Appendix B: Scenario 2

1.1   DESCRIPTION

This scenario covers the analogue 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 a study conducted with one source substance 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) or absence of effect is predicted. The predicted strength of the effects may be similar or based on worst case.

1.2   ASSESSMENT ELEMENTS FOR SCENARIO 2

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

Table B1: Assessment elements (AEs) for Scenario 2

AE #	AE TYPE	AE TYPE
AE A.1	Common	Characterisation of source substance
AE A.2	Common	Link of structural similarity and differences with the proposed prediction
AE A.3	Common	Reliability and adequacy of the source study
AE 2.1	Scenario-specific	Compounds the test organism is exposed to
AE 2.2	Scenario-specific	Common underlying mechanism, qualitative aspects
AE 2.3	Scenario-specific	Common underlying mechanism, quantitative aspects
AE 2.4	Scenario-specific	Exposure to other compounds than to those linked to the prediction
AE 2.5	Scenario-specific	Occurrence of other effects than covered by the hypothesis and justification
AE A.4	Common	Bias that influences the prediction

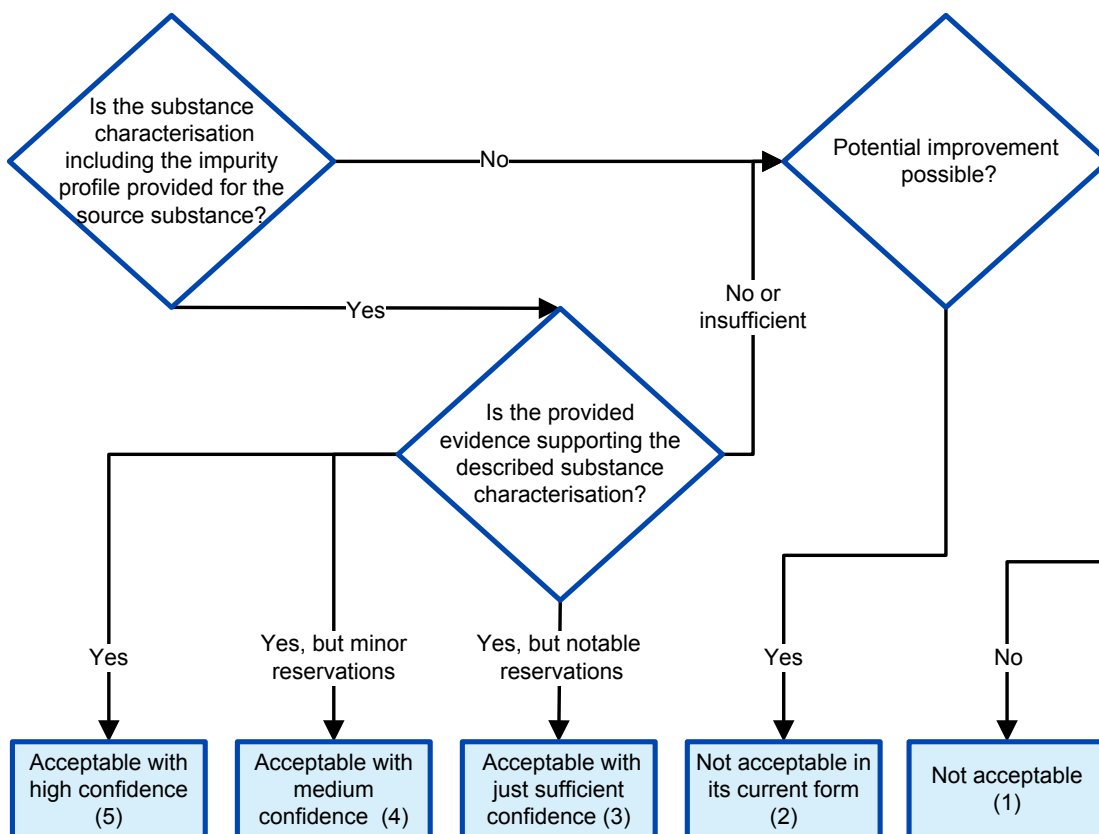## AE A.1 CHARACTERISATION OF SOURCE SUBSTANCE

PURPOSE

The substance which is used as the source substance[1] needs to have a clear substance characterisation.

It has to be assessed whether:

- the chemical identity of the analogue is sufficiently clear for a meaningful assessment of the proposed read-across; and

- the impurity profile is clear.

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

ASSESSMENT OPTIONS

Flowchart:

Is the substance characterisation including the impurity profile provided for the source substance?
- No → **Potential improvement possible?**
  - Yes → Not acceptable in its current form (2)
  - No → Not acceptable (1)
- Yes → **Is the provided evidence supporting the described substance characterisation?**
  - 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)
  - No or insufficient → Potential improvement possible?

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1 The test material actually used in a specific source study is addressed in AE A.3.

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 an analogue approach, the information provided on the identity of the source substance must establish a clear picture of its chemical structure. It is important that not only the chemical structures but also the impurity profiles of all source and target substances are well defined to establish the read-across hypothesis, since differences in impurities or stereochemistry can affect the activity and chemical properties. 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 group members, not only the substances which are registered.

