Appendix F: Scenario 6

1.1 DESCRIPTION

This scenario covers the category approach for which the read-across hypothesis is based on different compounds which have the same type of effect(s). For the REACH information requirement under consideration, the effects obtained in studies conducted with different source substances are used to predict the effects that would be observed in a study with the target substance if it were to be conducted. The same type of effect(s), are observed for the different source substances; this may include absence of effects for every member of the category. No relevant differences in strengths of effect(s) are observed for several source substances.

1.2 ASSESSMENT ELEMENTS FOR SCENARIO 6

The assessment elements (AEs) for this scenario consist of six AEs common to the category-approach and five scenario-specific AEs which depend on the mechanistic explanation (Table F1).

Table F1: Assessment elements (AEs) for Scenario 6

AE #	AE TYPE	AE TITLE
AE C.1	Common	Substance characterisation
AE C.2	Common	Structural similarity and category hypothesis
AE C.3	Common	Link of structural similarities and structural differences with the proposed regular pattern
AE C.4	Common	Consistency of effects in the data matrix
AE C.5	Common	Reliability and adequacy of the source study(ies)
AE 6.1	Scenario-specific	Compounds the test organism is exposed to
AE 6.2	Scenario-specific	Common underlying mechanism, qualitative aspects
AE 6.3	Scenario-specific	Common underlying mechanism, quantitative aspects
AE 6.4	Scenario-specific	Exposure to other compounds than to those linked to the prediction
AE 6.5	Scenario-specific	Occurrence of other effects than covered by the hypothesis and justification
AE C.6	Common	Bias that influences the prediction

AE C.1 SUBSTANCE CHARACTERISATION

PURPOSE

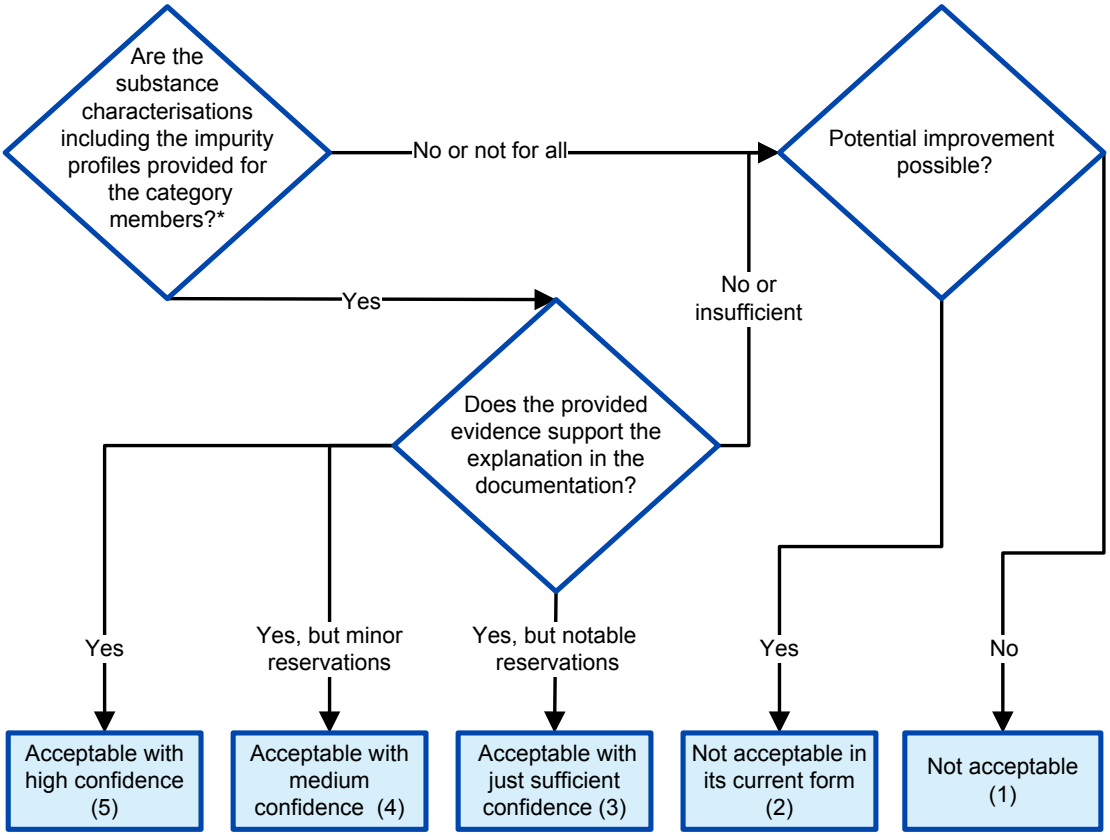
The substances which are members of the category need to have a clear substance characterisation[1].

It has to be assessed whether:

- the chemical identity of the category members is sufficiently clear for a meaningful assessment of 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.

ASSESSMENT OPTIONS



1 The test material actually used in a specific source study in AE C.5. The impact of impurities is addressed in AE 6.4.

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. This condition is usually met for the target substance, which is registered under REACH, since detailed information has to be provided on the identity, constituents and impurities of the registered substance.

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. It is 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. It is recommend in the ECHA guide "How to report on Read-Across" to follow the Guidance on identification and naming of substances under REACH (version 1.3, February 2014) for all category members, 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 (Guidance R.6.2.6.2):

- Name, CAS and/or EC number, chemical structure for the category members; and
- Impurities 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 toxicological properties. Whilst such changes are 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 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. The read-across hypothesis could be superficially convincing and could be supported by some data. Nevertheless, the read-across may still be invalid, because it does not take a difference in impurity profile of the source and target substances into account.

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."*

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.

EXAMPLE(S)[4]

C.1.a Example for an identity of a category member which is clear and unambiguous and allows for a meaningful read-across assessment

- A mono-constituent substance consists of 97.0-99.5% (typical 99.0%) substance A and 0.5-3.0% identified impurities (typical 1.0% water).

C.1.b Example for an identity of a category member which is clear and allows for a meaningful read-across assessment

- 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 well defined: i.e. Name, CAS and/or EC number, chemical structure and concentration ranges are available for all impurities.

In this case, the identity of the category member is clear and unambiguous for read-across purposes.

C.1.c Example for an identity of a category member which is unclear and does not allow for a meaningful read-across assessment for some predictions

- 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 and/or EC number, 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 of toxicological relevance.

In this case, the identity of the category member may not be clear for some predictions.

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

AE C.2 STRUCTURAL SIMILARITY AND STRUCTURAL DIFFERENCES WITHIN THE CATEGORY

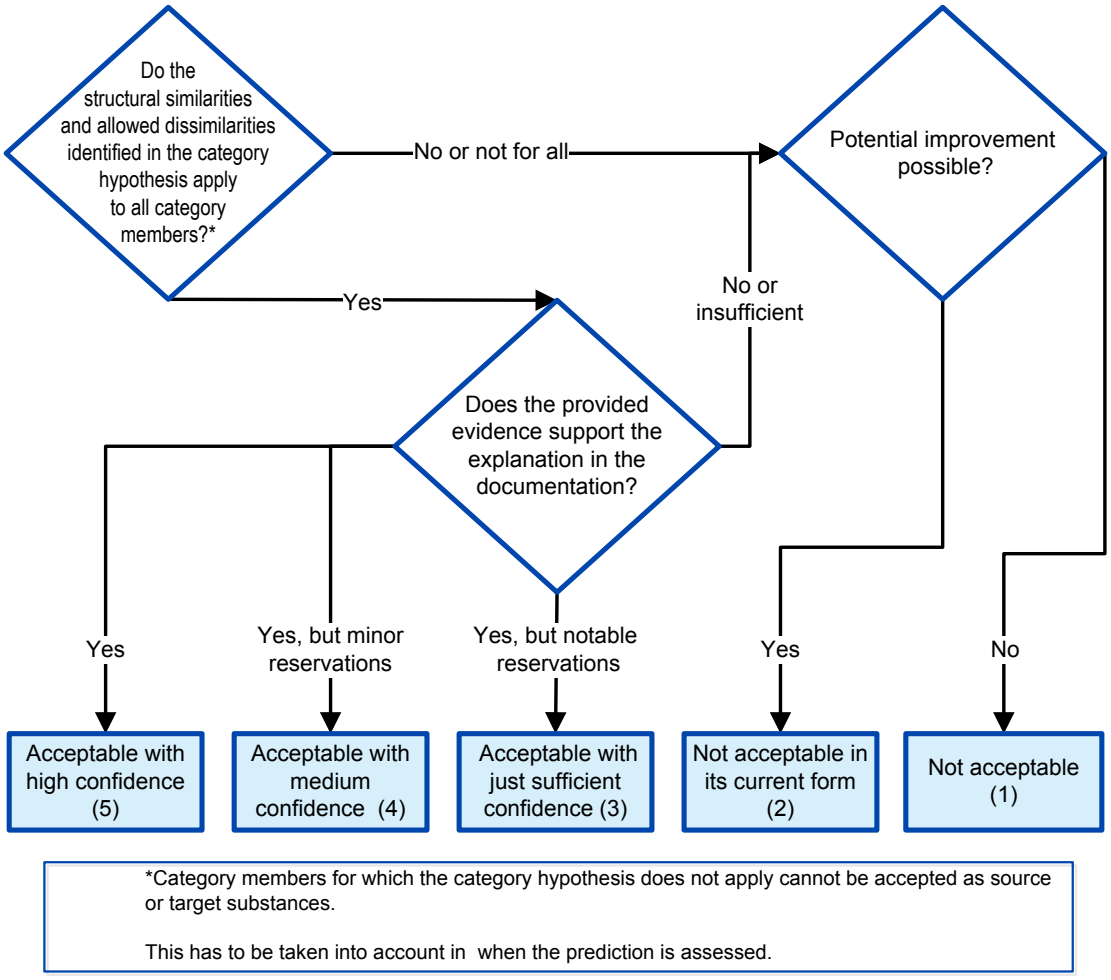
PURPOSE

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[5].

