# Essential Aspects of Read-Across for Repeated-Dose Toxicity Predictions

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## Introduction

Read-across based on chemical grouping is often proposed as means of **data gap filling for chemical safety assessments**. However, the lack of agreement on how to carry out read-across hampers acceptance by regulatory authorities.

One of the key aspects of performing a read-across is the confirmation that the source and target substance(s) belong to the same category and can be considered to be toxicologically similar. In read-across for repeated-dose toxicity, **similarity assessment** takes the form of comparing **chemical, toxicodynamic and toxicokinetic properties**. The level of evidence required to accept similarity arguments is not defined and may not be quantifiable. Thus, one is left with **uncertainties**. While there are many areas where dissimilarity can be identified and uncertainty can be defined, there is no agreement when dissimilarity is toxicologically relevant or the uncertainty warrants rejection of the prediction(s).

While read-across is conceptually simple, in practice it is difficult, especially for complex health endpoints such as repeated-dose toxicity. Essential aspects to applying read-across to repeated-dose endpoints have been identified and are summarised in this poster.

## Limitations Leading to Uncertainties

The limitations to read-across for repeated-dose toxicity include:

- **Difficulty to prove toxicologically-relevant similarity**. To justify similarity, consideration must be given not only to molecular structure and physico-chemical properties, but also biological similarity, similarity of the mechanism of toxicity, toxicodynamics, toxicokinetics, bioavailability and bio-modifications.
- The **availability of suitable *in vivo* data** to be read across
- The **lack of toxicokinetics understanding and data**
- The lack of toxicologically-relevant *in vitro* or alternative New Approach Methodology data to support the toxicodynamics and toxicokinetics.

These limitations lead to uncertainties in the read-across argument.

→ **How to reduce uncertainties?**

## Data Quality

Data quality must be addressed, specifically:

- The quality of the endpoint data to be read across and the supporting data, their reliability and relevance
- Understanding of the assays (study conditions, assay performance); especially new methods, their performance and related variabilities and uncertainties.

→ **How much detail to describe study and assay results is appropriate in the data matrix? How to assess quality?**

## Contribution of New Methods Data

Uncertainty of read-across predictions may be reduced by considering New Approach Methodology data. These may:

- Strengthen the mechanism plausibility
- Strengthen the Weight of Evidence for category membership
- Allow for targeted testing to define boundaries of categories.

→ **How can Adverse Outcome Pathways (AOPs) contribute to reducing uncertainties?**



## Essential Considerations for Read-Across

- Read-across arguments, i.e. the similarity rationale and the logic leading to the read-across must be
  - **transparent** and
  - thoroughly and adequately **documented**,

  so it can be retraced.
- **Uncertainty must be assessed and described** for the similarity and the read-across arguments.
- While it is not always possible to definitively state a mode-of-action, less uncertainty is linked directly to **strong mechanism plausibility**.

→ **How to strengthen the Weight of Evidence?**

→ **What level of uncertainty is acceptable for which purpose?**

Example of templates guiding the user through the collection of information required and systematic uncertainty assessment:

Schultz TW et al (2015) A strategy for structuring and reporting a read-across prediction of toxicity. *Reg. Toxicol. Pharmacol.* 72: 586–601

Comparison of substituents, functional groups, and extended structural fragments.

| | Target Substance | Analogue 1 | Analogue 2 |
|---|---|---|---|
| Name | | | |
| Key Substituent(s) | | | |
| Functional Group(s) | | | |
| Extended Fragment(s) | | | |
| Chemical Class: | | | |
| Chemical Sub-Class: | | | |
| Chemical Sub-Class: | | | |

Comparison of mechanistic plausibility and AOP-related event data.

| | Target Substance | Analogue 1 | Analogue 2 |
|---|---|---|---|
| Name | | | |
| Mechanistic Plausibility | | | |
| Adverse Outcome Pathway or Mode of Toxic Action: | | | |
| Molecular Initiating Event: | | | |
| Key Event 1 etc.: | | | |
| Key Event Relationship 1 etc.: | | | |

Template for assessing uncertainty associated with mechanistic relevance and completeness of the read-across.