The source substance should be described as comprehensively as possible and as a minimum[3] the following information should be provided (Guidance R.6.2.6.2):

- Name, CAS and/or EC number, chemical structure for the source substance; and

- Impurities profiles for the source substance (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 the source and target substances. This similarity is based on the main constituents of the source and target substances. However, toxicity may actually be determined by an impurity. The read-across hypothesis could be superficially convincing and could be supported by some data. Nevertheless, the read-across may still be invalid, because it does not take a difference in impurity profile of the source and target substances into account.

The relevance of the impurities for the prediction is assessed in AE 2.4

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

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

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

EXAMPLE(S)[4]

A.1.a   Example for an identity of the source substance which is clear and unambiguous and allows 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[5] (typical 1.0% water).

A.1.b   Example for an identity of the source substance which is clear and allows 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[5] 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 source substance is clear and unambiguous for read-across purposes.

A.1.c   Example for an identity of the source substance which is not clear and does not allow 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[5] is not provided.

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

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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.

5 The impurity profile of the actual test substance is addressed in A.3; the impact on the prediction is addressed in AE 2.4.

AE A.2 LINK OF STRUCTURAL SIMILARITY AND DIFFERENCES WITH THE PROPOSED PREDICTION

PURPOSE

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

It has to be assessed whether:

- the scientific hypothesis establishes the structural similarities and differences of source and target;

- structural similarities and differences are linked with the possibility to predict similar properties; and

- the provided evidence supports the proposed link between structural similarities and the possibility to predict.

ASSESSMENT OPTIONS
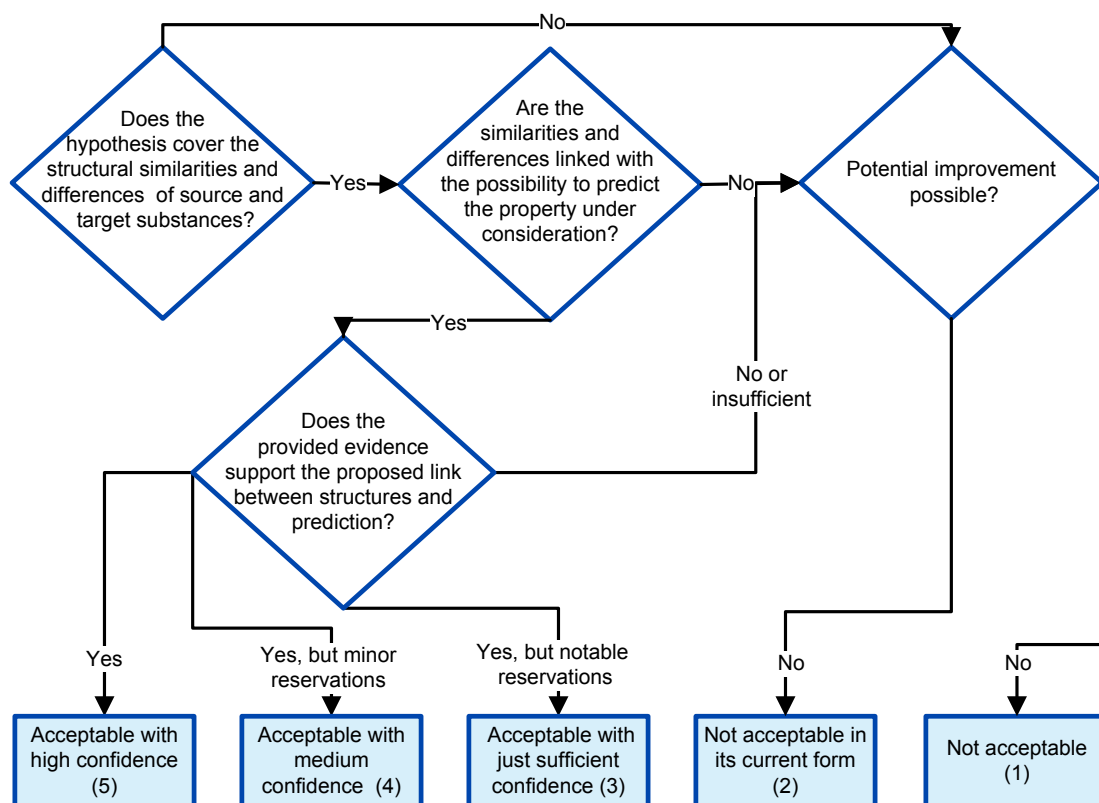
EXPLANATION

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

It should be understood:

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

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

The explanation should be based on recognition of the structural aspects the two structures have in common and the differences between the two structures. The possibility for predictions of similar properties should be linked to the common structural aspects.

EXAMPLE(S)[4]

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

*   Substances A and B are both alpha-olefins.

*   Substance A has a linear structure, substance B is branched.

*   The hypothesis does not address the branching of substance B.

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

AE A.3 RELIABILITY AND ADEQUACY OF THE SOURCE STUDY

PURPOSE

The source study needs to match the default REACH requirements in terms of reliability and adequacy as requested for any other key study.

It has to be assessed whether:

- The study design reported for the source study is adequate and reliable for the purpose of the prediction based on read-across:

  - The study design should cover the key parameters in the corresponding test method referred to in Article 13(3);

  - The study design should cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3); 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 identification), it may be assumed that the study results are adequate for the purpose of classification and labelling and/or risk assessment.

