RAAF Appendix ENV-B

Scenario 2

Description

This scenario covers the analogue approach for which the read-across hypothesis is based on different compounds which have similar properties. For the REACH information requirement under consideration, the property investigated in a study conducted with one source substance is used to predict the properties that would be observed in a study with the target substance if it were to be conducted. Similar properties or absence of effect is predicted. The predicted property may be similar or based on a worst case approach.

Assessment elements for Scenario 2

The assessment elements (AEs) for this scenario consist of four AEs common to the analogue-approach and five scenario-specific AEs which depend on the mechanistic explanation (Table B1).

Table B1: Assessment elements (AEs) for Scenario 2

ASSESSMENT ELEMENTS (AEs) FOR SCENARIO 2			Applicability to a predicted property		
AE #	AE type	AE Name	Degradation	Bioaccumulation	Environmental effects
AE A.1	Common	Characterisation of source and target substances	X	X	X
AE A.2	Common	Link of structural similarities and structural differences with the proposed prediction (presence of hypothesis)	X	X	X
AE 2.1	Scenario-specific	Degradation	X	X	X
AE 2.2	Scenario-specific	Bioaccumulation potential		X	X
AE 2.3	Scenario-specific	Common underlying mechanism, qualitative aspects			X

ASSESSMENT ELEMENTS (AEs) FOR SCENARIO 2			Applicability to a predicted property		
AE #	AE type	AE Name	Degradation	Bioaccumulation	Environmental effects
AE 2.4	Scenario-specific	Common underlying mechanism, quantitative aspects			X
AE A.3	Common	Impact of impurities on the prediction	X	X	X
AE A.4	Common	Consistency of properties in the data matrix	X	X	X
AE A.5	Common	Reliability and adequacy of the source data	X	X	X
AE A.6	Common	Bias that influences the prediction	X	X	X

AE A.1 Characterisation of source and target substances

Purpose

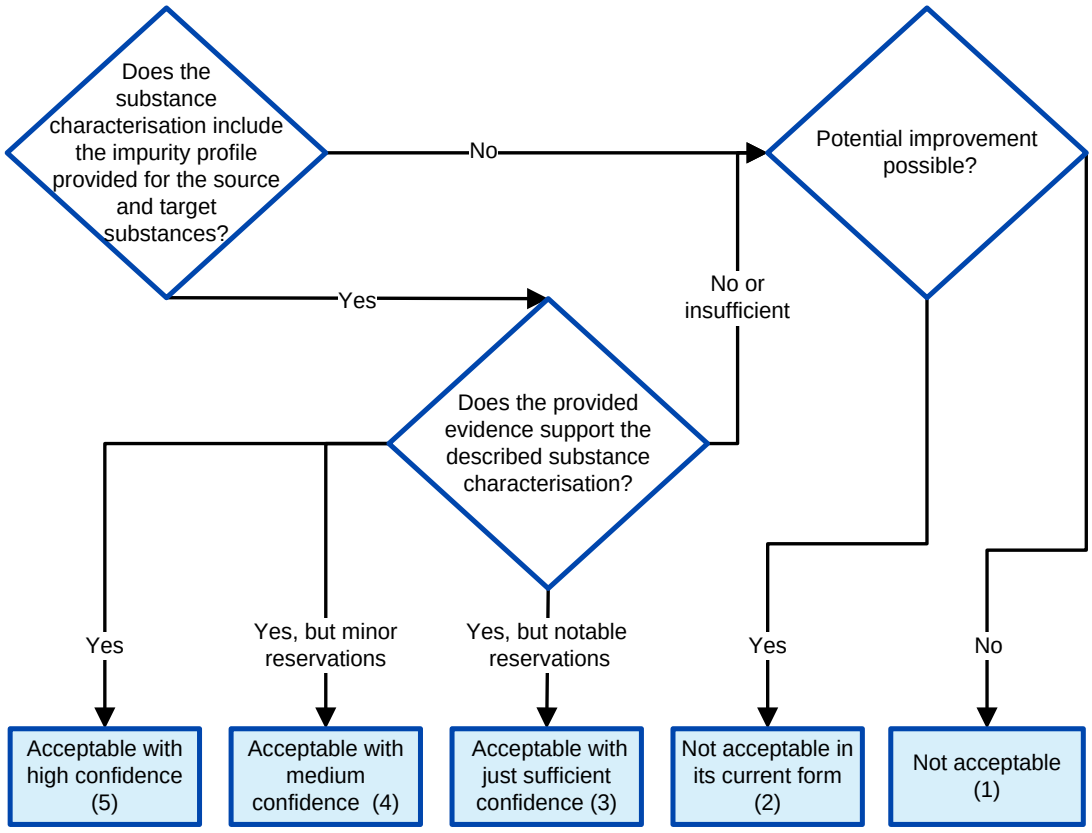
The source and target substances[1] need to have a clear substance characterisation.

It has to be assessed whether:

- the chemical identity of the target and source substances is sufficiently clear for assessing the proposed read-across; and
- the impurity profile is clear.

The current AE only looks at the basic information which allows the comparison of chemical structures to be started.

Assessment options



[1] The test material actually used in a specific source study is addressed in AE A.5.

Explanation

Structural similarity[2] is a necessary pre-requisite for any prediction based on read-across under REACH. To assess the structural similarity, the chemical identities of the target and source substances have to be clear.

If an adaptation based on read-across is used within an analogue approach, the information provided on the identity of the source substance must establish a clear picture of its chemical structure. Two-dimensional diagrams of chemical structure may be sufficient for simple cases (e.g. linear alkanes, etc.). However, in more complex cases three-dimensional energy minimised structures may need to be considered (e.g. when bridges between benzene rings are different), along with the size of particular functional groups, electron density/polarity, etc.

It is important that not only the chemical structures but also the impurity profiles of all source and target substances are well defined to establish the read-across hypothesis, since differences in impurities or stereochemistry can affect the activity and chemical properties.

In ECHA's practical guide "How to report on Read-Across" it is recommended to follow ECHA's *Guidance on identification and naming of substances under REACH* (version 1.4, June 2016) also for the source substances, not only for the substances which are registered.

The source substance should be described as comprehensively as possible and as a minimum[3] the following information should be provided (ECHA's *Guidance on information requirements and chemical safety assessment, Chapter R.6.2.6*):

- name, CAS and/or EC number, chemical structure for the source substance; and
- impurity profiles for the source substance (with identifiers as defined above).

[2] Structural similarity alone is not sufficient to justify a prediction based on grouping and read-across. The prediction must be based on the structural similarity, which is to be linked to a scientific explanation of how and why a prediction is possible on the basis of this structural similarity. These aspects are addressed dependent on the scenario applied by different AEs.

The Board of Appeal stated in the summary of its decision A-006-20132 of 13 February 2014: "*that for a read-across adaptation to be assessed and potentially accepted by the Agency, registrants have to show with clear reasoning and supporting data, set out in the appropriate section of the registration dossier, that the substances involved in the read-across are structurally similar and are likely to have similar properties (or follow a similar pattern). Registrants should also explain how and why the similarity of properties is the result of the structural similarity. The Board of Appeal explained that inclusion of the above information in the dossier is essential to allow the Agency to carry out its role of evaluating whether the read-across proposal complies with the relevant provisions of the REACH Regulation.*"

[3] Depending on the property under consideration in the read-across approach, the requirements for the substance identity information for the source substance may vary. In some cases, small differences in constituents or impurities may have a strong impact on the properties, even if such differences do not matter in terms of the substance identity information required under REACH.

Importance of impurities

A mono-constituent substance under REACH is defined by the main constituent, impurities and additives (if appropriate).

Small changes in the impurity profile may have strong effects on the substance properties. Whilst such changes may not need to be described to be in compliance with Annex VI (i.e. they are allowed in the substance identity description) they may need to be addressed in the hypothesis and justification for a proposed read-across approach.

Read-across has to be based on the structural similarity of the source and target substances. This similarity is based on the main constituents of the source and target substances. However, toxicity may actually be determined by an impurity. Similarly, environmental fate properties may differ for the impurities, which may be important for example when assessing the (target) substance for its persistence. The PBT/vPvB assessment should be performed on each relevant constituent, impurity and additive present in concentrations ≥ 0.1 % (w/w). Therefore, read-across assessment should similarly consider if impurities >0.1 % (w/w) have been addressed.

Although a read-across hypothesis may seem convincing, it could still be invalid if it does not take into account a difference in impurity profile of the source and targets substances.

The relevance of the impurities for the prediction is assessed in AE A.3.

Examples[4]

A.1.a Example for an identity of the target substance which is clear

- Substance A is a mono-constituent substance.
- The main constituent is present at 70-90 % with a typical concentration of 85 %.
- The impurity profile[5] is well defined: name, CAS, EC, chemical structure and concentration ranges are available for all impurities.

In this case, the identity of the target substance is clear for read-across purposes.

A.1.b Example for an identity of the source substance which is not clear

- Substance A is a mono-constituent substance.
- The main constituent is present at 70-90 % with a typical concentration of 85 %.
- The impurity profile is not provided.

In this case, the identity of the source substance is not clear for read-across purposes.

[4] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.
[5] The impact of the impurity profile on the prediction is addressed in AE A.3.

AE A.2 Link of structural similarities and structural differences with the proposed prediction (presence of hypothesis)

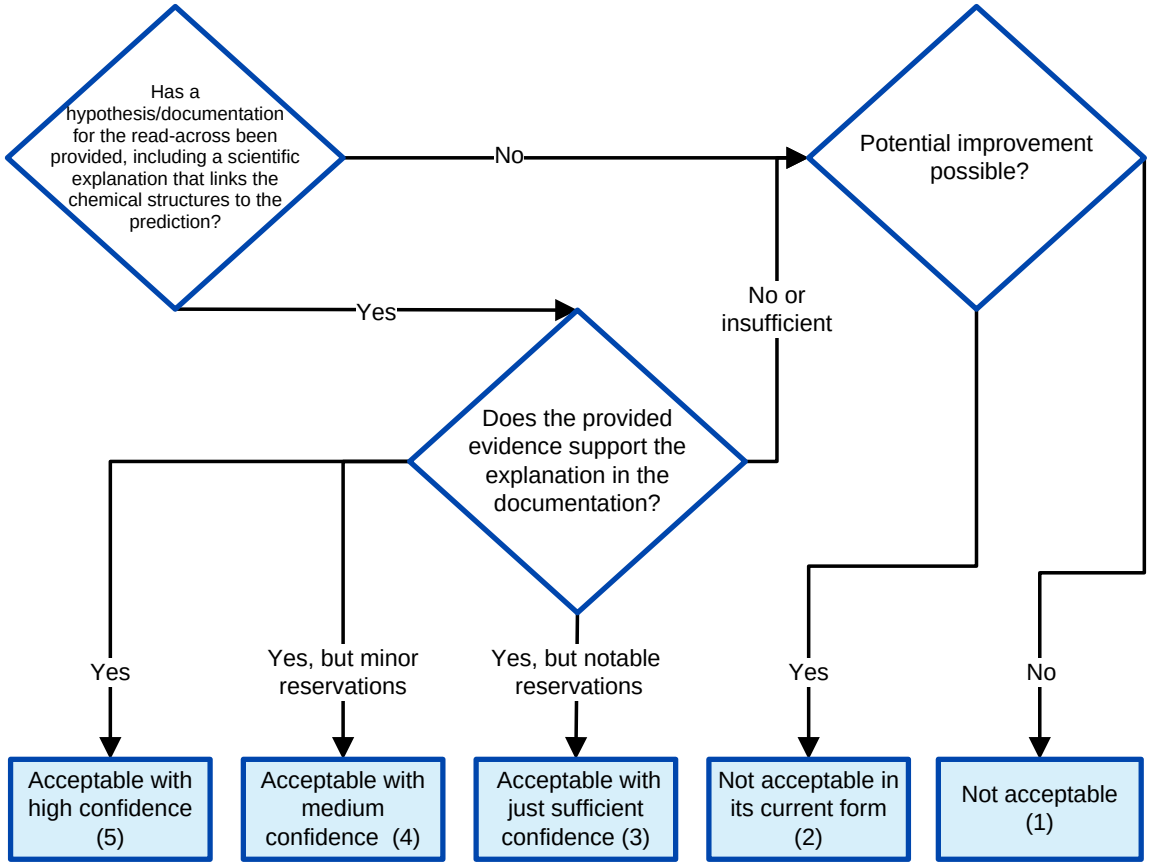
Purpose

The aim of this AE is to verify that the source and target substances are covered by the read-across hypothesis.

