathrm{SOIL}}}{PNEC_{\mathrm{SOIL}}} \qquad \textbf{Eq. 26.}$$

If the RQ is ≥1, the risk assessment proceeds to Phase II – Tier B.

4.2.6.2. Phase II Tier B Assessment for soil

Tier B Exposure assessment for soil

If a risk for soil organisms has been identified in Tier A, it is possible to refine the emission rate to influent wastewater by metabolism data as performed in Tier B for surface water (see section 4.2.3.2).

The refined emission rate to influent wastewater is used to recalculate the sludge concentration C_{SLUDGE} and the relevant PEC_{SOIL}, as described above for Tier A.

Tier B Effect Assessment for soil

If the RQ_{SOIL} from nitrogen transformation in Tier A is still ≥1, further evaluation of the PNEC may be possible in Tier B by extending the microorganisms Nitrogen Transformation Test (OECD 216) to 100 days (Table 12).

Table 12: Effect studies used for Tier B assessment for soil organisms

Study	Endpoint	AF	Guideline
Nitrogen Transformation (100 days – extension of Tier A study)	≤ 25% of control	*	OECD 216

* An assessment factor (AF) is not relevant for this endpoint – when the difference in rates of nitrate formation between the lower treatment (i.e., the maximum PEC) and control is ≤25% (negative or positive) at any sampling time before day 100, the substance can be evaluated as having no long-term influence on nitrogen transformation in soils.

Risk characterisation

The refined RQ_{SOIL} should be recalculated using the refined PEC_{SOIL} and the refined PNEC value if applicable. If a risk to the soil ecosystem cannot be excluded at this stage, the applicant should propose adequate precautionary and safety measures to protect soil ecosystems (see also section 7).

4.2.7. Groundwater

For substances with a $K_{\mathrm{FOC,SLUDGE}}$ ≤10,000 L kg^{-1} or for substances that are not readily biodegradable, the main route into the groundwater is considered to be via bank filtration. Entry into groundwater via the porewater in agricultural soil is considered when the soil assessment is triggered and the average $K_{\mathrm{FOC,SLUDGE}}$ is between 1,000 and 10,000 L kg^{-1}, except for substances that are readily biodegradable. It is assumed that the exposure of groundwater via sewage sludge incorporated into soil can be disregarded if the $K_{\mathrm{FOC,SLUDGE}}$ is >10,000 L kg^{-1} with reference to the high sorption affinity of these active substances to the soil.

4.2.7.1. Phase II Tier A assessment for groundwater (via bank filtration)

Tier A Exposure assessment for groundwater

The groundwater PEC (PEC_{GW}) is based on the PEC_{SW} as calculated in phase I (see Equation 1-3) and is estimated by a simple Equation 27.

$$PEC_{GW} = 0.25 \cdot PEC_{SW} \qquad \textbf{Eq. 27}$$

Tier A Effect assessment for groundwater

The $PNEC_{GW}$ is based on the $PNEC_{SW}$ (see 4.2.3.1) and an additional AF. Groundwater ecosystems are fundamentally different to surface water ecosystems and therefore more vulnerable as they lack the ability to recover from perturbations (EMA/CVMP/ERA/103555/2015). Consequently, an additional AF of 10 should be applied to extrapolate the $PNEC_{GW}$ from the $PNEC_{SW}$ (Equation 28).

$$PNEC_{GW} = \frac{PNEC_{SW}}{10} \qquad \textbf{Eq. 28}$$

Parameters used in Equation 27-28:

Parameter	Description	Unit	Default value / reference
PEC_{GW}	Predicted environmental concentration in groundwater	[mg L^{-1}]	-
PEC_{SW}	Predicted environmental concentration in surface water	[mg L^{-1}]	-
$PNEC_{GW}$	Predicted No Effect Concentration for the groundwater compartment	[mg L^{-1}]	-
$PNEC_{SW}$	Predicted No Effect Concentration for the surface water compartment	[mg L^{-1}]	-

Risk characterisation

The RQ for the groundwater compartment is determined using the PNEC for groundwater (Equation 29).

$$RQ_{GW} = \frac{PEC_{GW}}{PNEC_{GW}} \qquad \textbf{Eq. 29}$$

If the RQ is ≥1, a Phase II Tier B assessment is required, and risk refinement options may be used as described below.

4.2.7.2. Phase II Tier B assessment for groundwater (via bank filtration)

Tier B Exposure assessment for groundwater

If the RQ_{GW} is ≥1, further evaluation is needed in Tier B using one or both options below.

Refinement of PEC_{GW} using $PEC_{SW\text{-}REFINED}$

A $PEC_{SW\text{-}REFINED}$ as described in section 4.2.3.2 can be calculated (using Equation 6) and used for the $PEC_{GW\text{-}REFINED}$ estimation by Equation 27.

Refinement of PEC_{GW} using groundwater model 'simulation model bank filtration' (SiMBafi)

Refinement of PEC_{GW} may also be performed by a groundwater modelling for a realistic worst-case scenario according to SiMBaFi – a bank filtration simulation model. The model and a detailed description can be downloaded here: www.uba.de/simbafi

The following parameters are needed:

- $PEC_{SW\text{-}REFINED}$ as described in section 4.2.3.2.
- Adsorption of the active substance to soil derived from batch equilibrium test (OECD 106). SiMBaFi requires the non-oc-normalised K_F as input. The lowest K_F derived from soil ($K_{F,SOIL}$) should be used (n=3). If 4 or more soils are available, the geometric mean may be used. K_F derived from sludge ($K_{F,SLUDGE}$) cannot be used.
- Degradation as DT_{50} value derived from an OECD 308 study (total system, calculated using single first order kinetics, normalised to 12°C, highest value of 2 test systems).

Table 13: Fate studies required for groundwater risk assessment

Study	Endpoint	Guideline
Fate properties (4.2.1.2)		
Adsorption - Using a Batch Equilibrium Method in soil	$K_{F,SOIL}$ [L kg^{-1}]	OECD 106
Aerobic Transformation in Aquatic Sediment Systems	DT_{50} value [d^{-1}] (total system, SFO, 12°C normalisation, highest value of 2 test systems)	OECD 308

For the calculation of the PEC_{GW} the "realistic worst-case scenario" determined in SiMBaFi should be used, i.e., a groundwater flow time of 5 days between the surface water and the groundwater well. For the calculation, four steps are needed as described below:

Calculation of retardation:

$$Rf = 1 + \left(\frac{1-n}{n}\right) \cdot \rho s \cdot K_{F,SOIL} \qquad \textbf{Eq. 30}$$

Calculation of flow time for the active substance

The flow time of the active substance is calculated using the groundwater flow time of 5 days between the surface water and the production well considering the retardation (Equation 31). As the distance between surface water and production well is fixed, no flow velocities have to be calculated.

$$t_{AS} = t_{GW} \cdot Rf \qquad \textbf{Eq. 31}$$

Calculation of concentration at production well

This step considers elimination by biological degradation of the active substance during their transport from the surface water to the production well with an exponential equation (Equation 32):

$$PEC_{PRODUCTION\ WELL} = PEC_{SW-REFINED} \cdot e^{\left(\frac{-\ln 2}{DT_{50}} \cdot t_{AS}\right)} \qquad \textbf{Eq. 32}$$

As the percentage of bank filtrate at the production well is assumed to be 100%, the resulting PEC_{GW} equals the calculated concentration in the production well (Equation 33).

$$PEC_{GW-REFINED} = PEC_{PRODUCTION\ WELL} \quad \textbf{Eq. 33}$$

Parameters used in Equation 30-33:

Parameter	Description	Unit	Default value /
*R*f	Retardation factor	[-]	-
n	Porosity – the default value is typical for an aquifer composed of sand and gravel	[-]	0.35
*ρ*s	Solid density – the default value representing characteristic density for quartz as the main component of porous aquifer systems.	[g cm^{-3}]	2.65
$K_{F,SOIL}$	Adsorption coefficient (not oc-normalised)	[L kg_{dw}^{-1}]	See Table 3. Determined
t_{AS}	Flow time of the active substance	[d]	-
t_{GW}	Groundwater flow time – the default value representing a realistic worst case for flow time between surface water and production well	[d]	5
$PEC_{PRODUCTION\ WELL}$	Predicted environmental concentration at production well	[mg L^{-1}]	-
$PEC_{SW-REFINED}$	Predicted environmental concentration in surface water, refined in Phase II Tier B	[mg L^{-1}]	See section 4.2.3.2
DT_{50}	Half-life for biological transformation, water/sediment total system	[d]	-
$PEC_{GW-REFINED}$	Predicted environmental concentration in the groundwater after entry by bank filtration, refined in Phase II Tier B	[mg L^{-1}]	-

Risk characterisation

The refined RQ_{GW} should be recalculated using the refined PEC_{GW} (Equation 33) and the PNEC value from Phase II Tier A.