ASSESSMENT OPTIONS

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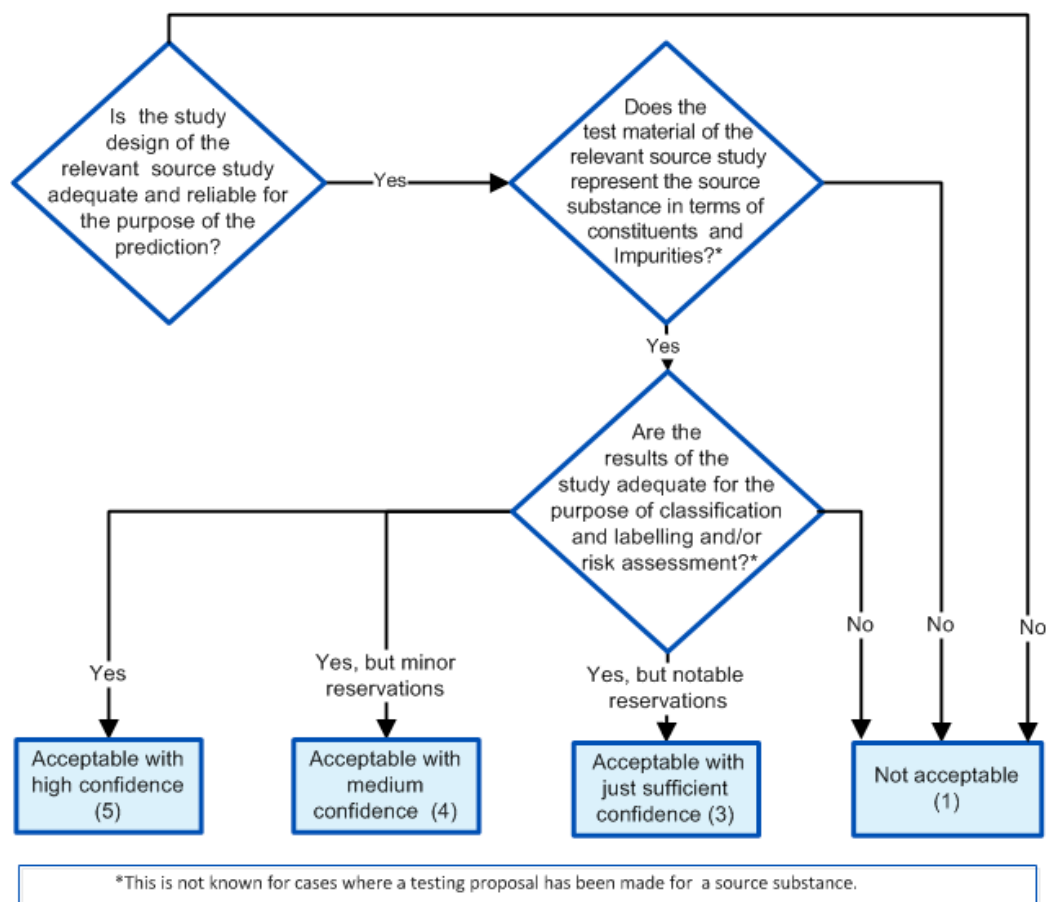
EXPLANATION

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 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, 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 assessing expert 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."*

EXAMPLE(S)[4]

A.3.a   Example for a source study not meeting the REACH information 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.

A.3.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.

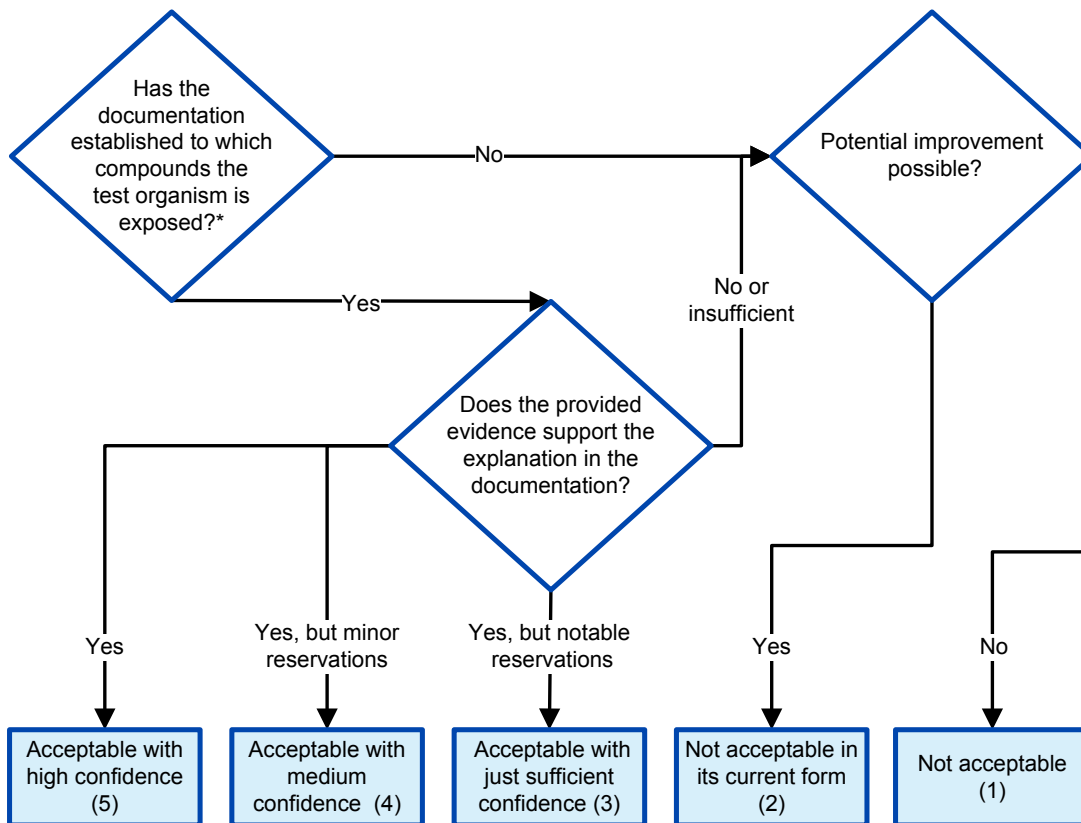## AE 2.1   COMPOUNDS THE TEST ORGANISM IS EXPOSED TO