It has to be assessed whether:

- the scientific hypothesis (explanation) establishes the structural similarities and differences of the source and target substances;
- structural similarities and differences are linked with the possibility to predict similar properties; and
- the provided evidence supports the proposed link between structural similarities and the possibility to predict.

Assessment options



Explanation

The hypothesis as to why the prediction of similar properties is possible should reflect the structural similarity of the source and target substances.

It should be understood:

1. Which structural moieties and/or characteristics the source and target substances have in common (for instance, they contain a mono-chloro phenyl moiety or they are primary alcohols of alkanes); and

2. Which structural differences exist (*e.g.* a linear alkyl group may be present at the para position and/or the *meta*-position of the mono-chloro phenyl ring that contains 1-10 carbon atoms or the chain length of the primary alcohols may vary from C7 to C14).

The explanation should be based on recognition of the structural aspects that the two structures have in common and the differences between the two structures. The possibility

for predictions of similar properties should be linked to the common structural aspects. The importance of structural similarities and dissimilarities is influenced by the property to be predicted, i.e. different weight can be given to structural (dis)similarities for different properties.

The hypothesis must be supported by relevant physicochemical, (eco)toxicological and environmental fate data to provide sufficient evidence that the observed structural dissimilarity does not influence the property under consideration.

Regarding environmental read-across, the interrelated nature of the properties increases the complexity of the assessment. Depending on the property for which a given read-across is proposed, different sets of related properties that should support structural similarity need to be assessed. The specific assessment elements describe how to verify the important aspects of the hypothesis.

Example(s)[6]

A.2.a Example for a missing consideration of structural differences between source and target substances

- Substances A and B are both alpha-olefins.
- Substance A has a linear structure, substance B is branched.
- The hypothesis does not address the branching of substance B.

The explanation also has to address the impact of the branching on the prediction under consideration.

[6] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

AE 2.1 Degradation

Purpose

This assessment element addresses abiotic and biotic degradation processes and the purpose is two-fold:

1) address the link between structure and property if degradation is to be predicted;

2) address the degradation processes that can occur in the course of testing and alter the test material identity if bioaccumulation or environmental effect properties are predicted.

If the prediction is for the property “degradation”

It has to be assessed whether:

- the hypothesis provided explains how the prediction is derived from the relation between an observed (degradation) property and the structure and/or related properties;
- relevant factors, such as adhesion to vessel surfaces, absorption onto organic material, toxicity to microorganisms or loss through evaporation, which can reduce the availability of a compound during the test have been taken into account in the documentation; and
- the provided evidence supports the explanation

If the prediction is for the properties “bioaccumulation” and/or “environmental effects”

It has to be assessed whether:

- degradation of the parent compounds and the potential formation of degradation products from source and target substances has been explained in the documentation (the potential impact of these products is assessed in AE 2.2 and AE 2.3);
- identity and the rate of formation of such degradation products are provided; and
- the provided evidence supports the explanation.

Applicability of the assessment element

This AE applies to the following properties to be predicted:

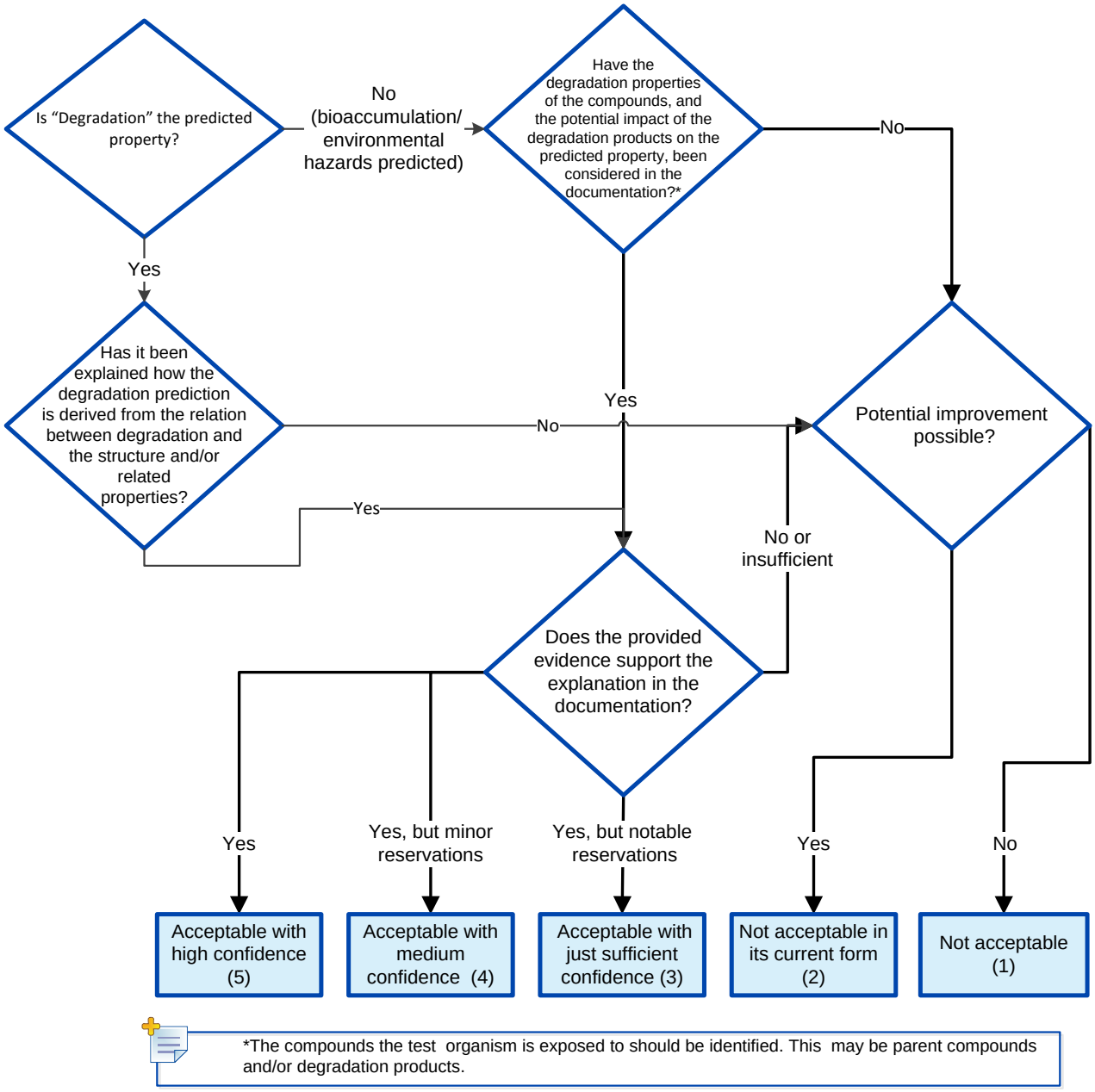
- Degradation:
  - Ready biodegradability
  - Simulation testing on ultimate degradation in surface water
  - Soil simulation testing

- Sediment simulation testing
- Hydrolysis

- Bioaccumulation in aquatic species (fish)
- Environmental effects

This assessment element is not applicable to the property “adsorption/desorption screening”.

Assessment options



Explanation

General: considerations on bioavailability for degradation

Bioavailability is important when assessing degradation. Compounds may not be available for a degradation process to take place if the substance is e.g. adsorbed to organic material or test vessels. Therefore, some important related properties are described below and should be considered when degradability of compounds are compared.

An important property to consider is the substance's adsorption/desorption capacity (Koc). Sorption is a parameter describing the availability of the substance for degradation which may be limited if absorbed to organic material, in line with ECHA's *Guidance on information requirements and chemical safety assessment Chapter R.6*.

Furthermore, the extent to which the test material is available for degradation also depends on volatility. Volatility should be considered when comparing the degradation potential between target and source substances.

Dissociation does not strictly fall under 'degradation' but is dealt with in this AE. The dissociated and non-dissociated species may have significantly different water solubilities and partition coefficients. A substance which ionises in water can have a significantly different bioavailability depending whether the dissociated or the neutral chemical species is present (ECHA's *Guidance on information requirements and chemical safety assessment Chapter R.7.c, Other indications of bioaccumulation potential*). In such cases, it is essential to know or estimate the pKa to evaluate the degree of ionisation of the source and target substance(s) in surface waters and under relevant environmental conditions (pH 4-9).

The prediction is for the property "hydrolysis" and "biodegradation"

If hydrolysis is predicted, it should be explained why structural differences between target and source substances do not impact the hydrolysis rate (e.g. structural differences influencing reactivity).

Similarly if biodegradation potential is predicted, the justification should provide an explanation why the target and source substances would degrade similarly. Regarding chemical structure, the type and extent of branching or substitution with organic functional molecular groups may affect the biodegradation potential and thus may need to be considered in the justification.

To support similarity in degradability, additional experimental and non-test data, qualitative information on degradation pathways, measured and expected degradation products and other evidence, in different environmental compartments and realistic conditions such as relevant pHs and temperatures may be available for the source and target substance(s) and they should support the hypothesis and justification given.

Furthermore, it is necessary to address the bioavailability of compounds to degradation, as described above in section "General: considerations on bioavailability for degradation". The bioavailability of the source substance needs to be considered in the test design of the source biodegradation study (as assessed in AE A.5). However, regarding prediction of the test result from such source study, it needs to be assessed if the target substance would be

as available to degradation as the source substance if the same test were performed with the target. Therefore, properties related to bioavailability should be compared between the target and source substance(s).

The prediction is for the properties “bioaccumulation” and/or “environmental effects”

Degradation properties and factors influencing degradation are also important in the assessment of the hypothesis for predicting bioaccumulation potential and environmental effects. Potential biotic and abiotic degradation processes (hydrolysis, photodegradation and acidic dissociation, biodegradation) occurring during test material preparation and during the test itself can have a significant impact on the results of bioaccumulation and ecotoxicity tests.

