Appendix C: Scenario 3

1.1 DESCRIPTION

This scenario covers the category approach for which the read-across hypothesis is based on (bio) transformation to common compound(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 some members of the category. There are differences in strength of the effect(s) 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 strengths of effects are observed/predicted.

1.2 ASSESSMENT ELEMENTS FOR SCENARIO 3

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 C1).

Table C1: Assessment elements (AEs) for Scenario 3

AE #	AE TYPE	AE TYPE
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 3.1	Scenario-specific	Formation of common (identical) compound(s)
AE 3.2	Scenario-specific	The biological targets for the common compound(s)
AE 3.3	Scenario-specific	Exposure of the biological target(s) to the common compound(s)
AE 3.4	Scenario-specific	The impact of parent compounds
AE 3.5	Scenario-specific	Formation and impact of non-common compounds
AE C.6	Common	Bias that influences the prediction

AE C.1 SUBSTANCE CHARACTERISATION

PURPOSE

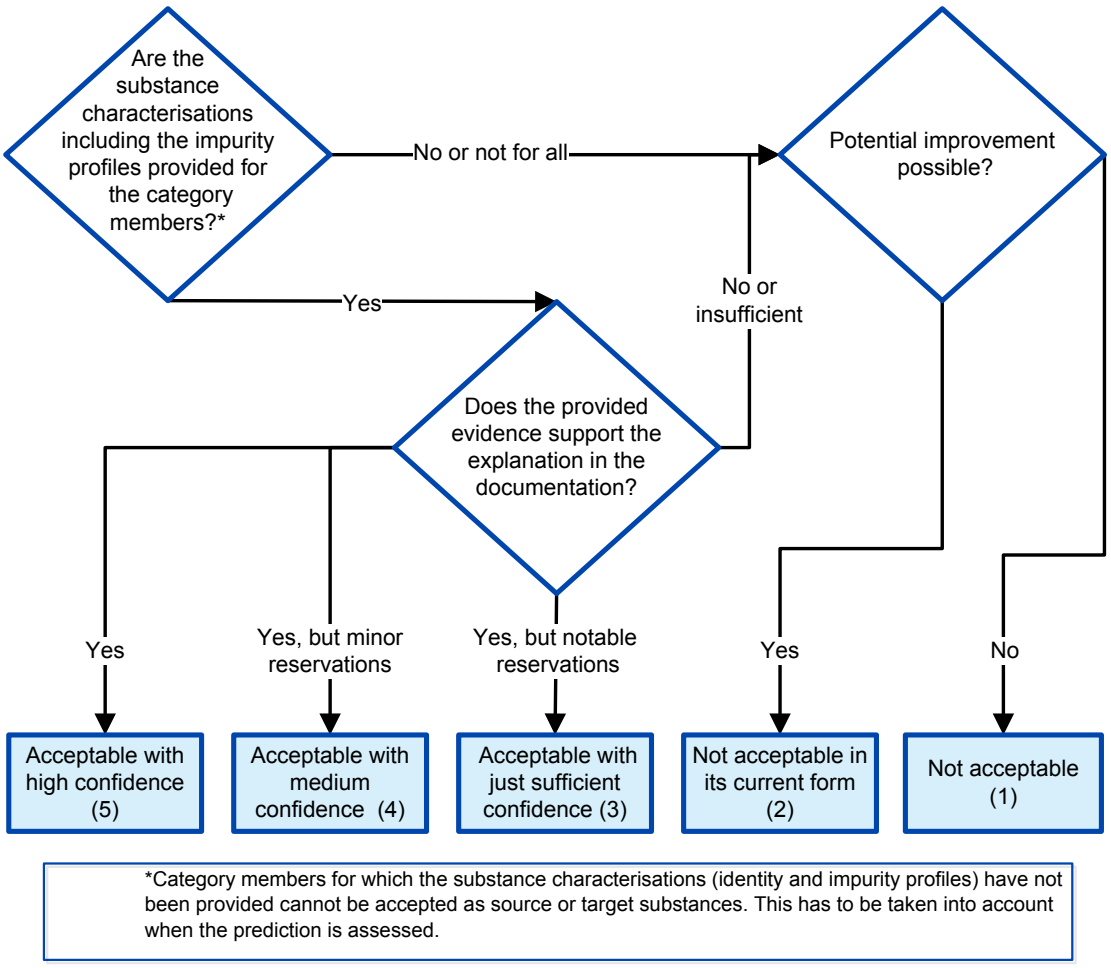
The substances which are members of the category need to have a clear substance characterisation[1].

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 is addressed in the AE C.5. The impact of impurities on the prediction is addressed in AE 3.4.

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 recommended 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 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. 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

