RAAF Appendix ENV-C

Scenario 3

Description

This scenario covers the category approach for which the read-across hypothesis is based on transformation to common compound(s).

For the REACH information requirement under consideration, the property investigated in studies conducted with different source substances are used to predict the property that would be observed in a study with the target substance if it were to be conducted.

Similar properties, are observed for the different source substances; this may include absence of effects for some members of the category. There are quantitative differences in the predicted property(ies) forming a regular pattern.

The prediction is based either on this regular pattern within the category, when the members are ordered by a variable related to the structural differences in the category, or on a worst-case approach.

The scientific explanation has to include the reason why differences in predicted properties are observed/predicted.

Although this scenario can in theory be applied, it is anticipated that this would be a very exceptional case as it would normally be difficult to build a strong, evidence-based hypothesis showing a trend based on transformation. Therefore, examples have not been developed for every AE.

Assessment elements for Scenario 3

The assessment elements (AEs) for this scenario consist of seven AEs common to the category-approach and four scenario-specific AEs which depend on the mechanistic explanation (Table C1).

Table C1. Appendix C – Assessment elements (AEs) for Scenario 3

ASSESSMENT ELEMENTS (AEs) FOR SCENARIO 3			Applicability to a predicted property		
AE #	AE type	AE Name	Degradation	Bioaccumulation	Environmental effects
AE C.1	Common	Characterisation of source and target substances	X	X	X
AE C.2	Common	Structural similarity and dissimilarity	X	X	X

ASSESSMENT ELEMENTS (AEs) FOR SCENARIO 3			Applicability to a predicted property		
AE #	AE type	AE Name	Degradation	Bioaccumulation	Environmental effects
		within the category (category description)			
AE C.3	Common	Link of structural similarities and structural differences with the proposed regular pattern (presence of hypothesis)	X	X	X
AE 3.1	Scenario-specific	Formation of common (identical) and non-common compounds	X	X	X
AE 3.2	Scenario-specific	Degradation of non-common compounds	X	X	X
AE 3.3	Scenario-specific	Bioaccumulation potential of non-common compounds		X	X
AE 3.4	Scenario-specific	Impact non-common compounds			X
AE C.4	Common	Impact of impurities on the prediction	X	X	X
AE C.5	Common	Consistency of properties in the data matrix	X	X	X
AE C.6	Common	Reliability and adequacy of the source data	X	X	X
AE C.7	Common	Bias that influences the prediction	X	X	X

AE C.1 Characterisation of source and target substances

Purpose

The substances which are members of the category need to have a clear substance characterisation[1].

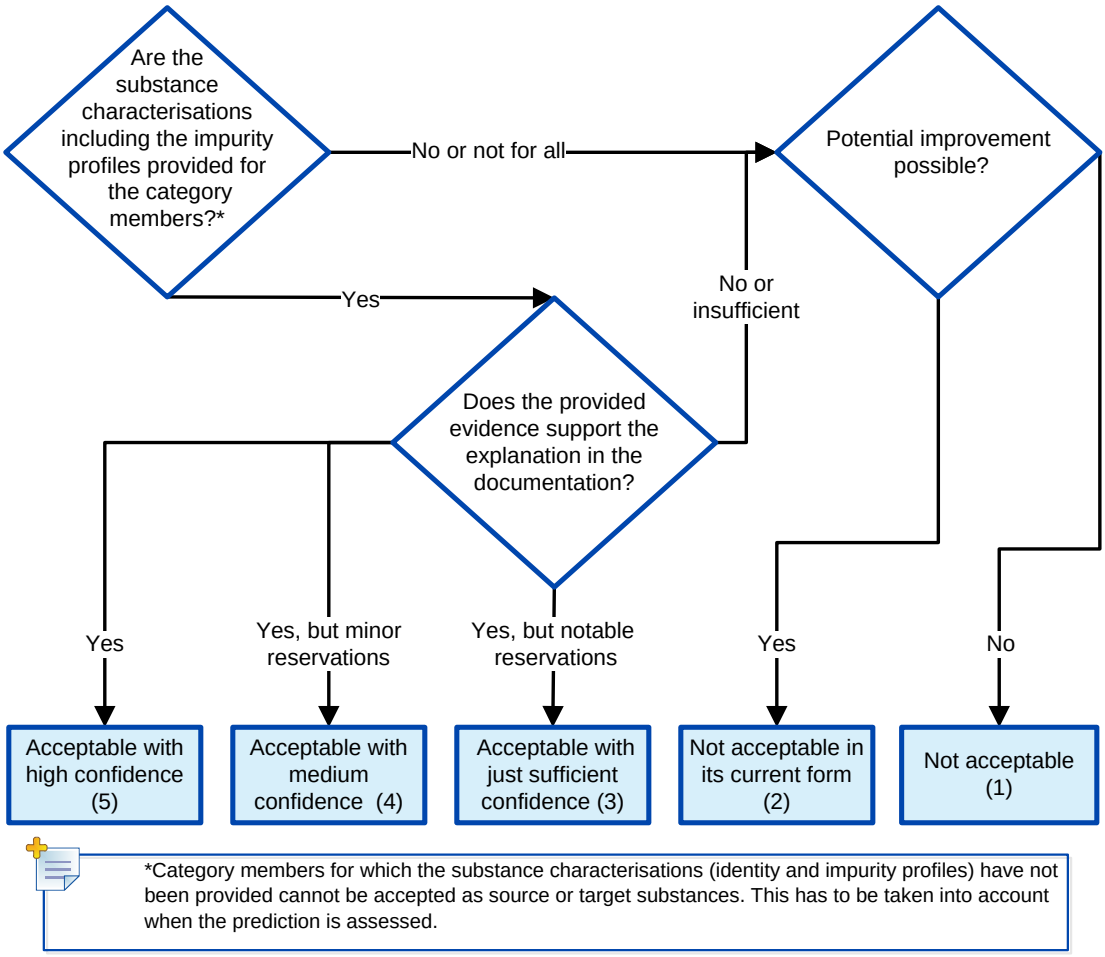
It has to be assessed whether:

- the chemical identity of the category members is sufficiently clear for assessing the proposed read-across; and
- the impurity profiles are clear for the category members.

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

[1] The test material actually used in a specific source study is addressed in AE C.6.

Assessment options



Explanation

Structural similarity[2] is a necessary pre-requisite for any prediction based on read-across

[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. In the different scenarios, this aspect is addressed in several 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.*"

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 a category approach, the information provided on the identity of the category members must establish a clear picture of the chemical structures of the constituents of the members of the category. 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 also important that not only the chemical structures, but also the impurity profiles of all category members are well defined to establish the category definition, 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 category members 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.6.2.6.2*):

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

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.* 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 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 of importance 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

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

% (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 C.4.

Example(s)[4]

C.1.a Example for an identity of a category member 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 category member is clear for read-across purposes.

C.1.b Example for an identity of a category member which is not clear

- Substance B is a mono-constituent substance.
- The main constituent is present at 88-96 % with a typical concentration of 92 %.
- The impurity profile consists of several impurities at 2-3 % and/or 'unknown' impurities <1 %. Name, CAS, EC, chemical structure and concentration ranges for all of these impurities are not available.
- Based on the manufacturing process of the substance it can be presumed that the impurity profile contains side products that may be relevant to environmental hazard identification.

In this case, the identity of the category member may not be 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 C.4.