This assessment element is required to understand to which compounds the organisms are exposed in a bioaccumulation or ecotoxicity tests, and to what degree. The rate of transformation should be evaluated against the duration of the test, and the media renewal, and the justification should cover all relevant substances and their potential transformation products that the test organisms are exposed to during a test.

Therefore, the identity and rate of formation of the potential degradation products should be provided. Their influence on the predicted bioaccumulation or environmental effect property is assessed in AE 2.2 and AE 2.3.

However, this assessment element is relevant only for compounds which have either a functional group that can hydrolyse or dissociate, or where there is the potential for photodegradation (e.g. double bonds in a compound used in an algae study), or for which there is an indication for considerable biodegradation e.g. from a ready biodegradability test.

Small changes in ready biodegradability are unlikely to indicate differences in degradation of the compounds in a bioaccumulation or ecotoxicity tests and therefore a comparison of ready biodegradability is not of high importance when assessing a prediction in bioaccumulation and environmental effect properties.

In contrast, slight changes in pH can considerably affect the form in which the substance is present in solution, especially if the dissociation constant (pKa) value is within the environmentally-relevant pH range. Thus, pKa is an important related property for degradation and should be compared among source and target substances.

Example(s)[7]

2.1.a Example for stable source and target substances in toxicity test

Substances A and B are structurally similar substances.

[7] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

- Substances A and B are stable and water soluble substances. It is reported that the substances do not hydrolyse, dissociate or photodegrade (the latter is particularly relevant for an algae study).

It should be checked whether the test material in the source study(ies) has been measured and considered in the determination of the toxicity value. It has to be verified if there is indeed no indication that the substance(s) degrade in the test medium or during test material preparation.

A biodegradation study (e.g. a ready biodegradability screening study) and hydrolysis study may give indications for potential degradation during the testing. Parameters such as test medium (aqueous, sediment, soil), test type (e.g. flow through versus static) and duration have to be taken into account in order to assess how relevant the information from a biodegradation study is for the stability assessment. If it could be verified that source and the target substances are sufficiently stable, then the test organisms are exposed to the starting material only and thus, only the presence and behaviour of these substances need to be taken into account for the read-across.

2.1.b Example for limited bioavailability of the target substance for the property ready biodegradability

Substances A and B are structurally similar substances.

- Substances A and B are linear aliphatic substances.
- Substance A is readily biodegradable.
- Substance B has a higher log Kow and lower water solubility compared to substance A, which may limit the bioavailability.

In this situation, the bioavailability of substance B is likely lower according to its physico-chemical properties. There is uncertainty in the prediction for substance B as it might not be available enough to exhibit the same ready biodegradability if the same test were performed using the same test conditions.

2.1.c Example for considerations of rapid hydrolysis of the source and target substances under acidic conditions for a prediction of environmental effects

Substances A and B are structurally similar substances.

- Substance A (source) and substance B (target) hydrolyse rapidly only under acidic conditions (pH 4).
- Substances A and B have a similar chemical reactivity based on theoretical chemical considerations.

Short-term aquatic toxicity tests for substance A were performed under slightly basic conditions and with analytical monitoring.

In this situation, it is verified that in short-term aquatic toxicity tests, the test organisms are exposed only to the starting material. Thus, the hypothesis should consider the presence, behaviour and toxicity of the source substance in the test system compared with the target substance.

AE 2.2 Bioaccumulation potential

Purpose

Under this assessment element, the uptake and bioaccumulation potential of source and target substances and any of their potential degradation products (from AE 2.1) are assessed when the bioaccumulation potential is predicted and also when environmental effects are predicted (a difference in bioaccumulation potential may affect the ecotoxicity).

If the prediction is for the property “bioaccumulation”

It has to be assessed whether:

- the hypothesis provided explains how the predicted bioaccumulation property is not influenced by the structural differences between source and target substances (parent compounds and potential degradation products identified in AE 2.1); the hypothesis should further explain why bioaccumulation can be predicted based on a related property (the independent variable); and

- the provided evidence supports the explanation.

If the prediction is for environmental effect properties

It has to be assessed whether:

- the hypothesis explains how the potential for bioconcentration and/or bioaccumulation of the target and source substances (parent compounds and potential degradation products identified in AE2.1) does not influence or underestimate the predicted environmental effect property of the whole compounds; and

- the provided evidence supports the explanation.

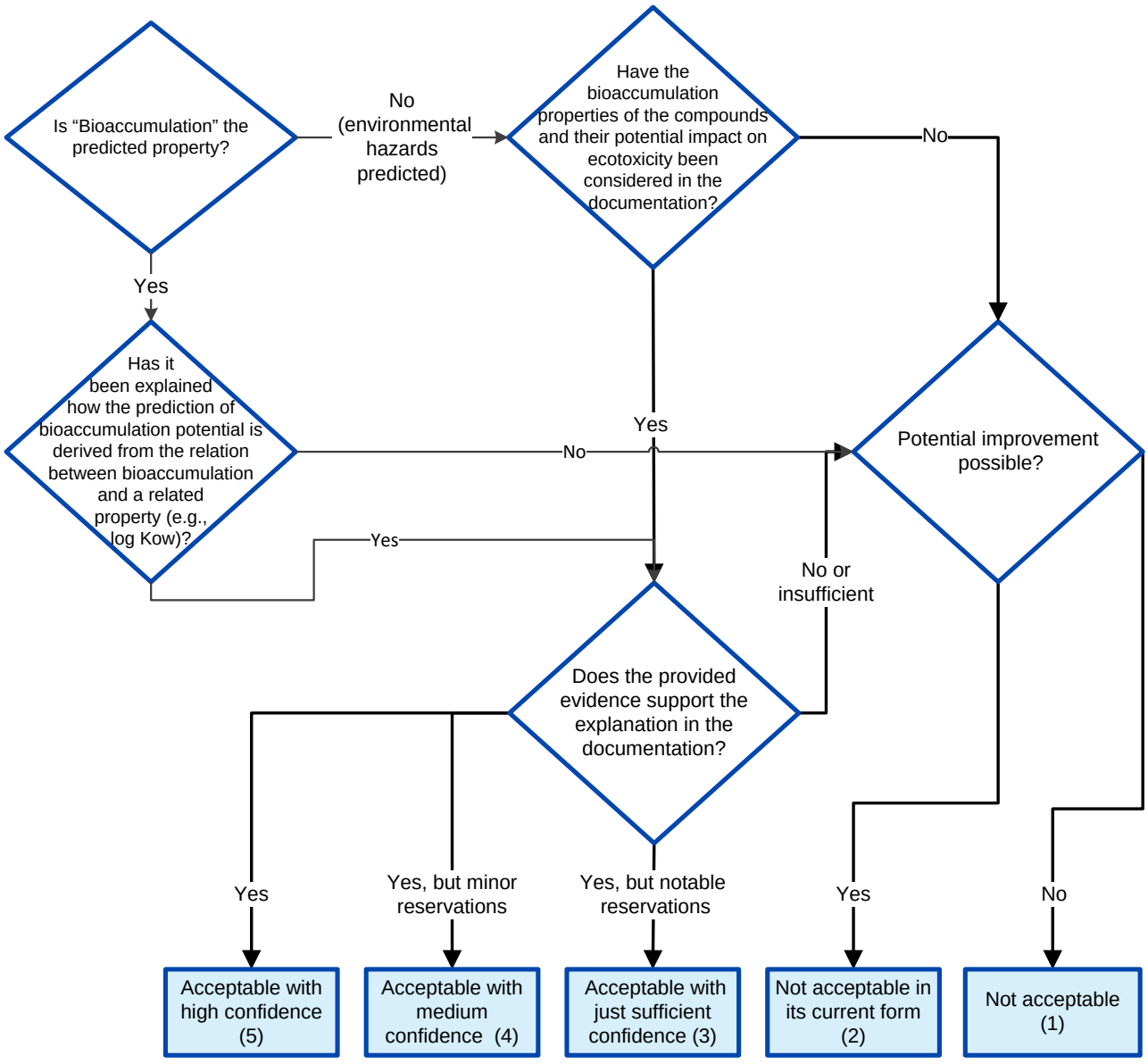
Applicability of the assessment element

This AE applies to the following properties to be predicted:

- Bioaccumulation in aquatic species (fish)
- Environmental effects

This assessment element does not apply to adsorption/desorption screening, hydrolysis, ready biodegradability and simulation testing.

Assessment options



Explanation

General: considerations on bioavailability (fate)

The bioaccumulation potential of source and target substances (both the parent compounds and potential degradation products identified in AE 2.1) has to be considered. Furthermore, as the degradation of the parent compound leads to lower concentrations of the parent substance in the test system, the influence of degradation of the parent substances on the bioaccumulation potential should also be assessed.

In bioaccumulation studies, the substance in the test system is generally measured and

therefore the tests for the source substance(s) per se accounts for such loss of the parent substance during the test. However, if there are nominal effect concentrations in ecotoxicity studies and for the target substances in general, it needs to be considered if the organisms would be exposed to the same degree if the same test with the target substance would be performed. The difference of degradation rate between the source and target substance has been assessed in AE 2.1.

In addition to the degradation of the parent compound, the related properties of the parent compounds and degradation products may inform about their behaviour in the test solution and potential to bioaccumulate. Factors such as loss due to volatility or adsorption to test vessels and low water solubility are important when considering how bioavailable the substance may be to organisms within a test. Therefore, such availability has to be compared between the compounds, especially if these have a low water solubility and/or a high bioaccumulation/adsorption potential. It should be explained if and how marked differences in the extent of test material availability impact or not impact the read-across.

Apart from substance properties, experimental conditions also need to be considered. The OECD Test Guideline 305 includes possibilities for both dietary and aqueous exposure routes. A direct comparison of both types of studies most probably cannot support similarity in bioaccumulation potential but the comparison should be made by study design.

Furthermore, BCF is dependent on lipid content of the tested fish (especially for lipophilic substances) and therefore the BCFs that are compared between compounds should be expressed as normalised to a fish with a 5 % lipid content (based on wet weight) as indicated by the OECD Test Guideline 305.

The prediction is for the property “bioaccumulation”

The justification should explain why the target and source substances (and their potential degradation products identified in AE 2.1) would bioaccumulate to a similar extent. It should be explained how bioaccumulation potential would be predicted from a bioaccumulation study on a source substance. Structure of a substance may provide an indication of a difference in bioaccumulation potential. Ionisable groups, sub-structures that could potentially bind to proteins and chain length may influence bioaccumulation potential and should be considered. In addition, the justification should also take into account related properties of the target and source substances that can be linked to bioaccumulation potential.

Bioaccumulation (bioconcentration factor BCF) of non-ionic organic compounds can generally be linked to lipid partitioning (log Kow) and should be considered in read-across of the bioaccumulation potential. The mechanistic basis for this relationship is the analogy of the partitioning process between lipid-rich tissues and water to that between n-octanol and water (whereby n-octanol acts as a lipid surrogate). In this approach, uptake is considered to be a result of passive diffusion through gill membranes and thus applies only to water exposure studies. Linear correlations give a good approximation of the log BCF for non-ionic, slowly metabolised substances with log Kow values in the range of 1 to 6.