PURPOSE

In this scenario, it is claimed that different compounds have the same effects for the property under consideration. Such different compounds maybe 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

| Has the documentation established to which compounds the test organism is exposed?* | Potential improvement possible? |
| 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) |

*The compounds the test organism is exposed to (after administration of target and source substance(s) 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 effect. To assess such a claim, it has to be clear to which compounds the test organism is exposed to.

In principle, these compounds can be:

- Target and source substances themselves;

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

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

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

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

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

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

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

EXAMPLE(S)[4]

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

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

- Substance A is used to predict a property of substance B.

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

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

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

- Substance B is absorbed, not (bio)transformed and eliminated unchanged in the urine.

- Substance B is used to predict a property of substance A.

In this situation, the test organisms are exposed to A, A1 and A2 after administration of A, and only to B after administration of B. The prediction needs to take into account the additional presence of A1 and A2.

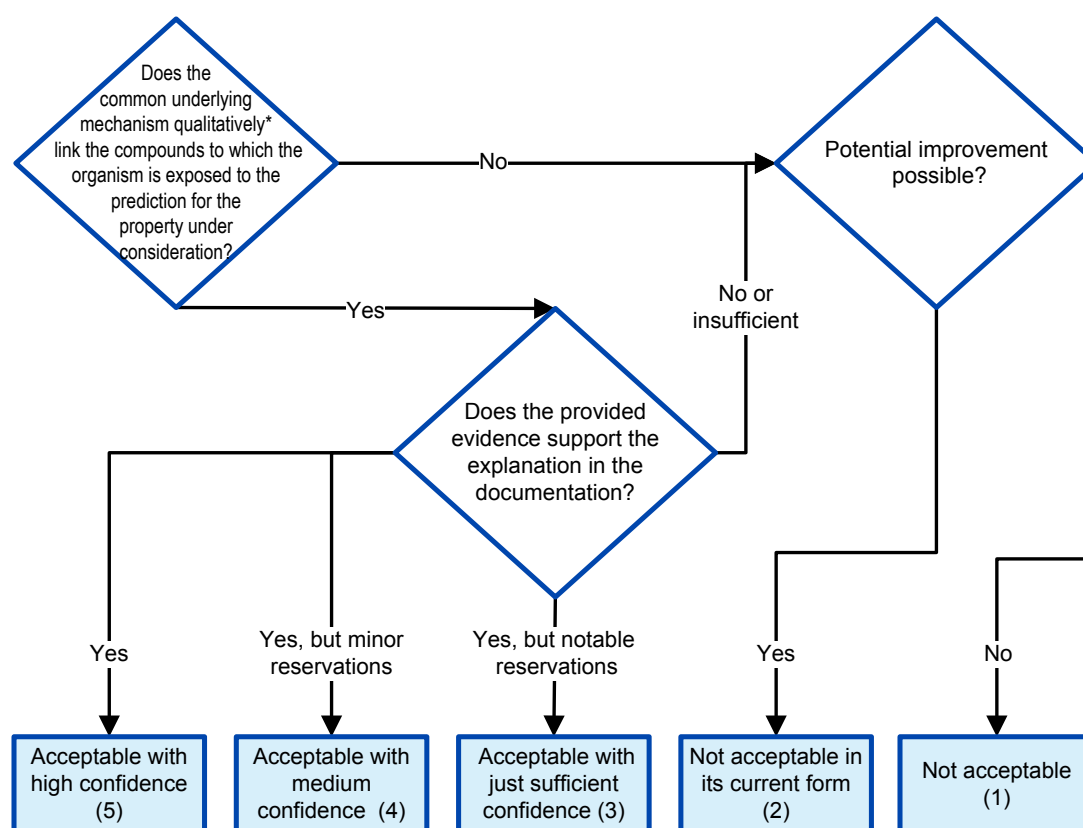## AE 2.2 COMMON UNDERLYING MECHANISM, QUALITATIVE ASPECTS

PURPOSE

The hypothesis/justification has to explain how the compounds the test organism is exposed to lead to the same type of effects/absence of effects.

It has to be assessed whether:

- the documentation has established a common underlying mechanism;

- this mechanism links the structures of the compounds under consideration with the possibility to predict qualitatively similar type of effects for the target substance for the property under consideration; and

- the provided evidence support the explanation.