AE C.2 Structural similarity and dissimilarity within the category (category description)

Purpose

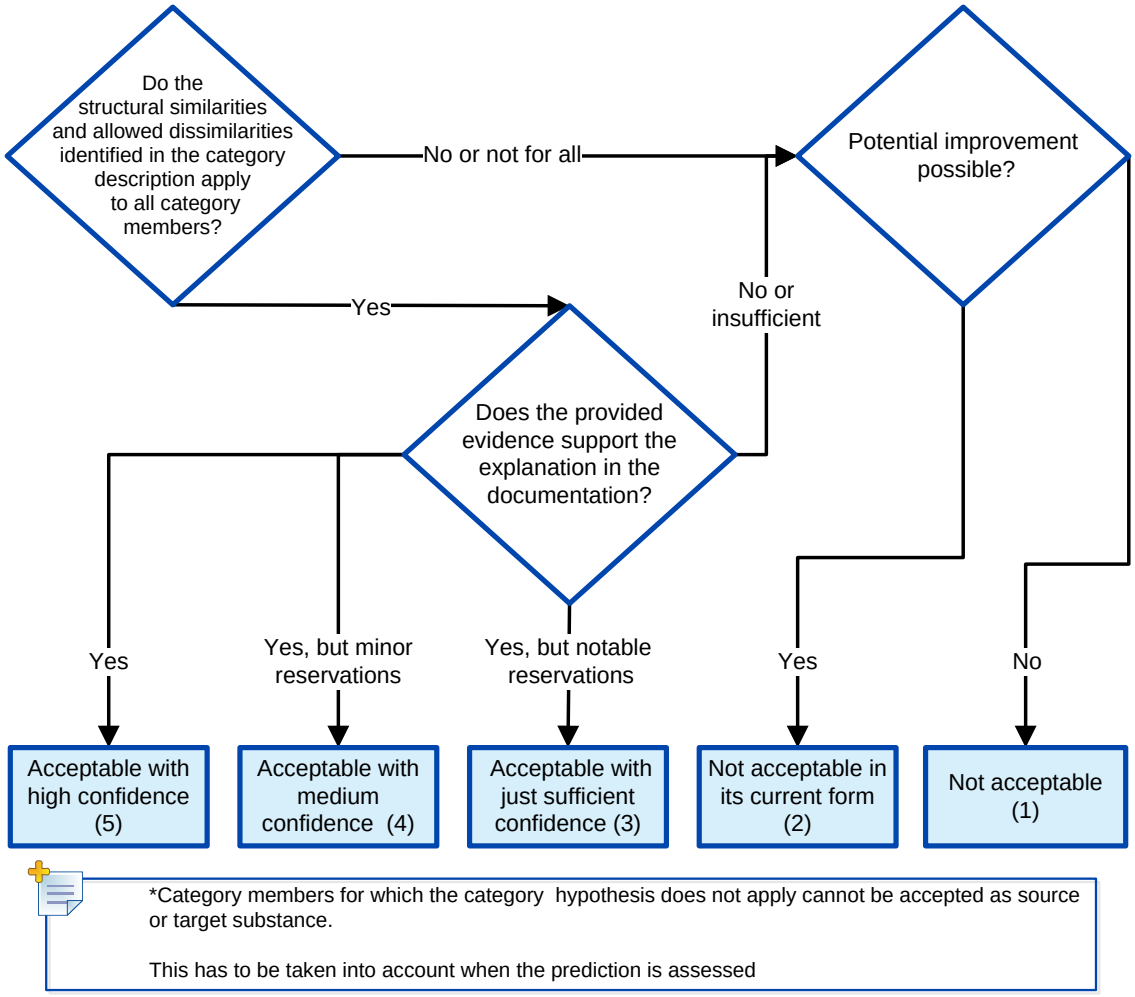
The aim of this AE is to verify that all category members indeed meet the criteria for structural similarities and allowed structural differences used for the category description[6].

It has to be assessed:

- whether the structural similarities identified in the description apply to all category members;
- which structural differences are allowed within the category; and
- whether the provided evidence supports the category description.

[6] The category definition includes the category description and hypothesis. The possibility of selection bias for category members is addressed in AE C.7.

Assessment options



Explanation

An explanation of the structural similarities and dissimilarities should be submitted and describe why the category is composed as it is. The starting point of read-across is structural similarity and it is a pre-requisite for any grouping and read-across approach under REACH. The category membership should be primarily based on chemical structure.

It should be understood:

- which structural moieties or characteristics the category members have in common;
- which structural differences are allowed by the category description;
- whether there are other criteria used to reduce the number of category members (such as physico-chemical criteria, data availability considerations);

The category description should address the structural similarities and dissimilarities of a

given group of substances (i.e. category boundaries), as it is the starting point for read-across.

It should be emphasised that category members for which the category description does not apply cannot be accepted as source or target substances. This has to be taken into account in the assessment elements relating to the assessment of the prediction.

Example(s) [7]

C.2.a Example for category members falling under a category description

- Substances A, B, C and D are substances with an n-alkyl chain and share the same functional group.
- The substances have different chain length, i.e. between 7 and 12 carbon-atoms in the chain).
- No other differences exist.

The category description has to explicitly address the difference in carbon-atom number. In this case, the boundaries of the category are clearly described, i.e. between 7 and 12 carbon-atoms in the chain.

C.2.b Example for a category member not covered by a category description

- Substances A, B, C and D are structurally similar halogenated substances.
- Substances A, B and C have the same number of halogenated substituents whereas substance D has more halogens in its structure.
- The potential impact of the number of halogenated substituents is not covered in the category description.

To include the substance D into the category, the category description would have to address the difference in number of halogenated substituents.

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

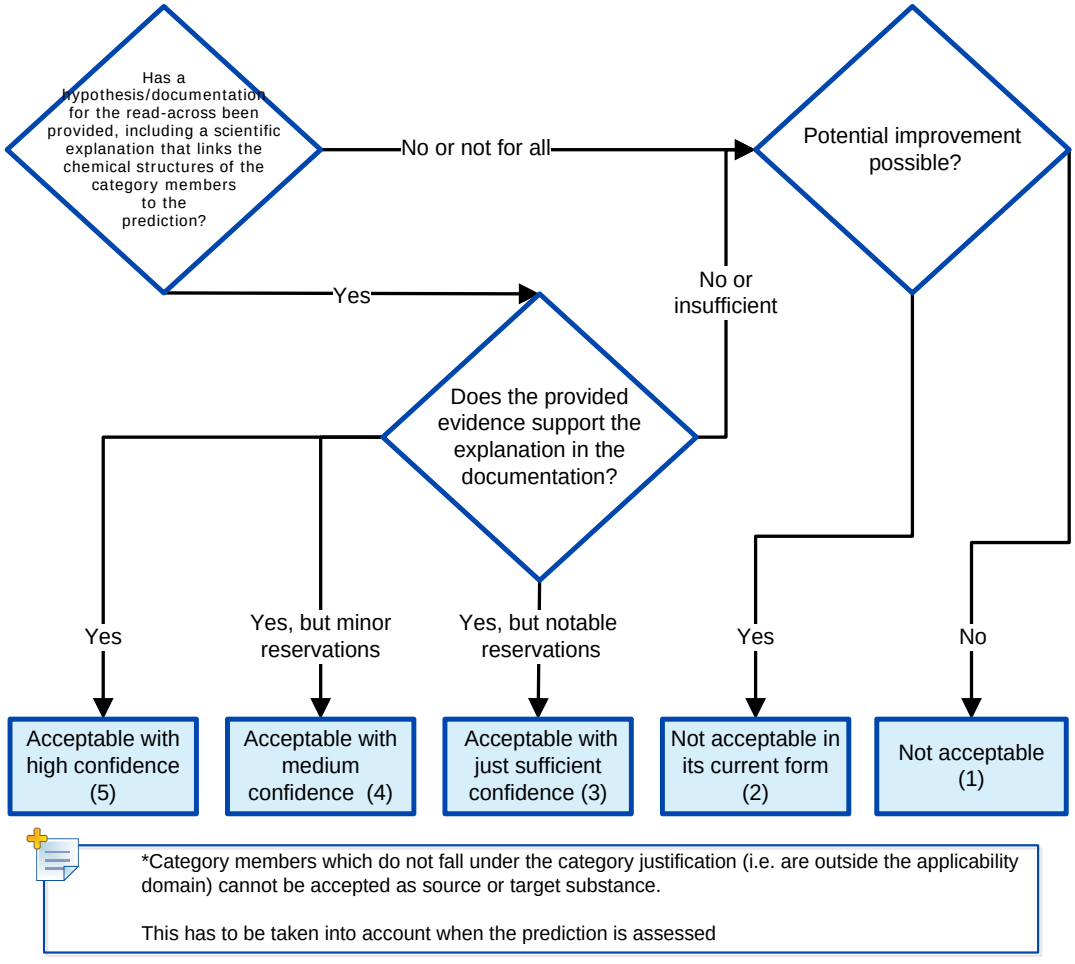
AE C.3 Link of structural similarities and structural differences with the proposed regular pattern (presence of hypothesis)