It has to be assessed:

- whether the structural similarities identified apply to all category members; and
- which structural differences are allowed within the category.

ASSESSMENT OPTIONS



5 The possibility of selection bias for category members is addressed in AE C.6.

EXPLANATION

It should be understood why the category is composed as it is. 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 (for instance, they all contain a mono-chloro phenyl moiety or they are all primary alcohols of alkanes);
- Which structural differences are allowed by the category hypothesis (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); and
- Whether there are other criteria used to reduce the number of the category members (such as physicochemical criteria, and data availability considerations).

It is recognised that knowledge of the chemical structures of the source and the target substances (see AE C.1) implicitly shows the common structural element and the allowed structural differences. However, the category definition should address the structural similarities and dissimilarities of a given group of substances, as it is the starting point for read-across. Depending on the prediction model used, the order within the category must be established based on the allowed structural (dis)similarities.

It should be emphasised that category members for which the category definition 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)[4]

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

- Substances A, B, C and D are alpha-olefins with a linear structure.
- The substances differ in the number of carbon-atoms in the chain (i.e. different chain lengths).
- No other differences exist.

The explanation has to address the difference in carbon-atom number.

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

- Substances A, B, C and D are alpha-olefins.
- Substances A, B and C have a linear structure whereas substance D is branched.
- Substances A, B, C and D differ in the number of carbon-atoms in the chain (i.e. different chain length).
- The branching of substance D is not covered in the category definition.

The explanation has to address the difference in carbon-atom number and also the branching of substance D.

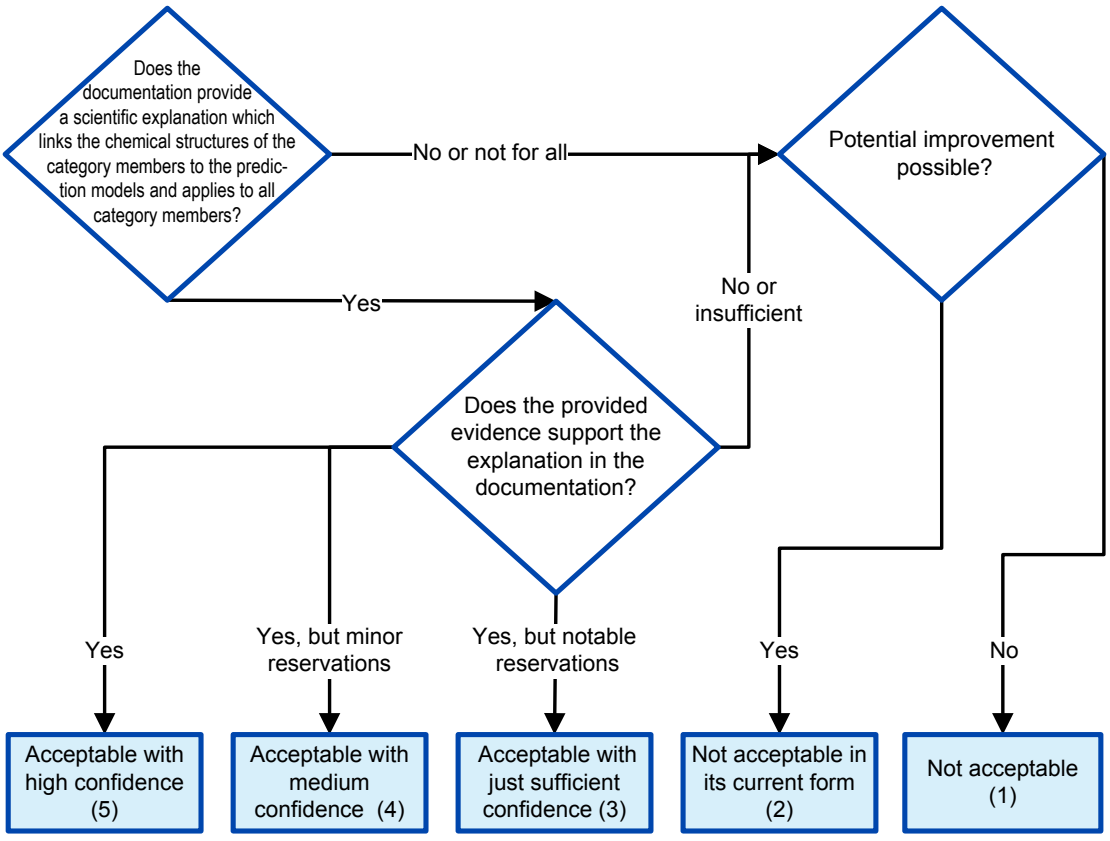
AE C.3 LINK OF STRUCTURAL SIMILARITIES AND STRUCTURAL DIFFERENCES WITH THE PROPOSED REGULAR PATTERN

PURPOSE

It has to be assessed:

- whether the documentation provides an explanation why 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 in increasing strength of effect due to kinetic differences);
- whether it is likely that all category members follow the proposed explanation and where the boundaries of the category are in this respect; and
- whether the provided evidence supports the explanation.

ASSESSMENT OPTIONS



*Category members which do not fall under the category justification (i.e. are outside the applicability domain) cannot be accepted as source or target substances.

This has to be taken into account when the prediction is assessed.

EXPLANATION

The scientific explanation why the category members should behave in a predictable manner is assessed. Such an explanation should apply to all the category members and form the boundaries of the category. There may be situations that the scientific explanation does not cover all category members or that this is not clear from the scientific explanation. A prediction cannot be based on the source substances which are not covered by scientific explanation. A prediction cannot be made for target substances that are not covered by the scientific explanation.

EXAMPLE(S)[4]

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 lengths C12, C14, C16 and C18 respectively.
- The category hypothesis only includes these esters (i.e. the borders of the category are formed by C12 and C18 esters) and 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).

C.3.b Example for an explanation not 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 lengths C12, C14, C16 and C18 respectively.
- The category hypothesis only includes these esters (i.e. the borders of the category are formed by C12 and C18 esters) and provides an underlying explanation why these substances are likely to behave similarly.
- Some predictions are made for members of the category using short chain esters (e.g. C4 acid with ethanol) with the justification that the data are not available for the long chain esters.

Prediction for the long chain esters based on studies conducted with a short chain ester cannot be used for prediction without further adequate explanation (i.e. in this case, short chain esters are outside the category boundaries and the predictive value is not clear).

C.3.c Example for an explanation applying to all category members (regular pattern)

- A category consists of metal salts for which the toxicity is governed by the metal ion, the inorganic anions are of no toxicological importance.
- The bioavailability of the metal ion in the category varies in a predictable manner and is claimed to be dependent on the water solubility at low pH.
- In vivo bioavailability studies are available for the most soluble salt, the least soluble salt and a salt with medium solubility, which confirms the hypothesis that the water solubility at low pH defines the bioavailability.

- The salt with the highest solubility (and the highest bioavailability) results in the strongest effect.
- The salt with the lowest solubility (and the lowest bioavailability) results in the lowest effect level.
- The salt with the medium solubility (and the medium bioavailability) has no data for the effect.

Prediction of the effect for this salt is proposed based on the relationship between solubility and toxicity.

