ICCS | INTERNATIONAL COLLABORATION ON COSMETICS SAFETY

Best Practice Guidance Document

Read-Across

June 2026

ICCS

Foreword

The International Collaboration on Cosmetics Safety (ICCS) established in 2023, is a global initiative, headquartered in New York, focused on advancing the adoption of animal-free assessments of cosmetics, and their ingredients, for human health and environmental safety.

ICCS brings together scientists and experts from cosmetics manufacturers and suppliers, industry and research associations, and animal protection organizations to drive greater global awareness and confidence in animal-free science through scientific research, capacity-building, and regulatory engagement. Building on nearly four decades of progress in the development, evaluation, and use of animal-free approaches, ICCS aims to accelerate the transition to animal-free safety science through widespread adoption and use.

ICCS publishes Best Practice Guidance documents and makes these available on the ICCS website, free of charge. These can be accessed at: https://www.iccs-cosmetics.org/education/best-practice-guidance.

For questions about the BPG, please contact ICCS at info@iccs-cosmetics.org.

About this Best Practice Guidance

This document was prepared by ToxMinds on behalf of the International Collaboration on Cosmetics Safety (ICCS). It has been extensively reviewed and informed by input from ICCS experts within the Priority Team: Best Practice Development and Priority Team: Human Health Systemic Toxicity and Developmental and Reproductive Toxicity, and the relevant Activity Teams organized under them.

Version

v1.0 (June, 2026)

v1.1 (September, 2026)

What's New in Version 1.1

Minor updates in this version include:

- Update to Figure 5 arrows to improve clarity.
- Addition of reference and suitability clarification (Ellison et al., 2025) on p30.
- Minor editorial revisions made throughout the document.

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Executive Summary

This Best Practice Guidance (BPG) document provides insights into using a read-across approach for both human health and environmental safety. The document aims to increase the use and acceptance of read-across by providing a structured workflow for safety assessors. The primary goal of this document is to inform hazard and safety assessments for both human health and environment without the need for new animal testing.

This BPG explains the key principles and steps involved in read-across based on the analogue and category approach, namely problem formulation, analogue identification and evaluation, suitability assessment and characterization and mitigation of potential uncertainties related to the chosen analogue(s). The importance of documentation and transparency is emphasized, with guidance on reporting input variables, assumptions, selection criteria, and uncertainties. The workflow consists of 10 steps and follows an iterative process.

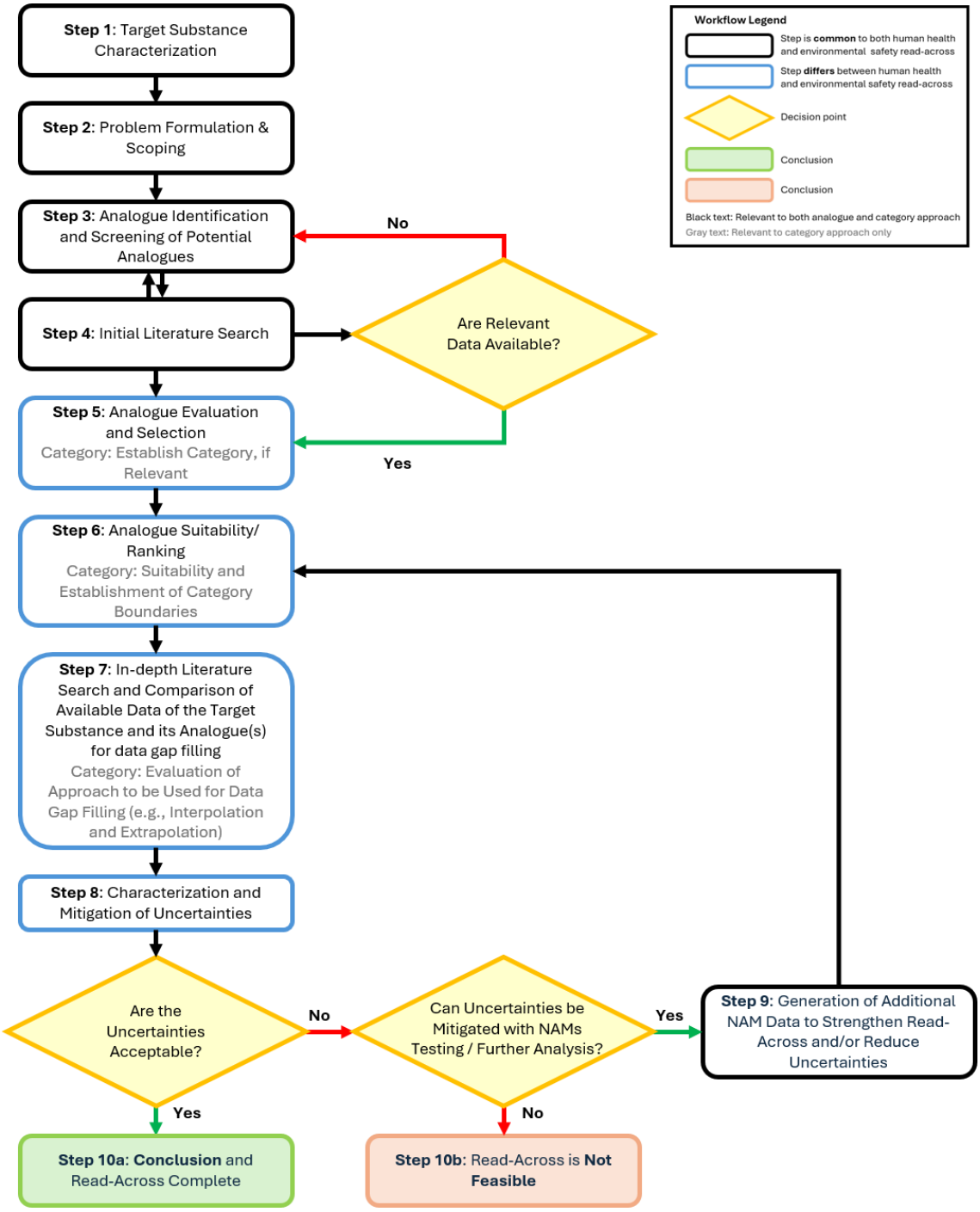
The objectives of each step of the workflow are listed below.