Purpose

It has to be assessed whether:

- the documentation provides an hypothesis (explanation) why and how the category members should behave in a predictable manner (*e.g.* based on no absorption due to molecular-weight considerations, or lacking reactivity towards biological material, regular pattern)[8]; and
- the provided evidence supports the proposed regular pattern.

Assessment options



[8] The category definition includes the category description and hypothesis.

Explanation

The hypothesis provides a scientific explanation why the category members should behave in a predictable manner (a regular pattern). Such hypothesis has to apply to all the category members.

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. 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.

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) [9]

C.3.a Example for an explanation applying to all category members

- The category is structurally defined as substances A, B, C and D which are esters of C4 acid and alcohols with chain length C12, C14, C16 and C18, respectively.
- The category description only includes these esters (i.e. the borders of the category are formed by C12 and C18 esters).
- The category hypothesis provides an underlying explanation why these substances are likely to behave similarly.

Prediction for the C14 and C16 esters may be based on studies conducted with the C12 and C18 esters (i.e. these esters are inside the borders of the category and prediction is based on interpolation).

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

AE 3.1 Formation of common (identical) and non-common compounds

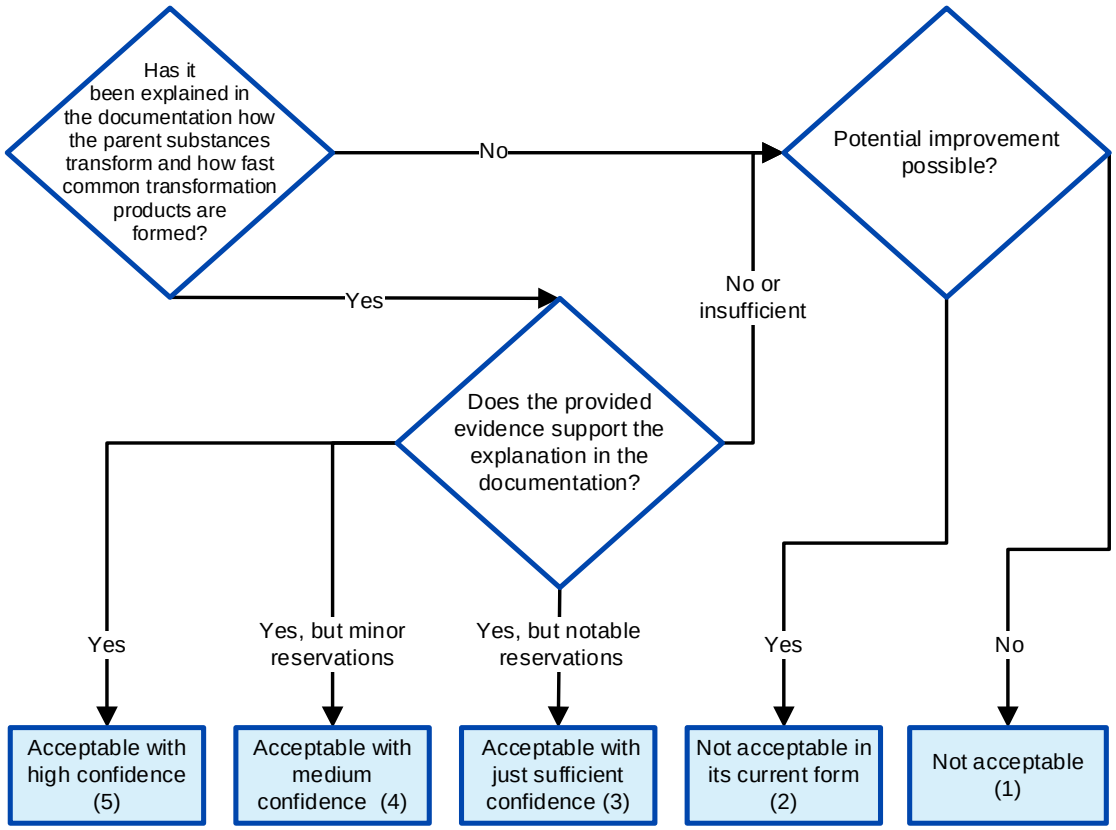
Purpose

This AE is used to assess the hypothesis that either the source substances transform into the target substance, the target substance transform into a source substance, or target and source substances transform into common compound(s).

For the category members, it has to be assessed whether:

- it is explained how the (identical) common product(s) are formed (i.e. the product(s) claimed to drive the impact on the property under consideration);
- it is explained how fast and to what extent the common transformation product(s) are formed;
- the (bio)availability of the target substance in comparison to that of the source substance(s) for formation of common compounds has been taken into account in the documentation;
- the hypothesis considers the impact of the rate of transformation of the parent substances into common compounds;
- the identity of the non-common compound(s) is explained (further degradation of non-common compounds is addressed in AE 3.2); and
- the provided evidence supports the explanation.

Assessment options



Explanation

In this scenario, the hypothesis for grouping substances is based on "*likelihood of common precursors and/or breakdown products, through physical or biological processes*" as given in Annex XI, Section 1.5. It is based on the assumption that either the source substances transforms into the target substance (or *vice versa*), or that the source and target substances undergo biological and/or chemical transformation in such a way that a common product (or common products) and non-common products are formed.

This AE deals with the formation of the common compound(s). In most cases, the scientific explanation needs to be supported with data on the source and target substances. Convincing evidence has to be provided that the common product(s) are formed relatively rapidly from the category members.

Factors limiting the availability of a substance for transformation to common compounds such as low water solubility can impact the transformation rate. Therefore it has to be assessed if the availability of the target substance is comparable to that of the source substance(s).

It should be assessed whether differences in the rate of formation of common compounds form a regular pattern.

Although this AE deals only with the formation of the common compound(s), indirect supporting evidence may also be derived by analysing the data matrix. Different effects are indicative for the formation of different compounds or a different rate of formation.

Different physical and biological processes may lead to common breakdown products. When environmental fate and effects are predicted, a hypothesis, where a common breakdown product is formed should include an explanation of the transformation pathway, for instance if rapid hydrolysis, dissociation or biodegradation occurs under environmentally relevant conditions.

Common breakdown products based on hydrolysis

Comparability of pathway and hydrolysis rate in similar conditions (pH, temperature)

It has to be assessed if the proposed hydrolysis pathways appear scientifically plausible and are comparable, or whether chemical knowledge or other sources (e.g. software) would indicate that different hydrolysis products could occur at a similar (relevant) pH and temperature.

Furthermore, the hydrolysis rate should be comparable at a similar pH and temperature for the compartment of concern. Note that in static and semi-static tests, the test organisms will be exposed mainly to the hydrolysis products if the hydrolysis rate is high enough, while in flow-through tests the exposure may be mainly to the parent substance, even if the hydrolysis is (relatively) fast.