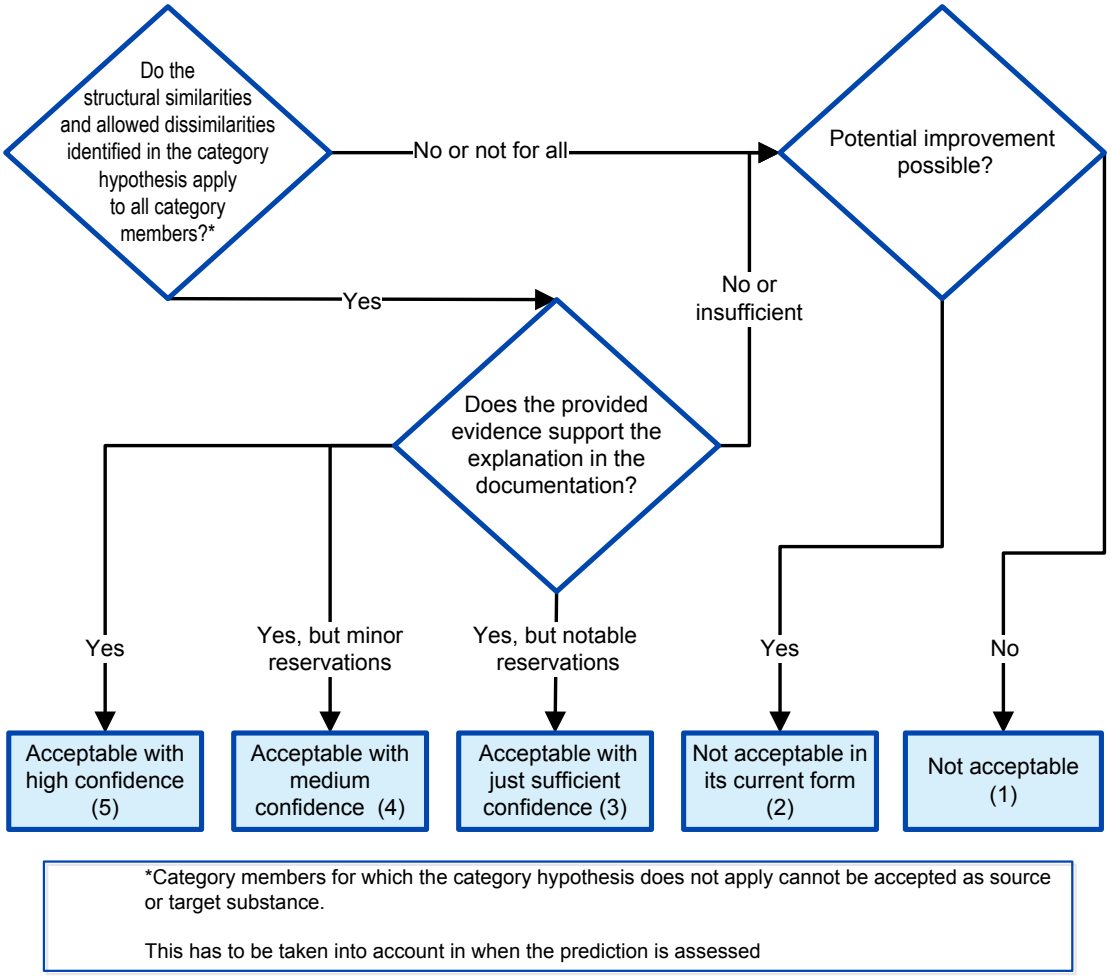
PURPOSE

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 3.6.

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 category members (such as physic-chemical criteria, 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 length).
- 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.
- The 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.

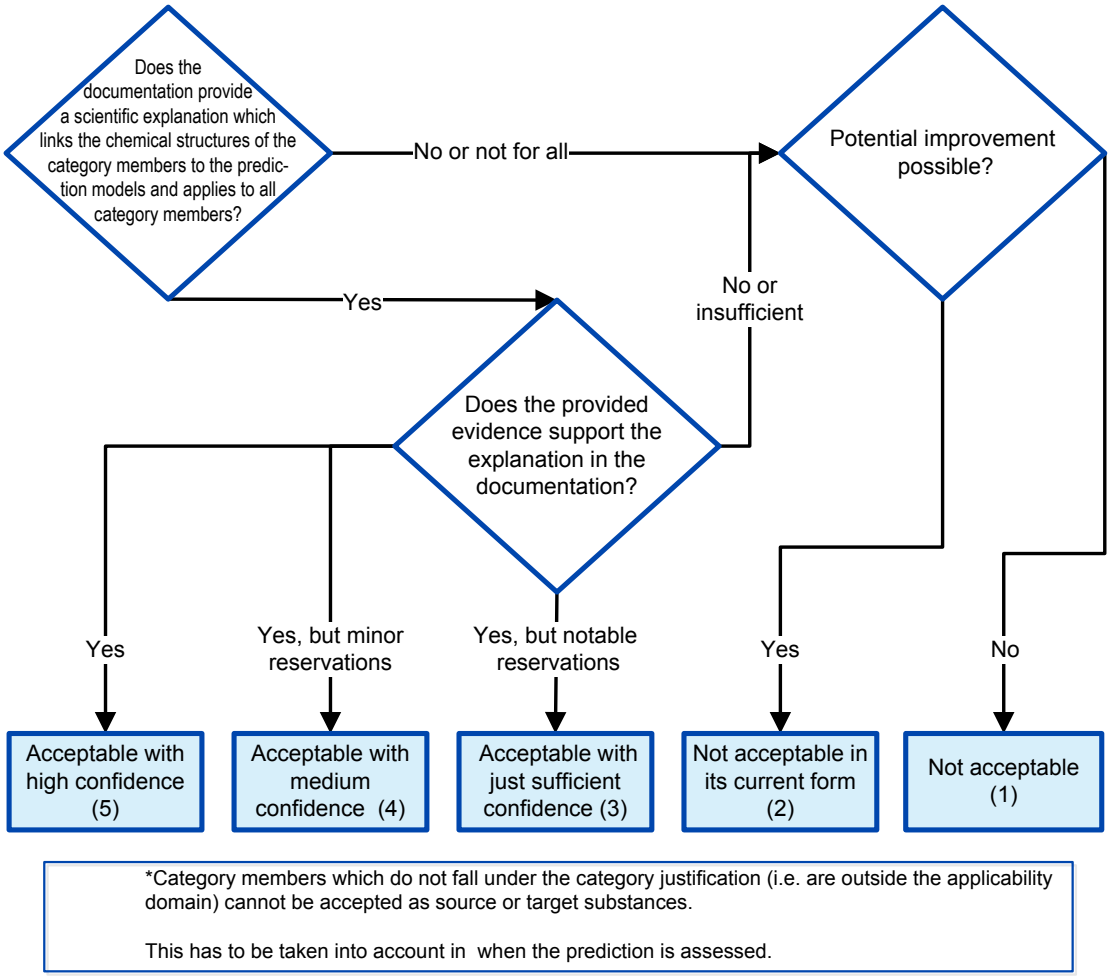
AE C.3 LINK OF STRUCTURAL SIMILARITIES AND STRUCTURAL DIFFERENCES WITH THE PROPOSED REGULAR PATTERN

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



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 length 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 length 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 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 (e.g. C4 acid with ethanol) 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

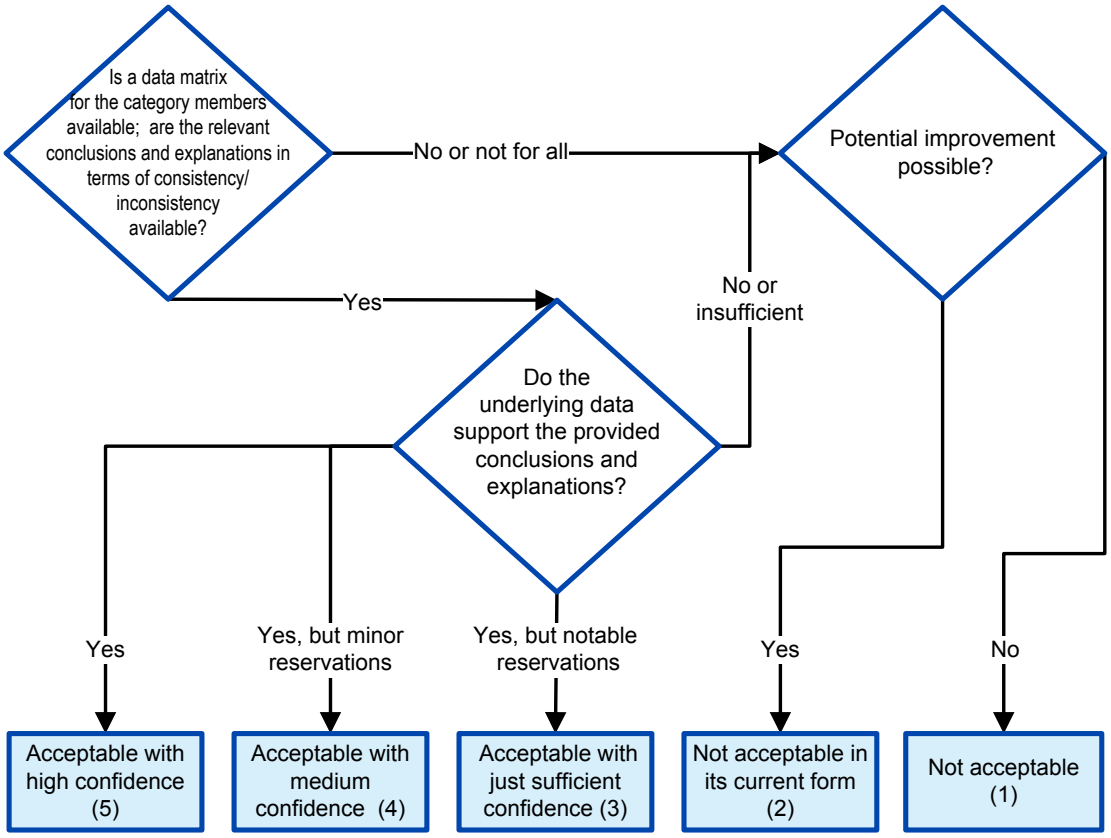
The category justification should include comparison of experimental data for the category members and a clear data matrix.

