Appendix D: Scenario 4

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 some members of the category. There are differences in strength of the effect(s) and they may form a regular pattern. The prediction is based on the 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 4

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 E1).

Table E1: Assessment elements (AEs) for Scenario 4

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 4.1	Scenario-specific	Compounds the test organism is exposed to
AE 4.2	Scenario-specific	Common underlying mechanism, qualitative aspects
AE 4.3	Scenario-specific	Common underlying mechanism, quantitative aspects
AE 4.4	Scenario-specific	Exposure to other compounds than to those linked to the prediction
AE 4.5	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
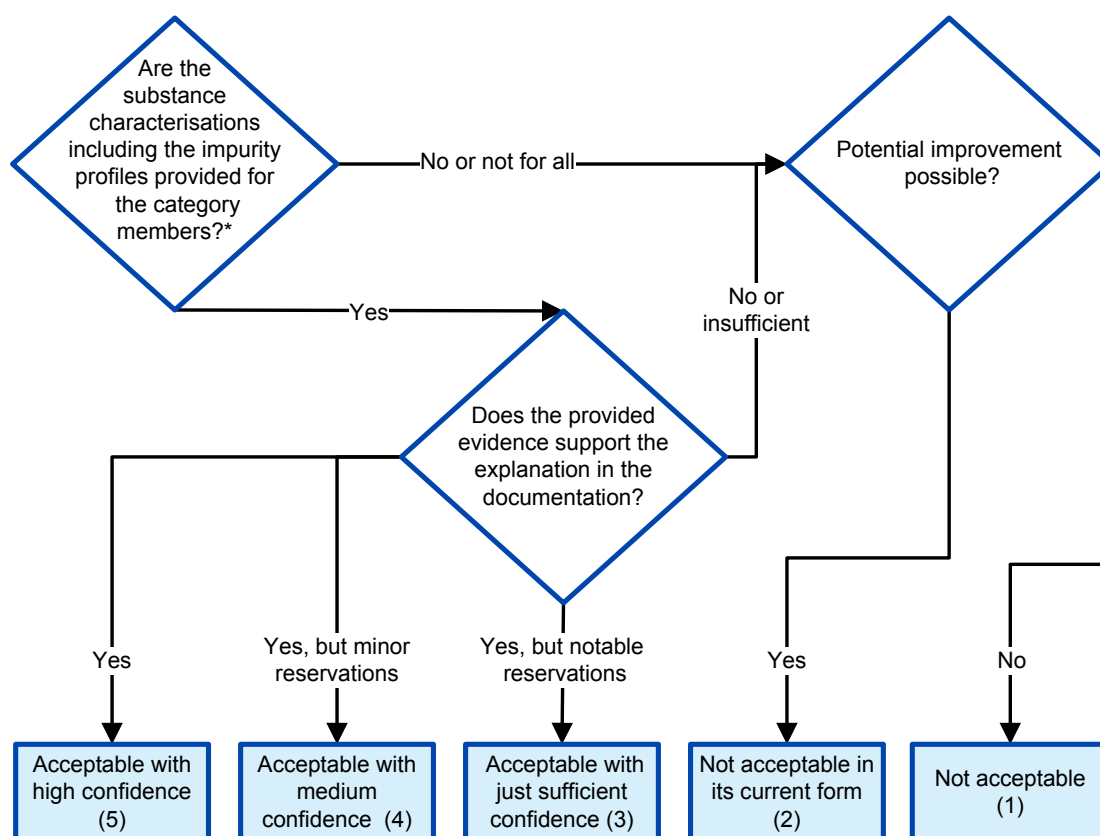
PURPOSE

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

```
                    Are the
                  substance
               characterisations
               including the impurity  ──No or not for all──►       Potential improvement
               profiles provided for                                      possible?
                 the category
                  members?*

                              ──Yes──►          No or
                                              insufficient

                                      Does the provided
                                      evidence support the
                                      explanation in the
                                      documentation?


         Yes        Yes, but minor     Yes, but notable          Yes              No
                     reservations       reservations

     Acceptable with  Acceptable with  Acceptable with    Not acceptable in   Not acceptable
     high confidence     medium         just sufficient    its current form        (1)
         (5)         confidence  (4)   confidence (3)           (2)
```

> *Category members for which the substance characterisations (identity and impurity profiles) have not been provided cannot be accepted as source or target substances. This has to be taken into account when the prediction is assessed.

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1 The test material actually used in a specific source study is addressed in AE C.5. The impact of impurities on the prediction is addressed in AE 4.4.

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 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.