When a risk to the groundwater ecosystem cannot be excluded, the applicant should propose adequate precautionary and safety measures to protect groundwater ecosystems (see section 7).

4.2.7.3. Phase II Tier A assessment for groundwater (via porewater; only when risk assessment for the soil compartment is triggered)

Tier A Exposure assessment for porewater

The PEC porewater (PEC_{PW}) is based on the PEC_{SOIL} as calculated in chapter 4.2.6 (see Equations 21-25) and is calculated by Equation 34.

$$PEC_{PW} = \frac{PEC_{SOIL} \cdot RHO_{SOIL}}{K_{SOIL-WATER} \cdot CONV_{m3/L}} \qquad \textbf{Eq. 34}$$

The partition coefficient between soil and water ($K_{SOIL-WATER}$) is calculated according to Equation 35.

$$K_{SOIL-WATER} = F_{WATER,SOIL} + \left(\frac{F_{SOLID,SOIL} \cdot Kp_{SOIL} \cdot RHO_{SOLID}}{CONV_{m3/L}}\right) \qquad \textbf{Eq. 35}$$

$$Kp_{SOIL} = F_{OC,SOIL} \cdot K_{FOC,SOIL} \qquad \textbf{Eq. 36}$$

Parameters used in Equations 34 – 36:

Parameter	Description	Unit	Default value / reference
PEC_{PW}	Predicted environmental concentration in porewater in agricultural soil after sludge application	[mg L^{-1}]	-
$PEC_{SOIL,SS}$	Predicted environmental concentration in wet weight soil in a steady-state situation	[mg kg_{ww}^{-1}]	See Equation 22
PEC_{SOIL}	Predicted environmental concentration in wet weight soil	[mg kg_{ww}^{-1}]	See Equation 21
RHO_{SOIL}	Bulk density of wet soil	[kg_{ww} m^{-3}]	1,700
$K_{SOIL-WATER}$	Soil-water partition coefficient	[m^3 m^{-3}]	See Equation 35
$F_{WATER,SOIL}$	Volume fraction water in soil	[m_{water}^3 m_{soil}^{-3}]	0.2
$F_{SOLID,SOIL}$	Volume fraction solids in soil	[m_{solid}^3 m_{soil}^{-3}]	0.6
Kp_{SOIL}	Solid-water in soil partition coefficient	[L kg_{dw}^{-1}]	See Equation 36
RHO_{SOLID}	Density of the solid phase	[kg m^{-3}]	2,500
$F_{OC,SOIL}$	Weight fraction organic carbon in soil solids	[kg_{oc} kg_{dw}^{-1}]	0.02
$K_{FOC,SOIL}$	Partition coefficient between organic carbon and water derived from soil	[L kg_{oc}^{-1}]	See Table 3. Determined using OECD 106
$CONV_{m3/L}$	Conversion factor from L to m^3	[L m^{-3}]	1,000

Risk characterisation

If the PEC_{PW} is higher than the PEC_{GW}, the highest value should be used for the calculation of the RQ for the groundwater compartment (RQ_{GW}) according to Equation 29 (see also section 4.2.7.1).

If the RQ is ≥1, risk refinement options should be used in Phase II Tier B as described below.

4.2.7.4. Phase II Tier B Assessment for groundwater (via porewater)

Tier B Exposure assessment for porewater

If a risk for groundwater organisms has been identified in Tier A, it is possible to recalculate with the refined PEC_{SOIL}, as described above for Tier B (see section 4.2.6.2).

Risk characterisation

The refined RQ should be recalculated using the refined PEC_{PW}. If a risk to the groundwater ecosystem cannot be excluded at this stage, advanced groundwater models such as PEARL can be used to refine the PEC_{PW} by simulation the leaching behaviour (see EMA/CVMP/ERA/418282/2005-Rev.1- Corr.). If not applicable, the applicant should propose adequate precautionary and safety measures to protect groundwater ecosystems (see Section 7).

4.2.8. Secondary poisoning

Secondary poisoning is a toxic effect on animals at trophic levels higher in the food chain (e.g. predatory fish, birds and mammals) resulting from consumption of contaminated prey (in the aquatic food chain e.g. aquatic invertebrates or fish). It is especially relevant for compounds that accumulate through the food chain, i.e. mainly lipophilic compounds. Thus, when log K_{ow} (or log D_{ow} for dissociating compounds) is ≥3, the potential for secondary poisoning should be evaluated. First, a bioconcentration factor in fish (BCF_{FISH}) should be determined experimentally (Table 14). It should be noted that a lack of accumulation in mammals does not exclude a potential for accumulation in fish and other aquatic species. Accumulation may occur as a result of decreased activity of enzymes involved in the transformation of xenobiotics in fish and/or lower trophic levels, differences in exposure routes (e.g. air via lungs vs. water via gills), differences in metabolism, different excretion routes, etc.

When the BCF_{FISH} is ≥100 L kg^{-1}, the potential for secondary poisoning should be further assessed. The BCF_{FISH}, together with mammalian toxicity data from the non-clinical safety assessment of the active substance are used to derive a $PNEC_{SW,\ SECPOIS}$. For some active substances, relevant mammalian or bird toxicity data can be found via a targeted review of public literature (see section 6). In cases where the BCF_{FISH} is >2000 L kg^{-1}, the B-criterion (and possibly also the vB-criterion) is considered fulfilled and a PBT/vPvB assessment is warranted (see chapter 5).

Input values

Inputs for the calculation of secondary poisoning potential are the BCF_{FISH} and the chronic mammalian toxicity data from the non-clinical part of the dossier.

Bioconcentration factor (BCF)

The BCF is determined in fish using the OECD 305 test guideline. Aqueous exposure is the preferred methodology when technically feasible, because a BCF value is obtained. Dietary exposure yields a biomagnification factor (BMF) from which a BCF can only be estimated using the depuration rate constant. In the aqueous exposure BCF test, the kinetic calculation of BCF (preferably based on simultaneously fitted uptake and elimination rates and taking dilution due to fish growth into account) is preferred over the steady state calculation (based on concentrations in fish and water). BCF values should be normalised to 5% lipid content. BCF values may need to be growth-corrected, depending on the weight of the fish. A minimised study design is also described in OECD 305, but this may only be

used for screening purposes. It may not be used to determine a steady-state BCF value because it cannot be determined whether steady state is reached (see OECD guidance document No. 264, 2017 for additional information). The kinetic BCF resulting from the minimised test should be considered as less accurate than the BCF from a full BCF study.

For ionisable substances the BCF test should be conducted at a stable pH consistent with the most bioaccumulative form of the test chemical (usually the non-dissociated form or the form with the most neutral molecule species). For more information see OECD Guidance Document No. 23 (OECD, 2019).

Table 14: Trigger for secondary poisoning assessment

Study	Endpoint	Guideline	Trigger for further assessment of secondary poisoning
Bioaccumulation in fish	BCF_{FISH} [L kg^{-1}]	OECD 305	≥100

Toxicity data

The first data to consider are the chronic (≥6 months) mammalian toxicity data from the non-clinical part of the dossier. If available and relevant, mammalian and bird toxicity data from the public literature may also be included. The lowest no observed adverse effect level (NOAEL) from the available repeated-dose toxicity studies (oral administration and chronic treatment are preferred, the use of other routes of administration should be justified) should be recalculated to a chronic no observed adverse effect concentration (NOAEC).[11]

If chronic studies are not available, the NOAEL values from sub-chronic studies (3-month or Developmental and Reproductive Toxicity Studies (DART)) or subacute studies (28-day) can also be used, provided that an appropriate additional AF of 3 and 10 respectively is applied to account for the limited exposure duration (see Step 3). Acute lethal doses (LD_{50}) and acute effect concentrations (LC_{50}) are only to be used if other data is not available. Due to the high uncertainty associated with the translation into chronic endpoints, an additional AF of 100 should be applied in Step 3. In accordance with Directive 2010/63/EU, repetition of *in vivo* toxicity data will not be required to complete the secondary poisoning assessment.

Calculation of secondary poisoning potential

Secondary poisoning potential can be calculated using different methodologies.