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 repeated-dose toxicity 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 (scenario 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 (scenario 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 is also 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. Also in scenarios 5 and 6, clustering may 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, also for these scenarios clustering 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	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 422 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); 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.

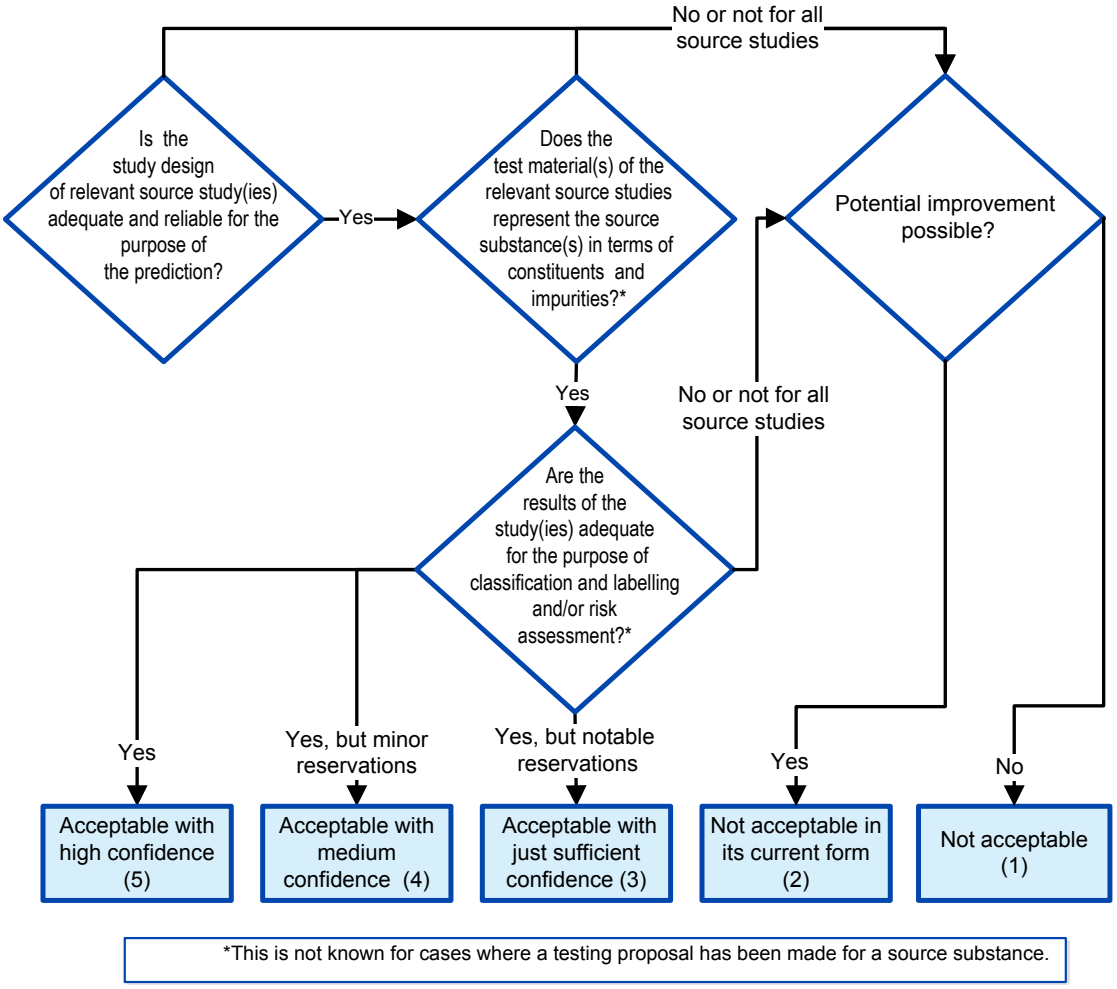
It has to be also assessed, whether:

- The study results are adequate for 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.

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), or the mode of the distribution of the source effects is applied to the target as well.

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 the 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 3.1 FORMATION OF COMMON (IDENTICAL) COMPOUND(S)