Step 1. Target Substance Characterization	Collect data on the target substance, including physico-chemical properties, as well as existing hazard and potency data.
Step 2. Problem Formulation & Scoping	Define the data gap(s), identify those (eco)toxicological or environmental fate endpoints for which a read-across approach will be used, and provide a clear statement of the assessment scope.
Step 3. Analogue Identification and Screening of Potential Analogues	Search for all potential analogue candidates using a variety of approaches, which may include similarity indices, matched molecular pair (MMP) analysis, comparison of functional groups and scaffolds, and other approaches.
Step 4. Initial Literature Search for Data Gap Filling	Conduct an initial literature search on the potential analogues identified in **Step 3** to select those for which there are available (eco)toxicological or environmental fate data suitable for filling the established data gaps.
Step 5. Analogue Evaluation and Selection	Evaluate the suitability of potential analogues for read-across purposes by comparing key attributes important for (eco)toxicological or environmental fate endpoints, including structural similarity, structural alerts, functional groups, physico-chemical properties and (bio)transformation/biodegradation. In case of multiple analogues, apply the grouping approach and determine whether a category can be established.

Step 6. Analogue Suitability/Ranking	Assess analogue quality based on the comparison of key attributes evaluated in **Step 5** and, finally, rank the analogues as Suitable, Suitable with Uncertainties, or Not Suitable. In case a category is established, determine the category member suitability as well as the category boundaries.
Step 7. Comparison of the (Eco)Toxicological or Environmental Fate Endpoint Data of the Target Substance and the Analogue(s)	Perform an in-depth literature search and compare the available data of the target substance and its analogue(s) to determine whether the target substance and the analogue(s) present a similar (eco)toxicological and/or environmental fate profile. For the category approach, determine the most appropriate strategy for data gap filling within the established category (e.g., interpolation or extrapolation).
Step 8. Characterization and Mitigation of Uncertainties	Specify and characterize potential uncertainties related to the selected analogue(s) or category members including uncertainties associated with category boundaries, trend consistency, and data gap filling approaches, and if necessary, define a targeted NAM-based strategy to mitigate them.
Step 9. Generation of Additional NAM Data to Strengthen Read-Across or Reduce Uncertainties	Conduct a NAM-based strategy to address the potential uncertainties with the overall aim to strengthen the read-across hypothesis.
Step 10. Conclusion and Documentation	Document the entire process in a transparent manner, including the similarity between the target substance and the analogue(s) or category members in all attributes considered, the reliability of the source study(ies) and any potential bias that could affect the read-across strategy.

This guidance is designed for safety assessors. It assumes a baseline understanding of read-across and safety assessment, and it follows Next Generation Risk Assessment (NGRA) principles. This BPG was developed through an iterative process involving literature reviews and expert input. The guidance outlined herein is based on current best practices and will be updated as scientific advancements occur.

This document provides background information on read-across and outlines the key steps in developing a read-across approach. By following the approach laid out in this BPG, safety assessors can confidently conduct a read-across assessment and identify resources for further details, if needed.



Overview of the Read-Across Process

Note: As needed, the safety assessor can iterate through the steps of this process, making the workflow flexible and suitable for different scenarios. NAM = New Approach Methodology.

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Acronym List

3Rs	Replace, Reduce or Refine
AEs	Assessment Elements
AOP	Adverse Outcome Pathway
BCF	Bioconcentration Factor
BPG	Best Practice Guidance
BSEP	Bile Salt Export Pump
CAS	Chemical Abstracts Service Registry Number
CMP	Chemicals Management Plan
CRED	Criteria for Reporting and Evaluating ecotoxicity Data
DEG	Differentially Expressed Gene
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
ECOSAR	Ecological Structure Activity Relationships
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EPI Suite	Estimation Programs Interface
EU	European Union
FET	Fish Embryo Acute Toxicity
GLP	Good Laboratory Practice
GenRA	Generalized Read-Across Approach
HPV	High Production Volume Challenge
HSE	Health and Safety Executive
HTS	High Throughput Screening
IATA	Integrated Approaches to Testing and Assessment
ICE	Integrated Chemical Environment
INCI	International Nomenclature Cosmetic Ingredient
ITS	Integrated Testing Strategy
LRI	Long Range Initiative
PBK	Physiologically Based Kinetic
MATE	Multidrug and Toxic Compound Extrusion
MMP	Matched Molecular Pair
MoA	Mode of Action
NAMs	New Approach Methodologies