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

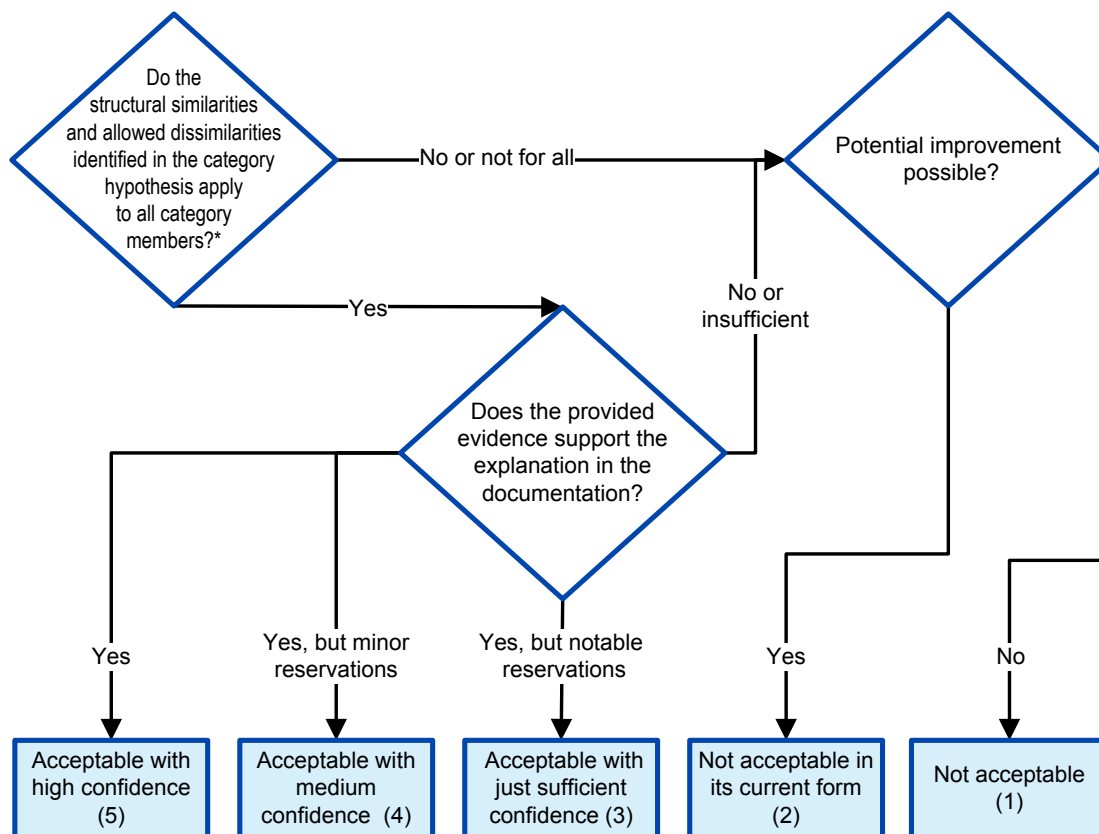## AE C.2 STRUCTURAL SIMILARITY AND STRUCTURAL DIFFERENCES WITHIN THE CATEGORY

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

Do the structural similarities and allowed dissimilarities identified in the category hypothesis apply to all category members?*

No or not for all

Yes

Potential improvement possible?

Does the provided evidence support the explanation in the documentation?

No or insufficient

Yes

No

Yes	Yes, but minor reservations	Yes, but notable reservations	Yes	No
Acceptable with high confidence (5)	Acceptable with medium confidence (4)	Acceptable with just sufficient confidence (3)	Not acceptable in its current form (2)	Not acceptable (1)

*Category members for which the category hypothesis does not apply cannot be accepted as source or target substances.

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

---

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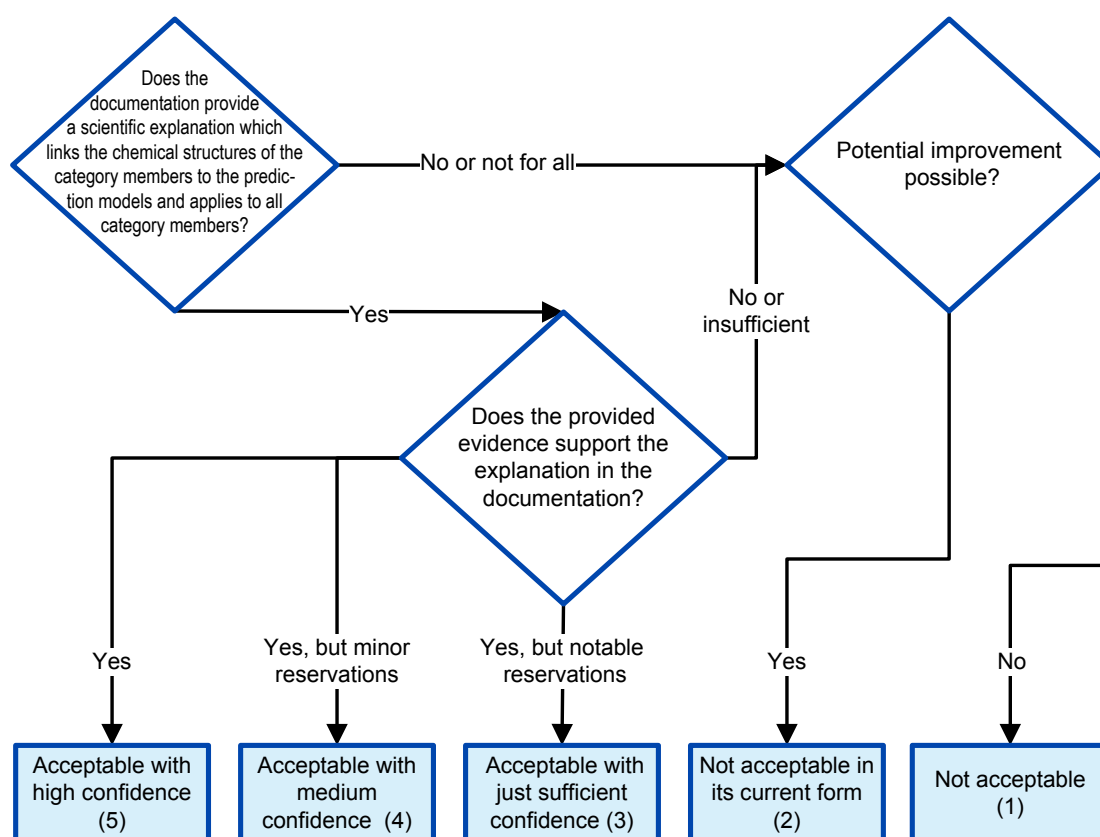
AE C.3 LINK OF STRUCTURAL SIMILARITIES AND STRUCTURAL DIFFERENCES WITH THE PROPOSED REGULAR PATTERN

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

Does the documentation provide a scientific explanation which links the chemical structures of the category members to the prediction models and applies to all category members?