For certain chemicals, for which the log Kow cannot be measured properly, a high adsorptive capacity (of which log Kp >3 may be an indication) can be considered instead (ECHA’s *Guidance on information requirements and chemical safety assessment Chapter R.7.c, Other indications of bioaccumulation potential*). Adsorption onto biological surfaces,

such as gills or skin, may also lead to bioaccumulation and an uptake through the food chain. Hence, high adsorptive properties may indicate a potential for both bioaccumulation and biomagnification.

For ionising substances, either the log D should be used instead of log Kow (if this parameter is suitable, i.e. no mechanism other than passive diffusion), or the log Kow of the neutral form could be applied for a worst case prediction. ECHA's *Guidance on information requirements and chemical safety assessment Chapter R.7.c*, gives further details: "*Fish BCFs of ionised substances can be estimated using appropriate QSARs (e.g. Meylan et al., 1999). In addition, the log BCF of an ionized substance may be estimated at any pH by applying a correction factor to the log BCF of the unionized form, based on the relationship between BCF and Kow. This factor would be derived from the Henderson-Hasselbach equation as log(10pH-pKa+1). However, this may lead to underestimates of the BCF in some circumstances, since the ionised form may be more accumulative than suggested by its Kow alone. For example, a correction factor of log(4pH-pKa+1) was found to be more appropriate for a group of phenolic compounds by Saarikoski and Viluksela (1982). Escher et al. (2002) also showed that the Kow is not always a good indicator of biological membrane-water partitioning for ionised organic chemicals when there is reactivity with cell constituents.[…]*"

Molecular weight and size are factors that could affect the bioaccumulation potential of chemicals. If a substance has a high molecular weight, the addition of an extra substituent that leads to an increase of the log Kow value does not necessarily lead to a higher BCF value. On the contrary, such an addition may cause the substance to be less easily taken up by the organism, which may result in a lower instead of a higher BCF value.

In such cases, the worst-case compound for read-across is a structurally similar compound with a slightly smaller molecular size. Therefore, molecular mass and size should be considered in read-across to confirm whether the source and/or target substance(s) have a decreased accumulation due to hindered passage across membranes. In addition, reduced bioavailability and difficulties in measuring exposure concentrations may occur for substances with low aqueous solubility, as well as failure to reach steady state because of slow membrane passage of large molecules.

Furthermore, it should be noted that branching or alkyl substitution sometimes enhances bioconcentration potential, for example due to a reduction in the biotransformation rate and/or a decrease in elimination (ECHA's *Guidance on information requirements and chemical safety assessment Chapter R.7.c*).

Biotransformation of compounds may largely alter bioaccumulation potential and therefore biotransformation of source and target substances should be considered when bioaccumulation is being predicted. Generally, biotransformation of a substance leads to lower bioaccumulation potential as often the transformation products are more water soluble and thus are more easily excreted than the parent compound. Small changes to molecular structure can be significant for the capability of fish to metabolise substances generally to more polar compounds, leading to a lower BCF value (ECHA's *Guidance on information requirements and chemical safety assessment Chapter R.7.c, Read-across and categories*). However, in some cases, transformation may also lead to an increased potential to accumulate. For example, if a substance is large and very lipophilic, break-down to a

smaller molecule may make it more available to organisms and increase its bioaccumulation potential.

Metabolism may be inhibited if a substituent is placed on the centre of metabolic action. If read-across is applied, it must be recognised that the presence of such a substituent on the substance to be evaluated may lead to a strongly reduced metabolism in comparison with the substance for which the BCF is known. As a consequence, the BCF value may be underestimated. If there are indications of metabolism for the analogue substance for which a BCF value is available, it must be examined if the same potential for metabolism is present in the substance and the species to be evaluated. If there are indications that the substances under evaluation are biotransformed, there might also be a need to consider the biotransformation products and their identity.

The prediction is for environmental effect properties

Bioaccumulation potential of target and source substances (and their potential degradation products identified in AE 2.1) also needs to be considered when environmental effects are predicted. It is assessed whether available and relevant experimental data on the potential for bioconcentration and/or bioaccumulation have been taken into account for the prediction.

The n-octanol water partitioning coefficient can be used as surrogate for some substance types and should be considered where applicable. AE 2.4 assesses whether the information supports the prediction or not.

Example(s)[8]

2.2.a Example for bioavailable source and target substance for the property bioaccumulation

Substances A and B are structurally similar substances.

- Substances A and B are soluble and hydrophilic substances.
- Substance B is the source substance and substance A is the target substance.
- Physico-chemical information is provided for the source and the target substances, including water solubility, log Kow, and vapour pressure. Log Kow is higher for the source B than for the target A.
- Evidence is provided that these substances are slowly metabolised by fish.
- Based on the information on physico-chemical and degradation properties for the substances together with information on the test conditions, it is predictable that the losses due to volatility and adsorption are minimal during the period of testing. The substances are likely to be fully bioavailable for aquatic organisms.

[8] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

In this situation, source and target substances are equally bioavailable to aquatic organisms and therefore it is expected that bioavailability does not influence the read-across and the prediction. The bioconcentration factor of organic substances can be assumed to increase with chain length and log Kow. Therefore, the bioaccumulation potential of the substance A can be predicted based on bioaccumulation data on B, which is a more hydrophobic substance, representing a worst case prediction.

2.2.b Example for dissociating source substances for the property bioaccumulation

Substances A and B are structurally similar substances.

- Substances A and B are structural isomers.
- Substances A and B dissociate at pH 4-9 to some extent.
- Substance A has a lower acid dissociation constant (pKa) value than substance B, indicating more dissociation than substance B.

In this situation, substance B is less dissociated and has therefore a higher potential to bioaccumulate. A predicted bioaccumulation potential for target substance B predicted from substance A may be underestimated, unless the dissociation has been considered in the BCF determination.

AE 2.3 Common underlying mechanism, qualitative aspects

Purpose

The read-across hypothesis should explain how the compounds to which the test organisms are exposed lead to the same effects/absence of effects.

It has to be assessed whether:

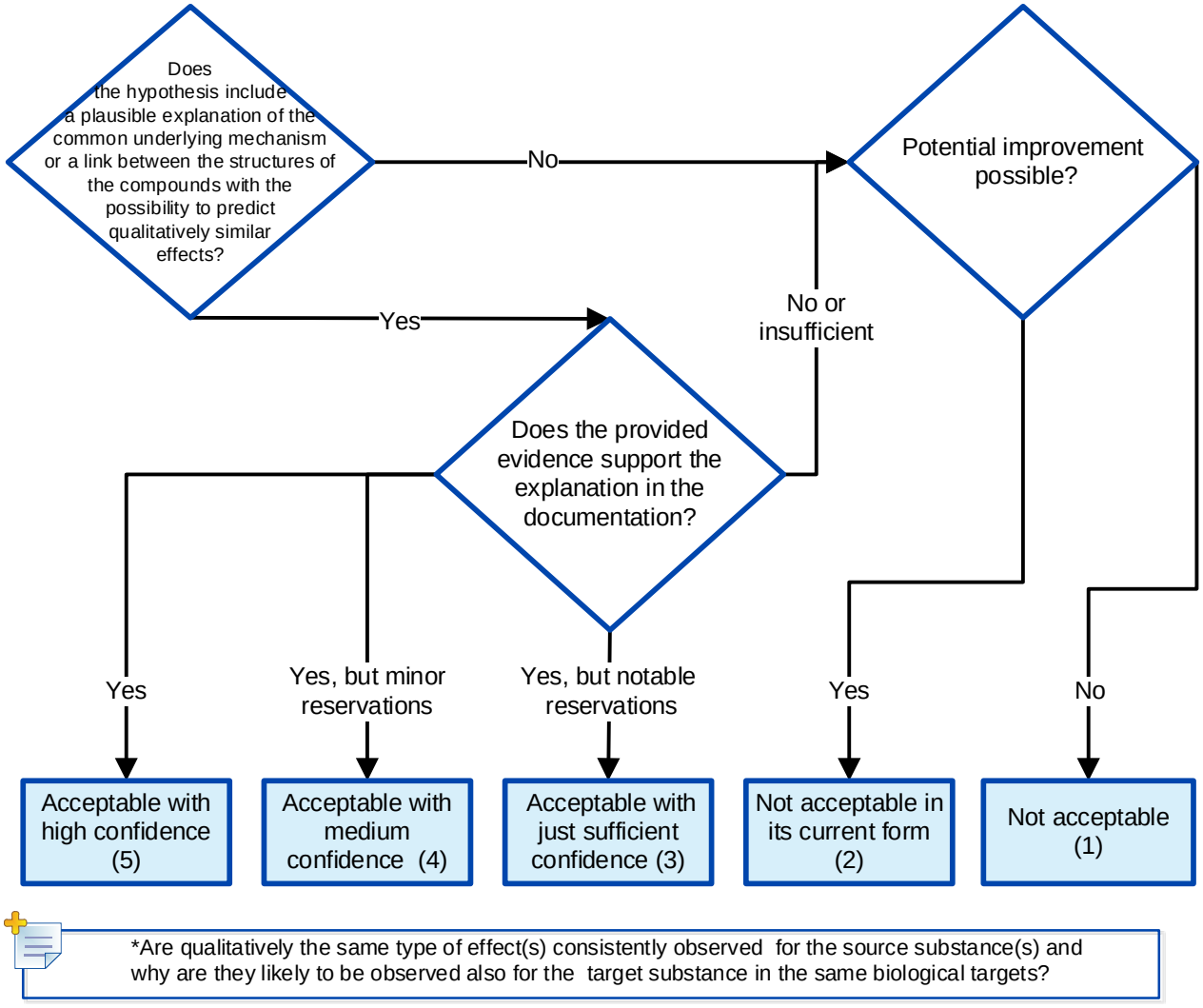
- a common underlying mechanism is established;
- the hypothesis links the structures of the compounds under consideration with the possibility to predict for the target substance qualitatively similar effects for the property under consideration; and
- the provided evidence supports the explanation.

Applicability of the assessment element

This AE applies to the following properties to be predicted:

- Environmental effects

Assessment options



Explanation

An underlying mechanism needs to be established which links the compounds to which the organisms are exposed with the prediction. The effects should be caused by a common mechanism. For all of the substances, this mechanism should link the structures of the compounds under consideration with the possibility to predict qualitatively similar effects for the target substance.

If there are functional groups/structural features present only in source or target substance, the hypothesis/justification should explain why the effects observed are still similar in spite of the observed structural differences. Different functional groups may lead to differences in the biological and physico-chemical properties of compounds and may thus affect the mechanism of action and the predicted property.

In certain cases, (small) structural differences can lead to different (bio)transformation of compounds, which in turn can lead to different mechanisms of action.

*C*omputational tools such as the OECD QSAR Toolbox may support the proposed mechanism and thus may increase the robustness of a case.