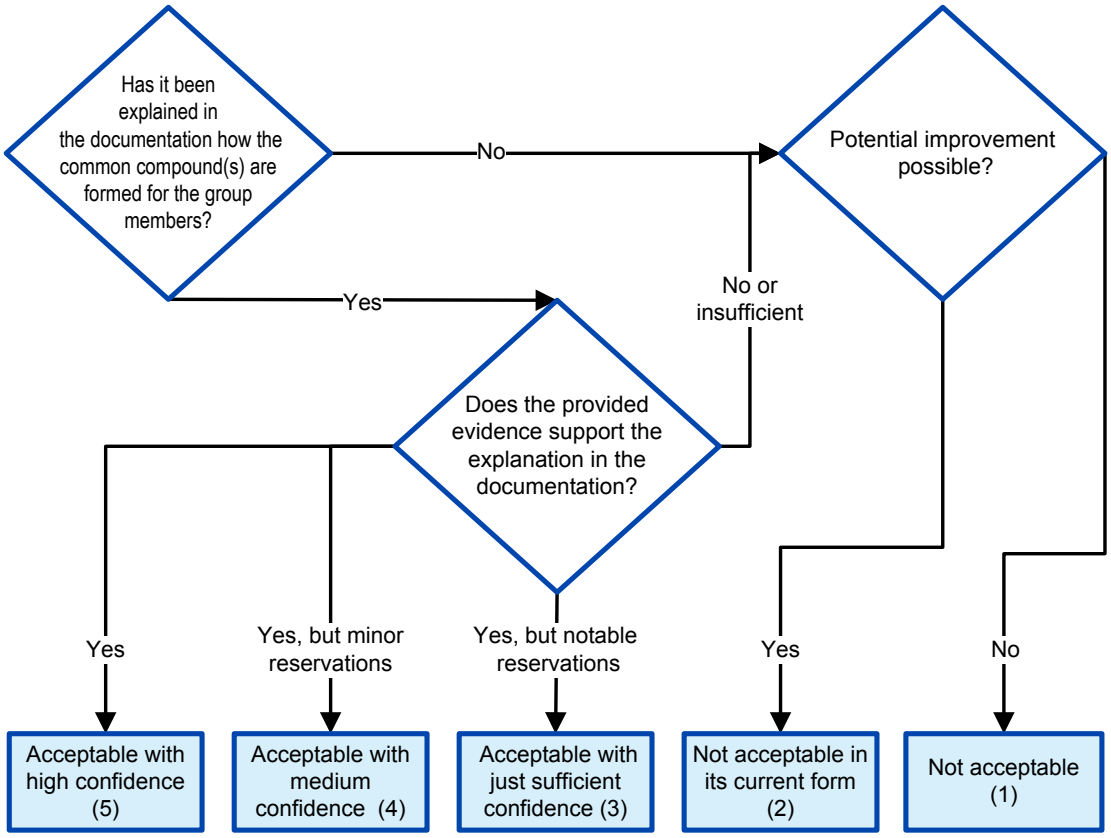
PURPOSE

This AE considers how the common compound(s) are formed from the members of the category.

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); and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



EXPLANATION

In this scenario, (bio)transformation of the source and target substances is claimed to result in local and/ or systemic exposure to common (identical) compounds or chemical reaction product(s). The prediction of effects or of absence of effect(s) is due to exposure to the common compound(s) only.

This AE is concerned with the formation of the common compound(s), as it is addressed in the scientific explanation. In most cases, the scientific explanation needs the support of case-specific evidence, e.g. experimental data with the source and target substances. Convincing evidence has to be provided that the

common product(s) are formed from the category members. However, detailed experimental data such as toxicokinetic studies may not need to be provided for each and every category member. Supporting information may include in vitro, in vivo, in chemico and in silico studies. The quantitative aspects of the kinetics of the (bio)transformation events are addressed in AE 3.3.

If the explanation for the formation of the common compound(s) is missing 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 has been conducted with category member(s) where the explanation is available and it is also available for the target substance. If the target substance is not covered by the hypothesis, prediction is not possible.

Although this AE is concerned only with the formation of the common compound(s), indirect supporting evidence may be derived also by analysing the data matrix provided by the registrant, i.e. effects in other parameters of multi-parameter studies or effects in studies on other properties. If there is the same clear set of effects in substances within the category, this fact does not contradict that the same common compound is formed and is causing the effects. If different compounds would have been formed and cause effects, a consistent pattern of effects would be unlikely. Different effects would indicate formation of different compounds or a different rate of formation.

EXAMPLE(S)[4]

3.1.a Example for common compounds

- Substances A, B, C and D are proposed to be converted to the common compound Z.
- Systemic exposure to Z is claimed to drive the toxicity profile of A, B, C and D.
- 28-day repeated-dose toxicity studies with substances A, B and D are used to predict the results of a 28-day study repeated-dose toxicity for the target substance C.

The explanation on how Z is formed[8] from A, B, C and D is necessary for the prediction. Supporting evidence is needed as well.

8 Other (bio)transformation pathways might exist which result in other compounds and are assessed in AE 3.5.

AE 3.2 THE BIOLOGICAL TARGETS FOR THE COMMON COMPOUND(S)

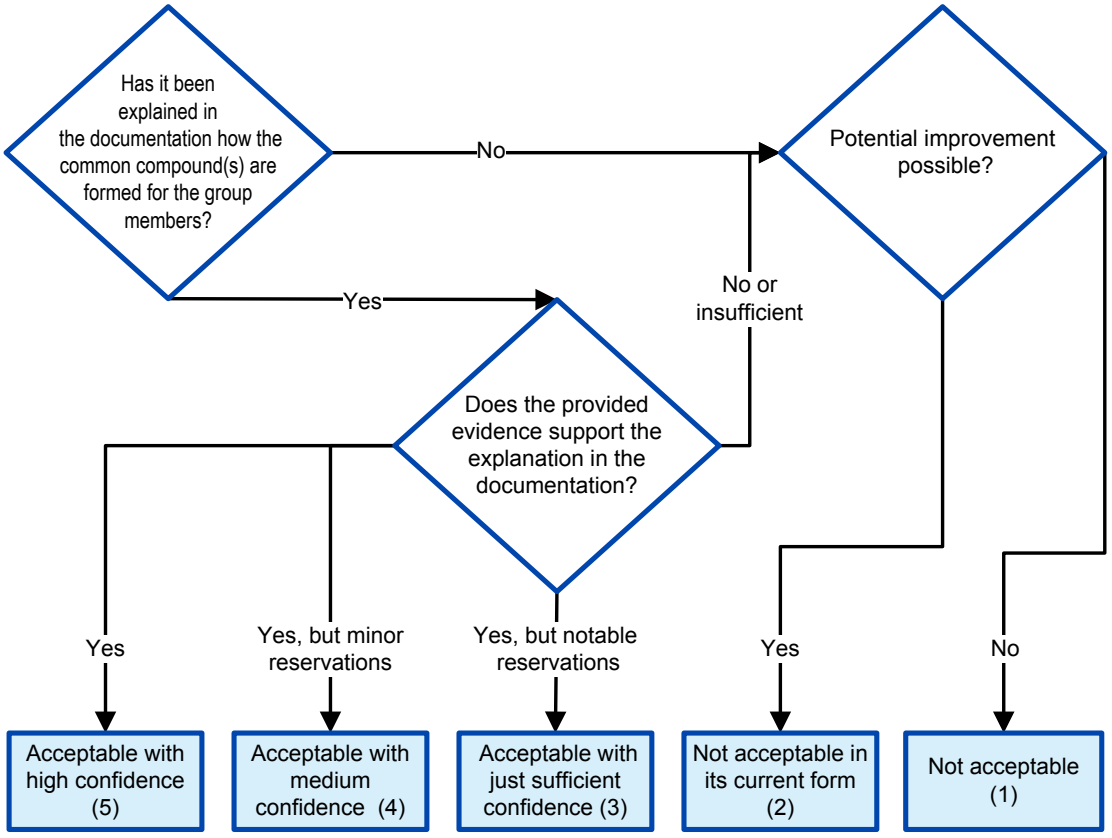
PURPOSE

The hypothesis claims that the common compound(s) have the same biological target(s) (and hence cause the same type of effects).