ASSESSMENT OPTIONS

Does the common underlying mechanism qualitatively* link the compounds to which the organism is exposed to the prediction for the property under consideration?

Potential improvement possible?

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)

*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 substances. This mechanism should link the structures of the compounds under consideration with the possibility to predict qualitatively similar effects for the target substance.

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

Prediction of absence of effect

Specific considerations are needed in the case of predictions of absence of effects. In the current AE, only the principle qualitative aspects of such a prediction are covered, but also 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. Two theoretical possible reasons to predict the same absence of effects for the target:

   a) Significant exposure of target tissues is expected/proven, but no relevant toxicity predicted. This prediction then 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. on 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.

Effects observed for other properties

The nature of the effects for other properties should be carefully considered. It is not only about whether effects for other properties are found, but also about which effects are found and whether and how they might relate mechanistically to the effect to be read-across.

The comparison of effects described for other properties (than the predicted property) available in the data matrix should be consistent and show the same or similar toxicity profiles when compared between related endpoints of the source or target substance and between the source and target substance. Since the analogue approach relies on only one source substance, it is of particular importance that the toxicity profiles are consistent. Therefore, information for the target substance has to be present which allows

assessing whether it is likely that the same type of effect will be observed than in the study proposed to be used as source study.

However, a consistent data matrix on its own is unlikely to be a sufficient justification without an associated common underlying mechanism for this scenario (see AE 2.5).

EXAMPLE(S)[4]

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

- Substances A and B 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.

- Substance B has the same structural features compared to A, and has in vitro evidence that it acts via the same receptor as A.

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

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

- Substances A and B both contain a double carbon-bond and are both metabolised to epoxides.

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

- The epoxide formed from substance B has a similar chemical reactivity based on theoretical chemical considerations.

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

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

- Substance A has a high molecular weight and is very water soluble.

- It has been demonstrated by an oral toxicokinetic study with radioactive labelling that it is poorly absorbed.

- Substance B has a very similar structure and in vitro information indicates that oral absorption is not expected

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

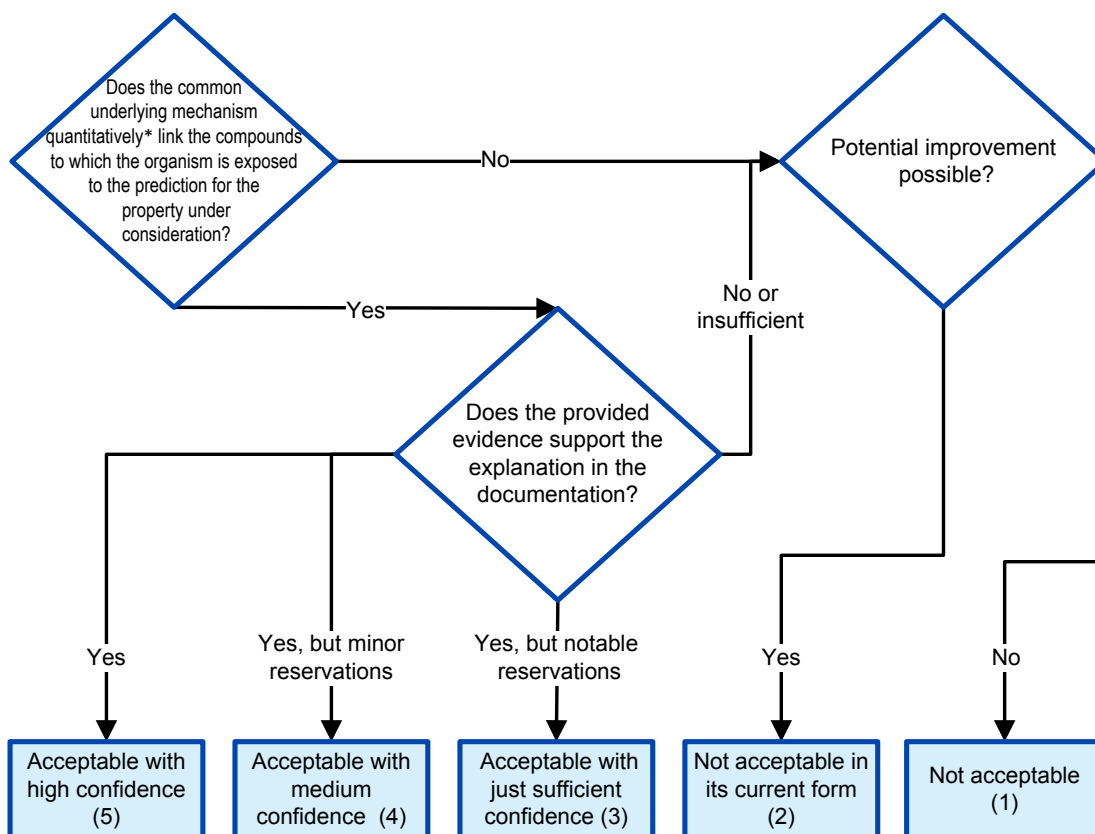## AE 2.3 FORMATION AND IMPACT OF NON-COMMON COMPOUNDS

PURPOSE

Under this scenario, there should be no biologically significant[6] quantitative differences for the same type of effects caused by the underlying mechanism or the differences should be used in a conservative prediction (i.e. the effects for the target substance are not likely to be under-predicted, worst-case approach).