No or not for all

Yes

Potential improvement possible?

No or insufficient

Does the provided evidence support the explanation in the documentation?

Yes

Yes, but minor reservations

Yes, but notable reservations

Yes

No

| Acceptable with high confidence (5) | Acceptable with medium confidence (4) | Acceptable with just sufficient confidence (3) | Not acceptable in its current form (2) | Not acceptable (1) |

*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 in 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 the 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 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 vary 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.
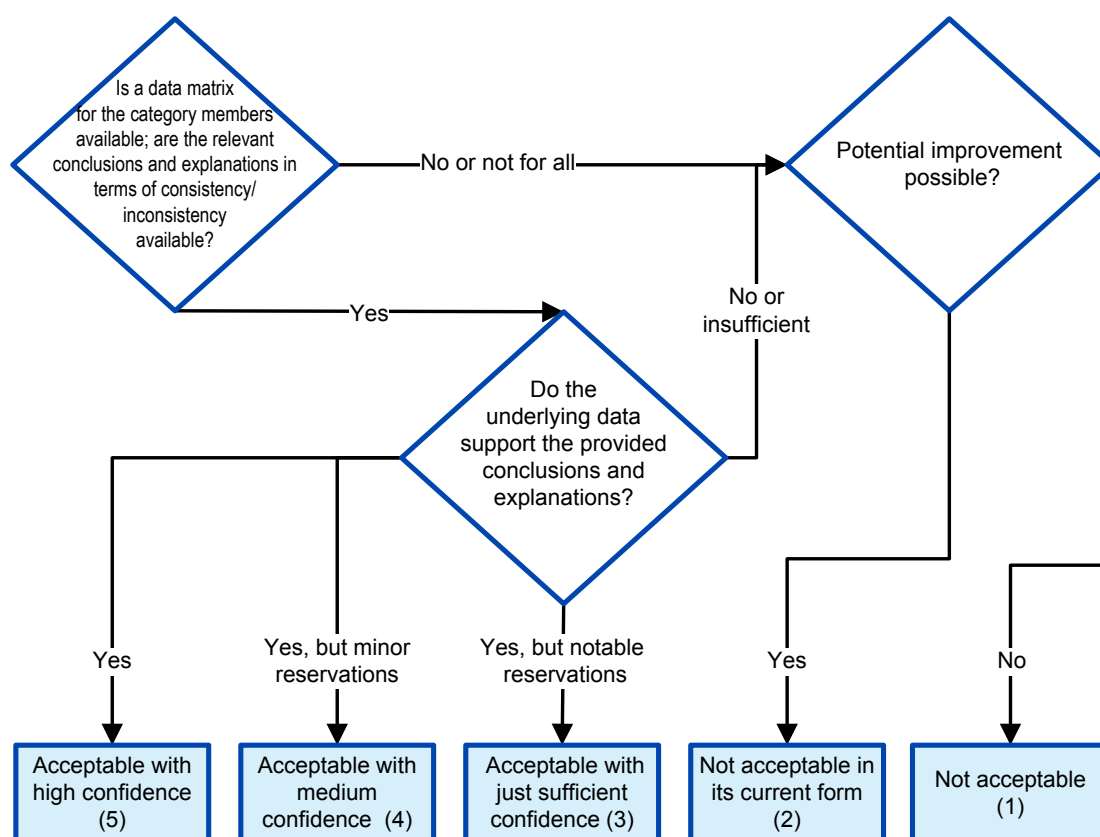
AE C.4 CONSISTENCY OF EFFECTS IN THE DATA MATRIX

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); and

    - 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.

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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

```
┌─ Is a data matrix ─┐
  for the category members
  available; are the relevant ──── No or not for all ────►  Potential improvement
  conclusions and explanations in                              possible?
  terms of consistency/
  inconsistency
  available?
        │
        └─ Yes ──►  Do the                          │
                    underlying data     No or
                    support the provided  insufficient
                    conclusions and
                    explanations?
```

Yes	Yes, but minor reservations	Yes, but notable reservations	Yes	No
Acceptable with high confidence (5)	Acceptable with medium confidence (4)	Acceptable with just sufficient confidence (3)	Not acceptable in its current form (2)	Not acceptable (1)