It has to be assessed whether:

- the same biological targets are affected in a consistent manner throughout the category, and by the common compounds; and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



EXPLANATION

The biological targets for the common compounds are supposed to be the same. It must be explained how the (bio)transformation of source and target substances to the common compounds results in exposure of the same biological targets. It has to be assessed whether the biological targets are indeed the same, e.g. if the same type of effects are observed for the category members.

It is also important to examine the proposed mechanism of formation of the common compound to

determine, if this provides a reliable basis for prediction of effects in the same target tissue(s) (see AE 3.1).

It is recognised that the strength of the effects will vary due to quantitative differences in exposure of the same biological targets and thus in quantitative differences in effects (including no-observable effects for some category members). The quantitative aspects are assessed in AE 3.3.

The fact that common compounds (derived from the members of the category and assumed to drive the effects) are formed does not always mean that the same biological target is exposed to these compounds. This may be the case when they are formed in different organs and exert their effect directly upon their formation. Another possibility is that due to differences in formation pathways, the distribution of the common compounds differ and consequently the exposure of possible target tissues.

The explanations should be supported by evidence. If the explanation for the induction of the same effects in the same biological targets is missing for one or more category members, it has to be assessed whether this has an impact on the prediction.

EXAMPLE(S)[4]

3.2.a Example for occurrence of effects in the same biological targets

- Substances A, B and D are oxidised to the common compound Z.
- Target substance C is also oxidised to Z.
- Substances A, B and D show nephrotoxicity; the toxicity differs in strength according to the rate of oxidation.
- Studies with substances A, B and D are used to predict nephrotoxicity also for C.

The supporting evidence (e.g. matrix/mechanistic evidence) supports that A, B, C and D have the same biological target.

3.2.b Example for occurrence of effects in different biological targets, although common compounds are formed

- Substances A, B and D are oxidised to the common compound Z and have been investigated in 90-day repeated-dose toxicity studies.
- Substance C is also oxidised to Z, no data are available for repeated-dose toxicity.
- Z is assumed to cause toxicity at the site of formation.
- A, B, and D are oxidised by a Cytochrome P450 isoform only present in the lung and C is oxidised via a different Cytochrome P450 isoform present in the liver.
- Substances A, B and D cause lung toxicity upon oral exposure.
- Substance C is predicted to cause lung toxicity upon oral exposure as well.

The prediction does not have a consistent mechanistic basis and therefore the same target organ has not been demonstrated for substance C.

3.2.c Example for a worst case prediction based on a common compound

- Substances A, B and C are converted to common compound Z in the liver with different rates resulting in varying strength of neurotoxic effects confirmed in 28-day repeated-dose toxicity studies; no other effects have been observed.
- The strength of neurotoxic effects correlates with the formation rates to Z.
- The common compound Z is water soluble and is proposed to drive the toxicity of A, B and C.
- Z has also been tested in both a 28-day study and a 90-day repeated-dose toxicity study, and causes only the same neurotoxicity, but at lower doses compared to A, B and C; no other effects are observed.
- The results of the oral 90-day repeated toxicity study with Z are used as a worst case to predict the results of 90-day repeated-dose toxicity studies with A, B and C.

Although differences in the exposure of organs/tissues (at least in the liver) to the common compound Z occur, the data set supports the worst-case assumption. This picture would change, if additional effects would have been observed for A, B or C.

AE 3.3 EXPOSURE OF THE BIOLOGICAL TARGET(S) TO THE COMMON COMPOUND(S)

PURPOSE

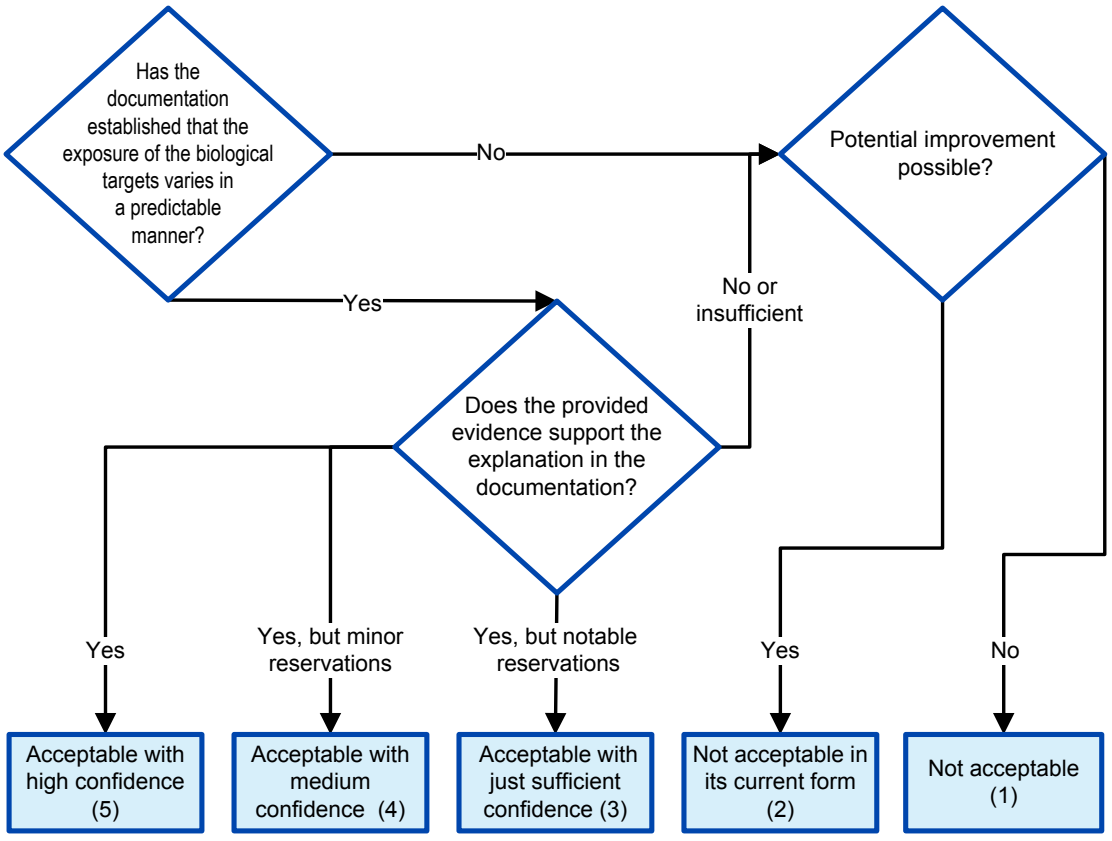
Under this scenario, it is proposed that the exposure of the biological targets to the common compound(s) vary in a predictable manner.