AE C.4 CONSISTENCY OF EFFECTS IN THE DATA MATRIX

PURPOSE

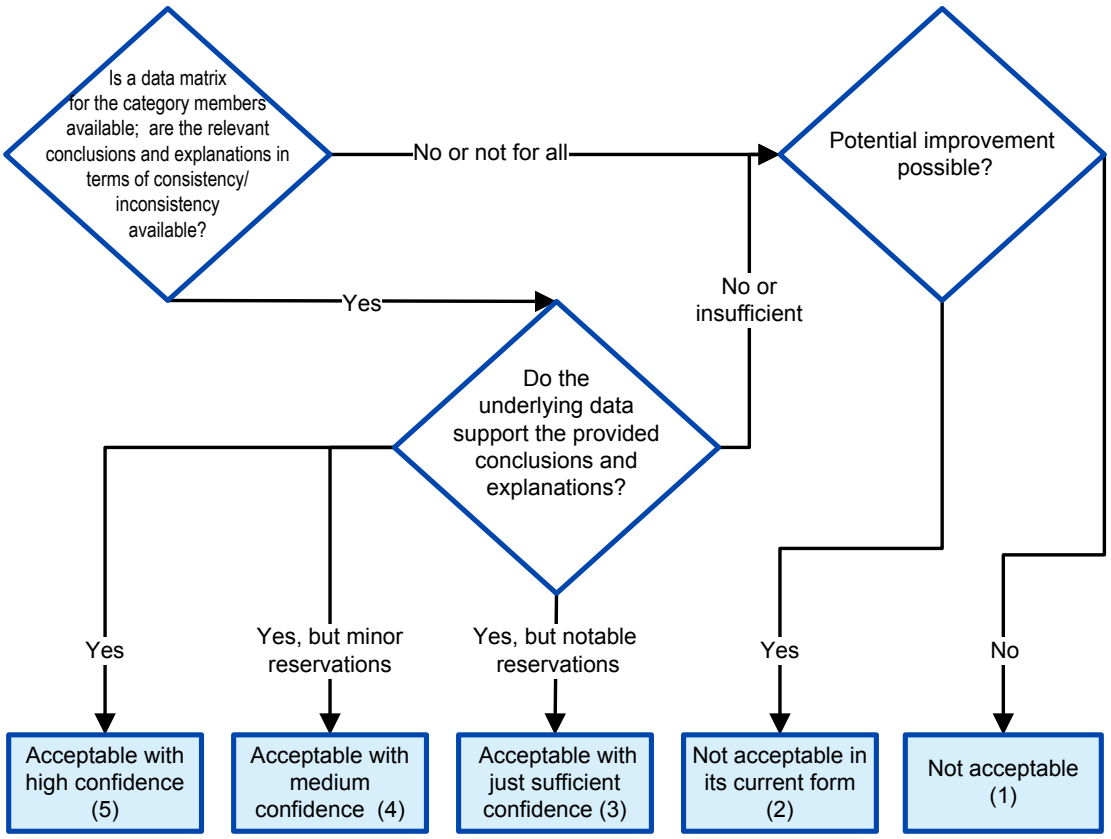
The category justification should include comparison of experimental data for the category members and a clear data matrix.

It has to be assessed:

- whether a data matrix has been provided which lists the category members in a suitable order versus their experimental data (e.g. for REACH information requirements) and which identifies data gaps;
- whether the properties of category members across the data matrix are consistent[6] in effects; 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 28-day and 90-day repeated-dose toxicity studies; reproductive toxicity screening tests and pre-natal developmental toxicity studies);
    - characteristics across all relevant properties (e.g. different reactivity towards genetic material may indicate different reactivity towards biological macromolecules which may influence the prediction for a 90-day study);
- the effects reported for the property under consideration differ in strength for the source substance and whether a basis for this difference is provided; and
- the underlying data support the provided conclusions and explanations.

6 "Consistency" means here, that the findings in studies reported in the data matrix support (or at least do not contradict) the prediction proposed.

ASSESSMENT OPTIONS



EXPLANATION

The data matrix should include a comparison of all available data within the category; per property for each category member; highlighting potential regular patterns within properties; and identifying data gaps.

Consistency of the effects in the matrix

There should be evidence from the data matrix that the effect to be predicted is consistently observed in the studies concerning this property among the category members. Depending on the hypothesis, the strength of observed effects is the same (scenarios 5 or 6) or a regular pattern is observed for the strength of effects, if ordered according to the allowed structural differences or according to an independent variable as defined by the hypothesis (scenarios 3 or 4).

Toxicological tests can reveal one well-defined effect (e.g. skin-irritation scores) or multiple, different effects (e.g. multiple, different biological targets identified in a 90-day repeated-dose toxicity study). In both cases, the results, of the study conducted with the source substance need to be used in the prediction of the target substance.

Occurrence of other effects

There are multi-parameter studies (e.g. a 90-day repeated-dose toxicity study) in which different effects can be observed that may or may not be mechanistically linked.

If several different effects are observed in the study to be read-across, it is typically the leading effect which will determine the hazard identification. However, other effects observed at the same or higher doses must be considered to determine the qualitative consistency of effects between studies conducted with different category members. Such effects might be observed in studies for the same property or for related properties. A careful analysis of the data matrix in this regard is required.

Effects in other (related) properties observed for members of the category may also be reported. Key issues to be considered are whether such effects occur for all category members or only for a few members. It also is important to determine whether such effects differ in their strength. Depending on the proposed read-across hypothesis this may indicate that the toxicological profiles of the category members differ and that there are different mechanisms acting. Thus, the prediction may not be valid.

No-effect levels based on different effects

The information given in a summary data matrix may not reflect the information given in the robust study summary (e.g. effects on which the NOAEL is based are not listed in the matrix, but if the robust study summaries are analysed, it is evident that different biological targets are observed for individual substances, which still lead to the same NOAELs). Numerical NOAEL values as such are not a suitable basis for establishing similarity of effect (see also previous paragraph). It is therefore 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.

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 must be justified.

For scenarios 5 and 6, there may be no independent variable which is determining the prediction, since the same type of effects and similar strength of effects 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 on the boundaries of the domain of the prediction.

Clustering of allowed structural differences over the range of effects (for the endpoint under consideration)

In the data matrix, clustering of the strength of an effect may be observed that is associated with some allowed structural differences among the category members. The nature of the clustering, and its relationship to the ability to predict the properties of the registered substance, must be scrutinised. For scenarios 3 and 4, where the read-across is based on trend analysis, clustering of the independent and/or the dependent variable may occur (the dependent variable is the property to be read-across). Clustering of these variables may seriously affect the reliability of the prediction; it may result in weaker trends and/or in artificial trends. Its occurrence and influence should therefore be carefully assessed. In scenarios 5

and 6, clustering may also influence the possibility to predict. Such clustering can demonstrate that the claim that the structural differences do not influence the property to be read-across is not valid and/or that it decreases the reliability of the prediction. So, for these scenarios, clustering also deserves careful assessment.

Sometimes clustering may facilitate a prediction. However, this depends among other things on the absolute and relative size of the clusters, the position of the cluster in the total range of data points, the range of the cluster and the support for the clustering by the mechanistic explanation for the read-across.

Relevance of inconsistencies for the prediction

Observed inconsistencies in the data matrix not in line with the proposed hypothesis do not in all cases have an impact on the prediction. However, they often reduce the confidence in the prediction.

EXAMPLE(S)[4]

Example C.4.a – Consistent effects in the data matrix

	SUBSTANCE A	SUBSTANCE B	SUBSTANCE C	SUBSTANCE D
OECD 407, rat, oral	Liver			Liver
OECD 422, rat, oral		Liver	Liver	
OECD 408, rat, oral	Liver	Annex VIII registration information not required	**Prediction?**	Liver

Example C.4.b – Inconsistent effects in the data matrix

	SUBSTANCE A	SUBSTANCE B	SUBSTANCE C	SUBSTANCE D
OECD 407, rat, oral	Liver			Liver, Heart
OECD 422, rat, oral		Heart (Liver)	Testis (Liver)	
OECD 408, rat, oral	Liver, Kidney	Annex VIII registration information not required	**Prediction?**	Liver, Heart

Example C.4.c - Unreliable prediction if based only on substance A or D; worst case may have been applied if substances A and D are the basis for the prediction; however, the grouping itself might be questionable

	SUBSTANCE A	SUBSTANCE B	SUBSTANCE C	SUBSTANCE D
OECD 422, rat, oral		liver hypertrophy	liver hypertrophy	liver hypertrophy, testicular atrophy
OECD 408, rat, oral	Liver hypertrophy, hepatic focal nodular hyperplasia	Annex VIII registration information not required	Prediction?	liver hypertrophy, testicular atrophy

Example C.4.d – A trend based on results in repeated-dose toxicity studies; in dependence of the decreasing formation of the common compound decreasing toxicity is observed. Observed effects are related to the same biological target

	SUBSTANCE A	SUBSTANCE B	SUBSTANCE C	SUBSTANCE D
OECD 407, rat oral	NOAEL 10 mg/kg/day LOAEL 50 mg/kg/day		NOAEL 30 mg/kg/day LOAEL 100 mg/kg/day	NOAEL 100 mg/kg/day LOAEL 300 mg/kg/day
OECD 408, rat, oral	NOAEL 3 mg/kg/day LOAEL 10 mg/kg/day	NOAEL 6 mg/kg/day LOAEL 15 mg/kg/day	**Prediction?**	NOAEL 20 mg/kg/day LOAEL 60 mg/kg/day
% hydrolysis to a common compound*	100%	50%	30%	10%

* based on differences in structural features

Using trend analysis, the NOAEL for substance C in a 90-day repeated-dose toxicity study is predicted based on this information to be 10 mg/kg/day.