In addition, adverse outcome pathways and results from *in vitro* methods may help to understand the mechanism/mode of action.

Other important information which may help identifying potentially different mechanisms of action between the source and target substances is the acute-to-chronic ratios: high acute-to-chronic ratios can be indicative of specific modes of action or different modes of action causing toxicity in short-term versus long-term studies. However, care should be taken if there is low water solubility which may influence acute-to-chronic ratios (e.g. testing at or above water solubility). Inconsistencies in the ratios or high ratios (normally ratio is somewhere around 10, ratio above 100 is high) can be indicative of a specific mode of action.

Although most experience with mechanisms of action comes from pelagic species, the approaches are in principle applicable to sediment and terrestrial species as well.

Prediction of absence of effect

Specific considerations are needed for predictions of the absence of effects. In the current AE, only the principle qualitative aspects of such a prediction are covered, but quantitative aspects are explained in the text below as well.

The prediction of absence of effects can have a basic explanation: absence of exposure due to lack of bioavailability. Information on uptake potential and lack of effects in long-term studies in different trophic levels is normally needed to demonstrate the absence of effects in aquatic toxicity tests. The supporting information (e.g. data matrix, information from human health properties) must not contradict such a claim. Please note that lack of effects in aquatic media does not necessarily provide sufficient evidence that effects would not occur in sediment or terrestrial environments (e.g. for highly adsorptive substances).

Supporting evidence

Information is needed about the chemical-biological interaction within the organism and mechanisms or modes of actions. Supporting evidence may come from human toxicological evidence, (Q)SAR profilers, *in vitro* data etc. The OECD QSAR Toolbox contains “mechanistic profilers” which can help identify mechanisms or modes of actions relevant for different regulatory properties for a target chemical or a list of target chemicals. Relevant profilers for environmental properties are:

- Acute aquatic toxicity MOA by OASIS (based on theoretical and empiric knowledge the following seven hierarchically ordered MOA are distinguished: Reactive Unspecified; Aldehydes; alpha, beta-Unsaturated alcohols; Phenols and anilines; Esters; Narcotic amines; Basesurface narcotics)

- Aquatic toxicity classification by ECOSAR (this profiler uses the class definitions from ECOSAR™ and identifies more than 100 chemical classes)

- Acute aquatic toxicity classification by Verhaar (this classification system is based on modified Verhaar scheme part of Toxtree. It separates a large number of small to intermediate organic chemicals into four distinct classes: (1) inert chemicals (baseline toxicity); (2) less inert chemicals, (3) reactive chemicals; and (4) specifically acting chemicals.

- Protein binding by OASIS (the scope of the profiler is to investigate the presence of alerts within target molecules responsible for interaction with proteins. The list of 101 structural alerts has been separated into 11 mechanistic domains. Each of the mechanistic domains has been separated into more than 2 mechanistic alerts. The profiling result outcome assigns a target to the corresponding structural alert, mechanistic alerts and domain)

- Protein binding by OECD (the profiler was developed by an analysis of direct acting structural alerts based on theoretical organic chemistry (the profiler does not contain metabolically / abiotically activated structural alerts). The protein binding by OECD profiler contains 16 mechanistic alerts covering 52 structural alerts. These data are supported by mechanistic chemistry and references to the scientific literature (the meta data)).

- For more information, see the illustrative examples addressing short and long-term aquatic toxicity (https://echa.europa.eu/support/oecd-qsar-toolbox).

Example(s)[9]

2.3.a. Example of a common underlying mechanism for source and target substances for aquatic toxicity

- Substances A and B are structurally similar substances.
- Long-term toxicity to fish for substance B is predicted.
- Both of the substances have been shown to be stable under test conditions.
- The OECD QSAR Toolbox profilers
    - Acute aquatic toxicity MOA by OASIS
    - Aquatic toxicity classification by ECOSAR
    - Acute aquatic toxicity classification by Verhaar

  all point towards an absence of a specific mode of action. Results indicate that all substances are neutral organics/baseline toxicants.

- Furthermore, the substances do not produce an alert for protein binding in the OECD QSAR Toolbox (Protein binding by OASIS, Protein binding by OECD), which is a further indication of the absence of elevated toxicity
- There is no indication in the open literature that the substances act through a specific mode of action.

[9] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

- Short-term fish and *Daphnia* studies are available for both substances (bridging study).
- Long-term *Daphnia* studies are available for substances A and B. These confirm the hypothesis of a similar (baseline) toxicity.
- Acute-to-chronic ratios are available for substances A and B and are 4 and 9.

In this example, it has been reported that there is no indication that the target substance would act by a different mode or mechanism or mode of action than the source substance(s). Long-term toxicity to fish can be predicted for substance B.

AE 2.4 Common underlying mechanism, quantitative aspects

Purpose

Under this scenario, there should be no significant quantitative differences for the same type of effects caused by the underlying mechanism or the differences should be used in a conservative prediction (i.e. the effects for the target substance are not likely to be under-predicted, worst-case approach).

It has to be assessed whether:

- the documentation establishes that the magnitude of effects does not vary between source and target substances, considering also the related properties that may influence the predicted property (e.g. bioaccumulation potential, log Kow); and
- the provided evidence supports the explanation.

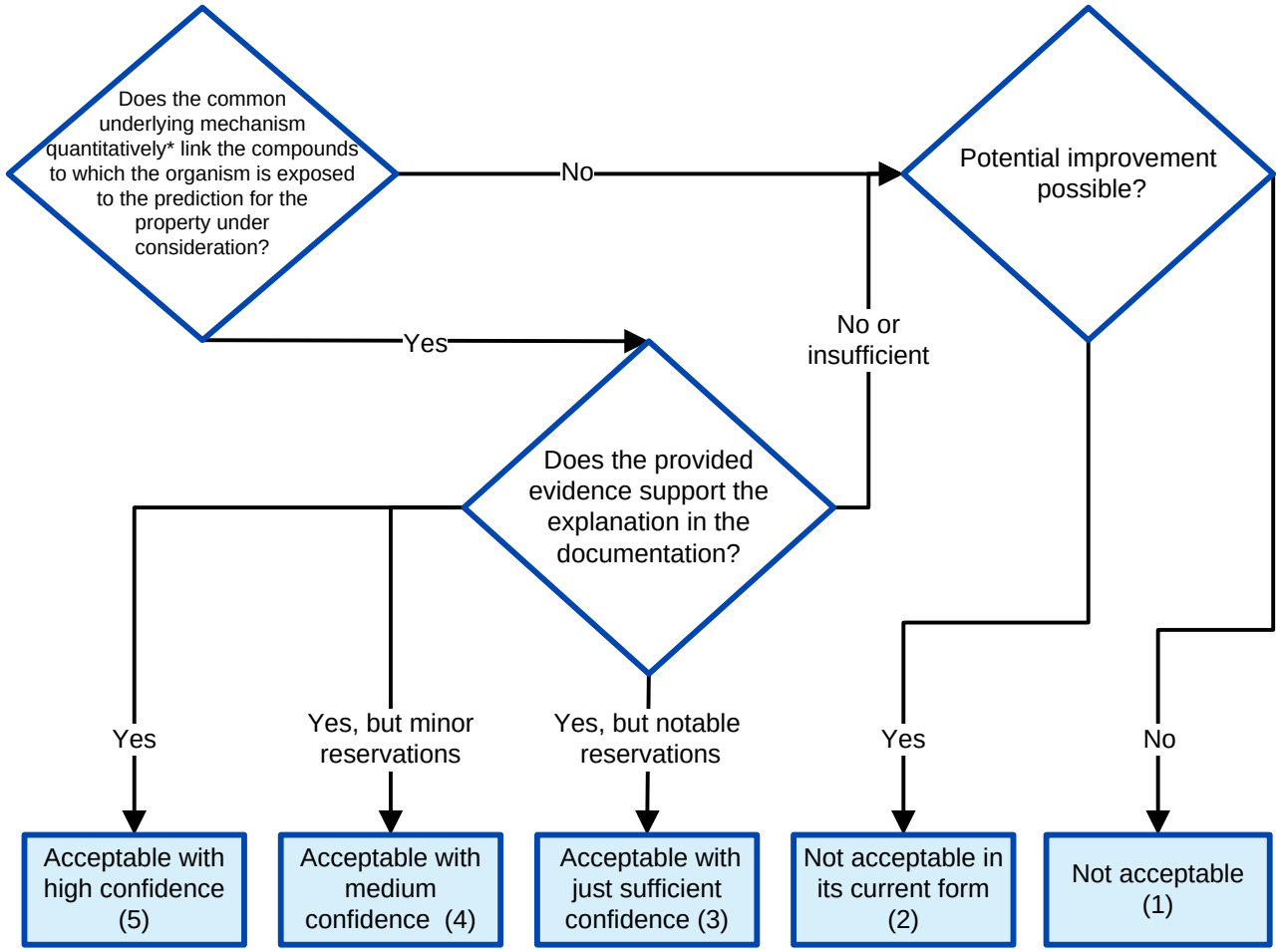
As a default, a prediction based on a regular pattern without a mechanistic explanation will not be acceptable.

Applicability of the assessment element

This AE applies to the following properties to be predicted:

- Environmental effects

Assessment options



Explanation

Quantitative differences

Under this scenario, there should be no significant quantitative differences for the effects caused by the underlying mechanism or the differences should be used in a conservative prediction (i.e. the effects for the target substance are not likely to be under-predicted, worst-case approach).

Prediction of environmental effects by read-across approaches should always consider potential differences in bioaccumulation properties of the substances (e.g. BCF, BAF, TMF, BSAF etc.). The purpose of this assessment element is to make sure that bioaccumulation potential is considered in relation to ecotoxicity.

The BCF reflects the potential of a substance that can be internalised and potentially reach the target sites where the toxic action takes place and biological effects are initiated. Assuming that the underlying mechanism of the compound is the same between source and target substance (as described in the assessment elements AE 2.3), differences in bioaccumulation may still cause differences in toxic potential simply by a producing higher concentration of the substance at the target sites of toxic action. Therefore, if the bioaccumulation potential is higher for the target substance, this should be taken into account.

Worst case

Sometimes a worst-case approach is claimed (i.e. the source is claimed to be a worst case and therefore the prediction is claimed not to under-predict the effects for the target(s)). Such an approach as a default does not fit to this scenario definition, since it necessarily means that source substances have biologically significant different effect levels. The analysis of the proposed prediction would then be handled under scenario 4.

However, there are cases where physico-chemical properties suggest that the uptake for the source substances will be higher than for the target substance. Still no effects are observed for the source substances. This situation is proposed to be a worst case for the prediction of no effects for the targets. This situation can be assessed under scenario 2 or 6, since there is no difference in effects predicted.

Predictions of absence of effects

If the prediction of absence of effects is justified by absence of uptake, the significance of possible small quantitative differences in uptake (and bioaccumulation) between the source and target substances need to be assessed.

If the prediction of absence of effects is justified by absence or undetectable interaction with biological targets, the mechanistic explanation and the supporting evidence should outline why this explanation applies to the target substance for the property under consideration.