It has to be assessed whether:

- the documentation established that the exposure of the biological targets to the common compound(s) is varying in a predictable manner;
- the prediction is derived from the relation between an observed property and the independent variable which determines the order within the category (prediction model[9]); and
- the provided evidence supports the explanation.

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

ASSESSMENT OPTIONS



9 The prediction model may be based on a regular pattern within the category or a worst case. If 'subgroups' have been proposed for certain property predictions, the prediction model must be different for the different subgroups. Prediction between these groups is not allowed.

EXPLANATION

Mechanistic explanation

Exposure of the biological target(s) to the common compounds derived from the source and target substances are assumed to be different under this scenario to explain observed differences in strength of effect(s) for all or some category members. Different blood area under the curve (AUC), peak concentration (Cmax), or time to reach Cmax (tmax) may be indicative of such circumstances. Reasons may be different rates in absorption, metabolism, distribution and/or elimination.

The prediction has to be supported by the explanation of how the kinetics of the (bio)transformation[10] of source and target substances lead to differences in the exposure of the biological target to the common compounds. If at equivalent doses, the kinetics[11] of the (bio)transformation to the common product differs between the source and target substance, the internal exposure to the common compound will differ and may cause differences in effects.

Therefore, the property under consideration for the target substance may be predicted on the basis of kinetic consideration. Such differences in kinetics are typically associated with the structural differences and/or physico-chemical properties of the category members.

Prediction model

The relation between structures/specific physico-chemical properties and the kinetic differences resulting in the observed differences in strength of effects has to be substantiated. This relationship can serve as a prediction model for the property under consideration. This prediction model must be specified in the documentation.

If the prediction model uses a statistical fit for the prediction, the fit has to be robust and statistically significant. The prediction model defines an independent variable, which is a property of the group members. Often this is an incremental structural feature (e.g. chain length); however, in many cases it is a physicochemical property, either measured or estimated. Bias may occur when the sensitivity of the measurement or estimation has a systematic deviation in relation to category members with certain structural features.

This possible occurrence of bias and its influence on the prediction should be addressed in the assessment. Trends with a high statistical significance can still go with an unacceptably wide error of the actual prediction. If needed the advice of a statistician may be taken. The statistical analysis must be consistent with the mechanistic explanation provided in the documentation.

If the prediction model is using a worst-case approach, the documentation is not using a statistical fit for the prediction. If the worst case for the prediction is claimed as a conservative approach to estimate the property under consideration, the mechanistic explanation should justify the claim that the chosen source substance(s) indeed represent a worst case. This claim also has to be analysed in detail in AE 3.4 – 3.5.

10 If other biotransformation pathways exist or are suspected (see AE 3.5) there might also be an influence of these pathways (leading to additional non-common compounds) on the kinetics of the pathway leading to the formation of the common compounds.

11 Often only blood kinetics are known. It is assumed that in many cases this can be taken as a surrogate for the internal exposure of biological targets. However, this may not be true for all cases.

Location of the target

To determine the confidence in the prediction is also necessary to analyse the position of the target's value for the independent variable with regard to the total range of values for the independent variables of the category members. This position can be within the range or represent one of the two boundaries of the range. Less confidence may be associated with the prediction, if the independent variable of the target represents a boundary. The mechanistic explanation should address the uncertainty for a prediction associated for a target being at a border of a trend.

Supporting information

The type of information needed to provide sound scientific explanations is case-specific. Quantitative kinetic data are very valuable in this regard. Predictions of absence of effects can only be made for some category members under this scenario and must be justified with appropriate data.

Differences in strength observed for related properties need to be analysed as well. They may be due to the impact of non-common compounds or the parent compounds (see AE 3.4 and 3.5).

EXAMPLE(S)[4]

3.3.a Example for a difference in metabolism resulting in differences in exposure at the biological target

- Substances A, B, C and D are (bio)transformed to the common compound Z.
- All substances have a similar absorption rate, but A has a faster metabolism rate to Z compared to B, C and D.
- As a result, the common compound Z has a higher Cmax in the blood after administration of A compared to the Cmax after administration of B, C and D (AUCs are assumed to be similar).
- This difference determines the exposure of the bone marrow to Z.
- The toxicity observed in the bone marrow is highest for substance A and varies slightly for B and D.

If A is used as the source substance to predict the bone marrow toxicity of C, it represents a worst case in this example. If B or D are used as the source substance, the bone marrow toxicity of substance C may be under-predicted.

3.3.b Example for a difference in absorption resulting in differences in exposure at the biological target

- Substances A, B, C and D are (bio)transformed to the common compound Z.
- All substances have information on oral absorption rates, which identify a pattern regularly decreasing from A to D (A > B > C > D).
- As a result, the common compound Z has a much higher AUC and Cmax in the blood for A compared to B, C and D; (assuming that metabolism rates are similar).
- This difference influences the exposure of the kidneys to the common compound Z.

- Decreasing severity of kidney toxicity was observed in 28-day repeated-dose toxicity studies (A > B > D)

The prediction model uses the quantitative relationship between the oral absorption and the severity of kidney toxicity.

AE 3.4 THE IMPACT OF PARENT COMPOUNDS

PURPOSE

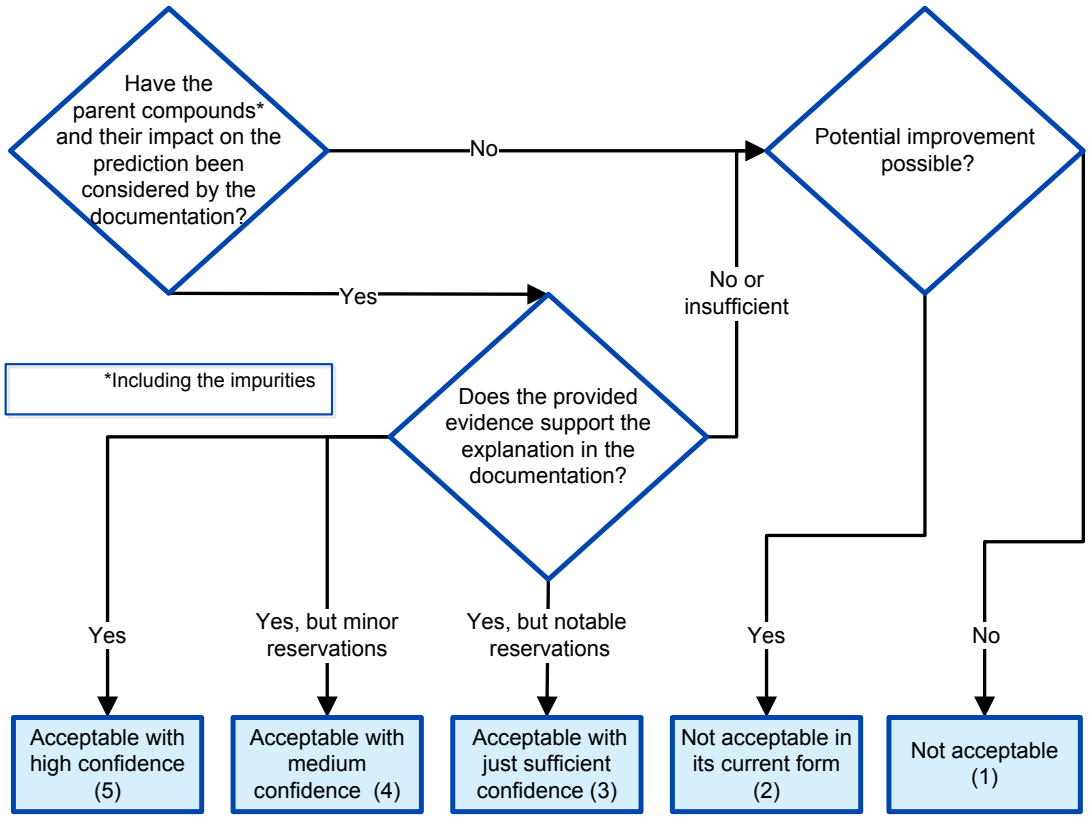
(Bio)transformation of parent compounds may not be immediate and/or complete. As a result, exposure of possible biological targets to the parent compounds may occur for source and/or target substances.