NRU	Neutral Red Uptake
OECD	Organisation for Economic Co-operation and Development
OPERA	OPEn structure–activity/property Relationship
OAT	Organic Anion Transporter
PAMPA	Parallel artificial membrane permeability assay
PBK	Physiologically Based Kinetic
PBT	Persistence – Bioaccumulation – Toxicity
(Q)SAR	(Quantitative) Structural-Activity Relationship
RAAF	Read-Across Assessment Framework
RADAR	Rapid Androgen Disruption Activity Reporter
RAJ	Read-Across Justification
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REACTIV	Rapid Estrogen Activity *In Vivo*
SCCS	Scientific Committee on Consumer Safety
SeqAPASS	Sequence Alignment to Predict Across Species Susceptibility
SMILES	Chemical Abstracts Service Registry Number
TK	Toxicokinetics
TSCA	Toxic Substances Control Act
US	United States
VEGA	Virtual Models for Property Evaluation of Chemicals within a Global Architecture
XETA	Xenopus Eleutheroembryonic Thyroid Assay

1 Introduction

1.1 Purpose and Scope

The purpose of this best practice guidance (BPG) document is to provide insight into the use of a read-across approach to inform human and environmental safety assessments.

The goal of this document is to increase the use and acceptance of read-across by providing a 10-step workflow that safety assessors can follow. This document provides guidance associated with the workflow to facilitate the development of a read-across approach, without the need for conducting additional animal testing.

The guidance covers key areas to consider when using a read-across approach, including problem formulation, analogue identification and analogue suitability assessment, as well as the characterization and mitigation of potential uncertainties related to the choice of the analogue(s), and the documentation of the read-across approach in a transparent manner.

The guidance is intended to be applicable to the use of an analogue and a category read-across approach for the evaluation of substances for both human health and environmental safety. While the focus is on assessing one substance at a time, the approach may also be applied to multi-constituents (e.g., botanicals) through grouping of constituents with similar chemistry or functionality.

The decision-making guidance refers to current best practices based on the state-of-the-science available at the date of publication (June, 2026). However, this area is one of rapid scientific advancements, and, as such, this guidance will be updated as deemed necessary

Finally, although a safety assessor may use this document to inform read-across-based assessments for regulatory purposes, agency-specific considerations are beyond the scope of this document.

1.2 Intended Audience

This guidance is intended to be used globally by safety assessors with some or limited experience in using a read-across approach in the context of a human health and/or environmental safety assessment. This guidance assumes that the safety assessor has a baseline understanding of read-across fundamentals.

1.3 Overview of Next Generation Risk Assessment (NGRA) Principles

This document covers both hazard and safety assessments using a read-across approach, aligned with NGRA principles. In 2017, nine NGRA principles were published by a working group convened under the auspices of the International Cooperation on Cosmetics Regulation (ICCR) (Dent *et al.*, 2018; ICCR, 2017). These principles,

outlined below, were identified to assist in developing integrated safety assessments without generating additional animal data.

1. Overall goal is safety assessment.
2. Assessment is exposure-led.
3. Assessment is hypothesis driven.
4. Assessment is designed to prevent harm.
5. Assessment follows an appropriate appraisal of all existing information.
6. Assessment uses a tiered and iterative approach.
7. Assessment uses robust and relevant methods and strategies.
8. Sources of uncertainty should be characterized and documented.
9. Logic of the approach should be transparently and explicitly detailed.

The subsequent chapters of this document and the workflow proposed (see **Executive Summary** or **Section 2.2.1**) are based on these nine principles. Details for each of these principles (i.e., how each relates to the goal of the risk assessment, how it should be conducted, and how it should be documented) are further described in Dent *et al.*(Dent *et al.*, 2018).

1.4 Method for Document Development

This BPG document was developed using an iterative process involving both a literature review of available guidance and best practices in read-across and input from experts experienced in read-across assessments.

The literature review resulted in an inventory of available read-across references and general approach methodology. Documents and reference materials from the Organisation for Economic Co-operation and Development (OECD), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), the United States Environmental Protection Agency (US EPA), the European Chemicals Agency (ECHA), the Scientific Committee on Consumer Safety (SCCS) and the European Food Safety Authority (EFSA) were reviewed and considered herein. Additional literature reviews were also conducted throughout the document development to fill gaps in the initial inventory. Key citations considered when developing this BPG document are presented in **Table 1**.

Table 1: Key Documents Reviewed in the Process of Best Practice Guidance Document Development

Organization/ Author	Title	Year	Reference
OECD	Guidance on grouping of chemicals. Report No. 80	2007	(OECD, 2007)
Wu *et al.*	A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the	2010	(Wu *et al.*, 2010)