For the aquatic food chain, in the guidance document for derivation of EQS under the Water Framework Directive (European Commission, 2018), the diet-based and dose-based methods have been replaced by a method that accounts for the energy content of food items of the predator considered in the assessment. Using this method, application of default Afs to convert from laboratory diet to natural diet can be avoided as it is described in other approaches (ECHA, 2023b; ECHA, 2016; EFSA 2023). Energy-normalised concentrations can be calculated from nonclinical study endpoints reported as a daily dose (NOAEL) or as a diet concentration (NOEC). Both approaches yield similar results. For HMPs, mammalian toxicity data will predominantly be obtained from the nonclinical part of the dossier where NOAEL values are reported. Therefore, the remainder of this section will elaborate on the method using these values.

[11] Note that in the stepwise approach below the NOAEC is expressed as the threshold concentration in fish ($NOEC_{FISH, CHRONIC}$).

Step 1: For the lowest NOAEL, the daily energy expenditure (*DEE*) of the test animals is determined based on the body weight (BW in kg) of the test animals and the regression between *DEE* (under field conditions) and BW of 46 mammalian species (Equation 37). Preferably, the time-weighted average (arithmetic mean) BW of the animals from the NOAEL dose is used. If this is not available, the initial weight of the animals from the NOAEL dose can be used, which is a conservative approach.

Step 2: The energy-normalised concentration $C_{\text{energy normalised}}$ is calculated with the obtained *DEE* using Equation 38, where *BW* is in kg. This will result in an energy-normalised concentration. If bird data is available, the same approach should be followed, but appropriate regression parameters should be taken from the guidance document for derivation of EQS under the Water Framework Directive.

$$logDEE = 2.9583 + 0.7149 \cdot log(BW) \qquad \textbf{Eq. 37}$$

$$C_{\text{ENERGY NORMALISED}} = DOSE \cdot \frac{BW}{DEE} = DOSE \cdot 0.0011 \cdot BW^{0.2851} \qquad \textbf{Eq. 38.}$$

Parameters used in Equation 37 and 38:

Parameter	Description	Unit	Default value / reference
DEE	Daily energy expenditure	[kJ d^{-1}]	-
BW	Average body weight	[kg_{bw}]	-
DOSE	Daily dose	[mg kg_{bw}^{-1} day^{-1}]	-
$C_{\text{ENERGY NORMALISED}}$	Energy-normalised concentrations	[mg kJ^{-1}]	-

Step 3: The energy normalised endpoint of the toxicity test is calculated into a threshold concentration in fish using Equation 39. If there is information that other food items lead to lower concentrations than those in fish (see WFD guidance (European Commission, 2018) for more details), these food items should be taken into account (e.g. mussels, aquatic invertebrates and plants).

$$C_{\text{FISH}} = C_{\text{ENERGY NORMALISED}} \cdot energycontent_{\text{FOOD ITEM,DW}} \cdot (1 - moisturefraction_{\text{DIET}}) =$$

$$6.08 \cdot DOSE \cdot BW^{0.2851} \qquad \textbf{Eq. 39}$$

$$\text{If } DOSE = NOAEL \text{ then } C_{\text{FISH}} = NOEC_{\text{FISH}} \qquad \textbf{Eq. 40.}$$

Parameters used in Equation 39 and 40:

Parameter	Description	Unit	Default value / reference
C_{FISH}	Concentration in critical food item (fish) expressed as wet weight	[mg kg_{ww}^{-1}]	-
$C_{\text{ENERGY NORMALISED}}$	Energy-normalised concentrations	[mg kJ^{-1}]	-

Parameter	Description	Unit	Default value / reference
$energycontent_{FOOD\ ITEM,DW}$	Energy content of the critical food item (default food item is fish)	[kJ kg_{dw}^{-1}]	21,000 (fish)
$moisturefraction_{DIET}$	Moisture fraction of the critical food item (default food item is fish)	[-]	0.736 (fish)
$NOEC_{FISH}$	NOEC for bird of mammal expressed as a concentration in fish wet weight	[mg kg_{ww}^{-1}]	-

To account for differences in exposure duration, AFs should be applied to the recalculated NOEC values, i.e. an AF of 1, 3, 10 or 100 for toxicity data from chronic, subchronic, subacute or acute studies, respectively (Equation 41), as mentioned in the section above on input values.

Step 4: The RQ is calculated then. First, the $PNEC_{BIOTA}$ is calculated by applying an AF of 10 to the lowest NOEC value (Equation 41). In the exceptional case of a very data-rich substance, a PNEC may be based on the HC_5 (hazardous concentration for 5% of species) according to the WFD EQS guidance (European Commission, 2018).

$$NOEC_{\mathrm{FISH,CHRONIC}} = \frac{NOEC_{\mathrm{FISH}}}{\mathrm{AF}} \qquad \textbf{Eq. 41.}$$

$$PNEC_{\mathrm{BIOTA}} = \frac{\text{lowest } NOEC_{\mathrm{FISH,CHRONIC}}}{10} \qquad \textbf{Eq. 42.}$$

The $PNEC_{BIOTA}$ (based on a concentration in fish) is then converted into a $PNEC_{SW,SECPOIS}$ (based on a concentration in water) by dividing it by the BCF_{FISH} and BMF. The BMF should represent biomagnification under field conditions. A BMF resulting directly from a dietary fish bioaccumulation test cannot be used without modifications (ECHA, 2023b). In the absence of field-measured or experimentally derived BMF values, default BMF values can be used (Table 15). When field-derived bioaccumulation factors (BAF) are available, they can be used instead of BCF and BMF values. Especially for strongly biomagnifying substances, it appears to be more appropriate to use BAF values for the proper trophic level, as accumulation over more than one trophic level is accounted for in the BAF (European Commission, 2018).

$$PNEC_{\mathrm{SW,SECPOIS}} = \frac{PNEC_{\mathrm{BIOTA}}}{BCF_{\mathrm{FISH}} \cdot BMF} \quad \text{or} \quad \frac{PNEC_{\mathrm{BIOTA}}}{BAF} \qquad \textbf{Eq. 43.}$$

Table 15: Default BMF values for organic substances for secondary poisoning assessment (not applicable to PBT/vPvB assessment) (Annex XIII to the REACH Regulation taken from ECHA, Chapter R.7c: Endpoint specific guidance, Version 4.0 – December 2023)

log K_{ow} of substance	Measured *BCF* (fish) [L kg^{-1}]	BMF
<4.5	<2,000	1
4.5 - <5	2,000 – 5,000	2
5 – 8	>5,000	10
>8 – 9	2,000 – 5,000	3
>9	<2,000	1

The RQ for secondary poisoning is calculated using PEC_{SW} and $PNEC_{SW,\ SECPOIS}$; see Equation 44.

$$RQ_{SW,SECPOIS} = \frac{PEC_{SW}}{PNEC_{SW,SECPOIS}} \qquad \textbf{Eq. 44}$$

Parameters used in Equations 41 - 44:

Parameter	Description	Unit	Default value / reference
$NOEC_{FISH,CHRONIC}$	Chronic NOEC for bird of mammal expressed as a concentration in fish wet weight	[mg kg_{ww}^{-1}]	-
$NOEC_{FISH}$	NOEC for bird of mammal expressed as a concentration in fish wet weight	[mg kg_{ww}^{-1}]	-
AF	Assessment Factor	[-]	See step 3 of calculation of secondary poisoning potential
$PNEC_{BIOTA}$	Predicted No Effect Concentration for biota expressed as a concentration in fish wet weight	[mg kg_{ww}^{-1}]	-
$PNEC_{SW,SECPOIS}$	Predicted No Effect Concentration for biota expressed as a concentration in water	[mg L^{-1}]	-

Parameter	Description	Unit	Default value / reference
BCF_{FISH}	Bioconcentration factor in fish	[L kg^{-1}]	-
BMF	Biomagnification factor	[-]	See Table 15
BAF	Bioaccumulation factor	[L kg^{-1}]	-
PEC_{SW}	Predicted environmental concentration in surface water	[mg L^{-1}]	-

If the RQ is ≥1, a risk of secondary poisoning is identified.

For medicinal products for human use, it is usually sufficient to consider the risk in the aquatic food chain. However, if the trigger for a terrestrial risk assessment is exceeded (see section 4.2.2), it is also necessary to determine potential effects on the terrestrial food chain. Guidance can be found Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38 (EMA/CVMP/ERA/418282/2005-Rev.1- Corr.1), pp. 31.

4.3. Tailored testing strategy for active substances with a specific mode of action (MoA)

For certain groups of active substances, a tailored testing strategy is required due to their specific MoA. In section 4.3.1 the tailored testing strategy for antibacterials is described, which is only required if the PEC exceeds the action limit in Phase I of the ERA. In section 4.3.2 the identification of EAS and their tailored testing strategy is described. For EAS the action limit does not apply, and a Phase II risk assessment should always be performed.