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

It has to be assessed whether:

- the systemic availability[12] of the parent compound and its impact on the prediction of the property under consideration has been addressed;
- the identified impurities (see AE C.1) have an impact on the prediction; and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



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

EXPLANATION

Possible influence of parent compounds

(Bio)transformation of parent compounds may not be immediate and/or complete. As a result, exposure of possible biological targets to the parent compounds may occur for source and/or target substances. This AE is related to AE 3.5 where the kinetics of the common compounds is covered, which is of course dependent on the(bio)transformation rates and extent of the parent compounds. However, the current AE covers the possible impact of the parent compounds on the predicted effects profile or on the prediction of absence of effects.

Guidance R.6, states that "in certain instances, the metabolism of the parent compound within barrier tissue (e.g. lung or gut tissue) occurs so rapidly that the initial primary metabolite is the predominant chemical found within the blood. Under these circumstances data from hazard identification studies conducted with that primary metabolite itself can be used to identify hazards for the parent compound."

If exposure to the parent compounds occurs for the source or target substance, it should be explained how this may impact the prediction of the property under consideration. Under this scenario, the parent compounds should not significantly influence the prediction. This means that the (bio)transformation of the parent compound should be rapid and complete or it is known that the parent compound is toxicologically silent. This might prove difficult to establish for higher tier human health endpoints for the target substance.

Supporting evidence

The type of information needed to provide sound scientific explanations is case-specific. Quantitative kinetic data are very valuable in this regard. Predictions of absence of effects can only be made for some category members under this scenario and must be justified with appropriate data.

The information presented in the data matrix may also provide indications for toxicity induced by the parent compounds. If observed effect levels for the same biological target are significantly different in studies on related properties or if different biological targets are observed, such differences may also be due to the impact of the parent compounds. Other possibilities would be the presence of other non-common compounds or kinetic differences.

Importance of impurities

Toxicity of the source and target substances 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 differences in impurity profiles into account.

EXAMPLE(S)[4]

3.6.a Example for a parent compound obviously not influencing the prediction

- Substances A, B, C, D and E are structurally similar and are (bio)transformed to the common compound Z.
- It is postulated that the toxicity is proportionate to Z, which is formed after absorption.
- The absorption of the parent compounds is decreasing with increasing carbon chain length.

- The toxicity of A, B, D and E has been determined in 90-day repeated-dose toxicity studies and are consistent with the hypothesised exposure to Z. In addition, a 28-day repeated-dose toxicity study for C is available.
- The prediction model uses the relation between the effects observed in 90-day repeated-dose toxicity studies and the absorption rates.
- The toxicity of C is predicted based on the trend between the effects and the absorption of A, B, D and E.
- The 28-day repeated-dose toxicity study conducted with C confirms the prediction.

In this situation, influence of the parent compound on the prediction for the target does not appear to be significant.

3.6.b Example for a missing explanation in the hypothesis about the impact of impurities.

- Substance A consists of the main constituent A, there is an impurity X of 5%.
- Substance B consists of the main constituent B, there is an impurity X of 3% and an impurity Y of 2%.
- The hypothesis only deals with A, B and X.
- Substance A is used as source substance to predict absence of effect, substance B is the target substance.
- Impurity Y is an impurity known to cause nephropathy at low doses.

The hypothesis also has to address the impurity Y with regard to the possible impact on the prediction of the property under consideration.