Organization/ Author	Title	Year	Reference
	suitability of analogs for SAR-based toxicological assessments		
ECETOC	Category approaches, read-across, (Q)SAR. Technical report No. 116	2012	(ECETOC, 2012)
OECD	Guidance on grouping of chemicals, Second Edition. Report No. 194	2014	(OECD, 2014)
Blackburn and Stuard	A framework to facilitate consistent characterization of read-across uncertainty	2014	(Blackburn and Stuard, 2014)
Patlewicz *et al.*	Building scientific confidence in the development and evaluation of read-across	2015	(Patlewicz *et al.*, 2015a)
Ball *et al.*	Toward Good Read-Across Practice (GRAP) guidance	2016	(Ball *et al.*, 2016)
ECHA	Read-across assessment framework (RAAF)	2017	(ECHA, 2017c)
ECHA	Read-Across Assessment Framework (RAAF). Considerations on multi-constituent substances and UVCBs	2017	(ECHA, 2017a)
Schultz *et al.*	Assessing uncertainty in read-across: Questions to evaluate toxicity predictions based on knowledge gained from case studies	2019	(Schultz *et al.*, 2019)
Helman *et al.*	Generalized Read-Across (GenRA): A workflow implemented into the EPA CompTox Chemicals Dashboard	2019	(Helman *et al.*, 2019)
Mihir *et al.*	Clustering a chemical inventory for safety assessment of fragrance ingredients: Identifying read-across analogs to address data gaps	2020	(Date *et al.*, 2020)
Lester and Yan	A matched molecular pair (MMP) approach for selecting analogs suitable for structure activity relationship (SAR)-based read across	2021	(Lester and Yan, 2021)
ECHA	Advice on using read-across for UVCB substances. Obligations arising from Commission Regulation 2021/979, amending REACH annexes	2022	(ECHA, 2022)
Alexander-White *et al.*	A 10-step framework for use of read-across (RAX) in next generation risk assessment (NGRA) for cosmetics safety assessment	2022	(Alexander-White *et al.*, 2022)
Lester *et al.*	Quantifying analogue suitability for SAR-based read-across toxicological assessment	2023	(Lester *et al.*, 2023)
SCCS	The SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation 12th revision. Report No. SCCS/1647/22	2023	(SCCS, 2023)
Muldoon *et al.*	Advancing chemical grouping: development and application of signature-based structure-activity groups for non-animal safety assessments	2025	(Muldoon *et al.*, 2025)
EFSA	Guidance on the use of read-across for chemical safety assessment in food and feed	2025	(EFSA, 2025)
OECD	Guidance on grouping of chemicals, Third Edition. Report No. 418	2025	(OECD, 2025b)

Abbreviations: ECETOC: European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA: European Chemicals Agency; EFSA: European Food Safety Authority; OECD: Organisation for Economic Co-operation and Development; SCCS: Scientific Committee on Consumer Safety.

This BPG will be updated iteratively as the field evolves.

1.5 Comparison with Other Guidance/Reference Documents on Read-Across

Over the years, several guidance documents as well as frameworks have been developed to describe how to conduct and apply read-across. At the international level, OECD provide general guidance on grouping of chemicals so that not every chemical needs to be tested for every required endpoint, with the ultimate goal to reduce unnecessary testing (OECD, 2007). This guidance was updated first in 2014 and then in 2025 to be more comprehensive and provide additional support for more specific cases like inorganic substances and nanomaterials (OECD, 2014; 2025b). Sector-specific regulatory agencies have provided guidance documents for the use of read-across. ECHA developed a systematic approach - the Read-Across Assessment Framework (RAAF) - to 1) define the most appropriate scenario for read-across, with scenarios 1 (biotransformation to common compounds) and 2 (different compounds with qualitative similar/worst case properties) being relevant for an analogue approach and scenarios 3 to 6[1] being relevant for a category approach; and 2) describe in detail the different assessment elements (AEs), applicable to each scenario (ECHA, 2017c). The EU's SCCS outlined recommendations on the approach and *in silico* tools to use for read-across purposes, suggesting to select those analogues with a similarity match of at least 70% (SCCS, 2023). In 2025, EFSA released detailed guidance for food and feed, which builds upon the RAAF document. EFSA proposes different approaches to identify analogues and provides exhaustive explanations on how to address uncertainties related to the choice of a given analogue (EFSA, 2025). Recently, an appraisal of the different guidance documents focusing on the respective approaches related to similarity justification, uncertainty characterization and regulatory applicability has been published (Hartung and Rovida, 2025).

In addition, several frameworks have been developed that have shaped the current practice. A framework outlining the use of expert judgement to assess the similarity in structure, reactivity, metabolism and physico-chemical properties has been described (Wu *et al.*, 2010). It has subsequently been refined by addressing uncertainties (Blackburn and Stuard, 2014). To reduce the need for expert judgement in analogue selection and enhance transparency, an approach known as matched molecular pair (MMP) analysis was implemented to streamline and automate the selection of analogues (Lester and Yan, 2021) and quantitatively evaluate the quality of each analogue (Lester *et al.*, 2023). Members of the Cefic Long-range Research Initiative (LRI) read-across team focused on the concept of Adverse Outcome Pathway (AOP) and High Throughput Screening (HTS)) data, such as ToxCast to address certain uncertainties when developing a read-across strategy (Patlewicz *et al.*, 2015a).

More recently, the US EPA developed a workflow based on an algorithmic approach, namely the Generalized Read-Across approach (GenRA), with the overall objective of

[1] Scenario 3 refers to (bio)transformation to common compound(s) with variations among the category members, while Scenario 5 refers to (bio)transformation to compounds with no variation among the category members. Scenario 4 refers to different compounds with the same type of effect(s), while Scenario 6 refers to different compounds with the same type of effect(s).

quantifying performance and uncertainty (Patlewicz and Shah, 2023). How to cluster and address the uncertainties related to read-across strategies has also been the focus of a framework proposed by Schultz and colleagues (Schultz *et al.*, 2015; Schultz *et al.*, 2019). Finally, a 10-step framework has recently been proposed: this consists of a tiered approach, which is exposure-driven and based on the identification of the mode of action (MoA) of both the target substance and the analogue (Alexander-White *et al.*, 2022).