The tailored testing strategy targets only the aquatic compartment, for which OECD ecotoxicity test protocols are available for a number of species and/or endpoints that may replace default test protocols, depending on the MoA.

However, for all active substances that require a Phase II risk assessment, where respective triggers for other compartments are met, a complete Phase II assessment is still required for those compartments, including fate studies.

4.3.1. Antibacterials

For active substances with an antibacterial MoA and no other known pharmacological targets, a tailored and targeted effect assessment should be performed for the aquatic compartment. Scientific knowledge and empirical data demonstrate that a tailored testing strategy focused on the effects on lower trophic levels including bacteria, algae and aquatic invertebrates is sufficiently sensitive for antibacterials and fish tests are not required. Table 16 lists the required effect studies for active substances with an antibacterial MoA in Tier A.

Table 16: Required effect tests in the tailored Tier A assessment for the aquatic compartment for active substances with an antibacterial mode of action (MoA)

Test	Test species[§]	Endpoint[*]
OECD 201	*Anabaena flos-aquae* (Cyanobacteria)	E_rC_{10} or NOErC
OECD 201	*Synechococcus leopoliensis* (Cyanobacteria)	E_rC_{10} or NOErC
OECD 201	*Raphidocelis subcapitata* [#](Green algae)	E_rC_{10} or NOErC
OECD 209	Activated sludge, respiration inhibition	EC_{10} or NOEC
OECD 211	*Daphnia magna* (Invertebrate)	EC_{10} or NOEC

[§] The test species recommended in the OECD 201 may be replaced by other species within the same taxonomic group provided it is scientifically and practically justified
[*]For the OECD 201 test, the average specific growth rate is the relevant endpoint to use. The culture should be in exponential growth during all time intervals of the experiment. For the OECD 211, various endpoints (e.g. related to survival or reproduction) are relevant. For both tests: The EC_{10} value is preferred over the NOEC value if a reliable dose/response curve is generated with concentrations around the EC_{10} and is hence used for the PNEC derivation when both are available.
 # Raphidocelis subcapitata formerly known as *Pseudokirchneriella subcapitata*

4.3.2. Endocrine active substances (EAS)

Some active substances may affect the reproduction or development of vertebrate or lower animals at concentrations below the trigger value of 0.01 µg L^{-1} in surface water. Many studies on the endocrine system published in the peer-reviewed literature document have shown that EAS can act *in vivo* at concentrations as low as pg L^{-1}. Changes of developmental and reproductive parameters can be major drivers of alterations in population growth. EAS exposure is linked to adverse changes in developmental and reproductive traits and effects in such endpoints are of particular relevance when assessing environmental risk.

Identification of EAS

If there is evidence that the active substance can exert an effect on development or reproduction by directly interacting or interfering with receptors, hormone levels or activities of oestrogens, androgens, thyroid hormones or other steroid hormones, that active substance should be assessed in Phase II regardless of the PEC. A tailored testing strategy that addresses its specific mechanism of action should be followed (see Table 17).

An active substance whose intended pharmacological action targets the endocrine system as described above is considered to be an EAS.

For other active substances, any existing information on potential non-intended endocrine activity should be obtained from the respective part of the dossier or, if relevant, other sources. This includes both *in vitro* and *in vivo* study-derived information. Endocrine-related effects relevant to the identification of an EAS include agonism, antagonism and modulation of relevant receptors, changes in steroid hormone levels and in steroidogenic tissues (adrenals and gonads), steroidogenic enzyme inhibition and direct interaction with the hypothalamic–pituitary–gonadal axis. Data should be evaluated using a weight-of-evidence approach to decide whether the substance should be considered an EAS and be assessed in Phase II following a tailored testing strategy. Data for evaluation could include:

In vitro data

- EC_{50}/IC_{50} in agonistic or antagonistic mode at levels < 10 µM at steroid hormone receptors
- IC_{50} at levels below 10 µM for inhibition of steroidogenic enzymes

In vivo data

- Endocrine-related adverse effects at the lowest observed adverse effect level (LOAEL) in pivotal toxicology, carcinogenicity or reproductive toxicology studies

Changes in steroid hormone levels and changes in steroidogenic tissues (adrenals and gonads) in mammals are considered relevant effects. Other relevant effects can include decreases in sperm function and reproductive capability, premature or delayed puberty, changes in oestrous cycles, carcinogenicity in endocrine organs and mammary glands, and changes in developmental landmarks, if there is evidence of an endocrine MoA. An integrated assessment with awareness of possible species-specific effects that do not predict environmental risk is expected. As examples, effects secondary to the role of inhibition or induction of drug metabolising isozymes or dopaminergic/anti-dopaminergic effects on the hypothalamo-prolactin axis would generally not be regarded as mechanisms which would warrant evaluation as an EAS.

Evidence from other sources

Evidence from scientific literature may be used to support the above listed criteria. Relevant information on altered parameters includes effects on development and reproduction such as intersex, sex ratio and feminisation or masculinisation of fish; effects on spawning for molluscs; and developmental effects on invertebrates, amphibia and/or fish. Where the evidence suggests endocrine related adverse effects at levels at or below the action limit of 0.01 µg L^{-1}, the active substance should be further assessed as an EAS and the trigger value does not apply.

Tailored testing of EAS

For all EAS, the assessment depends on the MoA of the compound. If it can be scientifically justified, the effect assessment may be tailored to specific groups of organisms of the aquatic compartment, e.g. fish and/or amphibians. Studies on environmental fate are required for all EAS. However, waiving of some effect tests may be applicable according to MoA, e.g. focus on specific long-term fish tests and, with justification, not include activated sludge (OECD 209) and/or algae (OECD 201).

In addition to substances identified as EAS, a tailored testing strategy should also be performed for active substances where the scientific literature shows evidence of endocrine related adverse effects at concentrations at or below the predicted PEC_{SW} as evidenced e.g. by intersex, sex ratio, feminisation or masculinisation, or effects at the population level in fish or amphibians. This information should be used to select the most appropriate chronic ecotoxicity study using apical endpoints.

A fish early life stage toxicity test (OECD 210) may not provide the most relevant ecotoxicological information for EAS since this test is rather short and it does not cover all relevant life stages like sexual maturation and reproduction. Thus, the design of a study should include the appropriate exposure time, the sensitive life-stage(s) and the relevant endpoints necessary to detect adverse effects and underlying modes of action. The evaluation of biomarkers may provide additional information for the interpretation of effects from apical endpoints but should not be used for the derivation of PNECs.

A tiered testing strategy should be followed; e.g. for suspected effects on the oestrogen or androgen receptors an *in vivo* screening test (OECD GD 148 or OECD 229 & 230) may be performed. These tests also evaluate secondary sexual characteristics in fathead minnow or medaka (OECD 229 or 230) or

gonad histopathology (OECD 229). As stated in these test guidelines, both are screening tests only, and therefore not suitable for a quantitative risk assessment. In case it is already known from e.g. mammalian toxicity studies that estrogenic or androgenic receptors are targeted, the screening assay (OECD 229 or 230) will become redundant. If effects are observed in such a screening test, long-term adverse effects should be characterised in a fish sexual development test or a fish full lifecycle test. If the MoA or the most sensitive endpoints are not known, a fish full lifecycle study should be performed. Where the MoA is known, it may still be necessary to perform a fish full lifecycle test, for instance, when the partial lifecycle tests do not cover all appropriate endpoints or life stages.

The table below summarises effect tests covering the most sensitive endpoints for the different MoA. The applicant should develop a test proposal based on MoA considerations, possibly covering test species other than those listed below. The appropriate PNEC is calculated by applying an AF of 10 to the lowest EC_{10} or NOEC for population relevant endpoints.

Table 17: Overview of recommended effect studies assessing apical endpoints for active substances with different endocrine mechanism of action and thyroid hormone agonist and antagonists.

Mechanism of Action	Recommended Effect Test
Oestrogen Receptor Agonist	Fish full lifecycle test (DRP no. 95 /OECD 240)
Oestrogen Receptor Antagonist	Fish sexual development test (OECD 234) or Fish full lifecycle test (DRP no. 95 / OECD 240)
Androgen Receptor Agonist	Fish sexual development test (OECD 234) or Fish full lifecycle test (DRP no. 95 / OECD 240)
Androgen Receptor Antagonist	Fish full life-cycle test (DRP no. 95 / OECD 240)
Aromatase Inhibitor	Fish sexual development test (OECD 234) or Fish full lifecycle test (DRP no. 95 / OECD 240)
Thyroid hormone agonists and antagonists	Larval amphibian growth and development assay (OECD 241)
Other mechanisms are subject to expert judgement	

It may be appropriate to conduct a range finding study to determine the appropriate concentrations of drug substance to use in the definitive study.