AE C.5 RELIABILITY AND ADEQUACY OF THE SOURCE STUDY(IES)

PURPOSE

The source study(ies) needs to match the default REACH requirements for any key study in terms of adequacy and reliability.

It has to be assessed for each source study whether:

- The study design reported for the source study is adequate and reliable for the purpose of the prediction[7] 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);
    - There is adequate and reliable documentation of the applied test method, i.e. a robust study summary should be provided; and
- The test material used represents the source substance as described in the hypothesis in terms of purity and impurities.

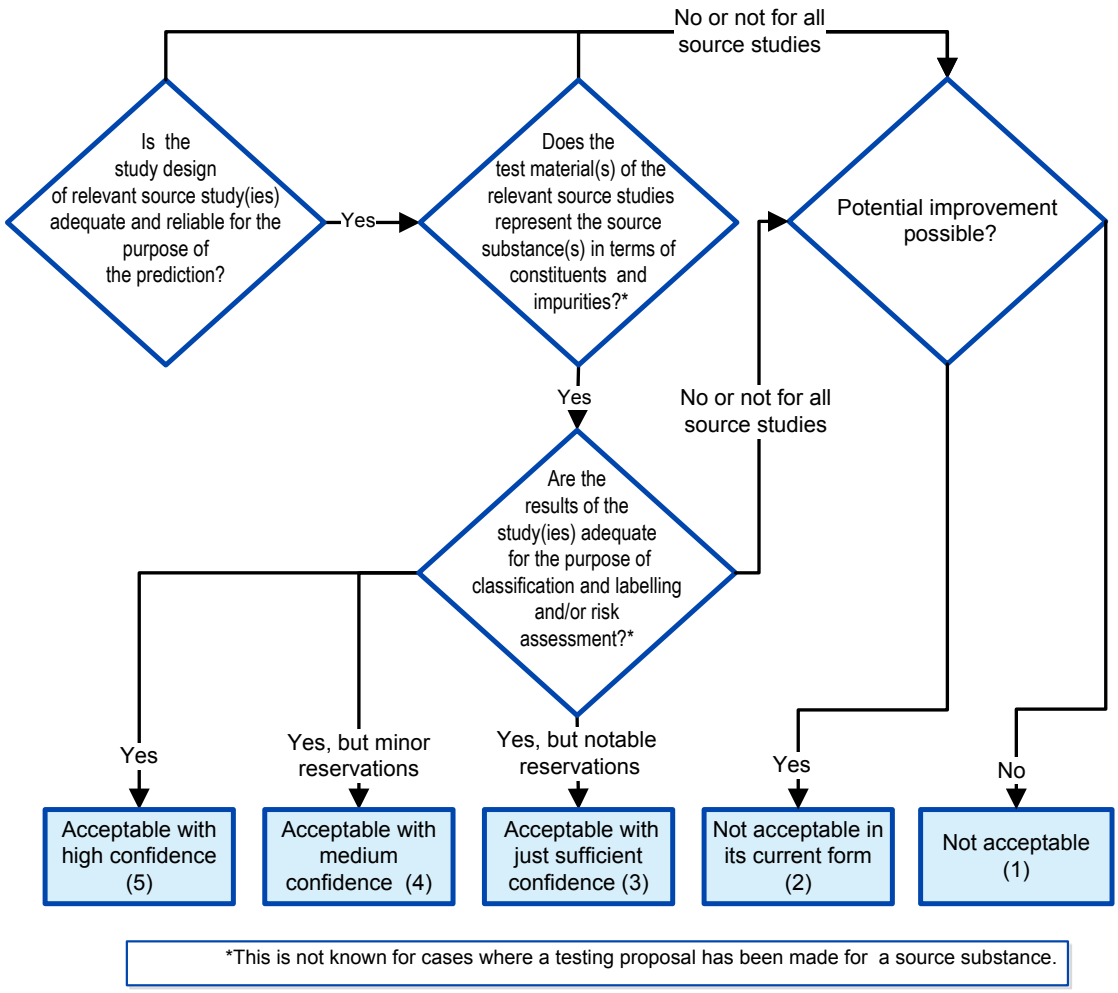
It has to be also assessed, whether:

- The study results are adequate for the purpose of classification and labelling and/or risk assessment. For example, this could include whether sufficient dose levels have been tested to enable the relevant determination of potency for a decision on classification and labelling, or whether a NOAEL/LOAEL has been identified from a study.

If all conditions listed above are met and the conclusions made are consistent with the reported results (e.g. clear identification of the critical effect(s), reliable NOAEL/LOAEL identification), it may be assumed that the study results are adequate for the purpose of classification and labelling and/or risk assessment.

7 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 effects differing in strength). 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.'*

These requirements are placed on the results of the read-across method. Therefore, 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.

Test substance versus source substance characterisation in the hypothesis

There should be no significant differences in the impurity profile for the test material in comparison with the source substance as covered in the hypothesis. If any such difference is identified, its impact on the prediction should be assessed.

Adequacy for C&L and risk assessment

If the source study is conducted with a test material representative of the source substance, and the study protocol is in accordance with the appropriate international guidelines and good laboratory practice (GLP),, sufficient dose levels have been tested to enable the relevant determination of potency for a decision on classification and labelling, and a reliable NOAEL/LOAEL has been identified from a study, the study results may be considered as adequate and reliable and can be used for risk assessment and/or C&L purposes. If the study has been conducted according to other methods the deviations need to be evaluated. The Klimisch scores (see below) used by the registrant in the endpoint study record may be helpful for this evaluation, if the assessor is able to verify the Klimisch classification of the registrant. A detailed reporting according to the criteria of a robust study summary is needed to assess the characteristics of the source study.

1 = reliable without restrictions: "studies or data [...] generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline [...] or in which all parameters described are closely related/comparable to a guideline method."

2 = reliable with restrictions: "studies or data [...] (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable."

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.

EXAMPLE(S)[4]

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

- The source substance was tested in a reproductive toxicity screening test according to OECD 421.
- This study is used to predict the results of a pre-natal developmental toxicity study according to OECD 414 for the target substance to meet the Annex IX requirement of a pre-natal developmental toxicity.

The key parameters of the source study are not appropriate to meet the information requirements of Annex IX, section 8.7.2. The source study is not adequate for the purpose of the intended prediction (but see last paragraph above).

C.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 pre-natal developmental toxicity study according to OECD 414 is proposed to be used to predict the pre-natal developmental 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 6.1 COMPOUNDS THE TEST ORGANISM IS EXPOSED TO