It has to be assessed whether:

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

- the provided evidence supports the explanation.

ASSESSMENT OPTIONS

```
         ┌─────────────────────┐
         │ Does the common     │
         │ underlying mechanism │
         │ quantitatively* link │───── No ─────►  Potential improvement
         │ the compounds to     │                     possible?
         │ which the organism   │
         │ is exposed to the    │
         │ prediction for the   │
         │ property under       │
         │ consideration?       │
         └─────────────────────┘
                 │ Yes
                 ▼
         ┌─────────────────────┐
         │ Does the provided   │── No or insufficient
         │ evidence support    │
         │ the explanation in  │
         │ the documentation?  │
         └─────────────────────┘
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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)

*Are quantitatively the same effect(s) consistently observed for the source substance(s) and why are they likely to be observed also for the target substance at a similar effect level for the same biological targets?

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

EXPLANATION

Quantitative differences

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

If quantitative differences[7] are evident from information in the data matrix, they can have the following origins in principle:

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

- Differences in the potency.

Worst case

In many cases, such quantitative differences will reduce the confidence in the prediction. In some cases, they might be used in a conservative manner. The mechanistic explanation should justify the claim that the chosen source substance(s) indeed represents a worst case. This claim also has to be analysed in the AEs 2.4 – 2.5.

If a worst-case approach is claimed, it has to be justified that the source substance leads to a more severe effect (at equivalent dose) than predicted for the target substance. This could be due to differences in toxicokinetics, toxicodynamics or both. Such differences have their basis in the structural (or compositional) differences between source and target substances. Therefore, the case-specific supporting evidence can have a toxicokinetic nature and/or a toxicodynamic nature.

Supporting evidence

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

The comparison of effects described for other properties (than the predicted property) available in the data matrix should be consistent and show the same or similar toxicity profiles. However, a consistent data matrix on its own is unlikely to be a sufficient justification without an associated common mechanism (see also AE 2.5).

Predictions of absence of effects

It is important to address possible differences in the toxicity profiles by carefully analysing the information in the data matrix.

If the prediction of absence of effects is justified by absence of exposure (biological targets are not reached), the significance of possible small quantitative differences in exposure between source and target may need to be assessed.

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7 Differences are meant to be biologically relevant. Statistical analysis of data in studies might also lead to quantitative differences observed when different study results are compared. Such differences

have to be in the range of the normal statistical variation typically observed in the study type under consideration.

If the prediction of absence of effects is justified by absence or undetectable interaction with biological targets, the mechanistic explanation and the supporting evidence should outline why this absence of interaction applies to the target substance for the property under consideration. For a complex higher tier study type (e.g. a pre-natal developmental toxicity study) with multitudes of possible biological targets, which also change during the development, this is challenging.

See also the general considerations covered in AE 2.2.

EXAMPLE(S)[4]

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

- Substances A and B 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.

- Substance B has the same structural features when compared to A, and has in vitro evidence that it acts via the same receptor with a similar potency.