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 (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 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. 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 in 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 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.
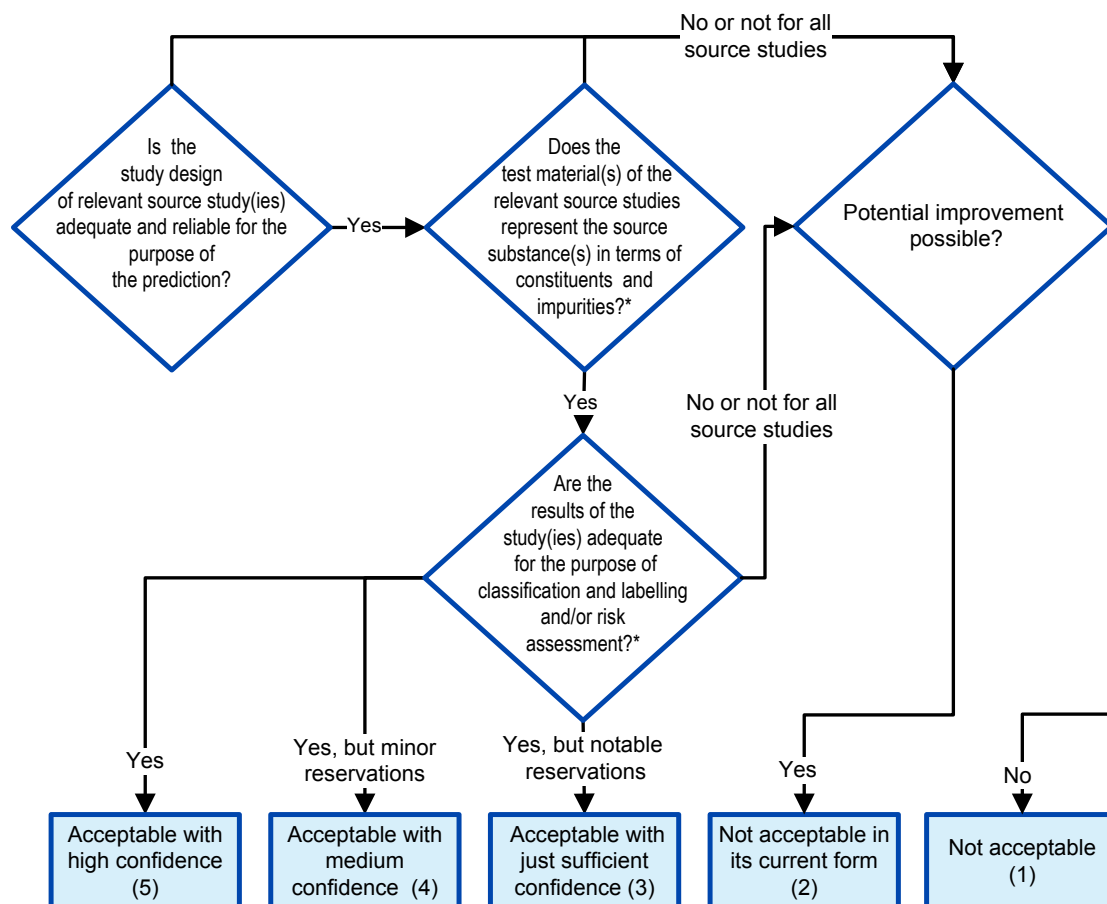
It has to be also assessed whether:

- 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.

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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

No or not for all
source studies

Is the
study design
of relevant source study(ies)
adequate and reliable for the
purpose of
the prediction?

Yes→

Does the
test material(s) of the
relevant source studies
represent the source
substance(s) in terms of
constituents and
impurities?*

Potential improvement
possible?

Yes

No or not for all
source studies

Are the
results of the
study(ies) adequate
for the purpose of
classification and labelling
and/or risk
assessment?*

Yes	Yes, but minor reservations	Yes, but notable reservations	Yes	No
Acceptable with high confidence (5)	Acceptable with medium confidence (4)	Acceptable with just sufficient confidence (3)	Not acceptable in its current form (2)	Not acceptable (1)

*This is not known for cases where a testing proposal has been made for a source substance.

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.

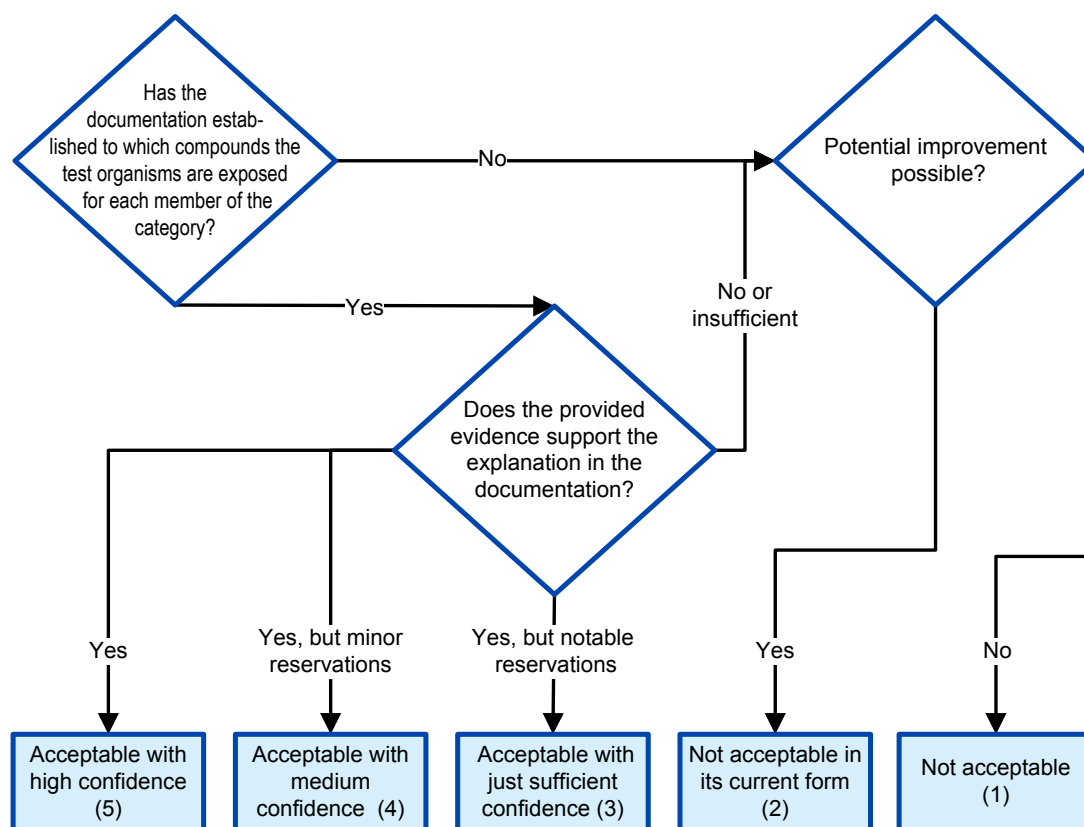## AE 4.1   COMPOUNDS THE TEST ORGANISM IS EXPOSED TO