This should be taken into account when evaluating ecotoxicity studies of category members, especially when parent substance and/or hydrolysis products can be expected to have an adverse effect. (See the *Guidance document on aquatic toxicity testing of difficult substances and mixtures,* OECD guidance series on testing and assessment Number 23 (2000)).

Similar quantity of common hydrolysis product

In some cases, such as read-across between a diglyceride and triglyceride, the quantity of the common hydrolysis product (stoichiometry) may differ and has to be taken into account for the further assessment.

Common breakdown products based on dissociation

Dissociation is the reversible splitting of a substance into two or more chemical species, which may be ionic (OECD TG 112, 1981). This hypothesis is basically applicable to salts. It has to be assessed to which extent the source and target substances dissociate, taking into account their water solubility.

Common breakdowns products based on biodegradation

Although this read-across based on biodegradation to common breakdown products can in theory be applied, it is anticipated that this would be a very exceptional case as it would

normally be difficult to build a strong, evidence-based hypothesis showing a trend based on biodegradation. Therefore, no examples have been developed.

Example(s)[10]

3.1.a Example for hydrolysis to common compounds for the property sediment toxicity

- Substances A, B, C and D hydrolyse into intermediate substances and eventually into the common compound Z at different rates.
- Exposure to substance Z is claimed to dominate the environmental hazard profile of A, B, C and D in the same way.
- It has been shown that non-common compounds do not contribute significantly to the effects.
- Studies with substances A, C and D do not reveal sediment toxicity and are used as source studies to predict sediment toxicity for target substance B.

The likelihood of the hydrolysis pathway including the identity of non-common compounds and the hydrolysis rates to intermediates and common compounds need to be characterised.

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

AE 3.2 Degradation of non-common compounds

Purpose

This assessment element addresses abiotic and biotic degradation processes which can occur in the course of testing, and have not been already addressed under AE 3.1.

If the prediction is for the property “degradation”

It has to be assessed whether:

- the hypothesis provided explains how the prediction of degradation potential follows a regular pattern, and is not influenced or underestimated by the non-common compounds; the relation between an observed (degradation) property and the structure and/or related properties may be used; 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:

- potential further degradation of the non-common compounds during a bioaccumulation or ecotoxicity test has been considered and the identity of the degradation products has been provided (the potential impact of these products is assessed in AE 3.3 and AE 3.4); and

- the provided evidence supports the explanation.

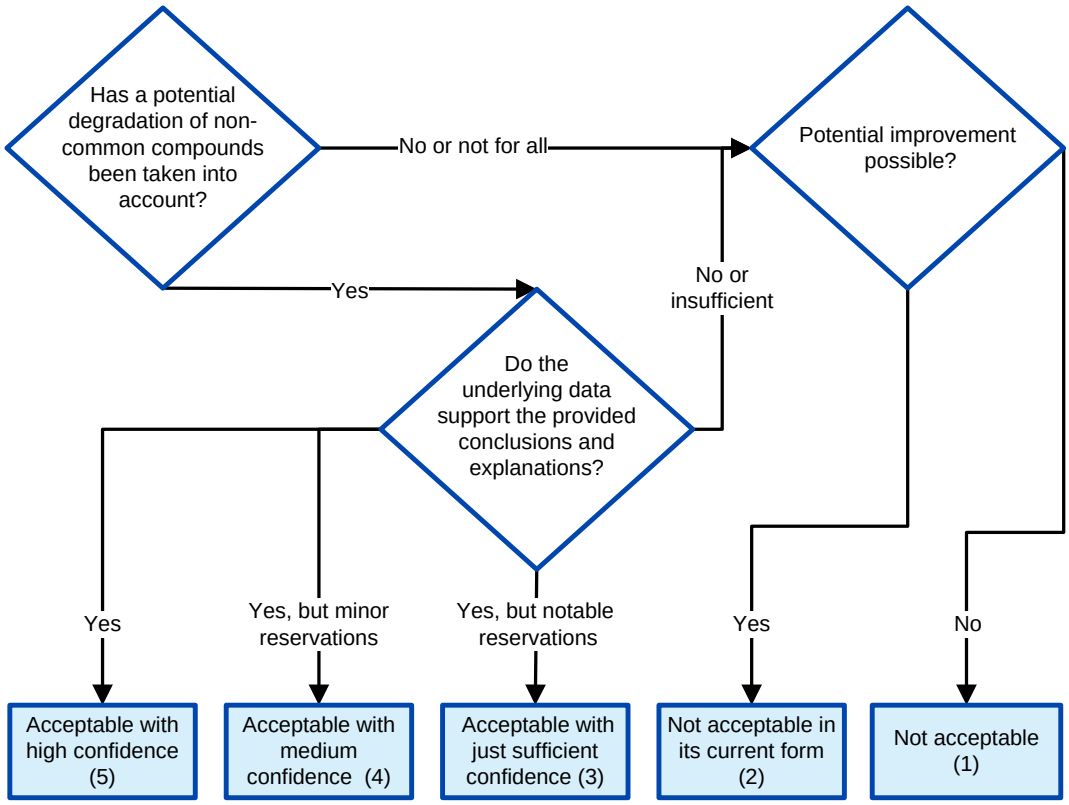
Applicability of the assessment element

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 on 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 "biodegradation"

This AE is related to AE 3.1 where the formation of the common compounds is covered, which is of course dependent on the transformation rates and extent of transformation.

Under the current scenario, the prediction for the property under consideration is claimed to be influenced only by the common transformation compound(s) of source and target substances. However, the persistency may also be influenced by non-common compounds including:

- parent substances;

- non-common degradation products resulting from abiotic degradation/transformation (e.g. hydrolysis, dissociation, phototransformation); and

- non-common degradation products resulting from biotic degradation/transformation (e.g. biodegradation, biotransformation).

The non-common compounds resulting from degradation can be the final degradation/transformation products or can be intermediate products that will further degrade/biotransform. Which of these non-common compounds are relevant usually depends on the rate of formation/disappearance, their bioavailability, bioaccumulation potential and (potential) strength of effects.

A justification should provide an explanation of why the degradation properties of the non-common compounds do not underestimate the predicted persistency of the whole target substance (a worst-case approach). 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.

Limited bioavailability of non-common compounds should be considered in the test design of source studies. Similarly, when predicting degradability of a target substance, it should be addressed in the justification how the potential differences in bioavailability of the non-common compounds (see section "General: considerations on bioavailability for degradation" above) may impact the predicted degradation property, if the same study that was performed with the source substance(s) would be performed with the target substance.

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/or non-common compounds and they should support the hypothesis and justification given.

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

Degradation properties and factors influencing degradation of non-common compounds are also important in the assessment of the hypothesis for predicting bioaccumulation potential and environmental effects.

Processes such as hydrolysis, photodegradation and biodegradation may alter the exposure to non-common compounds during the study (identity of non-common compounds as well as level of exposure). Consequently, potential degradation processes can have a significant impact on the results of bioaccumulation and ecotoxicity tests.

This assessment element is required to understand to which non-common compounds the organisms are exposed in a bioaccumulation or ecotoxicity tests.

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.

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.

Note: The impact of (potential) further degradation products on the predicted bioaccumulation property is assessed under AE 3.3 and on the predicted environmental effect under AE 3.4.

AE 3.3 Bioaccumulation potential of non-common compounds

Purpose

Under this assessment element, the uptake and bioaccumulation potential of non-common compounds (from AE 3.1) and any of their potential degradation products (from AE 3.2) are assessed both when the bioaccumulation potential is predicted and 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 follows a regular pattern, and is not influenced or underestimated by the non-common compounds such as the parent substance (as a result of incomplete transformation) and non-common degradation products (identified in AE 3.1 and AE 3.2); bioavailability of non-common compounds may be considered; 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 bioconcentration and/or bioaccumulation of non-common compounds such as the parent compounds and non-common degradation products (identified in AE 3.1 and AE 3.2) does not influence or underestimate the predicted environmental effect property of the whole compound; and

- the provided evidence supports the explanation.