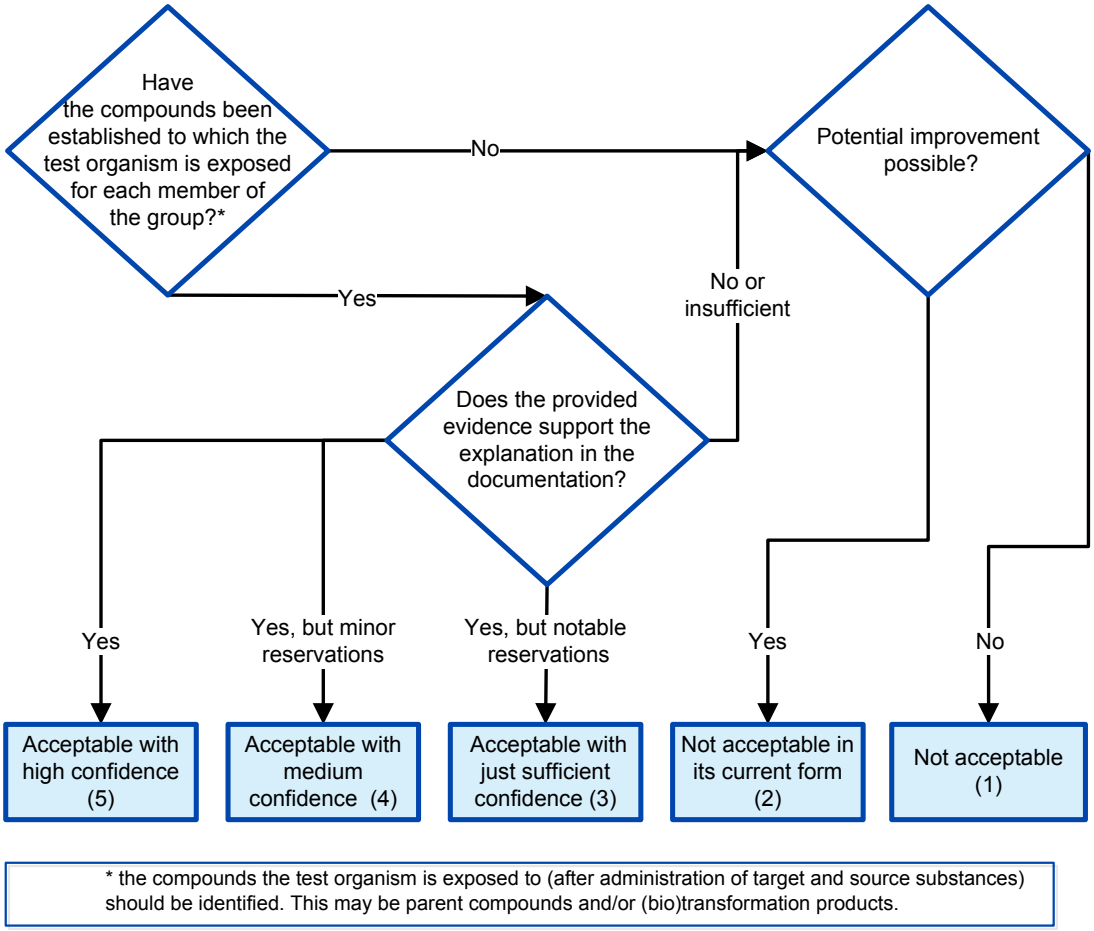
PURPOSE

In this scenario, the read-across hypothesis claims that the same effects are caused by different compounds (i.e. the source and target substances themselves and/or their (bio)transformation products).

It has to be assessed whether:

- the compounds to which the test organism is exposed (after administration of the source and the target substances) have been established in the documentation; and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



EXPLANATION

Under this scenario, it is claimed that the prediction for the property under consideration is possible because different compounds have the same effect. To assess such a claim it has to be clear to which compounds the test organism is exposed to. The validity of the read-across thus depends on the insight in the actual compounds to which the test organism is exposed.

In principle, these compounds can be:

- Target and source substance themselves;
- Source substance and (bio) transformation product(s) of the target substance;
- Target substance and (bio)transformation(s) product of the source substance;
- Target substance and source substance and their (bio) transformation(s) products; or
- (Bio) transformation(s) products of the source and target substance.

It therefore has to be assessed whether the compounds the test organism is exposed to (after administration of the source and the target substances) have been established and thus form the basis for the prediction for the property under consideration.

A special case is the prediction of absence of effects based on no exposure. It may be claimed that the test organism is not exposed to substances that cause an observable effect for the property under consideration in the source study (e.g. due to a lack of absorption). Absence of effects is then predicted also for the target substance. In such a case, it has to be clearly demonstrated that the test organisms are not exposed to the source substance and that the same applies for the target substance.

Supporting information should be presented for the presence of substances claimed to influence the prediction. Qualitative/quantitative kinetic information is valuable in this regard.

If some compounds to which the organism is exposed have not clearly been established for one or more category members, it has to be assessed whether this has any impact on the prediction. The prediction may not be affected if the source study(ies) has been conducted with category member(s) where the explanation is available and supported, and it is also available for the target substance. If the target substance is not covered by the hypothesis, prediction is not possible.

EXAMPLE(S)[4]

6.1.a Example for the presence of source and target substance only in the test organism

- Substances A, B, C and D are absorbed, not (bio)tranformed and eliminated unchanged in the urine.

In this situation, the test organism is exposed to substance A, B, C or D after administration of A, B, C or D and the prediction only has to take into account the presence of these substances.

6.1.b Example for the presence of source and target substances and their (bio)transformation products

- Substance A is absorbed and metabolised to A1 and A2.

- Substances B, C and D are absorbed, not (bio)tranformed and eliminated unchanged in the urine.

In this situation, the test organisms are exposed to A, A1 and A2 after administration of A, and only to B, C or D after administration of B, C or D. A prediction for the target substance A from a source study conducted with B, C or D needs to take into account the additional presence of A1 and A2.

AE 6.2 COMMON UNDERLYING MECHANISM, QUALITATIVE ASPECTS

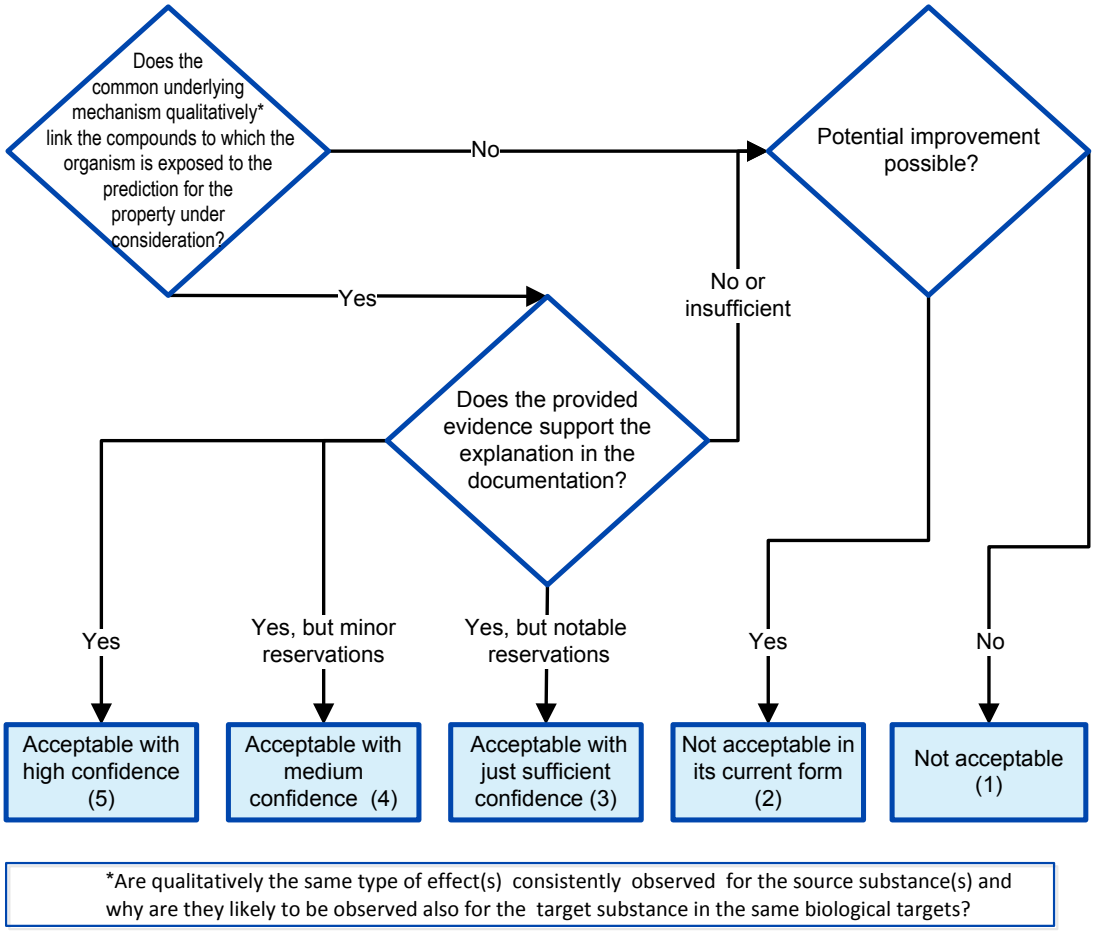
PURPOSE

The category hypothesis/justification has to be explained how the compounds the test organism is exposed to lead to the same or similar effects/absence of effects.

It has to be assessed whether:

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

ASSESSMENT OPTIONS



EXPLANATION

The underlying mechanism linking the compounds present in the organism to the prediction needs to be established. It needs to be a common mechanism causing the effects. 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.

The hypothesis/justification should explain why the effects observed with the category members are the result of a common underlying toxicological mechanism and that the same mechanism will determine the effect for the target substance for the considered property.

In vitro, in chemico and in silico studies (e.g. computational tools such as Derek, Meteor or OECD toolbox) may increase the robustness of a case, but usually are not sufficient as stand-alone information. Qualitative information obtained from in vivo or in vitro studies on the proposed mechanism is valuable.

Prediction of absence of effect

Specific considerations are needed in the case of 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 two basic explanations:

1. Absence of exposure due to lack of bioavailability. Kinetic information is needed to demonstrate absence of uptake or distribution. The supporting information (e.g. data matrix) must not contradict such a claim.