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

Decision tree:

- Has the documentation established to which compounds the test organisms are exposed for each member of the category?
  - No → Potential improvement possible?
    - Yes → Not acceptable in its current form (2)
    - No → Not acceptable (1)
  - Yes → Does the provided evidence support the explanation in the documentation?
    - No or insufficient → Potential improvement possible?
    - Yes → Acceptable with high confidence (5)
    - Yes, but minor reservations → Acceptable with medium confidence (4)
    - Yes, but notable reservations → Acceptable with just sufficient confidence (3)

* the compounds the test organism is exposed to (after administration of target and source substances) should be identified. This may be parent compounds and/or (bio)transformation products.

EXPLANATION

Under this scenario, it is claimed that the prediction for the property under consideration is possible because different compounds have the same type of 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 into the actual compounds to which the test organism is exposed.

In principle, these compounds can be:

- Target and source substances themselves;

- Source substances and (bio) transformation product(s) of target substance;

- Target substance and (bio)transformation(s) product of source substances;

- Target substances and source substances and their (bio) transformation(s) products; or

- (Bio) transformation(s) products of source and target substances.

It therefore 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 and thus form the basis for the prediction for the property under consideration.

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

EXAMPLE(S)[4]

4.1.a   Example for the presence of only source and target substances in the test organism

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

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

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

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

- Substances B, C and D are absorbed, not (bio)transformed 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 from a source study conducted with B, C or D needs to take into account the additional presence of A1 and A2.

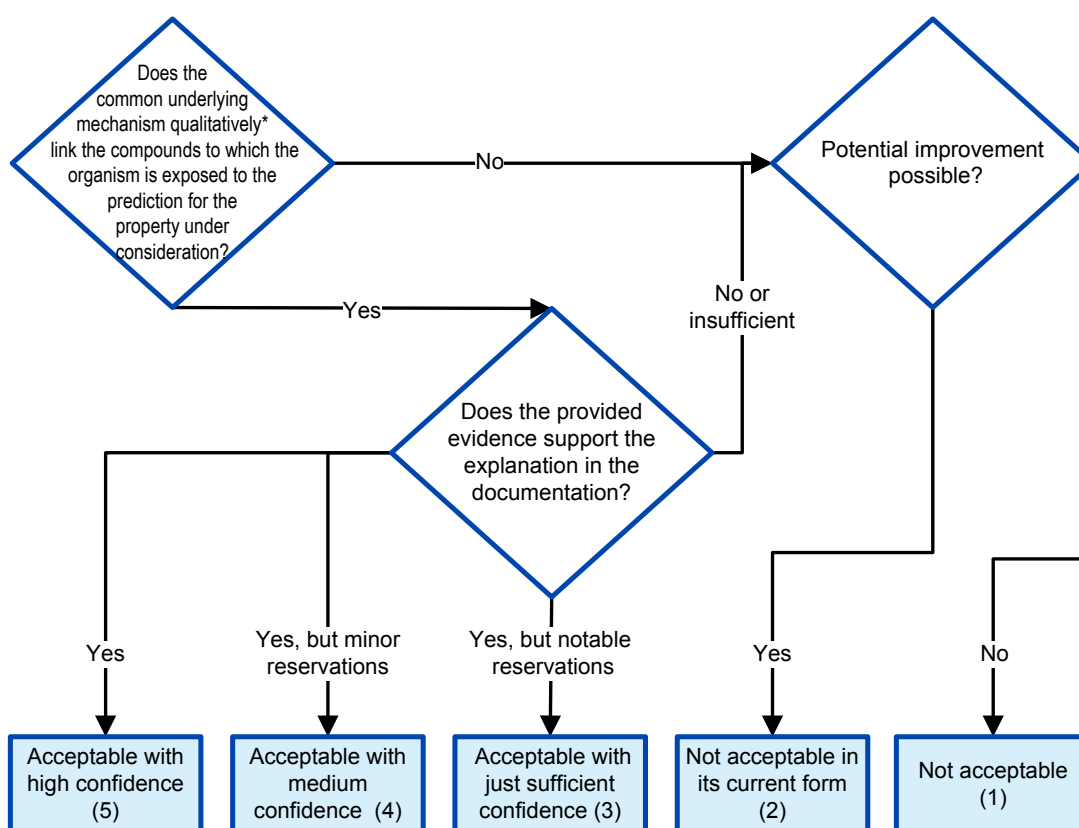## AE 4.2 COMMON UNDERLYING MECHANISM, QUALITATIVE ASPECTS

PURPOSE

The category hypothesis/justification has to explain why the compounds to which the test organism is exposed lead to the same type of effect(s) but with different strength of effect(s).