What sets this BPG apart from the pre-existing documents is that it provides a hands-on and operational workflow, in which the different steps have been developed on the basis of practical experience and using a pragmatic approach. It also defines terms like grouping, and category approach, that are sometimes confused. Importantly, the workflow takes into account the key concepts, principles and recommendations embedded in the various frameworks and guidance documents such as the use of structural and mechanistic similarity, analogue reliability criteria, systematic characterization of uncertainties and transparent documentation. By distilling these elements into a harmonized and structured process, this BPG offers safety assessors a practical tool to implement read-across in a manner that is scientifically robust and reproducible for both human health and environmental safety.

1.6 Overview of the Document

This document provides details on how to perform an assessment based on read-across, using a workflow based on 10 sequential yet iterative steps. It begins with background information, then follows the description of read-across based on the analogue approach, with the different steps outlined in the workflow (see **Section 2.2**), aiming to identify and evaluate analogues, characterize and mitigate uncertainties and to document read-across in a transparent manner. It also describes read-across based on the category approach (see **Section 2.3**), with the overall aim of describing how to define the category members (including inclusion and exclusion criteria) and how to address the data gaps, underlining the key differences with the analogue approach.

2 Read-across

2.1 Background and Key Elements of Analogue *versus* Category Approach

Read-across is a technique based on the principle that chemicals which are similar in their structure, reactivity, physico-chemical properties, and (bio)transformation/degradation have similar chemical and biological properties and, therefore, have a similar (eco)toxicological or environmental fate profile. Read-across can be either based on an **'analogue approach'** or on a **'category approach'** (ECETOC, 2012; ECHA, 2017c; OECD, 2025b). In addition, the term '**grouping**' is generally used when more than one chemical is evaluated at the same time, leading to the identification of multiple analogues or the formation of a '**category**' (OECD, 2025b). More specifically, in the case of the analogue approach, the focus is on predicting endpoint information for one specific '**target substance**' by using data from the same endpoint from one or more similar substances ('**analogues**' or '**source substances**') (**Figure 1**). In the case of the category approach, substances (**category members**), whose physico-chemical, toxicological and/or (eco)toxicological properties are likely to be similar or follow a regular pattern - often as a result of structural similarity - can be grouped as a 'category'; the properties of the individual substances in a category are evaluated using all the available information for a given endpoint for all the category members.

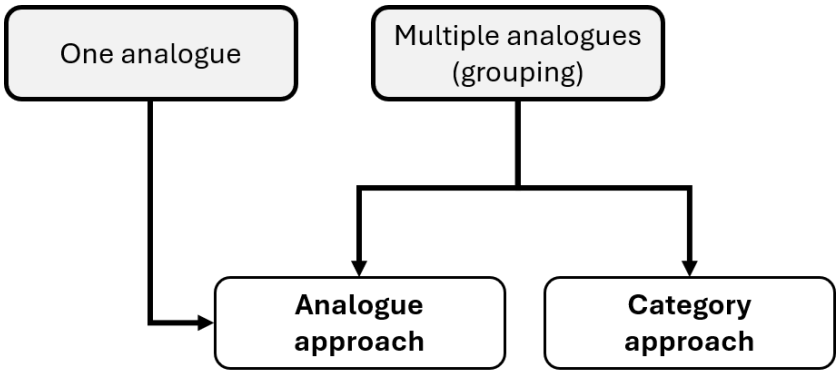


Figure 1: Analogue Approach *versus* Category Approach

Read-across is a foundational tool, critical to filling data gaps without any new and unnecessary (animal) testing, in a rapidly evolving regulatory context. There has been a significant shift from traditional, animal-based testing to the use of new approach methodologies (NAMs)[2], in order to integrate a broader understanding of toxicity mechanisms at molecular and cellular levels and support data-driven chemical management.

[2] The International Collaboration on Cosmetic Safety (ICCS) defines NAMs as any non-animal method, approach or combination thereof used to support safety assessments covering effects and exposure for humans and the environment (including fate) without new animal testing. https://www.iccs-cosmetics.org/iccs-nams-definition.

The use of read-across has been adopted by the UK Health and Safety Executive (HSE) according to the principles laid out by the US EPA High Production Volume (HPV) Challenge programs (OECD, 2002; US EPA, 2024a). In the European Union (EU), two regulations - the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (EU REACH, 2006) and the Cosmetic Product Regulation (EU, 2009) - have been put in place. Both mandate the consideration of NAMs, including read-across, to address information needs for hazard and risk assessment, in line with the 3Rs principle (Replace, Reduce or Refine) (Russell and Burch, 1959). Similarly, the US Toxic Substances Control Act (TSCA), as amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, has mandated US EPA to promote the consideration and development of NAMs in order to reduce animal testing (US EPA, 2024b). Finally, read-across is proposed by the Canada's Chemicals Management Plan (CMP) as an approach to be used to support regulatory safety decisions (Health Canada, 2025).

As outlined in **Section 1.4**, both regulatory agencies and the scientific community have developed guidance documents and frameworks to standardize the use of read-across. A common requirement across these efforts is the establishment of a rigorous and transparent approach to identify suitable analogues with (eco)toxicological or environmental fate data and an acceptable degree of similarity, and address the potential uncertainties related to the analogue selection.