2. Uptake occurs, but no effects are observed in the source study. There are two theoretical possible reasons to predict the same absence of effects for the target:

    a) Significant exposure of target tissues expected/proven, but no relevant toxicity predicted. This prediction can only be based on predicted insignificant interaction with biological targets. There needs to be supporting evidence proving such insignificant interaction in general terms for the property under consideration.

    b) Low or no significant exposure of target tissues due to metabolism/ distribution (including barriers, e.g. placenta)/ elimination. A prediction of no relevant toxicity could be based:

        i. on predicted lacking/low exposure. Kinetic information is needed to support this prediction;

        ii. insignificant interaction with biological targets in combination with predicted lacking/low exposure. Kinetic information is needed to support this prediction and supporting evidence proving such insignificant interaction in general terms for the property under consideration.

EXAMPLE(S)[4]

6.2.a. Examples of a common underlying mechanism for the source and target substances

- Substances A, B, C and D are absorbed, not (bio)transformed and eliminated unchanged in the urine.
- Substance A is known to be an antagonist of a receptor Z, and the structural basis for this receptor interaction is known.
- Substances B, C and D have similar structural features compared to A, and have in vitro evidence that they act via the same receptor as A.

Substances A, B, C and D are expected to induce the same qualitative effects via this receptor interaction.

6.2.b Example of a common underlying mechanism for metabolites of source and target substances

- Substances A, B, C and D all contain double carbon-bonds and are metabolised to epoxides.
- The epoxide which is formed from substance A binds to DNA and also causes genotoxicity in a gene mutation assay.
- The epoxides formed from substances B, C and D have a similar chemical reactivity based on theoretical chemical considerations.

Results from the gene mutation assay conducted with A are used to predict the results for substance B, C and D.

6.2.c Example of a common underlying mechanism explaining the absence of effects

- Substances A and B have a high molecular weight and are very water soluble.
- Substances C and D have molecular weights falling between those of A and B and are also very water soluble.
- It has been demonstrated by an oral toxicokinetic study with radioactive labelling with A and B that they are poorly absorbed.
- Substances C and D have a very similar structure and in vitro information indicates that oral absorption is not expected.

Results obtained in 90-day oral repeated-dose toxicity studies with A and B (no effects observed) are used to predict absence of effects in the same study type conducted with substance C and D.

AE 6.3 COMMON UNDERLYING MECHANISM, QUANTITATIVE ASPECTS

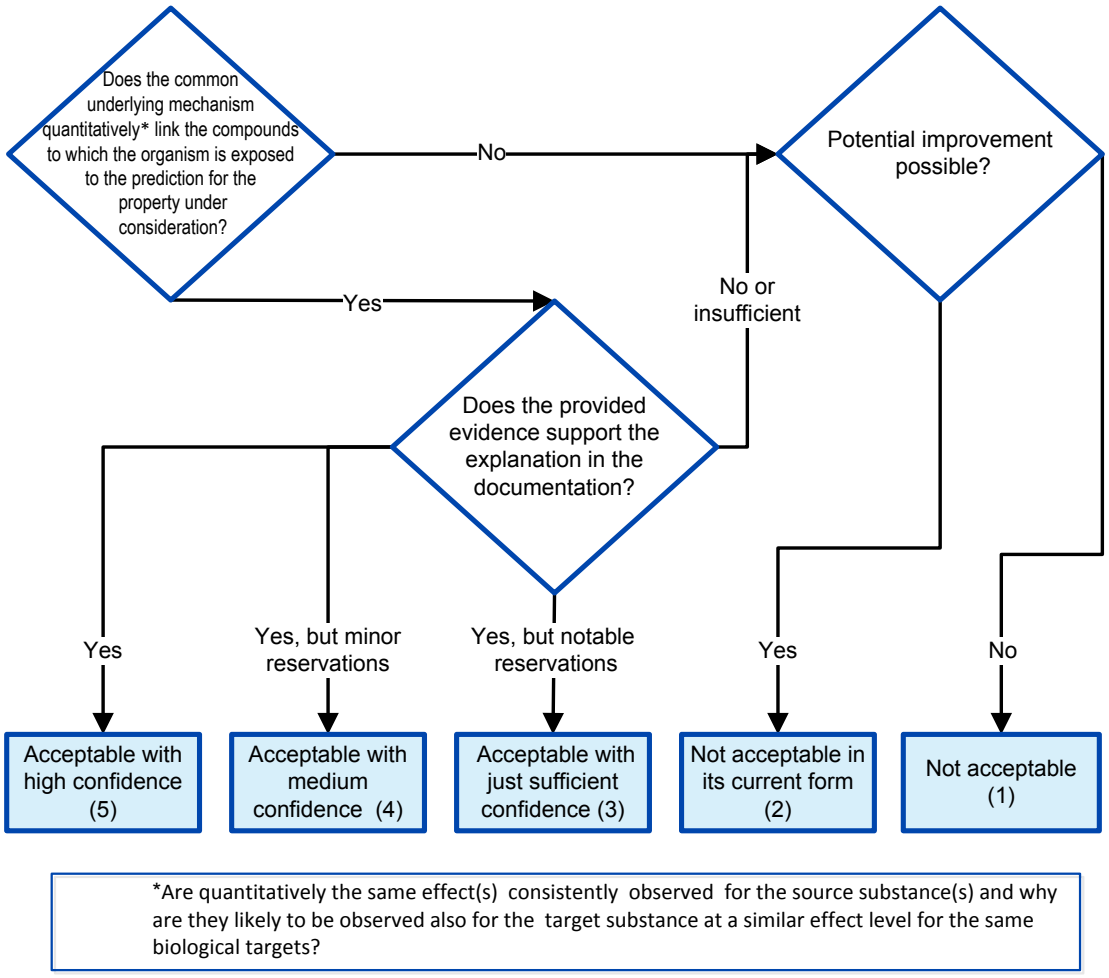
PURPOSE

Under this scenario, there should be no biologically significant[8] quantitative differences for the effects caused by the underlying mechanism. If there are slight differences observed in several source studies, a conservative approach should be followed in the prediction.

It has to be assessed whether:

- The documentation has provided an explanation why a common underlying mechanism leads to the same quantitative outcome (for the source and target substances) with regard to the prediction of the property under consideration; and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



8 here are allowable differences which are likely to be caused by statistical variations. These are not regarded as significant for this AE.

EXPLANATION

Quantitative differences

Under this scenario, there should be no biologically significant[9] quantitative differences for the effects caused by the underlying mechanism. If there are slight differences observed in several source studies, a conservative approach should be followed in the prediction.

There might be cases, where the same effects via the same common mechanism are triggered, but due to potency differences or kinetic differences there are quantitative differences in the effect. These circumstances are covered in scenario 4.

In principle, quantitative differences can have the following origins:

- Differences in the exposure (e.g. based on differences in absorption, distribution, metabolism, and excretion (ADME)); and/or
- Differences in the potency.

Worst case

Sometimes a worst-case approach is claimed (i.e. one 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 some source substances have biologically significant different effect levels. The analysis of the proposed prediction would then be handled under scenario 2 or 4 depending on the case.

However, there are cases where kinetic considerations lead to the conclusion that the exposure of the biological targets is higher for the source substance than for the target substances. Still no effects are observed for this source substance. This situation is proposed to be a worst case for the prediction of no effects for the targets. For the kinetic aspects, this can indeed be considered as a worst case, but the structural differences might still be more important with regard to the prediction. This situation can be assessed under scenario 6, since there is no difference in effects predicted. But this exemplifies that specific attention has to be focused on the assessment of worst-case approaches.

Supporting evidence

Case-specific supporting evidence may consist of information on the kinetics of uptake, metabolism, distribution and excretion of the source and target substances, if applicable. In vitro, in chemico and in silico studies (e.g. computational tools as Derek, Meteor and OECD toolbox) may increase the robustness of the case, but are not usually sufficient as stand-alone information.

Predictions of absence of effects

In cases where absence of effects is predicted, it has no meaning to speak of quantitative differences between the source substances, since according to the hypothesis no effects are observed in studies with the source substances and no effects are predicted to be observed in the same study type with the target substance. Still it is important to address other possible quantitative differences between the category

9 Differences addressed here are meant to be biologically relevant. Statistical analysis of data in studies might also lead to quantitative differences observed when different study results are compared. Such differences have to be in the range of the normal statistical variation typically observed in the study type under consideration.

substances by carefully analysing the information in the data matrix.

If the prediction of absence of effects is justified by absence of exposure (biological targets are not reached), the significance of possible small quantitative differences in exposure (e.g. ADME) between the source and target substances may 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. Since scenario 6 analyses structurally different compounds claimed to result in the same effects such an explanation needs to take into account the knowledge on the biological targets. For complex higher tier studies (e.g. a pre-natal developmental toxicity study), with multitudes of possible biological targets, which also change during the development, this is challenging.

See also general considerations covered in AE 6.2.