Applicability of the assessment element

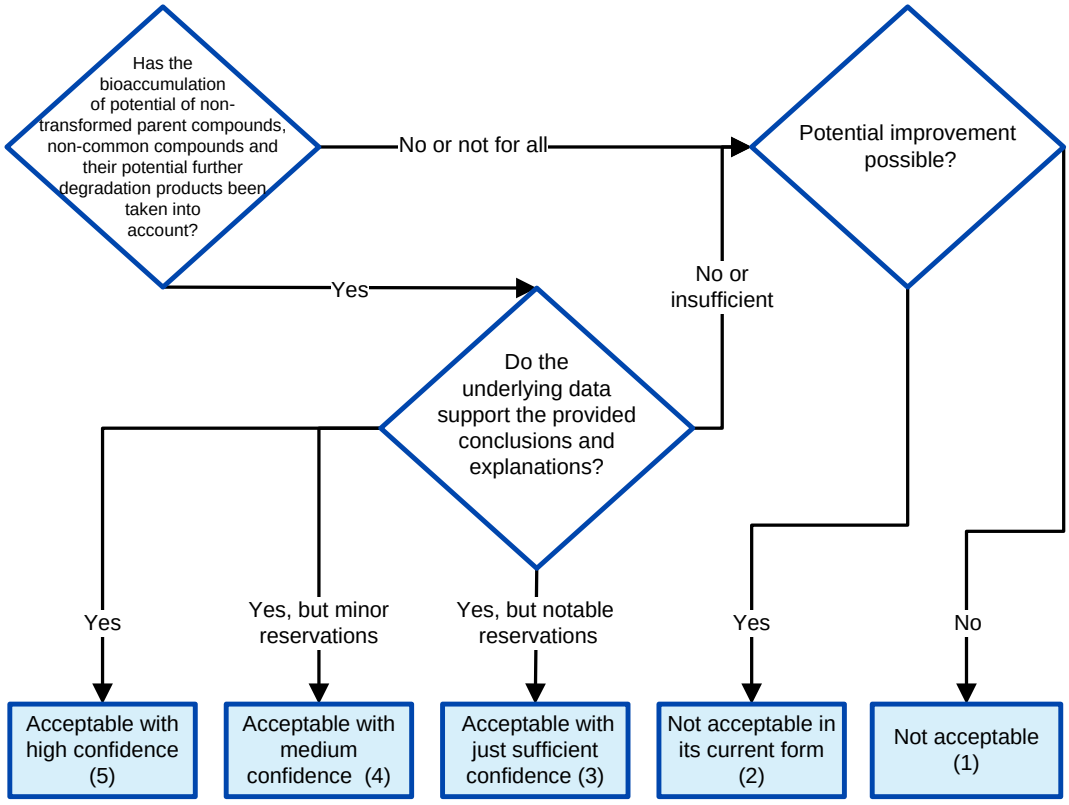
This AE applies to the following properties to be predicted:

- 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 non-common compounds needs to be assessed as the non-common compounds might have a (significant) potential to bioaccumulate. It should be explained why the presence of the parent substances and non-common transformation products in the test solution does not underestimate the predicted property or does not influence a regular pattern of the whole registered substance.

The related properties of the non-common compounds may be considered to inform about their behaviour in the test solution and potential to bioaccumulate. Factors such as the rate of transformation of the parent compound (see AE 3.1 and AE 3.2), 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 non-common 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 do 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”

This AE is related to AE 3.1 where the formation of the common compounds is covered, which is of course dependent on the transformation rates and extent of transformation.

Under this scenario, the prediction for the property under consideration is claimed to be influenced alone by the common transformation compound(s) of source and target substances. However, there may be exposure to non-common compounds including:

- parent substances;

- non-common degradation products resulting from abiotic degradation/transformation (e.g. hydrolysis, dissociation, phototransformation); and

- non-common degradation products resulting from biotic degradation/transformation (e.g. biodegradation, biotransformation).

The non-common compounds resulting from degradation can be the final degradation/transformation products or can be intermediate products that will further degrade/biotransform. Which of these non-common compounds are relevant usually depends on the rate of formation/disappearance, their bioavailability, bioaccumulation potential and (potential) strength of effects.

A justification should provide an explanation why the (potential) bioaccumulation of the non-common compounds does not underestimate the predicted bioaccumulation potential of the whole target substance (a worst-case approach).

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.

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 bioavailability of chemicals. In these cases, 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 the category members may be considered when bioaccumulation of non-common compounds is being predicted. Generally, biotransformation of a substance leads to lower bioaccumulation potential as the transformation products are often 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 category members 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 non-common compounds 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 of non-common compounds 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. Whether the information on bioaccumulation potential of the non-common compounds may underestimate the hazards or not is assessed in AE 3.4.

AE 3.4 Impact of non-common compounds

Purpose

This assessment element addresses the impact of exposure to parent compounds, non-common compounds and (potential) degradation products of non-common compounds. Transformation of parent compounds may not be immediate and/or complete. As a result, exposure to the parent compounds, non-common compounds and/or (potential) degradation products of non-common compounds may be different between (some) source and target substances.

It has to be assessed whether:

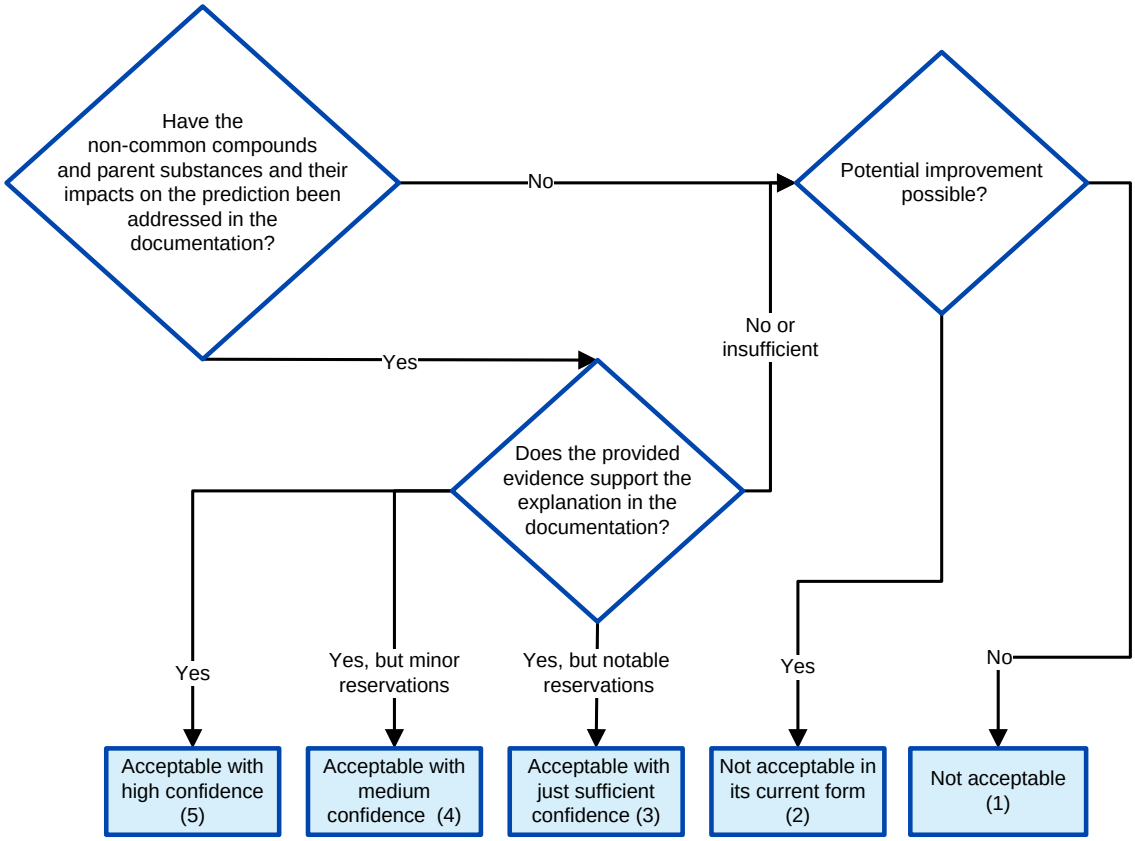
- the impact of the parent compounds on the environmental effect property under consideration has been addressed;
- the formation of non-common compounds (including possible intermediates) through the possible pathways and their potential impact on the predicted property under consideration have been considered, also taking into account the rate at which they are formed; 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