Based on all the documents available and the experience acquired so far, the objective of the present document is to outline the best practice on how to use read-across to evaluate human health and/or environmental safety. Specifically, **Section 2.2** describes read-across based on the analogue approach, while **Section 2.3** describes the category approach.

The main features of both approaches described in this BPG are summarized in **Table 2**.

Table 2: Key Elements Characterizing the Analogue Approach and the Category Approach

Key Element	Analogue Approach	Category Approach
Naming of Substances Used in Approach	'Target substance' (substance for which there is a data gap) and one or more 'analogues/source substances' (the substance(s) providing experimental data)	Category members (a substance can be 'target substance' for one endpoint and 'source substance' for another endpoint within the same category)
Hypothesis	The target substance and the analogue(s) are sufficiently 'similar' (e.g., physico-chemical properties and (eco)toxicological endpoints), that an endpoint value for the target substance can be predicted from data on one or more analogues.	The category members present 'similar' physico-chemical properties and/or (eco)toxicological endpoints or follow a regular trend - often as a result of structural similarity or common precursors/breakdown products
Identification of Analogues/Category Members	Search for analogues* (unsupervised approach)	• Supervised approach** based on pre-existing knowledge (e.g., categories already established by Regulatory Authorities) • Unsupervised approach (search for analogues)
Data Gap Filling	Direct read-across	• Interpolation • Extrapolation • Direct read-across (one category member can be the source substance for one endpoint and the target substance for another endpoint)

*The supervised approach can also be used for the analogue approach when pre-existing knowledge is available, although this is less common.
**It should be pointed out that a category established by Regulatory Authorities might not follow the BPG workflow.

Considering the commonalities between the analogue and category approach, the workflow described in Section 2.2.1 can be applied to both, with some key differences. More specifically, **Steps 1**, **2**, **9** and **10** are the same for both approaches, with no significant differences. Therefore, these steps in Section 2.2 use the terminology of the analogue approach, but they are applicable to the category approach when the terminology is adapted (e.g., analogues = category members). **Steps 3-8** present some differences that are described in detail in Section 2.3.

2.2 Analogue Approach

As a general rule, when performing a safety assessment, a literature search is initially conducted to identify (eco)toxicological or environmental fate data on the target substance and detect potential data gaps. In the absence of data on the endpoint of interest, read-across may be applied.

2.2.1 Overview of the Workflow

A stepwise approach can be used to identify potential analogues with (eco)toxicological or environmental fate data and evaluate the suitability of the selected analogues for read-across. In **Figure 2**, the 10-step workflow for read-across is illustrated. The majority of the steps are common for both human health and environmental safety. Since **Steps 5-8** differ slightly between human health and environmental safety, they are indicated by blue boxes in **Figure 2** and described in separate sub-sections.

The same workflow is used for the category approach with some key differences, which are indicated by the grey text (**Steps 5-7**) in **Figure 2** (see also **Section 2.3** for additional details).