EXAMPLE(S)[4]

6.3.a Example for prediction of a similar effects, presence of absorption, consistent data matrix information and common mechanistic explanation

- Substances A, B, C and D are absorbed at similar rates and extent.
- Substances A, B and D are agonists of a receptor that is driving the toxicity expressed by increased incidence of histopathological findings in the liver in 28-day repeated-dose toxicity studies. The data matrix is consistent with respect to the effect levels.
- All substances are tested in vitro and show similar potency towards the receptor.
- The data matrix supports that the liver toxicity is the leading effect for hazard identification and the overall pattern is consistent quantitatively throughout the data matrix.

The studies with A, B and D are used to predict the outcome of the 28-day repeated-dose toxicity study for substance C.

6.3.b Example for prediction of absence of effects, presence of absorption, consistent data matrix information and common mechanistic explanation

- Substances A, B, C and D are all fatty acids in the range of C16 to C22.
- All of them are absorbed and A, B and D each has been tested in a 28-day repeated-dose toxicity study at the limit dose of 1 000 mg/kg/day. Observed effects are interpreted as non-adverse.
- The mechanistic explanation is using the facts known about fatty acid absorption and metabolism supported by in vitro information obtained with the registered substances.

The studies with A, B and D are used to predict the absence of effects for substance C.

6.3.c Examples of a common underlying mechanism for the source and target substances, quantitative differences in other studies, but not relevant for the prediction

- Substances A, B, C and D are absorbed, not (bio)transformed and eliminated unchanged in the urine.
- Substances A, B and D are known to be antagonists of a receptor, and the structural basis for this action is known.
- Substance C has the same structural features, and has in vitro evidence that it acts via the same receptor with a similar potency.
- Substance A has a five-times higher absorption rate and consequently also a higher absorbed amount compared to B, C and D.
- Substances A, B, C and D have been tested in 28-day repeated-dose toxicity studies. Substance A has a lower effect level (100 mg/kg/day) compared to B, C and D (300, 380, 350 mg/kg/day respectively).
- Substances A, B and D have also been tested in pre-natal developmental toxicity studies showing no effects for the pups, but evidence of maternal toxicity in line with the 28-day repeated-dose toxicity study results, in particular for A.

The pre-natal developmental toxicity study result for substance C is proposed to be predicted based on the source studies with A, B and D predicting no effects for the pups.

6.3.d Example of a common underlying mechanism for metabolites of source and target substances, kinetic differences have a quantitative impact on the prediction

- Substances A, B, C and D all contain a double C-bond and are metabolised to epoxides.
- The epoxides formed from substances A, B, C and D bind to DNA and cause gene mutation in bacterial cells.
- Substance A did not cause gene mutation in an in vivo gene mutation assay.
- The epoxide formed from substance B, C and D have a similar chemical reactivity based on theoretical chemical considerations.
- It is known from in vitro experiments that S9 mix metabolises substances A, B, C and D at similar rates to the corresponding epoxides.
- However, the further detoxification of A-epoxide occurs immediately after formation of the epoxide and is tenfold faster compared with B, C and D, resulting in lower epoxide concentration derived from A in the liver and circulating in the organism.

The hypothesis is, that the in vivo result obtained with substance A can be used to predict the same in vivo result for substance B. Due to the metabolic differences, the hazard of B, C and D may be under-predicted.

AE 6.4 EXPOSURE TO OTHER COMPOUNDS THAN TO THOSE LINKED TO THE PREDICTION

PURPOSE

Other compounds than those linked in the hypothesis to the prediction may be formed via other (bio) transformation pathways or may be intermediates/metabolites of the identified pathway.

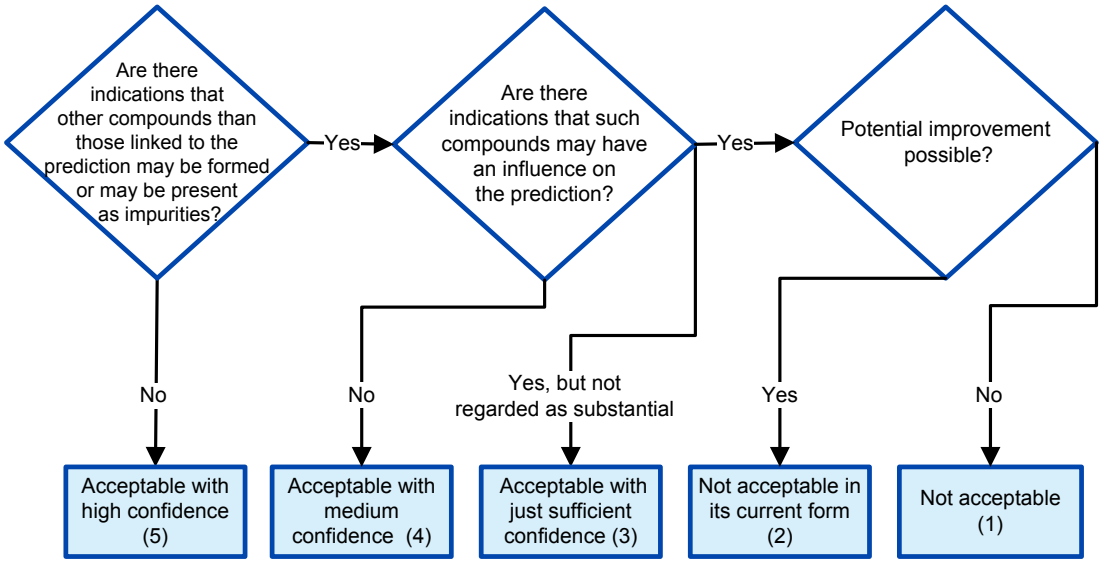
In addition, the impurity profiles[10] associated with the source and target substances may have an impact on the prediction.

The other compounds may have been identified by the hypothesis, but not linked to the prediction. Another possibility is that the occurrence of such compounds has been identified by the assessing expert.

It has to be assessed whether:

- other compounds than those linked to the prediction may be present (parent substance including impurities, also see AE C.1), or formed (e.g. via other (bio)transformation pathways or as intermediates); and
- indications are available that such compounds could influence the prediction of the property under consideration.

ASSESSMENT OPTIONS



10 See substance characterisation, as addressed in AE C.1 for the source substance or registration dossier for the target substance.

EXPLANATION

In AE 6.1, the hypothesis has been assessed qualitatively with regard to compounds the organism is exposed to. This AE investigates whether indeed all compounds possibly influencing the prediction have been addressed. It is supposed to provide a check on the robustness of hypothesis with regard to the influence of other compounds the test organism may be exposed to.

The confidence in the prediction may be decreased if such compounds are either present as parent substances (including impurities) or formed by (bio)transformation from the source and/or target substance and have not been considered by the hypothesis. In addition to the information assessed in AE 6.1, this AE requires an insight into the possible toxicological properties of possible other compounds. For the acceptance of the read-across approach, the other compounds should not influence the considered property. The lack of influence on the predicted property may be due to insignificant exposure of the biological target(s) or absence of relevant interaction with biological targets. The strength of the proposed mechanistic explanation and the associated evidence in the data matrix must be balanced against the uncertainties from un-characterised toxicity of other compounds than those linked in the hypothesis to the prediction.

For the prediction of absence of effects, the results obtained for the source substances indicate that no other compounds that can influence the considered property are present. However, it still needs to be addressed by the scientific explanation that this is also the case for the target.

Importance of impurities

Toxicity of category member(s) may actually be determined by an impurity. The read-across hypothesis could be superficially convincing and could have some supporting data. Nevertheless, the read-across may still be invalid, because it does not take a difference in impurity profile into account.

EXAMPLE(S)[4]

6.4.a Example for a compound which has been identified in the hypothesis but its impact on the prediction has not been addressed

- A category has been established consisting of substances A, B, C and D.
- The category members B, C and D are absorbed and eliminated unchanged.
- According to the hypothesis substance A is absorbed and rapidly metabolised to A1 and A2.
- The hypothesis is that the toxicity of A is caused by A1 and the toxicity is similar to B, C and D, which are structurally similar to A1.
- The results of a 90-day repeated-dose toxicity study conducted with substance A are used to predict the effects in 90-day repeated-dose toxicity studies for the substances B, C and D.
- There is toxicokinetic information available to the assessing expert which indicates that A2 is formed via a pathway competing with the formation of A1 and thereby reducing the systemic concentration of A1 upon exposure to A compared to the situation considered in the hypothesis (no competing pathway).

This situation may lead to under-prediction of the toxicity of B, C and D at equivalent doses of A. An additional question is whether A2-toxicity itself also influences the prediction (see next examples).