| Factor | Uncertainty (low, medium, high) | Comment |
|---|---|---|
| The problem and premise of the read-across | | **Example:** The endpoint to be read across, developmental toxicity, for the category of branched carboxylic acids is well-studied and well-understood. The scenario of the read-across hinges on the inhibition of beta-oxidation of the acid and the subsequent build up of acid in the embryo leading to histone deacetylase inhibitors, increased cell adhesion and concomitant reduced cell motility, prevention of convergent extension during ontogenetic development. |
| **In vivo data read across** | | |
| Number of analogues in the source set | | **Example:** There are 3 suitable category members with *in vivo* apical endpoint data usable for read-across. |
| Quality of the *in vivo* apical endpoint data read across | | **Example:** High quality empirical data from standard test guidelines for the stated regulatory endpoint exists for 1 category member. Similar non-standard test data of lower quality exists for 2 other category members. All these data are consistent in regards to qualitative description of effects and, where available, similar in quantification. |
| Severity of the apical *in vivo* hazard | | **Example:** Potency data for the *in vivo* apical endpoint (25 mg/kg/day) is limited to a single source substance. |
| **Evidence to biological argument for RA** | | |
| Robustness of analogue data set | | **Example:** The available data from *in silico*, *in chemico* and *in vitro* studies for the category members were judged to be reliable and conducted under the appropriate conditions. |

Example of structured assessment of a read-across argumentation: the ECHA Read-Across Assessment Framework (RAAF):

| | |
|---|---|
| AE C.1 | Substance characterisation |
| AE C.2 | Structural similarity and differences within the category |
| AE C.3 | Link of structural similarities and structural differences with the proposed regular pattern |
| AE C.4 | Consistency of effects in the data matrix |
| AE C.5 | Reliability and adequacy of the source study(ies) |
| AE C.6 | Bias that influences the prediction |

| SCENARIO 4 | SCENARIO 6 | ASSESSMENT ELEMENT TITLE |
|---|---|---|
| AE 4.1 | AE 6.1 | Compounds the test organism is exposed to |
| AE 4.2 | AE 6.2 | Common underlying mechanism, qualitative aspects |
| AE 4.3 | AE 6.3 | Common underlying mechanism, quantitative aspects |
| AE 4.4 | AE 6.4 | Exposure to other compounds than those linked to the prediction |
| AE 4.5 | AE 6.5 | Occurrence of other effects than covered by the hypothesis and justification |

## Transparent Documentation

1. Statement of the regulatory endpoint(s)
2. Statement of the read-across hypothesis
3. List of all the substances
4. Data matrices of relevant:
   - common chemical factors
   - *in vivo*, toxicokinetic, metabolic, *in vitro* data
   - structure-activity information
5. Statement of uncertainty
6. Statement of the conclusions

## Similarity Assessment

### Category Membership

1. Chemical similarity
   - Physico-chemical and molecular properties
   - Substituents, functional groups and fragments
   - 2D molecular similarity
2. Biological and toxicological similarities
   - Structural alert, toxicophores and Molecular Initiating Events (MIEs)
   - AOP and key intermediate events
   - *In vitro* data relevant to the apical endpoint
3. Toxicokinetics and bio-modifications
   - Metabolic pathways
   - Activation or degradation

→ **When is similarity sufficient and toxicologically relevant?**

## Assessment and Description of Uncertainties

- Data uncertainty and Weight of Evidence associated with the fundamentals of chemical, transformation / toxicokinetic and toxicological similarity
- Uncertainty associated with mechanistic relevance and completeness of the read-across

→ **Guidance needed for specific details in read-across execution and documentation?**

## Conclusions

To carry out read-across in practice with a view to supporting risk assessment, more information and work is needed to address the

- Definition of uncertainties and the level of uncertainties acceptable
- Role of New Approach Methodology Data to strengthen the Weight of Evidence.

Ongoing work illustrating the use of the RAAF will be helpful to guide the optimal way to present and document the read-across data and arguments, in view of regulatory submission.

* The views expressed are solely those of the authors and the contents of this poster do not necessarily represent the views or position of the European Commission.

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