If there is still uncertainty as to which test is most appropriate based on the possible mode(s) of action of compound, the applicant is encouraged to seek scientific advice regarding the detailed study design, particularly before conducting fish or amphibian tests.

5. PBT/vPvB assessment

PBT/vPvB substances are substances which will persist in the environment, bioaccumulate in organisms and are toxic to environmental organisms. Due to their physico-chemical characteristics, it is not possible to predict the environmental fate of these substances or the kind of adverse effects that could occur over long periods of time. Chronic exposure and long term cumulative adverse effects may lead to uncertainty when calculating the PEC via established exposure models, and/or establishing the PNEC from standard laboratory tests. Because the PBT/vPvB assessment is a hazard assessment, every

active substance should be assessed for its PBT/vPvB properties regardless of its PEC. A tiered PBT/vPvB testing strategy should be followed, beginning with a PBT/vPvB screening assessment (determination of log K_{ow}), followed by a definitive PBT/vPvB assessment when the trigger value of log K_{ow}>4.5 is met. The definitive PBT/vPvB assessment consists of sequentially testing and evaluating persistence, bioaccumulation, and toxicity.

Annex XIII of the REACH regulation (Regulation (EC) No 1907/2006) lays down the criteria for the identification of PBT and vPvB substances (see Table 18). To ensure a harmonised approach, these criteria together with the methodology in the current REACH guidance on PBT/vPvB assessment should be followed (Guidance on information requirements and chemical safety assessment Chapter R.11: PBT/vPvB Assessment (ECHA 2023c), and Chapters R.7a, R.7b, and R.7c on endpoints specific guidance) (ECHA 2017, 2023a-b). The REACH guidance documents in their current versions may be obtained from the ECHA website.

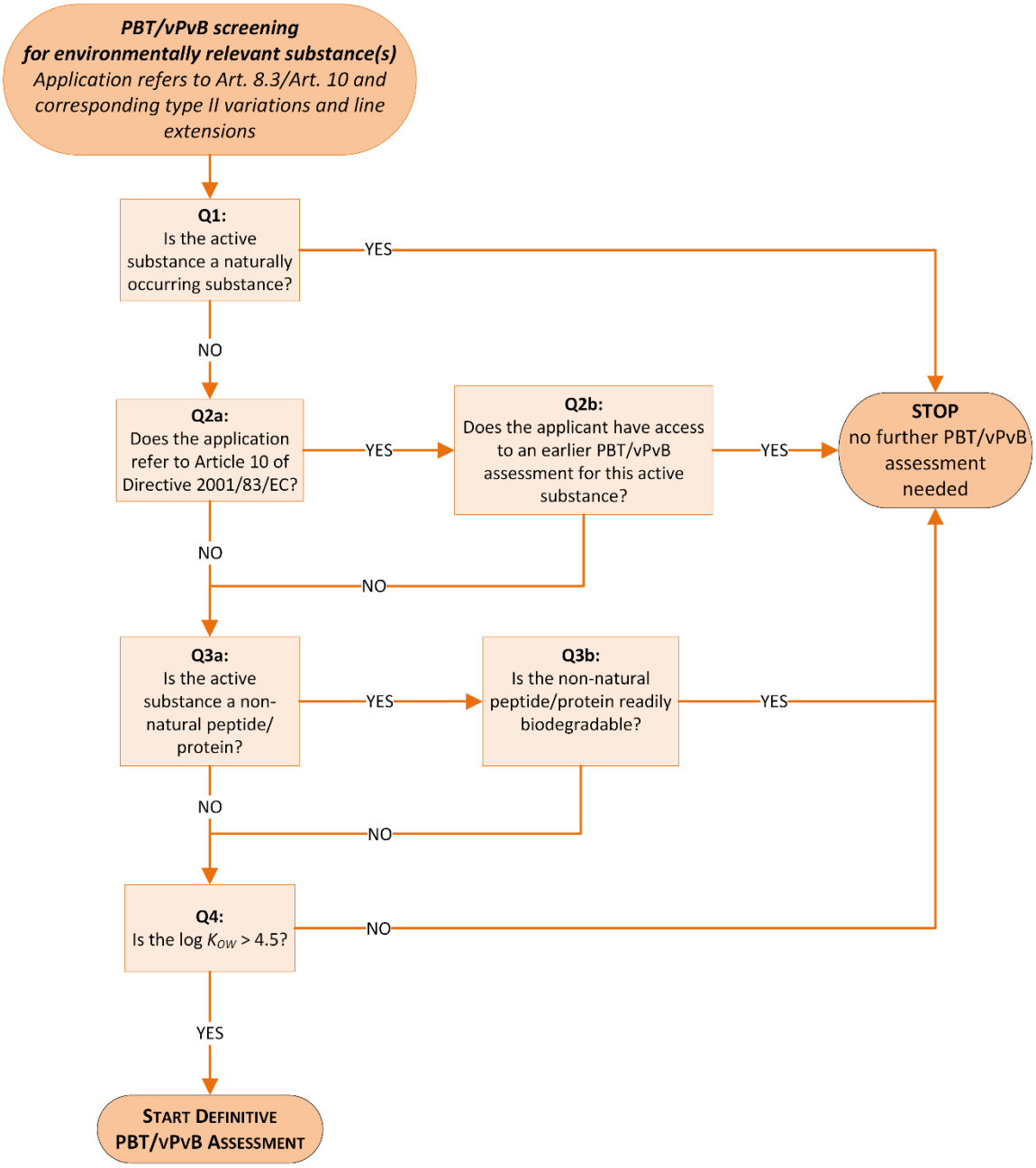
When a Phase II risk assessment is triggered and the log K_{ow} for the active substance is ≥3, a BCF should be determined experimentally according to OECD 305 to evaluate the potential for secondary poisoning (see section 4.2.8). When this study results in a BCF-value > 2000, and the T-criterion according to Table 18 is fulfilled, a simulation degradation study should be performed to check whether the substance should be classified as a PBT substance. In case of a BCF-value >5000, a simulation degradation study should be performed and evaluated against the vPvB criteria.

Note that in the risk assessment, data may be generated which can also be used for the PBT/vPvB assessment. Depending on the comprehensiveness and results of the data generated for the risk assessment, further studies may need to be conducted to conclude on PBT/vPvB (see Table 19). As is the case for the risk assessment, the PBT/vPvB assessment is performed for the pharmacologically active substance (e.g. in case of a pro-drug, the PBT/vPvB assessment may be required for the active metabolite).

5.1. PBT/vPvB Screening

A PBT/vPvB screening assessment should be performed for all active substances. The PBT/vPvB screening assessment consists of a decision tree (Figure 3 below). The questions in the decision tree are described in detail below Figure 3. The outcome of decision tree may be that the PBT/vPvB screening assessment is sufficient, or that a definitive PBT assessment is required. When no definitive PBT assessment is needed, the applicant should justify this decision in the ERA report.

Figure 3: PBT/vPvB Screening Decision tree (Q: question)



<u>Questions in PBT/vPvB Screening Decision tree (Figure 3):</u>

Q1: Is the active substance a naturally occurring substance?

See Q1 of the Phase I Risk Assessment Decision Tree (Figure 2, section 4.1).

Q2a: Does the application refer to Article 10 of Directive 2001/83 EC as amended?

See also Q2a of the Phase I Risk Assessment Decision Tree (Figure 2, section 4.1).

Q2b: Does the applicant have access to an earlier PBT/vPvB assessment for the active substance?

To avoid unnecessary repetition of studies, applicants are encouraged to share their data. If the current applicant has access to a relevant PBT/vPvB assessment performed by another MAH, the study reports may be submitted together with written consent from the owner of the data, as part of the PBT/vPvB assessment of the new application. In case data sharing is not agreed, see also Q2b of the Phase I Risk Assessment Decision Tree (Figure 2, section 4.1). The applicant may also submit a relevant PBT/vPvB assessment performed within another regulatory framework (e.g. for the chemical safety assessment under REACH), if sufficient information is provided (see section 3.1.4).

If the reference PBT/vPvB assessment is not in line with the current guideline (e.g. studies are missing) the applicant should conduct the missing/insufficient studies and update the PBT/vPvB assessment.

Q3a: Is the active substance a non-natural peptide/protein?

See also Q3a of the Phase I Risk Assessment Decision Tree (Figure 2, section 4.1).

Q3b: Is the non-natural peptide/protein readily biodegradable?

For non-natural peptides/proteins, an additional screening step should be performed to demonstrate that they quickly degrade in the environment.