6.4.b Example for a compound not identified and thereby not addressed by the hypothesis

- According to the hypothesis, substance A is absorbed and rapidly metabolised to A1.
- The hypothesis is that the toxicity of A is caused by A1 and the toxicity is similar to B.
- The structurally-similar substance B is absorbed and assumed to be eliminated unchanged.
- The results of a 90-day repeated-dose toxicity study conducted with substance A is used to predict the effects in a 90-day repeated-dose toxicity study with substance B.
- There is toxicokinetic information available to the assessing expert that a metabolite B1 is formed from B.
- It is known that B1 causes a different tissue toxicity as caused by A1.

The study conducted with A cannot be used to predict the results of a study conducted with B.

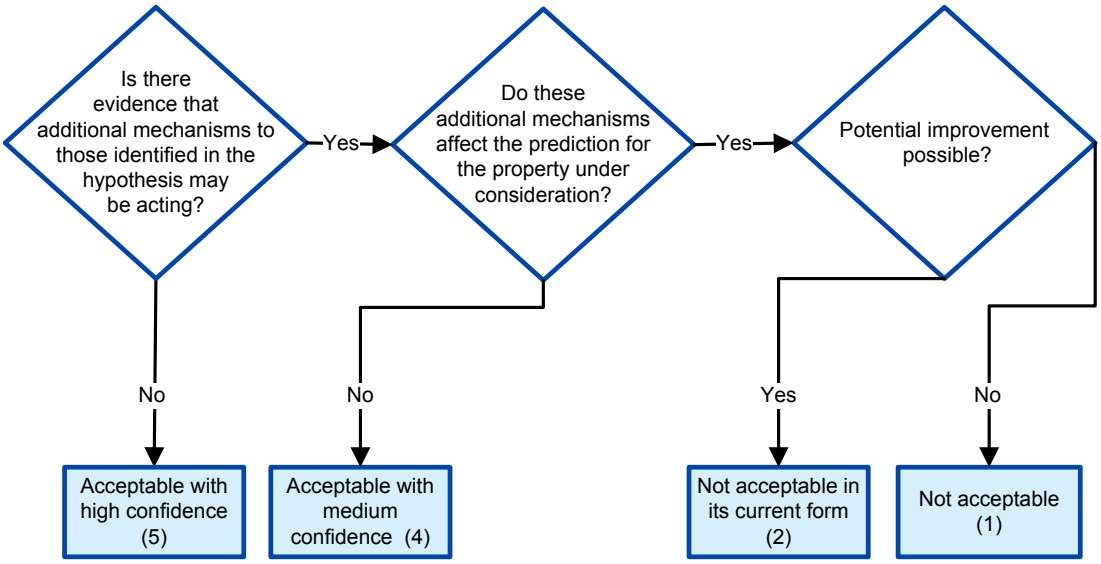
AE 6.5 OCCURRENCE OF OTHER EFFECTS THAN COVERED BY THE HYPOTHESIS AND JUSTIFICATION

PURPOSE

It has to be assessed whether:

- additional mechanisms than those identified in the hypothesis may be acting:
    - on the basis of mechanistic insights; or
    - derived from information in the data matrix; and
- these additional mechanisms affect the prediction for the property under consideration.

ASSESSMENT OPTIONS



EXPLANATION

There is the possibility that additional mechanisms than those identified in the hypothesis are acting, could cause toxic effects and are not covered by the prediction. In some cases, mechanistic insights (e.g. known receptor interaction, or known specific interactions with biological targets) may lead to the postulation of such other effects.

In the data matrix, quantitative and qualitative evaluation of the effects which have been reported may be indicative of such additional mechanisms. Effects other than those linked to the hypothesis have to be evaluated on a case-specific basis. Occurrence of such other effects may be not relevant if, for example, they are observed at clearly higher dose levels than the effects associated with the common underlying mechanism. The strength of the proposed mechanistic explanation and the associated evidence in the data matrix must be balanced against the uncertainties arising from possible other mechanisms and/or any

inconsistencies in the data matrix.

When the absence of effects is predicted, observation of effects in related studies conducted with the target substance invalidates the prediction if no further explanations are provided.

EXAMPLE(S)[4]

6.5.a Example for the absence of indications for other mechanisms

- Substances A, B, C and D cause neurotoxicity observed in 28-day studies which included functional observational batteries, no other effects were observed.
- In available pre-natal developmental toxicity studies for substances A and D, neurotoxic effects were noted and no other effects were observed in maternal animals.
- 90-day repeated-dose toxicity studies conducted with substances B and D are used to predict the toxicity of substances A and C in a 90-day repeated-dose toxicity study.
- The chemical structures of the substances do not indicate that other mechanisms are acting (no expert concern and no alerts in (Q)SAR analysis).

Based on this data set, it is considered in the explanation that other mechanisms are unlikely to influence the prediction.

6.5.b Example for indications of other mechanisms

- Substances A, C and D cause neurotoxicity observed in a 28-day repeated-dose toxicity studies which included functional observational batteries, no other effects have been observed.
- Substance B caused dose-dependent neurotoxic effects similar to A, C and D in a 28-day repeated-dose toxicity study. At the highest dose in this study, decrease of relative thymus weight was observed. This finding was accompanied with a decrease of the white blood cell count.
- A 90-day repeated-dose toxicity study conducted with substances A and D is used to predict the toxicity of substance B in a 90-day repeated-dose toxicity study.
- The chemical structures of the substances do not indicate that other mechanisms are acting (no structural alerts in (Q)SAR analysis).
- However, the mechanism of the thymus weight decrease is not known but it cannot be excluded that in studies with longer duration than 28 days, immunotoxicity may appear at lower dose levels

Based on the specific facts of the case, there may or may not be a concern for an additional mechanism. This may require further explanation for the thymus toxicity/ white blood cell effects.

AE C.6 BIAS THAT INFLUENCES THE PREDICTION

PURPOSE

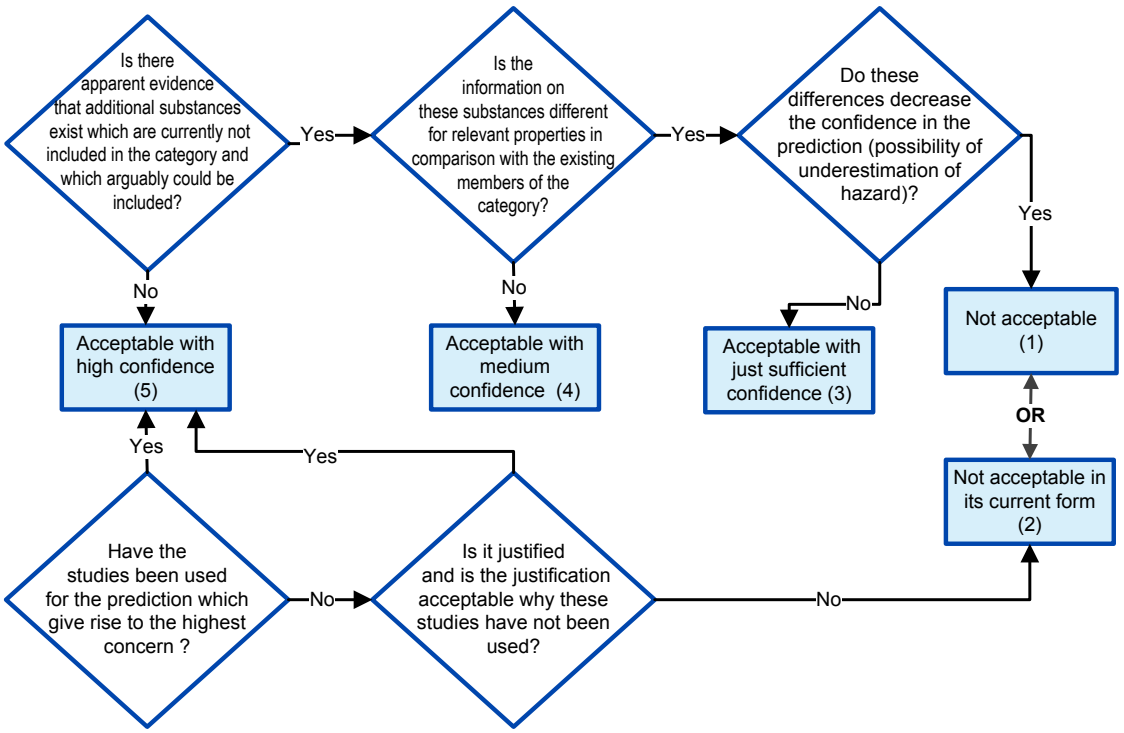
It has to be assessed whether:

- 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 biologically significantly different for relevant properties in comparison with the existing members of the category; 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. 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 definition; and

- when improper criteria in the category definition 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 Annex I, section 1.1.4, normally the study giving rise to the highest concern shall be used to establish derived no-effect levels (DNELs). If such a study is not used, this shall be fully justified. This applies to the selection of key studies for predictions based on read-across.