- Substance A has a 10-fold higher absorption rate and the absorbed amount compared to B is also higher.

- Substances A and B are expected to induce the same qualitative effects via this receptor interaction. At equivalent doses, the maximum concentration of A at the receptor sites is expected to be higher and to be reached faster compared to B.

If A is the source substance, it represents the worst case. If B is source substance, the hazard will be under-predicted.

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

- Substances A and B both contain a double carbon-bond and are metabolised to epoxides.

- The epoxide formed from substance A reacts with DNA in vitro but does not cause mutagenicity in an in vivo gene mutation assay.

- The epoxide formed from substance B has a similar chemical reactivity compared to A based on theoretical chemical considerations.

- It is known from in vitro experiments that S9-mix metabolises substances A and B at similar rates to the corresponding epoxides. However, the further detoxification of the epoxide formed from A is occurring immediately after formation. The detoxification of the epoxide formed from A is 10-fold faster compared with the epoxide formed from B. This results in a lower epoxide plasma concentration derived from A compared to the epoxide plasma concentration derived from B.

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

AE 2.4 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[8] 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 formed (e.g. via another (bio) transformation pathway or as intermediates) or are present as impurities (see AE A.1); and

- indications are available that such compounds could influence the prediction of the property under consideration.

ASSESSMENT OPTIONS

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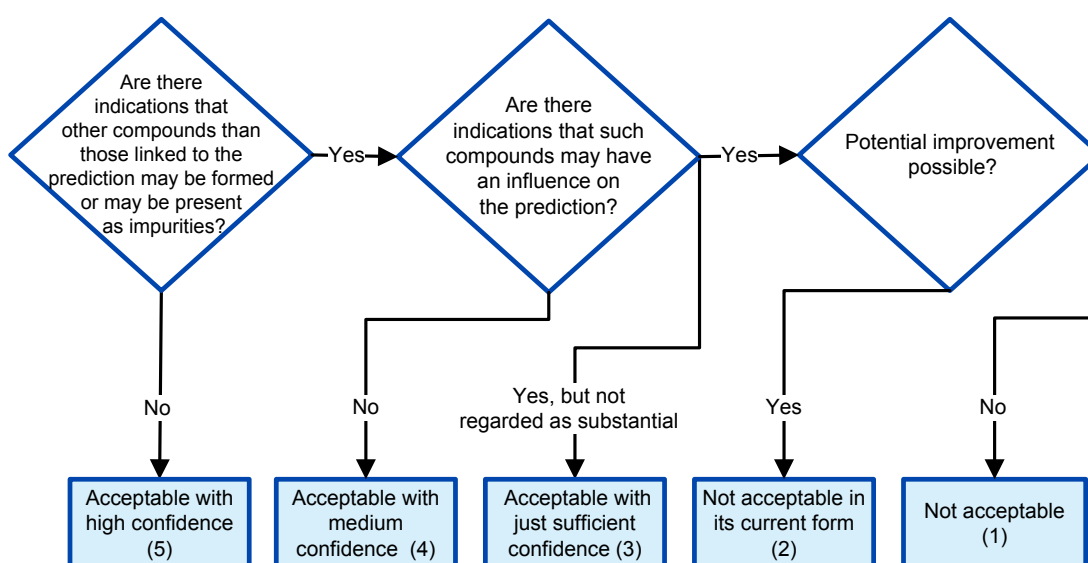
8 See substance characterisation, as addressed in AE A.1 for the source substance or registration dossier for the target substance.

EXPLANATION

In AE 2.1, the hypothesis has been assessed qualitatively with regard to compounds to which the organism is exposed. 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.

The confidence in the prediction may be decreased if such compounds formed by (bio)transformation from the source and/or target substance and have not been considered by the hypothesis. In addition to the information assessed in AE 2.1, this AE requires an insight into the possible toxicological properties of possible other compounds.

For the acceptance of the read-across approach, the other compounds should not influence the considered property. The lack of influence on the predicted property may be due to insignificant exposure of the biological target(s) or absence of relevant interaction with biological targets. The strength of the proposed mechanistic explanation and the associated evidence in the data matrix must be balanced against the uncertainties from the un-characterised toxicity of other compounds than those linked in the hypothesis to the prediction.

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

Importance of impurities

Toxicity of the source and target substance 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]

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

- According to the hypothesis substance A is absorbed and rapidly metabolised to A1 and A2.

- The hypothesis is that the toxicity of A is caused by A1 and the toxicity of A1 is similar to B.

- The structurally-similar substance B is absorbed and eliminated unchanged.

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

- Toxicokinetic knowledge reveals that A2 is formed via a pathway competing with the formation of A1 and thereby reducing the systemic concentration of A1 upon exposure to A.

This situation may lead to under-prediction of the toxicity of B at equivalent doses of A and B.

2.4.b   Example for a compound which was not identified and thereby not addressed by the hypothesis

- According to the hypothesis, substance A is absorbed and rapidly metabolised to A1.

- The hypothesis is that the toxicity of A is caused by A1 and the toxicity of A1 is similar to B.

- The structurally-similar substance B is absorbed and eliminated unchanged.