When it can be demonstrated that the non-natural peptide/protein is excreted by humans in amounts <10% of the dose, or it is demonstrated to be readily biodegradable in an OECD 301 test, the PBT screening assessment is sufficient and no definitive PBT assessment is warranted.

Q4: Is the log K_{ow} >4.5?

The PBT/vPvB screening ends with the determination of an octanol/water partitioning coefficient (log K_{ow}) of the active substance. In case of a dissociating substance, partitioning should be determined at three different pH values and the log D_{ow} for the neutral molecule (ion-corrected log D_{ow}) should be determined (see section 4.2.1.1). When the trigger value of log K_{ow} >4.5 is met, a definitive PBT/vPvB assessment should be performed.

5.2. Definitive PBT/vPvB assessment

5.2.1. PBT/vPvB Criteria

For industrial chemicals, the criteria for the assessment of P, B and T properties (Table 18) are specified in REACH Annex XIII. However, the specific classifications for the T-criterion under (b) and (c) in the table below are included for completeness but are applicable only for chemicals already classified under REACH according to the Regulation EC No 1272/2008 (Classification, Labelling and Packaging (CLP) Regulation). They are not used in the context of preclinical hazard assessment for human pharmaceuticals, as human pharmaceuticals are not within the scope of the CLP Regulation.

For most active substances the toxicity data available in the dossier for a human pharmaceutical can be used to assess whether the T-criterion for an ERA is fulfilled.

Table 18: PBT and vPvB criteria

Property	PBT criteria	vPvB criteria
Persistence	A substance fulfils the persistence criterion (P) in any of the following situations: (a) the degradation half-life in marine water is longer than 60 days; (b) the degradation half-life in fresh or estuarine water is longer than 40 days; (c) the degradation half-life in marine sediment is longer than 180 days; (d) the degradation half-life in fresh or estuarine water sediment is longer than 120 days; (e) the degradation half-life in soil is longer than 120 days.	A substance fulfils the "very persistent" criterion (vP) in any of the following situations: (a) the degradation half-life in marine, fresh or estuarine water is longer than 60 days; (b) the degradation half-life in marine, fresh or estuarine water sediment is longer than 180 days; (c) the degradation half-life in soil is longer than 180 days.
Bioaccumulation	A substance fulfils the bioaccumulation criterion (B) when the bioconcentration factor in aquatic species is higher than 2,000.	A substance fulfils the "very bioaccumulative" criterion (vB) when the bioconcentration factor in aquatic species is higher than 5,000.
Toxicity	A substance fulfils the toxicity criterion (T) in any of the following situations: (a) the long-term no-observed effect concentration (NOEC) or EC_{10} for marine or freshwater organisms is less than 0.01 mg/L; (b) substance meets the criteria for classification as carcinogenic (category 1A[12] or 1B[13]), germ cell mutagenic (category 1 or 1B), or toxic for reproduction (category 1A[14], 1B[15] or 2[16]) according to Regulation EC No 1272/2008[17] (c) there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Regulation EC No 1272/2008.	

(Annex XIII to the REACH Regulation taken from ECHA, Chapter R.11: PBT/vPvB assessment, Version 4.0 – December 2023)

[12] Substances known to have carcinogenic potential for humans (epidemiological and/or animal data)
[13] Substances presumed to have carcinogenic potential for humans (animal studies)
[14] Known human reproductive toxicant (human evidence)
[15] Presumed human reproductive toxicant (animal studies)

5.2.2. Performing the PBT/vPvB assessment

The most recent REACH guidance on PBT/vPvB assessment (R.11) should be followed where possible, and deviations should be scientifically justified. Note that the screening approaches used in REACH such as ecotoxicity QSARs are not applicable to human pharmaceuticals because of the specific modes of action. In order to avoid unnecessary animal testing, testing for the P, B and T criteria is conducted sequentially. For medicinal products for which a Phase II risk assessment is performed, most data is already available, and a stepwise approach is not necessary, with the exception of persistence data which is not available in Tier A unless a soil assessment is required in the ERA (see Table 2). In Table 19 an overview of studies is given to assess the different properties. Note that other information may be used as supportive information to conclude on these properties; for more information see the REACH guidance on PBT assessment.

Table 19: Overview of OECD test guidelines that stipulate which data can be used to assess PBT/vPvB properties

Property	Test guidelines	Study Type
Persistence	OECD 301 (or OECD 310)	Screening test for Ready Biodegradability
	OECD 307	Aerobic and anaerobic transformation in soil
	OECD 308	Aerobic and Anaerobic Transformation in Aquatic Sediment Systems
	OECD 309	Aerobic Mineralisation in Surface Water - Simulation Biodegradation Test
Bioaccumulation	OECD 305	Bioaccumulation in Fish: Aqueous and Dietary Exposure
Toxicity [a] [b]	OECD 201	Freshwater Alga and Cyanobacteria, Growth Inhibition Test
	OECD 211	Daphnia magna Reproduction Test
	OECD 210	Fish, Early-life Stage Toxicity Test

[a] Note that the list of test guidelines assessing toxicity is not exhaustive. The most common toxicity tests for three trophic levels to be tested are given.
[b] Note that the T criterion may also be fulfilled by evidence other than toxicity for marine or freshwater organisms (See Table 18).

5.2.2.1. Persistence

If the active substance is readily biodegradable (OECD 301), it is generally considered not persistent, and no further testing is required. If this is not the case, an OECD 307, OECD 308 and/or OECD 309 study should be performed to evaluate the P criterion. When the water solubility of a substance is very low, carrying out a sediment simulation study (OECD 308) or soil simulation study (OECD 307) may be preferable. The latter is also required if a terrestrial risk assessment is triggered and may be used for assessing persistence. For further guidance on testing strategies, see REACH guidance R.11.

[16] Suspected human reproductive toxicant (some evidence from humans or experimental animals, not sufficiently convincing to place the substance in category 1)
[17] Regulation on classification, labelling and packaging (CLP-Regulation (EC) No 1272/2008)

Persistence studies should reflect environmental temperatures in Europe and therefore preferably be conducted at 12°C. According to the REACH PBT/vPvB assessment guidance R.11 (ECHA, 2023c) if studies are conducted at different temperatures, DT_{50} values should be extrapolated to 12°C.

The Arrhenius equation (Equation 45) can be used to extrapolate DT_{50} values from the experimental temperature (e.g. 20°C) to 12°C.

$$DT_{50,T1} = DT_{50,T2} \cdot e^{\left(\frac{E_A}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right)} \qquad \textbf{Eq. 45}$$

Parameters used in the Arrhenius equation:

Parameter	Description	Unit	Default value
$DT_{50,T1}$	Degradation half-life value at reference temperature	[d]	–
$DT_{50,T2}$	Degradation half-life value at test temperature	[d]	–
E_A	Activation energy for degradation	[J mol^{-1}]	65,400
R	Gas constant	[J mol^{-1} K^{-1}]	8.314
T_1	Reference temperature (12°C)	[K]	285
T_2	Test temperature (e.g. 20°C)	[K]	–

The default value for activation energy *(E_A)* should be 65.4 kJ mol^{-1} [18] corresponding to a Q_{10} of 2.58, as specified in the EFSA guidance for use in the Forum for the co-ordination of pesticide fate models and their use (FOCUS) (EFSA, 2007).

In the OECD 308 water sediment simulation study for most persistent substances, removal from the aqueous phase is determined by dissipation due to partitioning to sediment rather than by true degradation. For this reason, transformation half-life values for the total system are considered more reliable than half-life estimates for the water phase and the sediment phase separately. Thus, dissipation half-life values for the single compartments water and sediment in water-sediment simulation studies, when determined in separate modelling, should only be used for the assessment of persistence when justified. For adsorptive substances, the sediment half-life can be reasonably estimated from the half-life for the total water-sediment system.

The formation of non-extractable residues (NER) should not per se be considered as degradation. While irreversibly bound NER (e.g. biogenic bound NER, covalently bound NER) can be seen as safe sink, potentially reversible NER (heavily sorbed, physically entrapped) pose a potential risk for the environment and should be considered in the assessment of persistence. For further information on the characterisation and further consideration of NER, see the most recent versions of ECHA guidance R.11 and R.7b (ECHA 2023c, ECHA 2023a).

Degradation studies should be preferably performed with radio-labelled substances and using the best possible extraction methods. Only in exceptional cases may acceptable degradation data be produced

[18] This value is the latest revised value and should be used instead of the one recommended value in the 'CVMP/VICH revised Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH Guidelines 6 and 38' of 68.9 kJ mol^{-1}.

using an unlabelled test substance (EMA/CVMP/ERA/349254/2014; Reflection paper on poorly extractable and/or non-radiolabelled substances), since the mass balance requirement cannot be met.