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 the same type of effects for the target substance for the property under consideration; and

- the provided evidence supports the explanation.

ASSESSMENT OPTIONS

```
Does the                                                        Potential improvement
common underlying                                                    possible?
mechanism qualitatively*
link the compounds to which the          ─No─────────────►
organism is exposed to the
prediction for the
property under
consideration?

                         ─Yes─►           No or
                                       insufficient
                  Does the provided
                  evidence support the
                  explanation in the
                  documentation?

  Yes        Yes, but minor    Yes, but notable      Yes           No
             reservations       reservations

Acceptable with  Acceptable with  Acceptable with  Not acceptable in  Not acceptable
high confidence      medium        just sufficient  its current form       (1)
    (5)         confidence  (4)   confidence (3)        (2)
```

*Are qualitatively the same type of effect(s) consistently observed for the source substance(s) and why are they likely to be observed also for the target substance in the same biological targets?

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 the same type of effects for the target substance.

The hypothesis/justification should explain why the effects observed with the group 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 in vivo or in vitro on the proposed mechanism is valuable.

Prediction of absence of effect

Under this scenario, prediction of absence of effects can only apply to some group members. Specific considerations are needed for predictions of 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]

4.2.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, and have in vitro evidence that they act via the same receptor.

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

4.2.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 which are metabolised to epoxides.

- The epoxide which is formed from substance A binds to DNA and causes genotoxicity in a gene mutation assay.

- The epoxides formed from substances B, C and D have similar chemical reactivities based on theoretical chemical considerations.

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

AE 4.3 COMMON UNDERLYING MECHANISM, QUANTITATIVE ASPECTS

PURPOSE

Under this scenario, quantitative differences for the same type of effects are expected to be caused by the underlying mechanism.

It has to be assessed whether:

- the documentation established that the strengths of the same type of effects vary 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[8]);

- the prediction model is consistent with the common mechanism; 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


```
Does the underlying mechanistic explanation qualitatively link the compounds to which the organism is exposed to the prediction for the property under consideration?

No → Potential improvement possible?

Yes → Does the provided evidence support the explanation in the documentation?

No or insufficient

No →

Yes → Acceptable with high confidence (5)
Yes, but minor reservations → Acceptable with medium confidence (4)
Yes, but notable reservations → Acceptable with just sufficient confidence (3)
Not acceptable in its current form (2)
Not acceptable (1)
```

* Are qualitatively the same type of effect(s) consistently observed for the source substance(s) and why are they likely to be observed also for the target substance in the same biological targets?

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8 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

Quantitative differences

Under this scenario, quantitative differences for the same type of effects are expected to be caused by the underlying mechanism.

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.

The prediction needs to be supported by the explanation of how the kinetics and/or potency determines the differences in effect(s) observed for the property under consideration. The explanation should be based on the differences in chemical structures and how they influence either the kinetic properties and/or the potency of the compounds to which the organism is exposed.

Prediction model

The relation between chemical structures and the proposed mechanism 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 has to be analysed in detail also in AEs 4.4 – 4.5.

Location of the target

To determine the confidence in the prediction it 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. In vitro, in chemico and in silico studies (e.g. computational tools such as Derek, Meteor and OECD toolbox) may increase the robustness of the case, but usually are not sufficient as stand-alone information.

If potency differences are proposed to be the reason for observed differences in strength of effect(s), quantitative data explaining the mechanism are very valuable.

Predictions of absence of effects can only be made for some group members under this scenario and must be justified with appropriate data (see AE 4.4).

Differences in strength observed for related properties need to analysed as well. They may be due to the impact of non-common compounds or the parent compounds (see AE 4.4 and 4.5).

EXAMPLE(S)[4]

4.3.a   Example for prediction of the same type of effect(s), but different strength of effect(s), and common mechanistic explanation

- Substances A, B, C and D are absorbed at similar rates and extent.

- Substances A, B and D are agonist of a receptor that is driving the toxicity expressed by increased incidences of histopathological findings in the liver in 28-day repeated-dose toxicity studies.

- All substances are tested in vitro and show increasing potencies from A to D towards the receptor related to a known structural feature of the substances.

- 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.

4.3.b   Examples of a common underlying mechanism, quantitative differences in effect, worst case claimed

- 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 receptor interaction is known.

- Substance C has the same structural features, and has in vitro evidence that it acts via the same mechanism with a similar potency compared to D.

- 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 bw) compared to B, C and D (300, 380, 350 mg/kg bw respectively).

- 90-day repeated-dose toxicity studies are available for A and D. A has a lower effect level (30 mg/kg bw) compared to D (150 mg/kg bw).

It is proposed to predict the 90-day repeated-dose toxicity study results for B and C based on the study obtained with A and a worst-case approach is claimed. On the basis of this quantitative prediction, the substances B and C are proposed to be classified in line with the classification of A.

4.3.c   Example of a common underlying mechanism for metabolites, kinetic differences may have a quantitative impact on the prediction

- Substances A, B, C and D all contain a double carbon-bond and are metabolised to epoxides.

- The epoxides formed from substances A, B, C and D cause cell death in vitro.

- It is known from in vitro experiments that S9-mix metabolises substances A, B, C and D at decreasing rates (from A to D) to the corresponding epoxides due to known structural differences.