Possible influence of parent and non-common compounds

This AE is related to AE 3.1 where the formation of the common compounds is covered, which is of course dependent on the transformation rates and extent of transformation.

Under this scenario, the prediction for the property under consideration is claimed to be influenced alone by the common transformation compound(s) of source and target substances. However, there may be exposure to non-common compounds including:

- parent substances;
- non-common degradation products resulting from abiotic degradation/transformation (e.g. hydrolysis, dissociation, phototransformation); and
- non-common degradation products resulting from biotic degradation/transformation (e.g. biodegradation, biotransformation).

The non-common compounds resulting from degradation can be the final

degradation/transformation products or can be intermediate products that will further degrade/biotransform. Which of these non-common compounds are relevant usually depends on the rate of formation/disappearance, their bioavailability, bioaccumulation potential and (potential) ecotoxicity.

It should be explained whether exposure to non-common compounds may impact the prediction of environmental effects, using information assessed in previous AEs, such as AE 3.3 Bioaccumulation potential of non-common compounds.

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 effect is initiated. Assuming that the underlying mechanism of the non-common compounds is the same, differences in bioaccumulation may still cause differences in toxic potential simply by producing a difference in concentration of the substance at the target sites of toxic action. Therefore, if the bioaccumulation potential is higher for the non-common compounds of the target substance, this should be taken into account.

Under this scenario, non-common compounds should not impact the prediction of the property under consideration.

For non-common compounds and parent compounds, it should be shown that these do not significantly contribute to the toxicity. If a non-common compound or parent compound is known/predicted to have an effect, it should be negligible compared to the effect of the common compounds. If the non-common compounds or parent compounds have an impact on the read-across, it should be explained how this impacts the prediction.

Possible influence of common compounds formed at different rates

In this scenario a trend among category members is observed that is only caused by differences in the formation of common compounds. As common compounds are formed at different rates, the impact of these compounds may be different as well. It should be addressed what the impact of the rate of transformation to common compounds on the predicted property (environmental effects) is during the time course of the test.

Supporting evidence

Experimental and/or other evidence on the rate of formation and the non-common compounds formed is dealt with in AE 3.1 In the current assessment element, first it is assessed whether information on potential effects/absence of effects of the non-common compounds has been provided for all relevant degradation/transformation products of the source and target substances.

Whether non-common compounds are relevant or not is usually determined based on the rate of formation/disappearance, their bioavailability, bioaccumulation potential and (potential) ecotoxicity.

The information presented in the data matrix may provide indications for toxicity induced by the parent compounds or non-common transformation products. A trend in the effects studies for the category members is in line with common compounds being formed at different rates and triggering the effects. For this scenario it may be different to distinguish between a trend caused by the common compounds (formed in the studies at different

rates) or by the parent compounds (disappearing from the studies at different rates). The latter would indicate that the case does not fit the current scenario.

If non-common compounds (degradation/transformation products) trigger significant effects, a regular pattern of effects would be more unlikely.

The type of information needed to provide sound scientific explanations is case-specific. Reliable bioaccumulation data (ideally on the parent compounds and common compounds) are very valuable in this regard. Typically, experimental information will only be available for some parent compounds and/or the common compound. *In silico* studies (e.g. computational tools such as the OECD QSAR Toolbox) may increase the robustness of the case.

AE C.4 Impact of impurities on the prediction

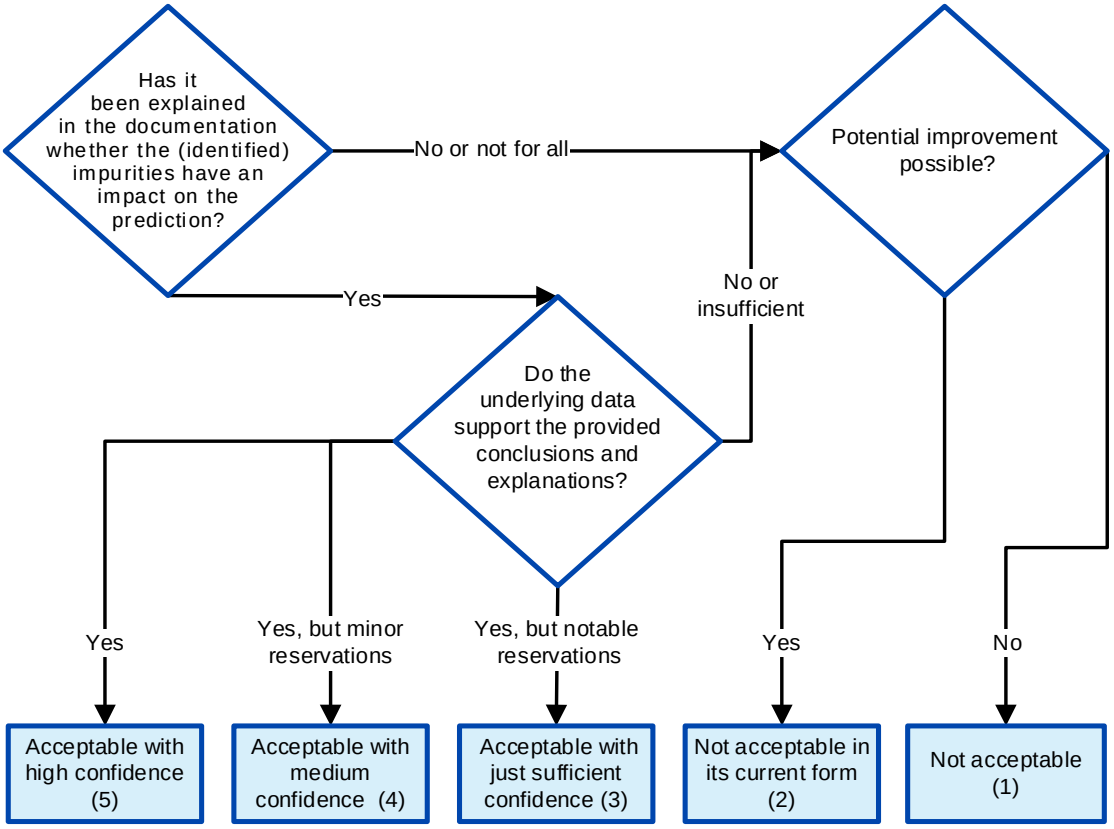
Purpose

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 C.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]

C.4.a Example of impurities not influencing the prediction

	Substance A	Substance B	Substance C	Substance D
Impurities	x (1-3 %) y (1-3 %)	x (1-3 %) y (1-3 %) **z (5-7 %)**	x (1-3 %) y (1-3 %)	x (1-3 %) y (1-3 %)

- Substances A, B, C, and D are structurally very similar.
- All substances are quickly hydrolysed to substance T.
- Exposure to substance T is claimed to dominate the acute fish toxicity of substances A to D in the same way.
- The substances A, B, C, and D 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 the other substances; 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 the hydrolysis product T 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.