- There is toxicokinetic information available to the assessing expert that another metabolite A2 is formed from A.

- It is known that A2 causes a different toxicity pattern compared to A1.

- The results of a 90-day repeated-dose toxicity study conducted with substance B are used to predict the effects in a 90-day repeated-dose toxicity study with substance A.

The contribution of A2 to the toxicity profile of A is not addressed by the prediction.

AE 2.5   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.

- these additional mechanisms affect the prediction for the property under consideration.

ASSESSMENT OPTIONS
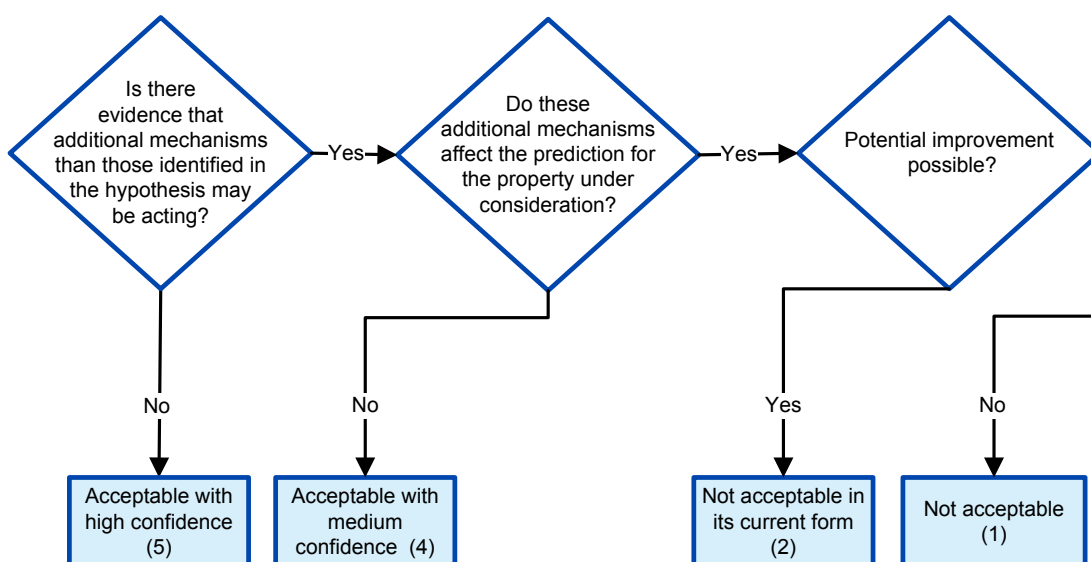
EXPLANATION

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

In the data matrix, quantitative and qualitative evaluation of the effects which have been reported may be indicative of such additional mechanisms. Effects other than those linked to the hypothesis have to be evaluated on a case-specific basis. Occurrence of such other effects may be 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.

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

EXAMPLE(S)[4]

2.5.a   Example for the absence of other mechanisms

- Substance A causes neurotoxicity which was observed in a 28-day repeated-dose toxicity study that included a functional observational battery, no other effects were observed.

- The structurally-similar substance B causes similar neurotoxic effects, no other effects were observed in the 28-day study with B.

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

- A 90-day repeated-dose toxicity study conducted with substance A is used to predict the toxicity of substance B in a 90-day repeated-dose toxicity study.

- The chemical structures of the substances do not indicate that other mechanisms are acting (no expert concern and no alerts in (Q)SAR analysis).

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

2.5.b   Example for indications of other mechanisms

- Substance A causes neurotoxicity which was observed in a 28-day repeated-dose toxicity study that included a functional observational battery, no other effects were observed.

- Substance B caused dose dependent neurotoxic effects similar to A in a 28-day study. At the highest dose in this study, a decrease of relative thymus weight was observed. This finding was accompanied with a decrease of the white blood cell count.

- A 90-day repeated-dose toxicity study conducted with substance A is used to predict the toxicity of substance B in a 90-day repeated-dose toxicity study.

- The chemical structures of the substances do not indicate that other mechanisms are acting (no structural alerts in (Q)SAR analysis).

- However, the mechanism of the thymus weight decrease is not known and it cannot be excluded that in studies with a longer duration than 28-days immunotoxicity may appear at lower dose levels.

Based on this data set, the prediction may only be acceptable if further explanation for the thymus toxicity is provided.

AE A.4 BIAS THAT INFLUENCES THE PREDICTION

PURPOSE

It has to be assessed whether:

- it is clear from the documentation how the source substance(s) have been chosen, for example, what methods/tools have been used to map the field of potential source substance(s), 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 analogue approach and which arguably could be used;

- there is readily-available information from these additional substances;

- this information is biologically significantly different for relevant properties in comparison with the existing analogue(s); and

- these differences decrease the confidence in the prediction (possibility of underestimation of hazard).

It also has to be assessed whether:

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

ASSESSMENT OPTIONS
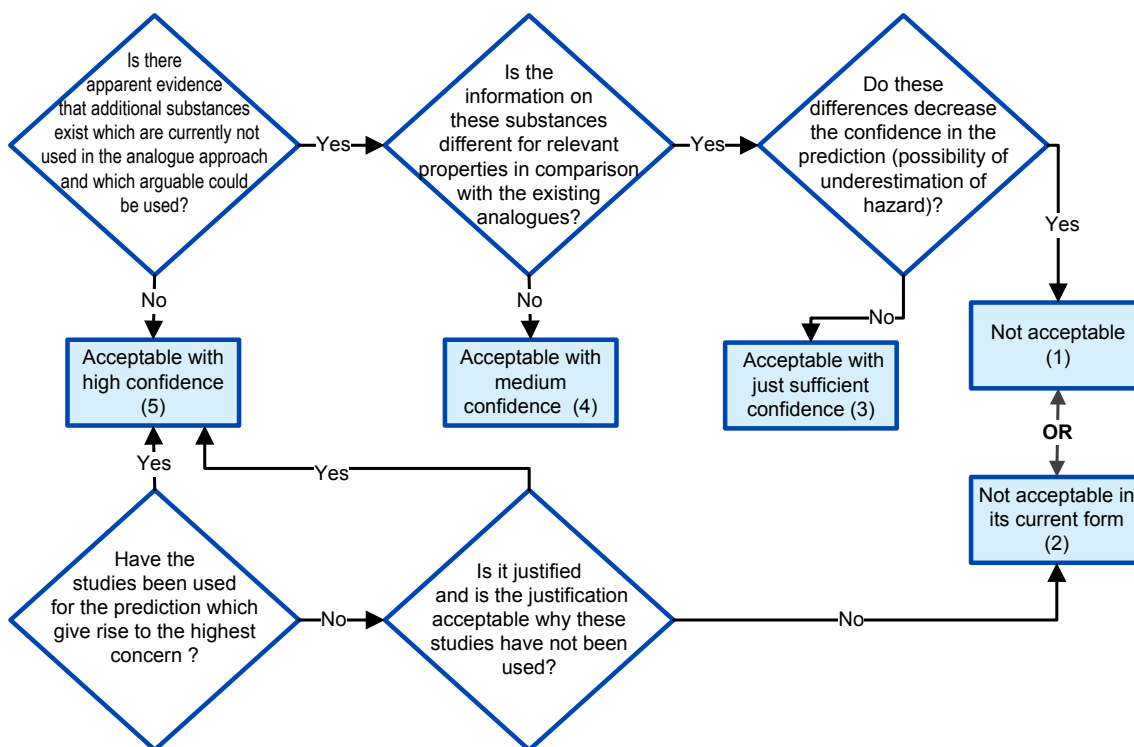
EXPLANATION

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

Such a situation may occur:

- when there are multiple possible analogues with equivalent structural similarity; or

- the assessing expert has knowledge of such additional structurally-similar analogue(s).

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

In addition there might be selection bias for the study used for the prediction when several studies are available in the data matrix. According to 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.