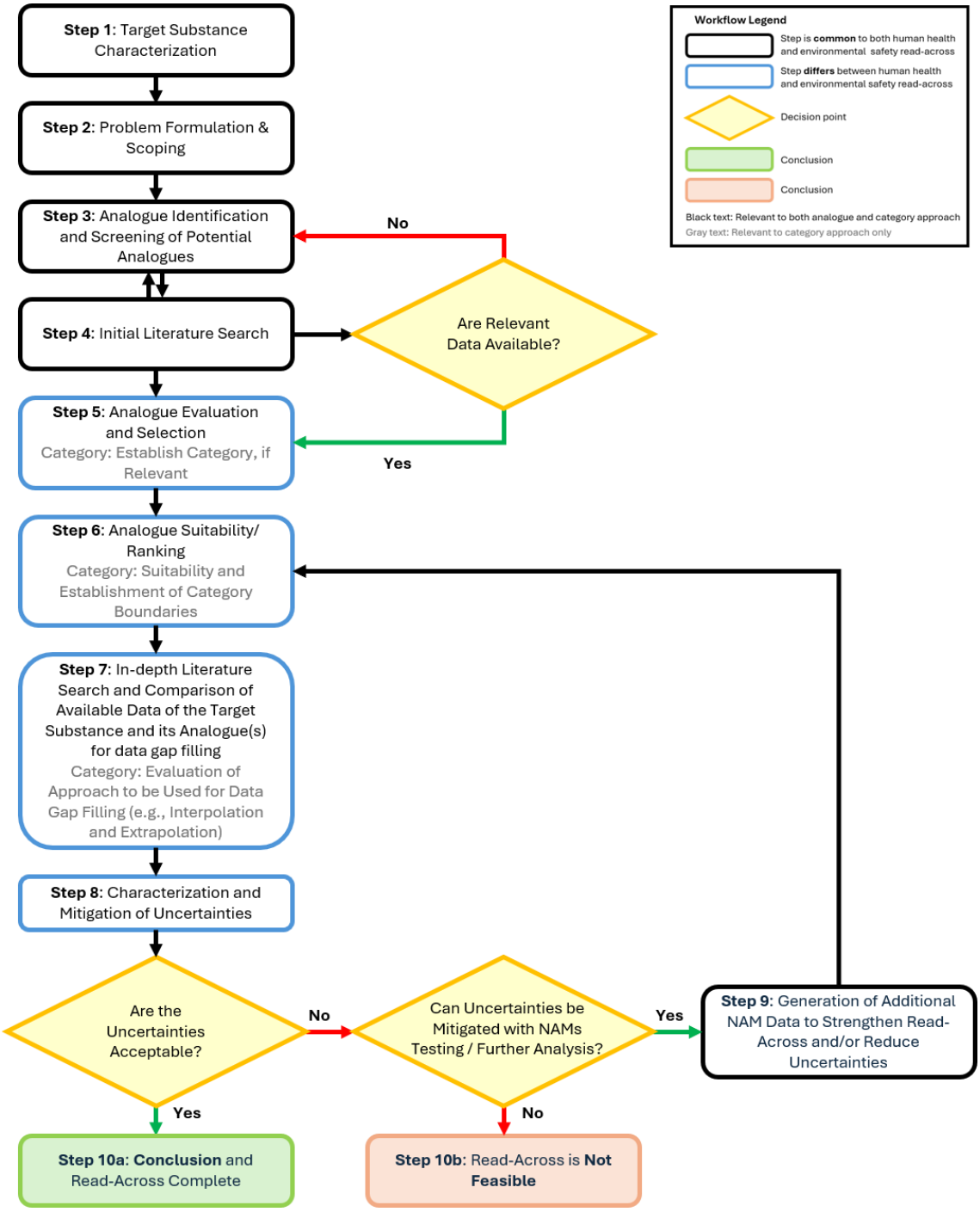


Figure 2: Overview of the Read-Across Process

Note: As needed, the safety assessor can iterate through the steps of this process, making the workflow flexible and suitable for different scenarios. NAM = New Approach Methodology.

The sections below provide details on each step of the workflow, that allows the safety assessor to proceed in a systematic manner to conduct a read-across-based assessment using the analogue approach.

2.2.2 Step 1. Target Substance Characterization

Step 1 Objective: Collect data on the target substance, including physico-chemical properties, as well as existing hazard and potency data.

The initial step in any hazard assessment is the characterization of the substance of interest, referred to as the 'target substance'. A basic data matrix to characterize the substance of interest is provided in **Table 3,** however, it should not be deemed to be exhaustive. Compositional information on the substance must be gathered, such as the name, CAS number[3] and/or the SMILES[4], the degree of purity and the presence of potential impurities; in case of a multi-constituent substance, a representative structure may be used, or each structure can be evaluated individually. In addition, data on physico-chemical, (eco)toxicological, and/or environmental fate properties must be collected. Conditions in which experimental values for properties (e.g., temperature used in assessing solubility) exist should also be captured for completeness. If *in silico* models are used to predict these properties, the platform and version of the model(s) must also be captured (OECD, 2023).

2.2.2.1 Examples of sources to gather experimental data

Physico-chemical properties and (eco)toxicological and/or environmental fate data may be collected from a wide range of sources, including the following databases (non-exhaustive list):

- **PubChem** (https://pubchem.ncbi.nlm.nih.gov/) is a public database developed by the US National Institute of Health (NIH). It provides information on chemicals including their structure, physico-chemical properties and a range of biological and (eco)toxicological and environmental fate data.
- **ECHA CHEM Database** (https://chem.echa.europa.eu/) is a public chemicals database maintained by ECHA. It contains the information submitted under the REACH Regulation, including physico-chemical, (eco)toxicological and environmental fate data.
- **ChemSpider** (https://www.chemspider.com/) is a public chemical structure database developed by the Royal Society of Chemistry. It provides access to curated chemical identifiers, structures, and selected physico-chemical property data from multiple sources.
- **eChemPortal** (https://www.echemportal.org/echemportal/) is a public resource developed by OECD. It provides access to publicly available datasets of chemicals (including physico-chemical, (eco)toxicological and environmental fate data) from multiple national and international programs.

[3] Chemical Abstracts Service Registry Number

[4] Simplified molecular-input line-entry system (representing a line notation to encode molecular structures)

- **Cosmetic Ingredient Review (CIR) Database** (https://cir-reports.cir-safety.org/) is a public resource, which provides the safety reports - at different stages of the CIR review process - of cosmetic ingredients.
- **AICIS (Australian Industrial Chemicals Introduction Scheme) Database** (https://services.industrialchemicals.gov.au/search-assessments/) is a public database, which provides safety assessments of chemicals developed by the Australian Authorities.
- **ICE (Integrated Chemical Environment)** (https://ice.ntp.niehs.nih.gov/) is a public database developed by the US National Toxicology Program (NTP). It provides free access to curated (eco)toxicological and/or environmental fate data, including high-throughput screening information.
- **EnviroTox** (https://envirotoxdatabase.org/): is an open access repository of aquatic toxicity data for chemicals and species, which is also designed to calculate the Ecotoxicological Threshold of Concern.
- **ECOTOX** (https://cfpub.epa.gov/ecotox/): is a public database, developed by the US EPA. It provides toxicity data on aquatic and terrestrial species.
- **ChemEXPERT** (https://www.toxplanet.com/) is a commercial database providing a single search interface covering a simultaneous search in more than 500 global regulatory as well as scientific databases.

2.2.2.2 Examples of in silico tools to predict specific endpoints

Some endpoints can be predicted using *in silico* tools, as extensively described in a practical guide from ECHA (ECHA, 2016). It is good practice to use at least two different (Q)SAR ((Quantitative) Structural-Activity Relationship) models (ECHA, 2025).