AE C.5 Consistency of properties in the data matrix

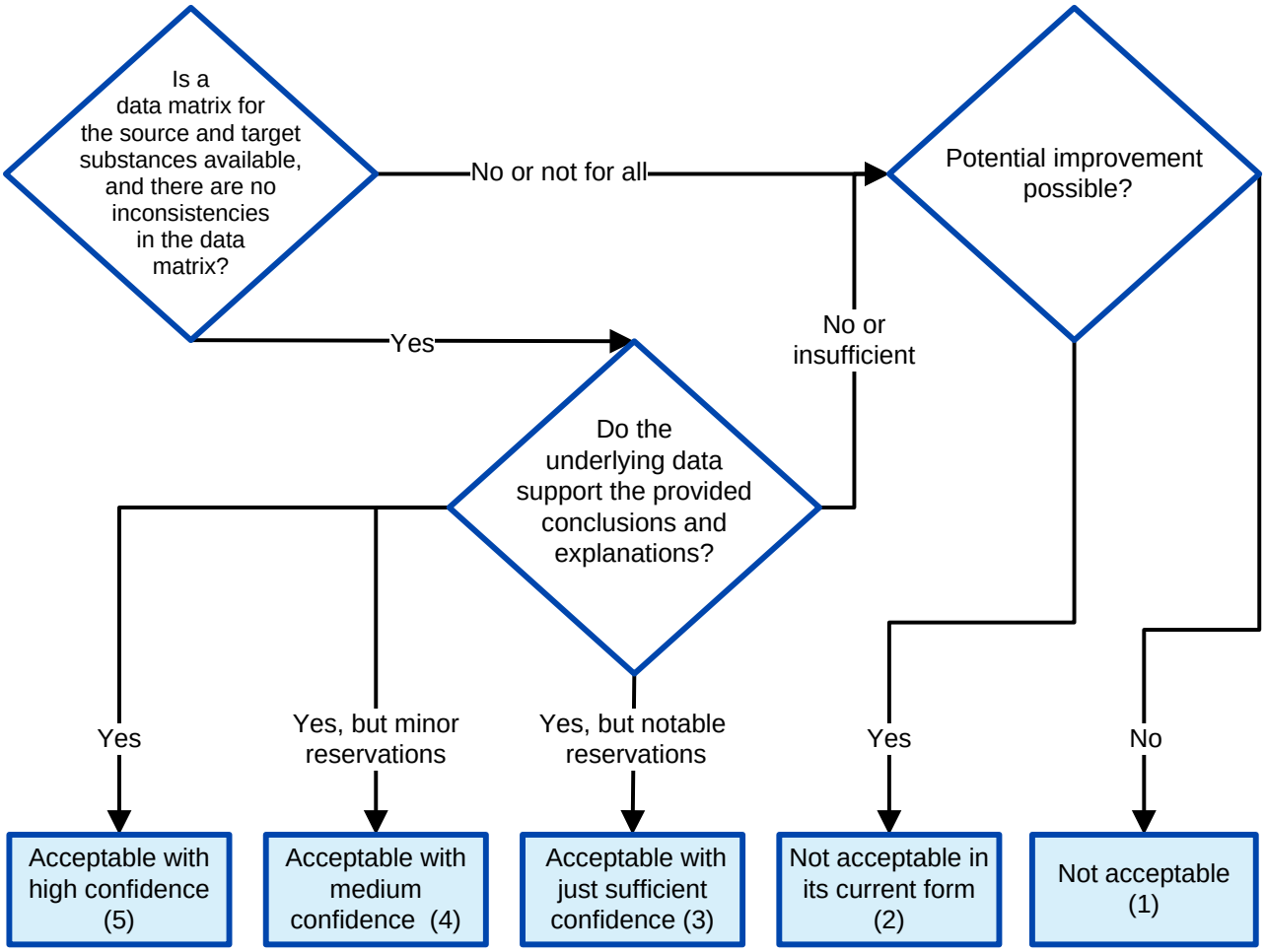
Purpose

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 category members and which identifies data gaps;
- the properties of category members 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 vary in a predictable manner and whether a basis for this regular pattern 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 category, per property for each category member;
- highlight potential regular patterns 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 there is a regular pattern for the property to be predicted. Depending on the hypothesis, the magnitude of the property is the

same (Scenario 5 or 6) or a regular pattern is observed if ordered according to the allowed structural differences or according to an independent variable as defined by the hypothesis (Scenarios 3 or 4). 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 category members 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.

Order within the category

For Scenarios 3 and 4, the independent variable (identified in the hypothesis to describe a regular pattern) determines the order within the category. Often it is a quantifiable structural property (*e.g.* the number of carbon atoms in an alkyl side chain). It could also be a physicochemical property which is directly related to the structural property (e.g. log Kow).

Whether or not a regular pattern is observed for the property under consideration may depend on which independent variable is chosen. Therefore, the choice of the independent variable should normally be justified.

For Scenarios 5 and 6, there may be no independent variable which is determining the prediction, since similar magnitude of properties are observed or predicted for all members of the category. However, a description of the category according to physicochemical properties (related to the structural properties) may still be valuable. There may be cases

where an order (according to a chosen variable) is very informative for checking the boundaries of the domain of the prediction.

Other supporting evidence provided in the technical dossier

Supporting evidence may refer to human toxicological evidence, toxicokinetic assessments, 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]

C.5.a Inconsistent information in the data matrix

- Substances A, B, C and D all hydrolyse to common compound Z. It is postulated that the toxicity is proportionate to Z, which is formed after hydrolysis. The information on other related properties (e.g. log Kow) reported in the data matrix presents an overall consistent quantitative pattern throughout the category.

	Substance A	Substance B	Substance C	Substance D
Hydrolysis half-life	1 h	4 h	7 h	15 h
Short-term toxicity on aquatic invertebrates, EC50	EC50 0.04 mmol/l (10 mg/l)	EC50 0.038 mmol/l (10 mg/l)	EC50 0.036 mmol/l (10 mg/l)	EC50 0.0035 mmol/l (1 mg/l)
Short-term toxicity on fish, LC50	LC50 0.004 mmol/l (1 mg/l)	LC50 0.0042 mmol/l (1 mg/l)	LC50 0.0036 mmol/l (1 mg/l)	Prediction by trend analysis based on hydrolysis rate

The toxicity is similar for substances A, B and C in short-term tests on aquatic invertebrates, but the toxicity is higher for substance D, despite that the common compound Z, which was hypothesised to induce the toxicity, is formed in a lower rate than from the substances A, B and C. Inconsistencies in the data matrix raise concern regarding the reliability of the predictions of short-term toxicity to fish for substance D and hypothesis that the common compound Z would cause alone the toxicity.