Critical body burden approach

To account for differences in bioaccumulation potential when assessing read-across approaches in properties related to aquatic toxicity, the Lethal Body Burden (LBB) and for sub-lethal endpoints Critical Body Burden (CBB) approaches can be applied. An LBB or CBB can be measured directly during an ecotoxicity study in which biological effects and chemical body burdens are measured in the same test organisms. However, internal substance concentrations are rarely measured in ecotoxicity studies and therefore the LBB or CBB may be estimated indirectly.

Indirect estimates can be made on the basis of bioconcentration and effect concentrations, so that LBB = LC50 x BCF and CBB = NOEC x BCF. This approach allows estimation of the toxic potential of the target substance based on NOEC/EC50 of the source substance(s) and BCF of the source and target substances. The methodology for body burden approaches is explained in ECHA's *Guidance on information requirements and chemical safety assessment Chapter R.7b (Appendix 7.8-3)*.

If the CBB approach is applied, it should be applied to the component of the substance is driving the toxicity (parent or a potential degradation product). Furthermore, care should be taken when CBB is used for poorly water soluble substances which reach steady state within a longer time period when compared to duration of a test. A justification should also cover what uncertainties may arise if different species have been used in bioaccumulation studies and in the ecotoxicity study of the source substances.

Supporting information

The type of information needed to provide sound scientific explanations is case-specific. Reliable bioaccumulation data are very valuable in this regard. In silico studies (e.g. computational tools such as the OECD QSAR Toolbox) may increase the robustness of the case.

Differences in strength observed for related properties (e.g. environmental effects in another species or compartment) need to be analysed as well.

Considerations for toxicity to sediment organisms

When assessing the read-across argumentation on toxicity in other than aquatic environments, the relevant related properties may be different. For example, regarding sediment toxicity, the following needs to be adequately addressed, for both source and target substance(s).

Adsorption and binding behaviour (partitioning) are usually assessed using Log Kow, Log Koc, dissociation-ionisation and water solubility. Also, vapour pressure can have an influence on the observed sediment toxicity if there is a significant loss of test material during the test duration. These parameters have to be addressed and need to be taken into account when looking at a trend and for verification if the data gap filling was done from the more toxic to the less toxic substance.

Uptake processes are important, namely uptake through the pore water or through direct contact with the substance and dietary uptake. Different sediment test organisms have different living strategies (e.g. burrowing, sediment surface scavengers) and feeding strategies (e.g. filter feeders, sediment ingesters), resulting in differences in the main route of exposure (water or dietary) causing toxicity for these organisms.

These processes and differences in living/feeding strategies make it sometimes difficult to read-across between substances. Differences between the key physicochemical properties may increase/decrease the relevance of one route of exposure over the other for the source and target substances. Moreover, sediment characteristics can vary widely across tests (e.g. artificial sediment vs. natural sediment), which can have a significant impact on the observed effects.

Example(s)[10]

2.4.a Example for influence of adsorption for the property sediment toxicity

- Substances A and B are structurally similar mono-constituent substances. The substance A is smaller molecule than B.
- Substance A is the source substance for predicting sediment toxicity of substance B.
- Physico-chemical information is provided for the source and the target substances including water solubility, log Kow, log Koc, pKa and vapour pressure.
- Substance A is volatile, while substance B is less volatile and physico-chemical properties indicate a higher potential for adsorption.
- There are several results available for sediment toxicity for substance A but some also for B. Most of them show toxicity, but variation is high between the substances and tests. The data matrix is not consistent for sediment toxicity. There seems to be an influence of organic matter content or other test material properties on the test result.

The volatility and bioavailability were not taken into account in the read-across justification and hypothesis. In this case, the given information and the inconsistency of effects in the data matrix suggest that the bioavailability and loss due to volatility have a significant influence on the test outcome. There is high uncertainty for a prediction of the sediment toxicity for target substance B. The read-across approach and reliability of the prediction can be improved using measured values for the test concentrations and by determining which factors determine toxicity and how (e.g. organic matter content).

2.4.b Example for different bioaccumulation potential for source and target substance and its impact on sediment toxicity

- Substances A and B are structurally similar chemicals that share the same functional groups. Substance B has a higher degree of branching including terminal tert-butyl groups.
- Sediment toxicity is predicted for substance B
- Substances A and B are screened to be potentially bioaccumulative based on the octanol-water partitioning coefficient (log Kow). Evidence on stability (no degradation in test media, minimal adsorption to test vessels) of the compounds A and B has been provided in the documentation.
- Substance A is rapidly transformed in fish and therefore the bioaccumulation potential in fish is low. In contrast, the bioaccumulation potential of substance

[10] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

B is high in fish due to the presence of terminal tert-butyl groups. Evidence for rate of transformation and bioaccumulation potential is provided.
- The transformation products are not relevant for sediment organisms based on evidence provided for high water solubility and low toxicity to sediment organisms of the transformation products.
- The evidence of substance B having a higher bioaccumulation potential in fish than substance A indicates that there might also be higher bioaccumulation potential of the substance B in sediment organisms.
- The explanation does not address the possible higher bioaccumulation potential of substance B, neither its possible impact on the predicted toxicity.

In this situation, there is uncertainty in the read-across and prediction for substance B as there may be significant bioconcentration, leading to a higher internal concentration and thus higher sediment toxicity.

2.4.c Example for predicting the property aquatic toxicity

- Substances A and B are mono-constituent organic substances including the same functional groups.
- Substances A and B are soluble and hydrophilic substances.
- Substance A is the source substance, substance B is the target substance.
- Physico-chemical information is provided for the source and the target substances including water solubility, log Kow, and vapour pressure. The target B has a higher log Kow and lower water solubility. Data on bioaccumulation is provided for neither of the substances.
- Based on the physico-chemical information for the substances together with information on the test conditions it is predictable that the losses due to volatility and adsorption are minimal during the period of testing. The substances are likely to be fully bioavailable for aquatic organisms.

In this situation, source and target substances are equally bioavailable to aquatic organisms and therefore it is forecasted that bioavailability does not influence the read-across and prediction. However, considering the differences in log Kow, the target substance B might concentrate to organisms to a higher degree and thus the toxic potential of the target substance might be underestimated. The read-across justification does not indicate an intention to correct the potentially underestimated toxicity value based on the Critical Body Burden approach and there are no reliable bioaccumulation data provided to apply such an approach. Therefore, the approach cannot be accepted.

AE A.3 Impact of impurities on the prediction

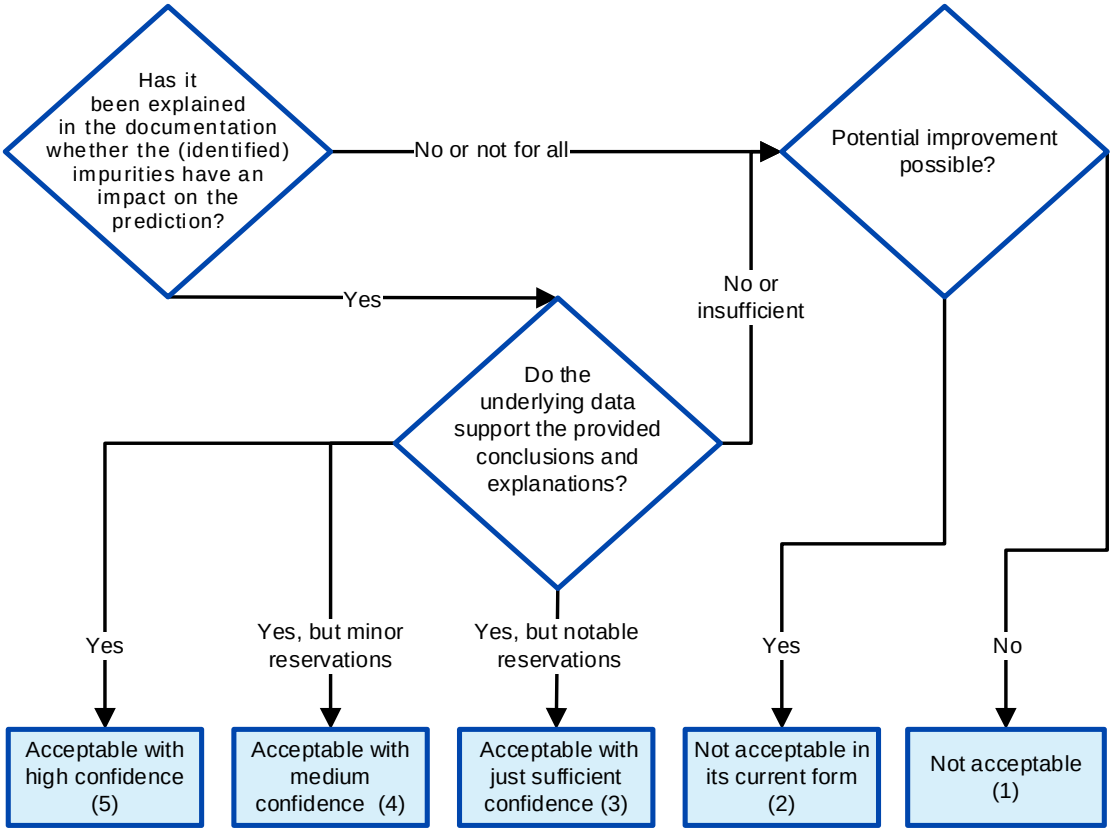
Purpose

The impurities[11] associated with the source and target substances may have an impact on the prediction.

It has to be assessed whether:

- the identified impurities have an impact on the prediction; and
- the provided evidence supports the explanation.

Assessment options



Explanation

Small changes in the impurity profile may have strong effects on the property that is predicted. The read-across justification should be clear whether it covers the impurities in addition to the main constituent of source and target substances. If the read-across

[11] See substance characterisation, as addressed in AE A.1 for the source substance or registration dossier for the target substance.

prediction covers only the main constituent, it might not be adequate for the whole target substance including the impurities. The properties of the impurity(ies) have to be addressed additionally.

The importance of impurities depends on the property that is predicted. A certain impurity might drive aquatic toxicity of a substance, while another one is important for the bioaccumulation potential.

Example(s)[12]

A.3.a Example of impurities not influencing the prediction

	Substance A	Substance B
Impurities	x (1-3 %) y (1-3 %)	x (1-3 %) y (1-3 %) **z (5-7 %)**
		Read-across from substance A

- Substances A and B are structurally very similar and acute fish toxicity is predicted from A to B.
- The substances share a common impurity x and y in similar concentration ranges (1-3 %).
- Substance B also has an impurity z that is not present in substance A; the concentration range of z is 5-7 %.
- It has been shown that the toxicity of z is at least one order of magnitude lower than the toxicity of substance A and the impurities x and y.

In this situation, the potential influence of maximum 7 % of impurity z will not impact the acute toxicity of B.

[12] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

A.3.b Example of an impurity not covered for the property bioaccumulation

- Substances A and B are structurally very similar; substance B has lower log Kow.
- Bioaccumulation for substance B is predicted based on substance A.
- Substance B has an impurity z in concentrations of ≤0.5 % that is not present in substance A.
- There is concern that this impurity might be a vPvB substance.
- The potential impact of this impurity is not addressed.