The highest sediment or total system DT_{50} value derived from the OECD 307 and/or 308 and/or 309 tests should be used for the PBT/vPvB assessment.

5.2.2.2. Bioaccumulation

The results of the OECD 305 (bioaccumulation in fish) study may be used for the assessment of bioaccumulation. This study is also required for risk assessment for secondary poisoning In Phase II (section 4.2.8). Since the B criterion is based on bioconcentration in aquatic species, the test species may also be species other than fish (e.g. mussels).

For comparison with the B and vB criteria, the measured bioconcentration value(s) (BCF) should be normalised to 5% lipid content, including a correction for growth dilution as recommended by the OECD test guideline 305 and REACH guidance (ECHA, 2023c).

Bioaccumulation studies should preferably be performed with radio labelled substances and using the best possible extraction methods. Remaining residues in biota should be considered after the experimental depuration phase.

For ionisable substances, the BCF test should be conducted at a stable pH consistent with the most bioaccumulative form of the test chemical (usually the non-dissociated form or the form with the most neutral molecule species). For more information see OECD Guidance Document No. 23 (OECD, 2019).

5.2.2.3. Toxicity

A substance fulfils the T criterion if it meets any of the toxicity criteria outlined in Table 18. Information on carcinogenicity, mutagenicity, reproductive and chronic toxicity for mammals should be available in other parts of the dossier and may also be obtained from the CLP inventory[19].This information should also be compared to the criteria in Table 18. Harmonised classifications in the CLP inventory can be used to conclude on the T criterion. In case the substance fulfils the T criterion based on classification in the CLP inventory, no additional toxicity testing is needed for the PBT assessment. Note that additional testing may still be necessary for the risk assessment.

When toxicity data, as mentioned above, does not show that the active substance fulfils the T criteria, normally the testing order based on chronic data is algae/cyanobacteria, then Daphnia and then fish. If the T-criterion is fulfilled (Table 18) by the chronic algae/cyanobacteria or Daphnia data, a chronic fish test is not necessary for the PBT assessment. If further aquatic toxicity studies other than the available studies are considered necessary to conclude on the T criterion, and if there are indications that representative species from one taxonomic group are more sensitive than species from other taxonomic groups, the most sensitive group should be chosen for chronic testing.

For those substances where a Phase II risk assessment is triggered, sufficient toxicity studies are already available to verify whether the T criterion is met.

6. Search and evaluation of data

6.1. Data Search

The Applicant should provide a targeted literature review on endpoints of significance to the ERA. This literature review is also expected, even if data or a previously performed ERA is obtained from another

[19] https://echa.europa.eu/nl/information-on-chemicals/cl-inventory-database

MAH, to identify new information on ecotoxicity of the active substance. All submitted data needs to be assessed for reliability (see section 6.2). If of acceptable quality, data from published literature on the active substance should be employed in the ERA, as

- an alternative or supplement to the recommended standard experimental studies;
- a support for a proposed tailored assessment strategy (see section 4.3); and/or
- an update when data or a previously performed ERA is obtained from another MAH, to identify new information on ecotoxicity of the active substance.

The applicant is requested to show how the literature search was performed, e.g. by stating the search engine and search terms used. To be acceptable for use in risk and/or PBT assessment, literature studies should be of sufficient reliability and include a description of all relevant aspects of the study. In addition to meeting reliability criteria, literature studies used as alternatives to experimental studies should be comparable in design to studies recommended in this guideline (e.g. OECD technical guideline study designs). The applicant should submit a list of all relevant studies accompanied by an overview of the reliability scores. GLP compliance is not an absolute requirement for studies in the published literature.

Public Assessment Reports (PARs or EPARs) and reviews or summary data from other regulatory frameworks cannot be used as data in the ERA dossier. Endpoints are owned by the company who submitted them in the original procedure and cannot be used by other applicants without this letter of access. If the applicant has a letter of access, the applicant also should have the study reports available and submit those.

6.2. Evaluation of studies

The approach used to assess the reliability and relevance of a study should be based on scientific argumentation and all studies, whatever their source, should be assessed in the same manner. A standardised assessment method designed for toxicological/ecotoxicological studies, such as the CRED method (Moermond et al, 2016), is therefore recommended. All studies should be assigned a reliability category and be accompanied by a short study summary. If the CRED method is used, all studies with reliability scores of 1 (reliable) and 2 (reliable with restrictions) should be used for the risk assessment. Studies with reliability 3 (unreliable) or 4 (not assignable due to lack of information) cannot be used.

7. Labelling and risk mitigation

When the possibility of environmental risks cannot be excluded, specific arrangements to limit the environmental impact shall be made. The applicant should propose and discuss a strategy for risk mitigation. Appropriate mitigation measures should generally aim at minimising the quantity discharged into the environment.

Precautionary and safety measures may consist of:

- An indication of potential risks presented by the medicinal product for the environment in the product information.
- Instructions on appropriate product storage and disposal.

  Appropriate disposal of unused pharmaceuticals, e.g. when shelf life has expired, is considered important to reduce the environmental exposure. In order to enhance protection of the

environment, it is therefore recommended that even medicinal products that do not require special disposal measures are appropriately labelled. See Table 20.

- Appropriate measures regarding the use of the medicinal product (e.g. to avoid the discharge of formulations such as patches and other devices into the sewage).

Precautionary and safety measures should be adequate given the anticipated use of the product and are to be included in the SmPC and patient leaflet.

Table 20: Proposed labelling aimed at minimising discharge of unused medicine into the environment

ERA category	SmPC section 5.3	SmPC section 6.6	Labelling (section 10)	PL (section 5)
No significant risk to the environment **or** **Current ERA data does not suggest a potential risk to the environment**	No statement	Any unused medicinal product or waste material should be disposed of in accordance with local requirements.	No statement	Do not throw away any medicines via wastewater <or household waste>. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment.
ERA has identified a potential risk to the environment.	*Information to be driven by conclusion of the assessment e.g.:* <Environmental risk assessment studies have shown that <act.subst> has the potential to be <persistent, bioaccumulative and toxic (PBT) >< very persistent and very bioaccumulative (vPvB)> to the environment.>* or <Environmental risk assessment	This medicinal product may pose a risk to the environment. (See section 5.3) Any unused medicinal product or waste material should be disposed of in accordance with local requirements.*	No statement*	Do not throw away any medicines via wastewater <or household waste>. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment.*

ERA category	SmPC section 5.3	SmPC section 6.6	Labelling (section 10)	PL (section 5)
	studies have shown that <act.subst> may pose a risk for *<environmental compartment(s)>*. >* (See section 6.6)			

* The actual information provided in the labelling and the PL should be considered on a case-by-case basis depending on the specific risk. In the package leaflet (PL), this could lead to a specific advice regarding disposal. In the labelling, a relevant statement, if any, should be as short as possible, e.g. "Disposal: Read the package leaflet".

8. Scientific advice from the EMA or national competent authorities

The applicant may request scientific advice on issues related to environmental risk assessment and on possible precautionary and safety measures to be taken with respect to the use and disposal of a medicinal product.

9. Structure of the ERA report

The ERA report should be presented in Module 1.6 of the eCTD dossier. The full study reports and references should be provided in the annex of the ERA.

The ERA report should start with a clear identification of the active substance, including company name/code, IUPAC name, CAS number, empirical formula, structural formula, SMILES code, and molecular weight.

There may be cases in which the absence of environmental studies could be justified, as specified in section 4.1. In these cases, the expert should provide a rationale for the absence of studies in addition to the identification as mentioned above.

The report should contain summaries of all studies used.

A dated signature of the author, information on the author's relevant educational, training and occupational experience, and a statement of the author's relationship with the applicant, shall be included.

10. References

Commission Regulation (EC) No 1085/2003 of 3 June 2003 concerning the examination of variations to the terms of a marketing authorisation for medicinal products for human use and veterinary medicinal products falling within the scope of Council Regulation (EEC) No 2309/93, Annex II.

Council Directive 2013/59/Euratom of 5 December 2013 laying down basic safety standards for protection against the dangers arising from exposure to ionising radiation, and repealing Directives 89/618/Euratom, 90/641/Euratom, 96/29/Euratom, 97/43/Euratom and 2003/122/Euratom

Directive 2001/83/EC (2001), Directive of the European Parliament and of the Council of 6th of November 2001 on the community code relating to medicinal products for human use.

EC (European Commission) (2018), Technical Guidance for Deriving Environmental Quality Standards. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No.27, Technical Report-2011–055.

ECETOC (2013), Understanding the relationship between extraction technique and bioavailability. 159 Technical Report No. 117, Brussels, May 2013, ISSN-0773-8072-117.