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

AE C.6 Reliability and adequacy of the source data

Purpose

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

It has to be assessed for each source study whether:

- the study design reported for the source study is adequate and reliable for the prediction[14] 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 has to be also assessed, whether:

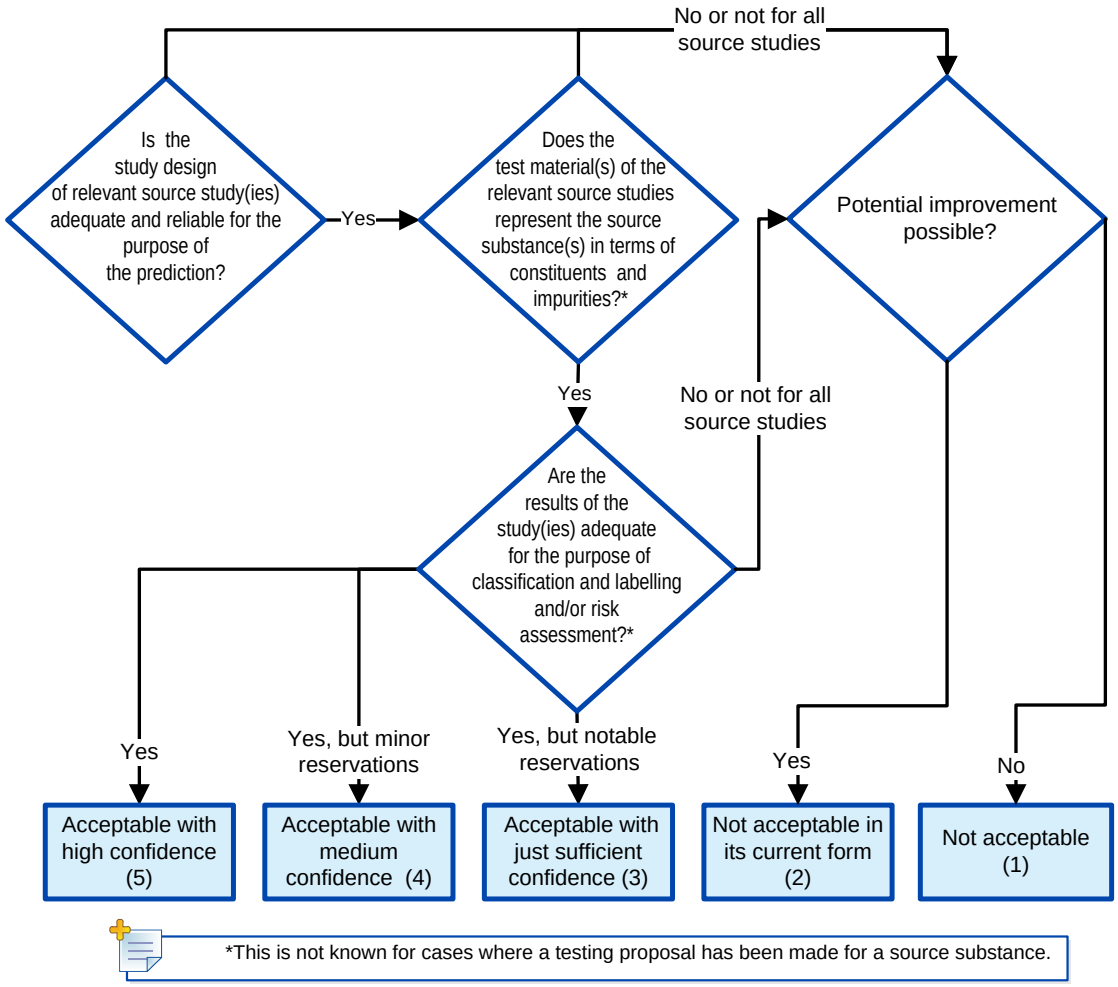
- the study results are adequate for classification and labelling and/or risk assessment. For example, this could include whether sufficient concentrations have been tested to enable the relevant determination of an effect concentration for a decision on classification and labelling, or whether a NOEC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

[14] For the further assessment, it should be noted how the prediction has been derived from the source studies for the property under consideration (one source study, several sources studies, prediction model for properties differing in value). These aspects are analysed in other AEs.

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.

Several source studies are used for the prediction

In the category approach, several source studies conducted with different source substances may be selected to predict the property under consideration. Therefore, all of these source studies have to be assessed with regard to the above-identified criteria. If one or several of the source studies fail to meet these criteria, it has to be assessed whether the overall weight-of-evidence provides sufficient coverage of the key parameters for the prediction.

The way several source studies are used to derive a prediction also has to be assessed. Possible ways would be a worst-case approach, averages (only when normal distribution).

Example(s) [15]

C.6.a Example for a source study not meeting the REACH 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.

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

C.6.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.

AE C.7 Bias that influences the prediction

Purpose

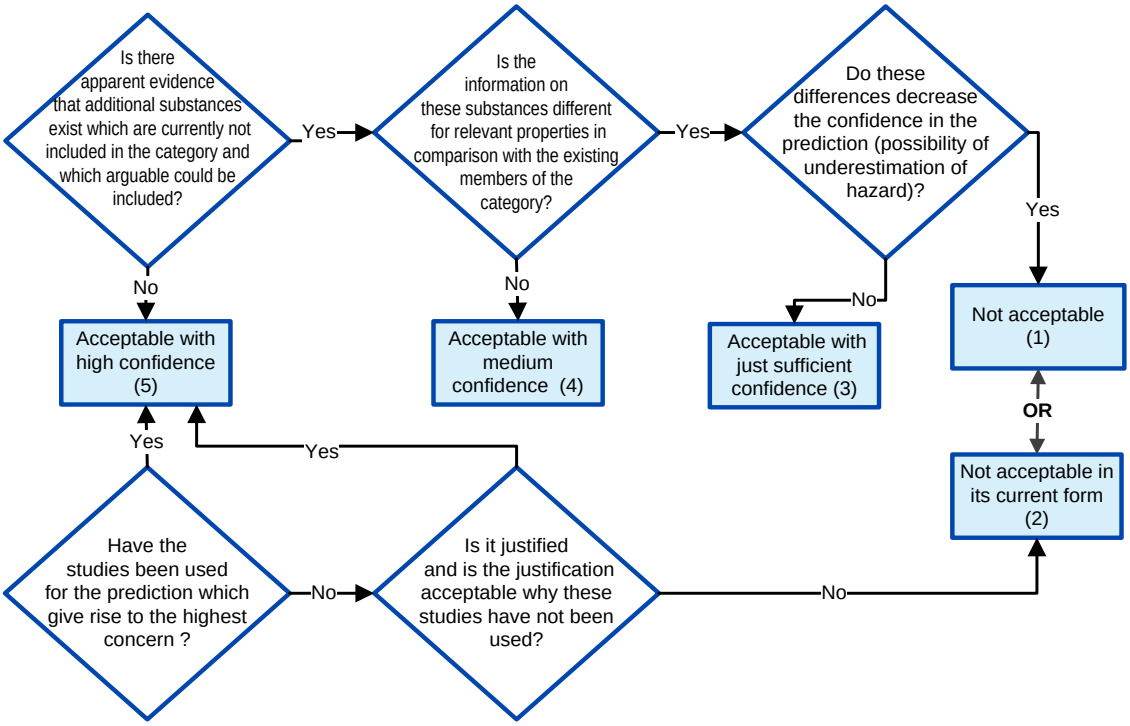
It has to be assessed whether:

- it is clear from the documentation how the category members have been chosen, for example, what methods/tools have been used to map the field of potential category members, which other substances have been considered and why they have been discarded;
- there are additional, structurally-similar substances which are currently not used in the category approach and which arguably could be included in the category;
- there is readily-available information from these additional substances;
- this information is significantly different for relevant properties in comparison with the existing members of the category;
- these differences decrease the confidence in the prediction (possibility of underestimation of hazard); and
- the prediction for the property under consideration is based either on interpolation of the closest source substances or is based on the worst-case source substance.

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. 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 category members of the category. Such a situation may occur:

- when there are additional substances with equivalent structural similarity which meet the category description; and
- when improper criteria in the category description have been used which reduce the category members to exclude certain (otherwise) suitable members and lead to biased selection of category members.

This situation may lead to a skewed estimation of effects for the properties under consideration. If consideration of all chemicals in the chemical space of the category leads to the conclusion that there is a difference in the prediction, with respect to the proposed prediction, with the possibility of underestimation of the hazard, the prediction may be considered 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.