In this situation, the read-across does not cover the impurity z that is in the target substance B and it needs to be assessed/considered elsewhere in the registration dossier.

AE A.4 Consistency of properties in the data matrix

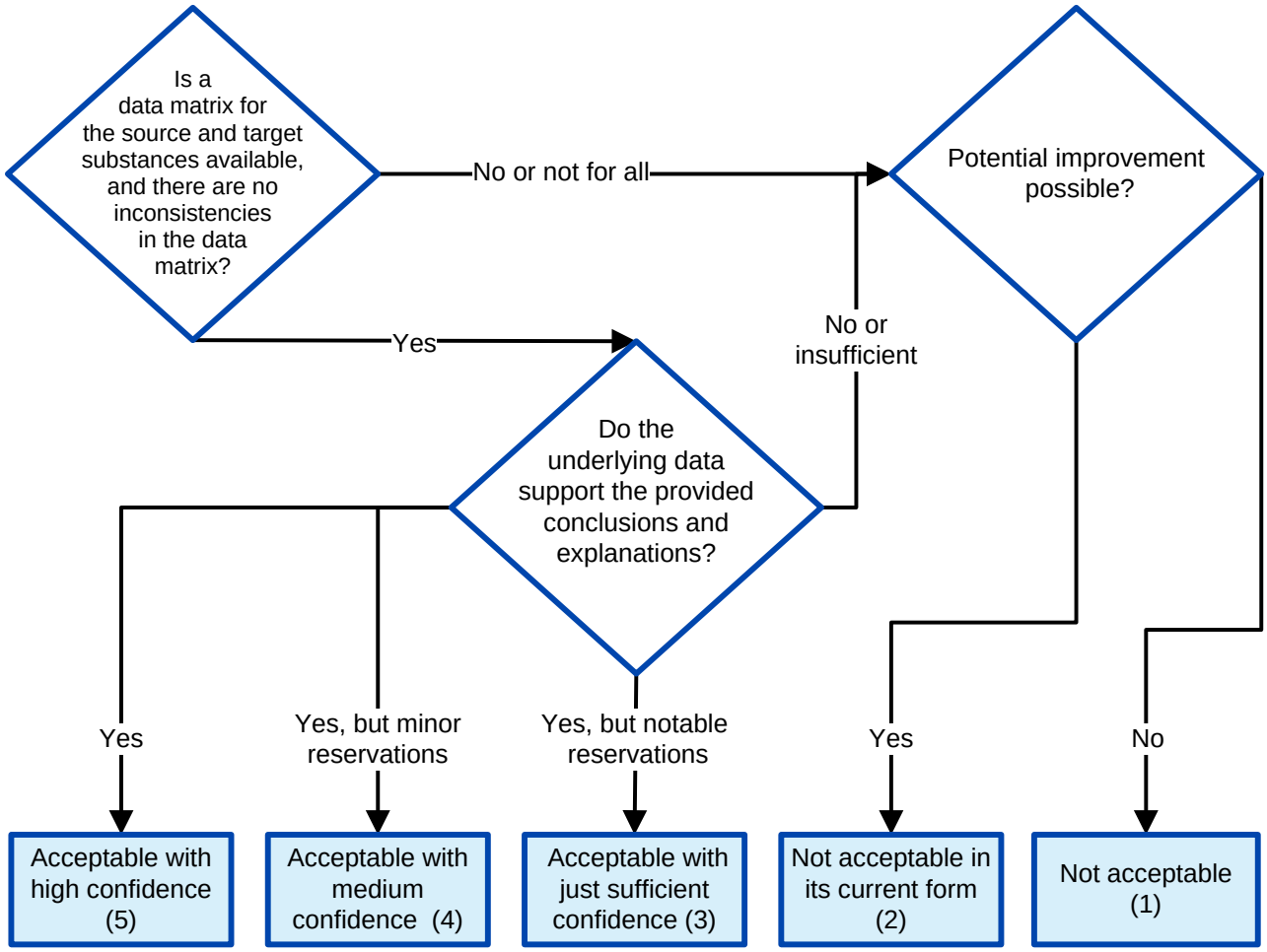
Purpose

A data matrix with experimental data for source and target substances is needed to support the read-across.

It has to be assessed whether:

- a data matrix has been provided which lists available reliable data for the source and target substances and which identifies data gaps;
- the properties of source and target substances across the data matrix are consistent; this has to be assessed in the following dimensions:
    - within the specific property which is under consideration for the prediction;
    - between the property under consideration and related properties (*e.g.* between short-term and long-term aquatic toxicity studies; log Kow and aquatic toxicity studies);
- the properties of source and target substances are the same or similar or a worst-case prediction is provided. If there are differences in critical properties for a given endpoint (such as log Kow for BCF or water solubility for aquatic toxicity), particular consideration has to be given to how this will affect the prediction; and
- the underlying data support the provided conclusions and explanations.

Assessment options



Explanation

The data matrix should:

- include a comparison of all available data within the source and target substances, per property for each substance;
- highlight similarity within properties; and
- identify data gaps.

The study results provided in the data matrix should be checked for adequacy and reliability; the test material used should be representative of source and target substances.

Consistency of the information in the matrix

There should be evidence from the data matrix that the properties related to the property to be predicted are consistent between the source and target substances. Depending on the

hypothesis, the same or similar type of effect and/or fate property(ies) are observed for target and source substances. For example, when environmental effects are predicted, ecotoxicological data from different trophic levels or environmental compartments are useful to support the hypothesis.

Note that the comparison of effect concentrations should be done on a molar basis, which relates effects to the number of molecules per quantity. Furthermore, test conditions and duration should be comparable, or it should be explained that differences do not impact the prediction.

Inconsistencies may indicate that the reactivity of the analogues differs and that there are different mechanisms acting. Thus, the prediction may not be valid. Note that for the evaluation of data consistency, the outcome of other AEs (e.g. presence of impurities, stability issues) has to be taken into account.

It should also be acknowledged that there is intra-species, inter-species and inter-lab variation, even for well conducted OECD test guideline studies on standard species. Therefore, even an acceptable grouping approach allows for a certain degree of variation.

No-effect concentrations based on different effects or different experimental conditions

The information given in a data matrix may not always reflect the information given in the robust study summary. For instance, the endpoint on which a NOEC is based should be reported in the data matrix (e.g. growth, survival, hatching rate) and target and source substances should be compared against the same biological endpoint.

Similar considerations apply to comparability of different test conditions. For example, sediment characteristics such as pH and organic carbon content may impact the bioavailability and thus ecotoxicity of compounds. Therefore, the comparability of test results from e.g. artificial versus natural sediment studies may not indicate similarity in toxic properties in a reliable manner. It is necessary to assess the underlying data in the study information to get a clear picture of the study results.

Other supporting evidence provided in the technical dossier

Supporting evidence may refer to human toxicokinetic and toxicological evidence, validated (Q)SARs, monitoring data etc. It should be assessed whether this information supports, does not support or even contradicts the proposed prediction.

Example(s)[13]

A.4.a Consistent information in the data matrix

- Substances A and B are structurally similar substances. Their structures differ in the carbon chain length, being longer for B. The information on other related properties (e.g. log Kow) reported in the data matrix is consistent between the source and target substances.

	Substance A	Substance B
Short-term toxicity on fish	LC50 0.16 mmol/l (20 mg/l)	LC50 0.11 mmol/l (15 mg/l)
Short-term toxicity on invertebrates (*Daphnia* sp.)	EC50 0.24 mmol/l (29 mg/l)	EC50 0.18 mmol/l (25 mg/l)
Growth inhibition (algae)	EC50 0.33 mmol/l (40 mg/l)	EC50 0.24 mmol/l (32 mg/l)
Long-term toxicity on invertebrates (Daphnia sp.)	NOEC 0.04 mmol/l (4.5 mg/l)	NOEC 0.01 mmol/l (1.5 mg/l)
Long-term toxicity on fish	**Prediction?**	NOEC 0.12 mmol/l (16 mg/l)

[13] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

Fish, daphnids and algae show toxicity in about the same range for source and target substances. Therefore, the effects in the data matrix are consistent or worst case prediction.

A.4.b Inconsistent information in the data matrix

- Substances A and B are structurally similar substances. Their structures differ in the carbon chain length, longer for B. The information on other related properties (i.e. log Kow) reported in the data matrix is consistent between the source and target substances.

	Substance A	**Substance B**
Short-term toxicity on fish	LC50 >0.93 mmol/l (>100 mg/l)	LC50 0.16 mmol/l (20 mg/l)
Short-term toxicity on invertebrates (*Daphnia* sp.)	EC50 >0.93 mmol/l (>100 mg/l)	EC50 0.24 mmol/l (29 mg/l)
Growth inhibition (algae)	EC50 >0.93 mmol/l (>100 mg/l) NOEC 0.28 mmol/l (30 mg/l)	EC50 0.33 mmol/l (40 mg/l) NOEC 0.041 mmol/l (5 mg/l)
Long-term toxicity on fish	NOEC 0.012 mmol/l (1.3 mg/l)	NOEC 0.5 mmol/l (61 mg/l)
Long-term toxicity on invertebrates (*Daphnia sp*.)	NOEC 0.12 mmol/l (13 mg/l)	**Prediction?**

For short-term toxicity test results, there is a consistent trend towards higher toxicity for Substance B. However, this trend is not observed for long-term fish toxicity studies, where Substance B shows lower toxicity than Substance A. Inconsistencies in the data matrix raise concern regarding the reliability of the predictions of long-term *Daphnia* toxicity for Substance B.

A.4.c Absence of short-term toxicity cannot support long-term toxicity prediction

- Substances A and B are structurally similar substances. Their structures differ in the carbon chain length, longer for B. The information on other related properties (i.e. log Kow) reported in the data matrix is consistent between the source and target substances

	Substance A	Substance B
Short-term toxicity on fish	LC50 >0.93 mmol/l (>100 mg/l)	LC50 >0.82 mmol/l (>100 mg/l)
Short-term toxicity on invertebrates (*Daphnia* sp.)	EC50 >0.93 mmol/l (>100 mg/l)	EC50 >0.82 mmol/l (>100 mg/l)
Growth inhibition (algae)	EC50 >0.93 mmol/l (>100 mg/l) NOEC >0.93 mmol/l (>100 mg/l)	ECr50 >0.82 mmol/l (>100 mg/l) NOEC >0.82 mmol/l (>100 mg/l)
Long-term toxicity to aquatic organisms	NOEC 0.3 mmol/L	**Prediction?**

Short-term aquatic toxicity tests showing no toxicity for substances with low water solubility are not adequate to establish similarity in inherent toxicity for long-term aquatic toxicity tests.

AE A.5 Reliability and adequacy of the source data

Purpose

The source study(ies) need(s) to be reliable and adequate as requested for any other key study.

It has to be assessed whether:

- the study design reported for the source study is adequate and reliable for the prediction based on read-across:
    - the study design should cover the key parameters in the corresponding test method referred to in Article 13(3);
    - the study design should cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3);
    - the study should be conducted according to design indicated in the corresponding test method referred to in Article 13(3), such as temperature and pH; and
    - there is adequate and reliable documentation of the applied test method, i.e. a robust study summary should be provided;
- the test material used represents the source substance as described in the hypothesis in terms of purity and impurities.