AE 3.5 FORMATION AND IMPACT OF NON-COMMON COMPOUNDS

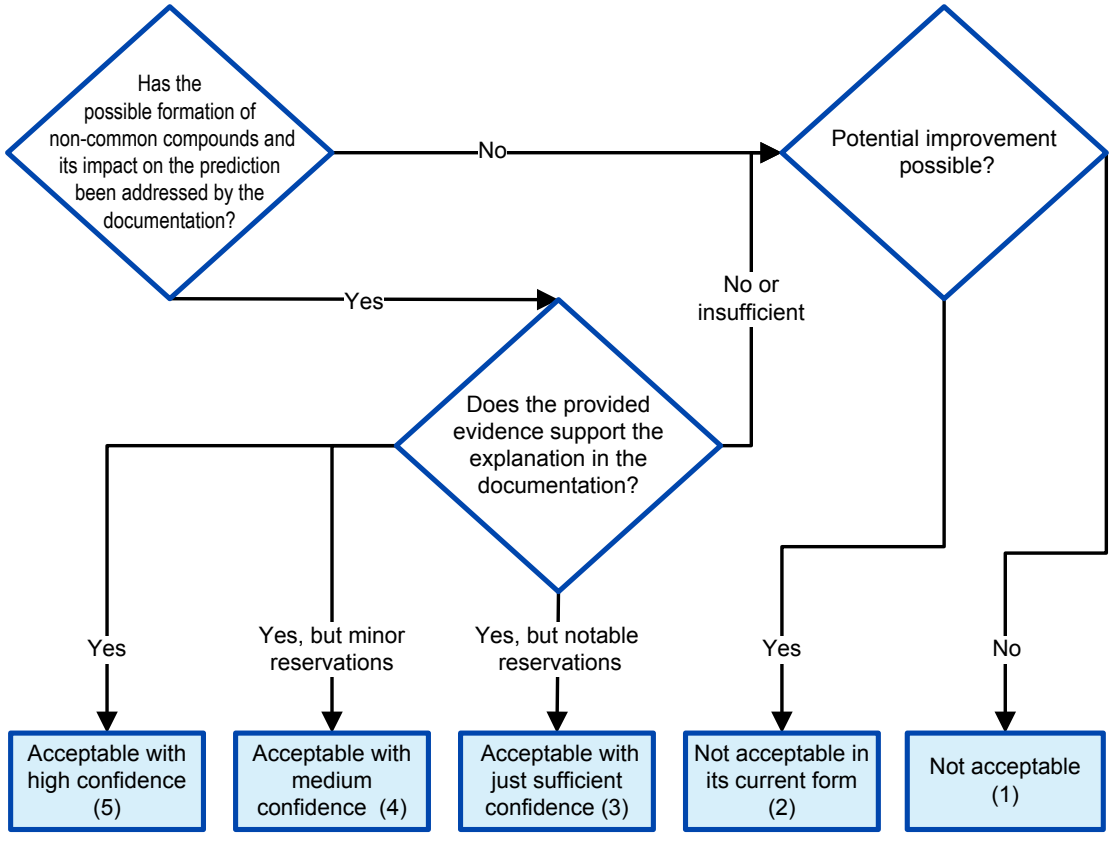
PURPOSE

The formation of common compound(s) often goes together with the formation of non-common compound(s) and possible intermediates which form the common compound(s). The source and/or target substance may also be (bio)transformed via other pathways[13] leading to other additional non-common compounds.

It has to be assessed whether:

- the formation of non-common compounds (including possible intermediates) via the possible pathways and their possible impact on the prediction property under consideration have been considered; and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



13 "Other pathways" are pathways not involved in the formation of the common compound(s) (which are claimed to drive the toxicity).

EXPLANATION

Under this scenario, the prediction for the property under consideration is claimed to be influenced alone by the common (bio)transformation compound(s) of source and target substances.

Non-common compounds may have the following possible sources[14]:

- formed via the pathway(s) leading to the common compounds;
- formed via (an)other pathway(s); and
- formed as intermediates (all pathways).

As a default, non-common compounds should not impact the prediction of the property under consideration. If a non-common compound is known to have a toxicity on its own, it should occur at such high doses that it is not considered to influence the prediction of the property under consideration.

Experimental evidence on kinetics and potential toxicity/absence of effects of the non-common compounds may be necessary for source and target substances.

The analysis of the data matrix may provide facts supporting or contradicting the claim. There might be effects on other parameters in multi-parameter studies or effects in studies on other properties. Occurrence of the same clear set of effects in source and target substances does not contradict that indeed one and the same common compound is formed and is triggering the effects. If non-common compounds are formed and trigger effects, a regular pattern of effects would be unlikely, if source and target substances are compared. Different effects would indicate formation of non-common compounds or influence of parent compounds or different rate of formation for the common compound.

EXAMPLE(S)[4]

3.5.a Example for formation of non-common compounds via the pathway leading also to the common compounds

- Substances A, B, C and D are esters of the same alcohol and long-chain fatty acids.
- After absorption, all substances are hydrolysed and the test organism is only exposed to the corresponding alcohol and the fatty acids. The fatty acids are the non-common compounds.
- It is explained that toxicity is fully determined by the alcohol and its rate of formation from the esters.
- Toxicity is increasing with the increasing hydrolysis rates from A to D.
- The fatty acids are proposed to be non-toxic.

Evidence for the non-toxicity of the fatty acids should be provided. The possible impact of the parent compounds on the prediction is assessed in AE 3.6.

14 If other pathways which compete with the formation of the common compound exist there may also be an influence on the kinetics of the formation of the common compounds.

3.5.b Example for non-common compounds formed via other pathways

- Substances A, B, C and D are converted to the common compound Z via the same pathway, but at different rates.
- An additional different pathway converts substance D to D1.
- Increasing systemic exposure to Z occurs after exposure to A, B and C and causes an increase in severity of specific histopathological effects in the liver.
- Studies conducted with substances A, B and C are used to predict the property of substance D.

The formation and possible impact of D1 on the prediction for the property under consideration should be addressed.

AE C.6 BIAS THAT INFLUENCES THE PREDICTION

PURPOSE

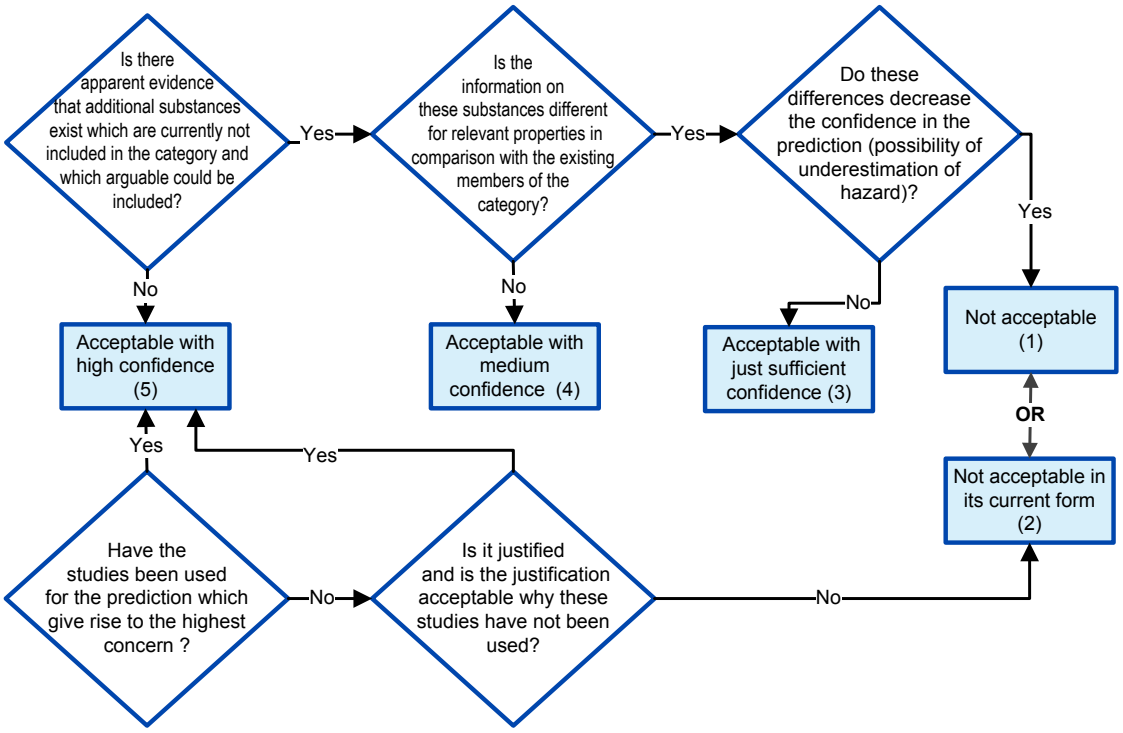
It has to be assessed whether:

- 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.