- There are 28-day repeated-dose toxicity studies conducted with C and D which demonstrate the same liver and lung toxicity, however, the strength of effects is higher for D than for C. The results obtained in a 90-day repeated-dose toxicity study conducted with A show similar strength of effects than observed in the 28-day repeated-dose toxicity study conducted with C.

- The hypothesis is that the results obtained in a 90-day repeated-dose toxicity study with substance A can be used to predict the results for a 90-day repeated-dose toxicity study for substance B. The basis of the prediction is that the toxicity is determined by the formation rate of the epoxide as measured in vitro with S9-mix.

This data set shows that the possible prediction model based on the chemical structures and the epoxide formation rates does not match the obtained in vivo results.

AE 4.4 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[9] 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
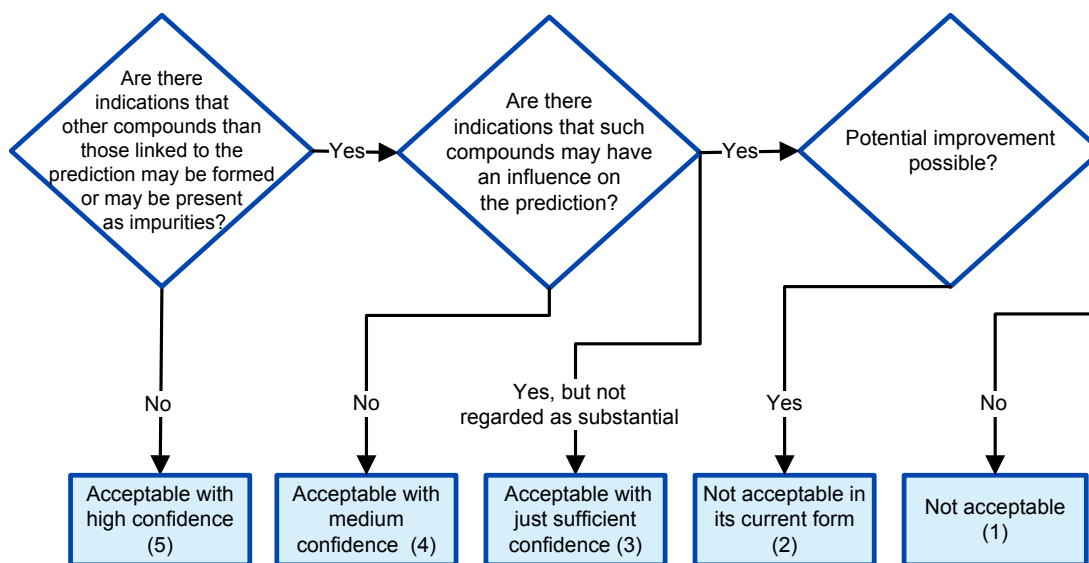
EXPLANATION

In AE 4.1, the hypothesis has been assessed qualitatively with regard to compounds the organism is exposed to. This AE investigates whether all compounds possibly influencing the prediction have indeed been addressed. It is supposed to provide a check on the robustness of the hypothesis with regard to the influence of other compounds the test organism may be exposed to.

---

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

Confidence in the prediction may be decreased if such compounds are either present as parent substances (including impurities) or formed by (bio)transformation from source and/or target substances and have not been considered by the hypothesis. In addition to the information assessed in AE 4.3, 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 the lack of 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.

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 supporting data. Nevertheless, the read-across may still be invalid, because it does not take a relevant difference in impurity profile of source and targets substances into account.

This AE also evaluates whether a substance chosen as worst case is indeed a worst case when considering other compounds present (including impurities) or formed.

EXAMPLE(S)[4]

4.4.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 A, C and D are absorbed and eliminated unchanged. Substance B is rapidly metabolised to B1 and B2.

- The absorption has been measured for all substances and decreases from A to D due to a known structural difference.

- There are quantitative differences in strength of the observed effects in 28-day repeated toxicity studies for A, C and D.

- The prediction model is that the toxicity is related to the absorption also for B.

- The results of a 90-day repeated-dose toxicity study conducted with substance A are used to predict the effects in a 90-day repeated-dose toxicity study for B assuming that B is absorbed to the same extent as A, i.e. as a worst-case approach.

This situation may lead to under-prediction of the toxicity of B, since B1 and B2 are not considered.

4.4.b   Example for an impurity which has not been addressed in the hypothesis

- A category consists of structurally-similar substances A, B, C and D.

- All substances cause dose dependent bone marrow toxicity in vitro. For A, C and D, this is the critical effect for the hazard identification in a 90-day repeated-dose toxicity study.

- The results of a 90-day repeated-dose toxicity study with A is used to predict the study results for B.

- Substance B has an impurity known to be a kidney toxicant, but the hypothesis does not consider this.

In this situation, it is unclear what role the kidney toxicity will have for a 90-day repeated-dose toxicity study with B.