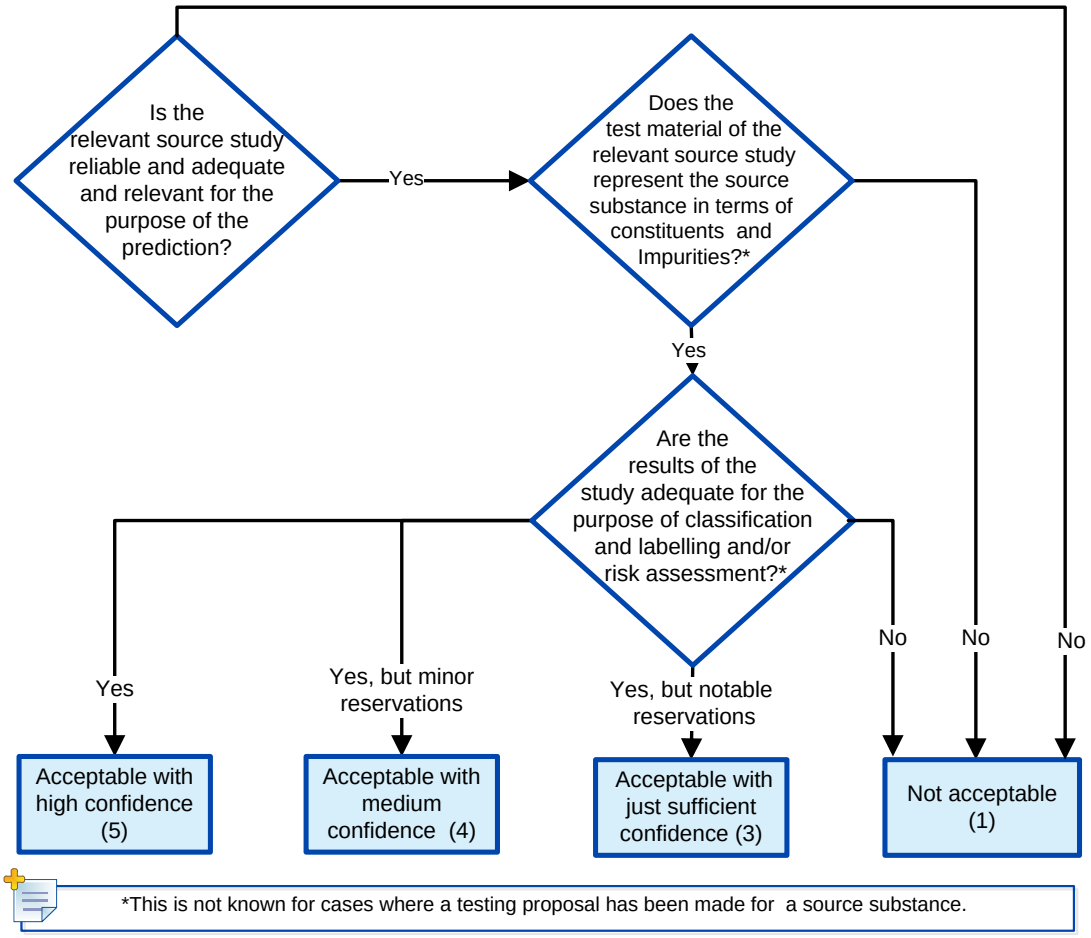
It also has to be assessed whether:

- the study results are adequate for classification and labelling and/or risk assessment. For example, this could include whether sufficient concentrations have been tested to enable the relevant determination of an effect concentration for a decision on classification and labelling, or whether a NOEC has been identified from a study.

If the conditions listed above are met and the conclusions made are consistent with the reported results (*e.g.* reliable effect concentrations and NOEC identification), it may be assumed that the study results are adequate for classification and labelling and/or risk assessment.

Although most emphasis will be on the source study used for the property under prediction, any study used in the read-across (data matrix) should in principle be reliable and adequate and the test material used should be representative of source and target substances. If studies with lower quality (reliability/adequacy) are used, the impact of such lower quality on the prediction has to be assessed.

Assessment options



Explanation

Requirements for source studies

Section 1.5 of Annex XI stipulates that the results of "Grouping of substances and read-across approach" should in all cases:

- '*Be adequate for the purpose of classification and labelling (C&L) and/or risk assessment,*
- *have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3),*
- *cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3) if exposure duration is a relevant parameter, and*
- *adequate and reliable documentation of the applied method should be provided.*'

The source study needs to meet all requirements placed on any key study used as stand-alone evidence to meet an information requirement under REACH. Therefore, an analysis of

the source study used for the prediction of a property needs to be conducted. The elements of the analysis are covered in the purpose section.

The Klimisch scores used by the registrant in the endpoint study record may be helpful as a starting point for this evaluation.

Test substance *versus* source substance characterisation in the hypothesis

The test material should be clearly defined. If there are any differences between the test material and the source substance, it should be clarified that the test material is representative of the source substance and its impact on the prediction should be assessed.

Example(s) [14]

A.5.a Example for a source study not meeting the REACH information requirements

- The source substance was tested in a Fish, Prolonged Toxicity Test: 14-Day Study (OECD 204).
- This study is used to predict the results of a long-term aquatic toxicity study in fish according to OECD 210 for the target substance to meet the Annex IX requirement of a long-term toxicity in fish.

The key parameters of the source study are not appropriate to meet the information requirements of Annex IX, Section 9.1.6. The source study is not adequate for the intended prediction.

A.5.b Example for a source study conducted with a test substance which significantly differs from the source substance as described in the read-across hypothesis

- The read-across hypothesis refers to a source substance, *para*-isomer, with a purity of 95 %, impurities are known.
- The structurally similar target substance is also a *para*-isomer with a purity of 90 %, impurities are known.
- A long-term aquatic toxicity study in fish according to OECD 210 is proposed to be used to predict the long-term fish toxicity study outcome of the target substance. The test material consists of a mixture of *para*-, *meta*-, and *ortho*-isomers of about 35, 20 and 35 %, respectively. 10 % are unknown impurities.

The test material does not represent the source substance as referred to in the read-across hypothesis and it is not explained in the read-across justification.

[14] The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

AE A.6 Bias that influences the prediction

Purpose

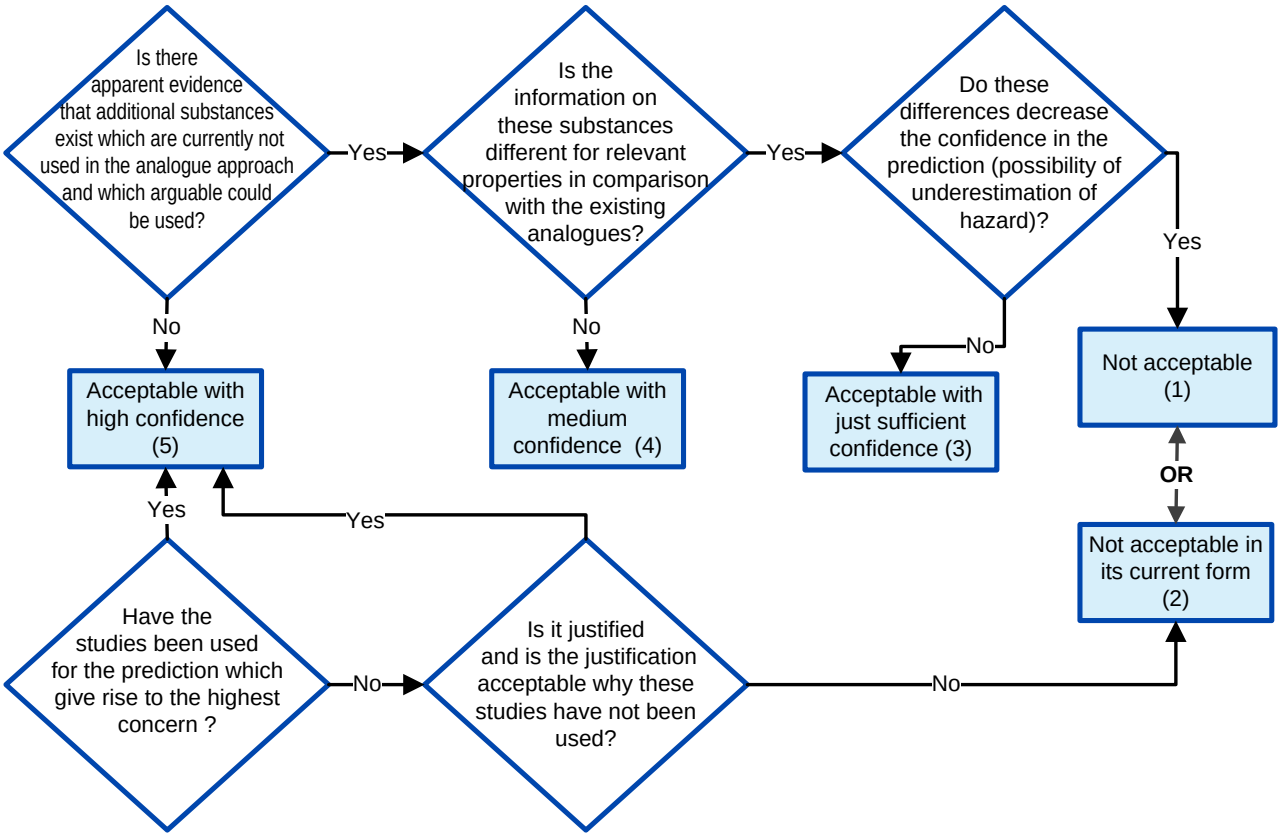
It has to be assessed whether:

- it is clear from the documentation how the source substance(s) have been chosen;
- there are additional, structurally-similar substances which are currently not used in the analogue approach and which arguably could be used;
- there is readily available information from these additional substances;
- this information is significantly different for relevant properties in comparison with the existing analogue(s); and
- these differences decrease the confidence in the prediction (possibility of underestimation of hazard).

It also has to be assessed whether:

- the study(ies) used for the prediction is(are) giving rise to the highest concern for the property under consideration when several studies are available in the data matrix. Justifications have to be provided if the studies giving rise to the highest concern have not been used.

Assessment options



Explanation

There might be information obtained from the dossier or from outside the dossier which triggers concern on selection bias with regard to the source substance(s).

Such a situation may occur:

- when there are multiple possible analogues with equivalent structural similarity that are not referenced in the dossier and/or it has not been satisfactorily justified why they have not been used; or
- the assessing expert has knowledge of such additional structurally-similar analogue(s) that are not referenced in the dossier.

If the studies conducted with the additional structural analogue(s) have significantly different results for the properties of the substance, then this may result in a difference in the prediction for the property under consideration. Consequently, the proposed prediction may be considered to be unreliable.

In addition there might be selection bias for the study used for the prediction when several studies are available in the data matrix. According to REACH Annex I, Section 3.1.5,

normally the study giving rise to the highest concern has to be used to draw a conclusion. If such a study is not used, this has to be fully justified. This also applies to the selection of key studies for predictions based on read-across.