

SEURAT-1 Proof-of-Concept Read-Across Case Study for Repeated-Dose Toxicity

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Introduction and Aim

The SEURAT-1 Research Initiative with the long-term goal of achieving "Safety Evaluation Ultimately Replacing Animal Testing" aimed at finding alternative approaches for the safety assessment for repeated-dose toxicity. Within the framework of proof-of-concept case studies for applied safety assessment, SEURAT-1 investigated the practical application of the read-across approach for repeated-dose toxicity.

The concept of category formation, chemical grouping and of read-across is used to support chemical safety assessment by filling data gaps. It is based on the inference of properties of a chemical substance, including its toxicity, from similar chemicals with known properties.

Read-Across Strategy

In a first step, a strategy for structuring and reporting read-across for repeated-dose toxicity predictions was developed (Schultz et al 2015). Templates guide the user through the collection of data for building the chemical category and constructing the similarity argument and support the transparent documentation. Emphasis was given to the description and assessment of the uncertainty of the similarity rationale, the read across data and the overall approach and conclusion, in order to make an informed decision about the read-across prediction result.

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.

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

Similarity Parameter	Data Uncertainty * (low, medium, high)	Strength of Evidence * (low, medium, high)	Comment
Substance Identification, Structure and Chemical Classification			Example: All category members have CAS numbers, similar 2D structure and belong to the same chemical class/subclass.
Physico-Chemical & Molecular Properties	Empirical: Modelled:		Example: All category members are appropriately similar with respect to key physicochemical and molecular properties. There is a high degree of consistency between measured and model estimated values.
Substituents, Functional Groups, & Extended Structural Fragments			Example: Substituents, functional groups and extended structural fragments are consistent across all category members.
Transformation/Toxicokinetics and Metabolic Stability	Empirical: <i>In vivo</i> ; <i>In vitro</i> ; Simulated:		Example: Based on <i>in vivo</i> and <i>in vitro</i> data for multiple category members, there is evidence for similar toxicokinetics and metabolic pathways. Comparison of results from empirical studies and model predictions indicate similar metabolism among all category members.
Potential Metabolic Products			Example: Based on <i>in silico</i> metabolic simulations, potential metabolic products are similar among all category members.
Toxicophores/Mechanistic Alerts			Example: Based on <i>in silico</i> profilers, all category members contain the same toxicophores.
Mechanistic Plausibility and AOP-Related Events			Example: Although no AOP is currently available for the hypothesized toxicity pathway, many category members have been tested for what is generally accepted as a mechanistically-relevant event leading to the <i>in vivo</i> apical outcome of interest (a citation could be provided).
Other relevant, <i>in vivo</i> , <i>in vitro</i> and <i>in silico</i> endpoints			Example: Although not part of the hypothesized toxicity pathway, many category members have been tested for recent acute oral toxicity and there is general agreement among the reported LC50 values.

With particular reference to regulatory submissions, the category formation and read-across process has to be **transparent, reproducible and clearly documented**; key principles of biological and chemical similarity need to be supported by scientific literature and data.

While there can be an over-arching rationale for grouping organic substances based on **molecular structure and chemical properties**, further information is often required to justify the chemical grouping and read-across prediction, including **considerations of bioavailability, metabolism and mechanistic plausibility**. Sources of uncertainty must be addressed and include a variety of elements divided into two main issues: uncertainty associated with the similarity justification and completeness of the read-across argument.

SEURAT-1 Read-Across Case Study Scenarios

Based on the outcome of a SEURAT-1 initiated workshop with external experts, case studies were conceived for four chemical similarity scenarios (Berggren et al 2015) to evaluate the practical application of the read-across strategy and uncertainty assessment, and in particular appraise the possibility of reducing uncertainty by taking into consideration "new approach" data. The case studies focussed on repeated-dose liver toxicity, where applicable.