ECHA (2016), Guidance on Information Requirements and Chemical Safety Assessment: Chapter R.16: Environmental exposure assessment. Version 3.0, 2016 (under revision)

ECHA (2017), Guidance on Information Requirements and Chemical Safety Assessment: Chapter R.7a: Endpoint specific guidance. Version 6.0, 2017 (under revision)

ECHA (2023a), Guidance on Information Requirements and Chemical Safety Assessment: Chapter R.7b: Endpoint specific guidance. Version 5.0, 2023

ECHA (2023b), Guidance on Information Requirements and Chemical Safety Assessment: Chapter R.7c: Endpoint specific guidance. Version 4.0, 2023

ECHA (2023c), Guidance on Information Requirements and Chemical Safety Assessment: Chapter R.11: PBT/vPvB assessment. Version 4.0, 2023

EFSA (2007), Opinion on request from EFSA related on the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. Scientific opinion of the panel on plant protection products and their residues (PPR-panel). The EFSA Journal 622: 1-33.

EFSA (2023), Guidance on the risk assessment for Birds and Mammals. EFSA Journal 2023;21(2):7790, 300 pp

EMA (2005a), Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38. (EMA/CVMP/ERA/418282/2005-Rev.1- Corr)

EMA (2006), Guideline on environmental risk assessments for medicinal products consisting of, or containing, genetically modified organisms (GMOs). (EMEA/CHMP/BWP/473191/2006 – Corr)

EMA (2016), Reflection paper on poorly extractable and/or non-radiolabelled substances. (EMA/CVMP/ERA/349254/2014)

EMA (2018), Guideline on assessing the environmental and human health risks of veterinary medicinal products in groundwater. (EMA/CVMP/ERA/103555/2015)

EMA (2023), Pre-authorisation procedural advice for users of the centralised procedure: Chapter 3.4.2. When do I have to submit an Environmental Risk Assessment (ERA)? (EMEA-H-19984/03 Rev. 104)

EMA (2023), Pre-authorisation procedural advice for users of the centralised procedure: Chapter 3.4.3. What should I submit if my medicinal product contains or consists of genetically modified organisms (GMOs)? (EMEA-H-19984/03 Rev. 104)

Moermond et al. (2016), CRED: Criteria for reporting and evaluating ecotoxicity data. Environ Toxicol Chem. 2016 May;35(5):1297-309. doi: 10.1002/etc.3259. Epub 2016 Mar 18. PMID: 26399705.

OECD (2019 as amended), Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, OECD Series on Testing and Assessment, OECD Publishing, Paris, https://doi.org/10.1787/0ed2f88e-en.

Annex

SimpleTreat manual

1. Model

Download the latest version of SimpleTreat from https://www.rivm.nl/en/soil-and-water/simpletreat. - Background information on the model can be found in Struijs (2014) and Struijs (2015).

2. Data entry – default Phase II data set

2.1 Substance properties

For the guidance in this section, we assume that experimental data on the active substance (AS) are available at a Phase II ERA level, which is considered the default situation. See section 3 for guidance when less data is available.

- Open SimpleTreat.
- Go to tab **Substance**. Enter the first four parameters and, where applicable, their temperature of determination (in Kelvin).
  - Molecular weight [g mol^{-1}]
  - Vapour pressure [Pa] and temperature of determination [K]
  - Water solubility [mg L^{-1}] and temperature of determination [K]
  - $K_{FOC,SLUDGE}$ [L kg_{oc}^{-1}], enter this value in the field 'Organic carbon partition coefficient (K_{OC})': Enter the K_{FOC} determined using activated sludge here, not the K_{FOC} determined in soil. For refinement of PEC_{SW} (see chapter 4.2.3.2 of the ERA guidance) the lowest K_{FOC} derived from sludge should be used (n=2). For soil risk assessment (see chapter 4.6.2.1 of the ERA guidance) the highest K_{FOC} derived from sludge should be used (n=2). If 3 or more types of sludge are available (n≥3) the geometric mean can be used.
  - Result of the ready biodegradability test (OECD 301): readily biodegradable or not readily biodegradable.

No data needs to be entered in the other fields. Hence, the fields for K_{ow}, D_{ow}, pK_a, H (Henry coefficient or Henry's law constant), Kp_s and Kp_a (partition coefficients in raw sewage and activated sludge) are left empty. The **Chemical class** selection box (pull down menu) is coupled to K_{ow}, D_{ow} and pK_a. If this data is omitted, the selection made in 'Chemical class' is not used.

- Go to tab **Biodegradation**. From the menu **Select biodegradation test method**, select 'Method 1: estimated from OECD/EU standardised biodegradability tests (OECD 301 series, 310, 302 series)'. Under **Select a degradation rate constant corresponding to a TGD-EU test result, or choose to enter a custom value**, select the line corresponding with the outcome of the OECD 301 test:
  - Ready biodegradable, fulfilling the 10 d window: 1 h^{-1},
  - Ready biodegradable, not fulfilling the 10 d window: 0.3 h^{-1},
  - Not biodegradable: 0 h^{-1}

2.2 Emitted concentration of AS

- Go to tab **Emission scenario**.
- In the field Emission rate chemical, enter the value calculated for $Elocal_{WATER}$ in [kg d^{-1}] using Equation 7 from the guideline.
- The other values in this tab are left at their default value.

2.3 Other settings

2.3.1 Model of operation

In the tab **Mode of operation**, all pre-selected options and parameters keep their default values. That means that a municipal STP is modelled, including a primary settler ('Include primary solids removal') with Surface aeration. The selection of default values for the operational parameters is detailed in the cited references (see Section 1).

3. Data entry - reduced data set

3.1 Other type of data on biodegradation

- A rate constant for biodegradation determined in an OECD 314B test can also be used in SimpleTreat modelling. It should be entered in tab **Biodegradation**, by selecting 'Method 2: chemical is biodegradable in activated sludge batch test (OECD 314B)' under **Select biodegradation test method.** The rate constant expressed in [h^{-1}] and temperature of determination should be entered in the fields below.
- In case no OECD 301, OECD 302 or OECD 314B study is available, and no other information on biodegradation of the substance in activated sludge is at hand (e.g. data from scientific literature), the substance is considered 'Not biodegradable' by default. This should be selected in the tab **Biodegradation**, in the pull-down menu under **Select a degradation rate constant corresponding to a TGD-EU test result or choose to enter a custom value**. Note that a 308 study cannot be used to estimate biodegradation of the AS in activated sludge.

3.2 Henry's Law constant

- If no value for *H* is available, a value for water solubility (*S*) and vapour pressure (*Vp*) should be entered, along with the temperature of determination for both parameters. In case no value for vapour pressure (*Vp*) is available, a default value of 10^{-6} can be used*. H* is then calculated from *S* and *Vp*.
- In case an experimentally determined value for *H* is available, it can be entered, in [Pa m^3 mol^{1}], along with its temperature of determination. In this situation, a value for *S* and *Vp* need not be entered. In case they are entered, the experimentally determined *H* is given priority over *H* calculated from *S* and *Vp* the in the calculations.

4. Results used for risk assessment

4.1 Refined risk assessment for surface water (see chapter 4.2.3.2 of ERA guidance)

Go to tab **Distribution** and select 'Table' in the top right corner. This tab shows the calculated distribution of the AS mass at steady state over the five compartments discerned in the modelled STP. The fraction emitted to water ($F_{STP,WATER}$) is used in Equation 9 of the guideline. Although this compartment is called 'water', this represents the effluent of the STP, which is emitted to the surface water adjacent to the treatment plant.

Note that SimpleTreat displays the fractions as percentages, divide the result by 100 before entry as $F_{STP,WATER}$ in Equation 9.

4.2 Phase II A – risk assessment for soil (see chapter 4.2.6 of ERA guidance)

To calculate the concentration in soil, the concentration in sludge is needed (C_{SLUDGE}).

Go to tab **Concentrations**. C_{SLUDGE} in [mg kg^{-1}] dry weight sludge is shown at the top of the left column ('Concentrations'), displayed as 'Combined sludge (C_{SLUDGE})'. This value should be used in Equation 21 of the ERA guidance.

5. Saving and exporting SimpleTreat results

- The results from a SimpleTreat run can be saved using File, Save in the menu bar.
- Under File, Options, a default directory for storing SimpleTreat files can be entered.
- Results can be exported (and then saved, if desired) to a spreadsheet (e.g. MS™ Excel) or pdf format. It is recommended to provide the SimpleTreat results with the ERA dossier.
- Under Calculation mode, the user can switch to calculations using an older version of SimpleTreat (version 3.1). This option is not needed for risk assessment of pharmaceuticals.