AE 4.5   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
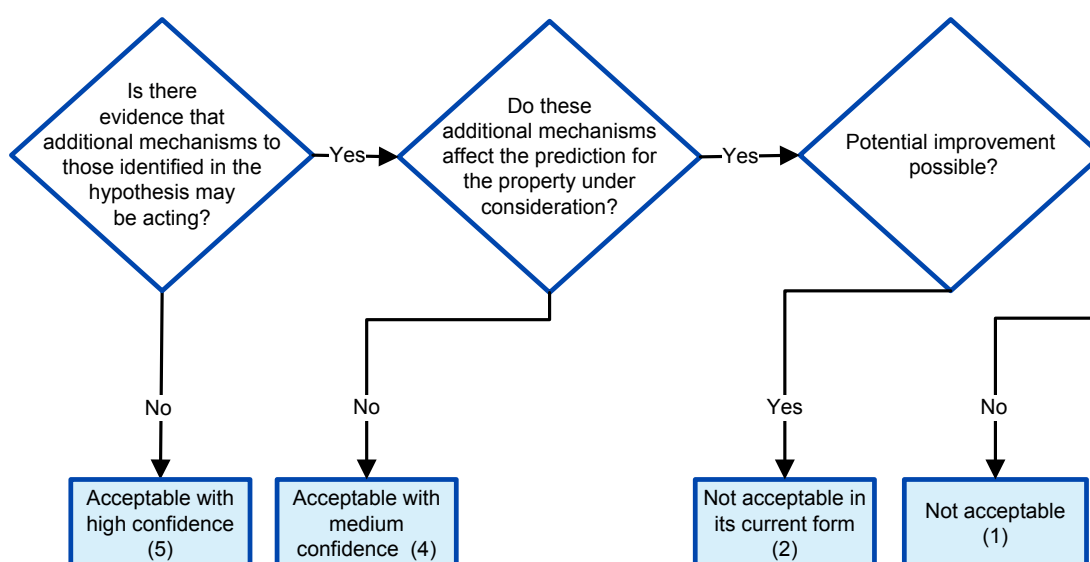
EXPLANATION

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. Other effects than those linked to the hypothesis have to be evaluated on a case-specific basis. Occurrence of such other effects may be non-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.

This AE also evaluates whether a substance chosen as worst case is indeed a worst case when considering the possibility that additional mechanisms may operate in the target substance.

EXAMPLE(S)[4]

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

- Substances A, B, C and D cause neurotoxicity observed in 28-day repeated-dose toxicity studies which included functional observational batteries, no other effects were observed. The strength of neurotoxic effects is linearly related to the activation of a nerve cell receptor in in vitro studies.

- In available pre-natal developmental toxicity studies for substances A and D, neurotoxic effects have been noted and no other effects have been observed in maternal animals.

- 90-day repeated-dose toxicity studies conducted with substance A and D are used to predict the toxicity of substances B 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.

4.5.b Example for indications of other mechanisms

- Substances A, C and D cause neurotoxicity observed in 28-day studies which included functional observational batteries; no other effects were observed. The neurotoxicity seen in the 28-day repeated-dose toxicity studies is linearly related to the activation of a nerve cell receptor seen in an in vitro assay.

- 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, a decrease of relative thymus weight was observed. This finding was accompanied with a decrease in the white blood cell count.

- 90-day repeated-dose toxicity studies conducted with substance A and D are 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
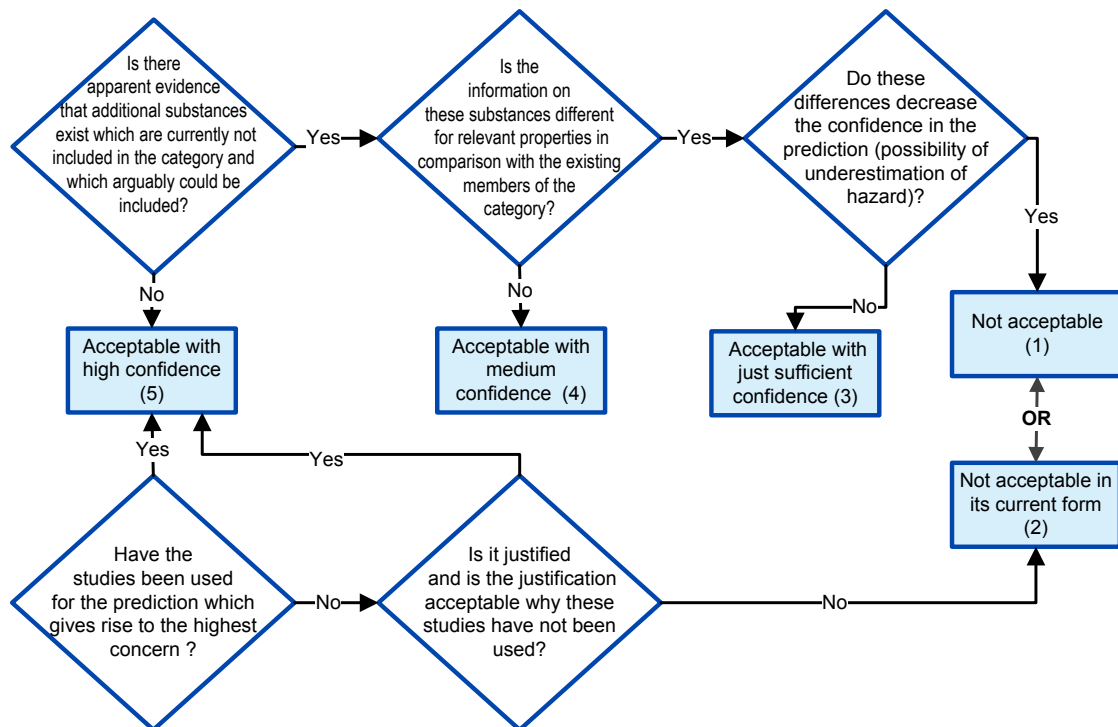
EXPLANATION

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

- when improper criteria in the category definition have been used which reduces the category members to exclude certain (otherwise) suitable members and leads 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.