The four scenarios consider similar chemicals:

- not requiring/undergoing metabolism to exert a potential adverse effect → **perfluorinated alkyl acids**
- metabolised to the same/similar toxicant (metabolite) → **β-unsaturated alcohols**
- with general low or no toxicity → **saturated alcohols**
- with markedly different potency or effects → **alkyl-substituted phenols**

Berggren E et al (2015) Chemical safety assessment using read-across: assessing the use of novel testing methods to strengthen the evidence base for decision making. Environ. Health Perspect. 123: 1232–1240

The case studies took into account available chemical, mechanistic, existing *in vivo* and non-test information, and carefully assessed the uncertainty, both for the category similarity and the overall read-across prediction. Furthermore, "new approach" data such as *in vitro* and *in silico* data from the SEURAT-1 initiative, as well as ToxCast, were evaluated for their ability to reduce the uncertainty and strengthen the read-across argumentation.

Templates guiding the user through

- the collection of data necessary to build and underpin the similar categories
- a systematic uncertainty assessment, both for the category similarity and the overall uncertainty of the read-across prediction.

Uncertainty of read-across

predictions can be reduced by added value in the form of increased WoE. This added value may come from suggestions of how targeted testing and "new-approach" data, using the logic of the SEURAT-1 conceptual framework, may be used to improve the read-across justification.

Factors Affecting Uncertainty: Mechanistic Relevance and Read-Across Completeness

1. What is the level of complexity of the read across endpoint? What is the purpose of the exercise? What is the over-arching premise and scenario?
2. Number of source chemicals and their relative applicability domain(s); is it an analogue-or category-based read-across?
3. Absence/presence of toxicity and relevant mechanisms, e.g. whether mechanisms can be defined for non/low toxicity compounds.
4. Quality of the *in vivo* apical endpoint data read across, performance (e.g., reliability, accuracy, precision, repeatability and reproducibility). Is the data to be read across sufficient to meet the purpose of the exercise?
5. Consistency in the severity of the apical *in vivo* hazard, consistent among the source chemicals?
6. Robustness of the (*in chemico*, *in vitro* and/or other) data sets. How extensive are the relevant events empirically measured/modelled? What is the method performance (reliability, reproducibility)?
7. Concordance of the *in chemico*, *in vitro* and/or other data with regard to the intermediate and apical effects and potency data. What is the temporal and dose-response relationship between mechanistically-relevant endpoints?
8. The overall Weight-of-Evidence (WoE) supporting the prediction. How many and how large are the mechanistically-related data gaps?

Scenario	Category Members	Weaknesses and Uncertainties	New Approach Data
• Perfluorinated alkyl acids	• Straight-chain perfluorinated carboxylic acids of intermediate size (C7-C10)	• Mechanistic plausibility of PPAR activation leading to repeated dose liver toxicity • Effect of analogues, species and sex on renal resorption and clearance	• Toxicogenomics and <i>in vitro</i> binding studies support PPAR-linked mechanism • ToxCast data and new approach virtual screening procedure confirm PPAR activity for PFOA/high molecular weight analogues
• β-Unsaturated alcohols	• Primary and secondary, straight-chain or branched, C3-C6	• Variation in potency • Variation in metabolic routes • Not many data	• New SEURAT-1 <i>in vitro</i> data, e.g. Hepatic Stellate Cell Activation, confirm mechanism, initiation of fibrosis
• Saturated alcohols	• Primary C5 to C13 alkanols	• Proving a negative prediction • Uncertainty associated with metabolism	• ToxCast data and <i>in silico</i> profilers confirm no specific bioactivity

Findings to Date and Conclusions

- Read-across potentially provides a solution to prediction of "complex" toxicities.
- Assessment of quality and quantity of the endpoint data is critical.
- Similarity must be assessed based on chemical, toxicokinetic and toxicodynamic considerations.
- Toxicodynamic uncertainty may be addressed by new methods information.
- Toxicokinetic uncertainty, especially metabolism, is often the limiting factor.
- SEURAT-1 contributed to developing read-across predictions:
 - A strategy and workflow/templates to support documentation
 - Case studies using "new approach" data to reduce uncertainty.