Tools that can be used to predict physico-chemical properties are described in **Section 2.2.6.3**.

Examples of *in silico* tools that can address (eco)toxicological endpoints include (non-exhaustive list):

- **VEGA (Virtual Models for Property Evaluation of Chemicals within a Global Architecture) HUB** is a publicly available platform, developed by the Istituto di Ricerche Farmacologiche Mario Negri; it consists of several models predicting a range of endpoints including mutagenicity, carcinogenicity, skin sensitization and environmental fate parameters (Benfenati *et al.*, 2013).
- **OPEn structure–activity/property Relationship App (OPERA)** is a publicly available and open-source data suite of QSAR models developed by the US EPA; it provides predictions for different endpoints related to human health and environmental fate (Mansouri *et al.*, 2018).
- **ECOSAR (Ecological Structure Activity Relationships)** is a computerized predictive system developed by the US EPA to estimate the acute (short-term) and

chronic (long-term) toxicity of industrial chemicals to aquatic organisms like fish, invertebrates, and algae (US EPA, 2022).

- **Leadscope Model Applier™** is a commercial platform**,** which provides toxicity predictions relevant to human health, including repeated dose toxicity, genotoxicity and carcinogenicity (Roberts *et al.*, 2000).
- **Lhasa Limited software** (https://www.lhasalimited.org) includes different commercial models, such as Derek Nexus and Sarah Nexus. It provides the prediction of endpoints related to human health, such as skin sensitization, genotoxicity and carcinogenicity.
- **ProtoPRED** is a commercial computational platform designed to predict a wide range of properties of a given chemical compound with respect to human health and environmental safety (ProtoPRED, 2025).

Table 3: Template of a Data Matrix for Substance Characterization[5]

Property/Endpoint	Target substance
Chemical information	
2D Structure	
Common name	
INCI name	
Synonyms	
CASRN	
Molecular formula	
SMILES	
Purity	
Known impurities	
Potential impurities	
Physico-chemical properties	
Molecular formula	
Molecular weight (or MW distribution, if applicable)	
Particulate size or size range (if applicable)	
Physical form	
Water solubility	
Partition coefficient	
Vapor pressure	
Pka (dissociation constant)	
Charge at physiological pH	

[5] Properties and endpoints can be added and removed, as required (e.g., local inhalation toxicity).

Property/Endpoint	Target substance
Mode of Action	
Biological activity	
Human health endpoints	
Toxicokinetics (absorption, distribution, metabolism, and excretion)	
Acute toxicity (oral, dermal, inhalation)	
Skin irritation and corrosion	
Serious eye damage and eye irritation	
Skin sensitization	
Repeated dose toxicity (oral, dermal, inhalation)	
Genetic toxicity	
Carcinogenicity	
Reproductive toxicity	
Developmental toxicity	
Environmental fate endpoints	
Hydrolysis	
Biodegradation	
Adsorption/desorption	
Bioaccumulation	
Ecotoxicity endpoints	
Short-term toxicity to fish	
Long-term toxicity to fish	
Short-term toxicity to aquatic invertebrates	
Long-term toxicity to aquatic invertebrates	
Toxicity to aquatic algae and cyanobacteria	
Toxicity to microorganisms	
Sediment toxicity to benthic organisms (e.g., *Chironomus*, *Lumbriculus* or *Corophium* spp)	
Short-term toxicity to soil macro-organism (e.g., earthworm)	
Long-term toxicity to soil macro-organism (e.g., earthworm)	
Toxicity to soil microorganisms (nitrogen transformation test)	
Toxicity to terrestrial plants	
Toxicity to birds	

2.2.3 Step 2. Problem Formulation & Scoping

Step 2 Objective: Define the data gap(s), identify those (eco)toxicological or environmental fate endpoints for which a read-across approach will be used, and provide a clear statement of the assessment scope.

Using the data matrix (**Table 3**), data gaps for the target substance can be identified, and addressed using NAMs, including a read-across approach. At this stage, problem formulation and scoping is essential to set the assessment scope and set the boundaries for the read-across hypothesis.

This step should consider the following elements:

1. **Define the data gap clearly** – specify which (eco)toxicological and/or environmental fate endpoints are missing and why they are required (e.g., regulatory tonnage band and safety assessment need).
2. **Formulate the read-across hypothesis** – identify for which endpoint the read-across approach will be used, outlining the potential analogue space (e.g., which type of substances could qualify as potential analogues), recognizing that read-across can be endpoint specific (e.g., analogues suitable for one endpoint may not be suitable for others).
3. **Establish the assessment context** – clarify the purpose of the read-across approach (e.g., regulatory submission and internal decision-making) and the decision context (e.g., cosmetic sector, pharma and chemical sector).

The choice of the best strategy to fill data gaps may be different for human health and environmental safety:

Human health: Endpoints such as serious eye damage and eye irritation, skin irritation and corrosion, skin sensitization, and genetic toxicity can typically be addressed using (combinations of) *in vitro* NAMs. For example, the ICCS skin sensitization BPG describes in detail how to apply NAMs to evaluate skin sensitization potential (https://www.iccs-cosmetics.org/education/best-practice-guidance/bpg-skin-sensitization-assessment-using-new-approach-methods). BPGs for serious eye damage and eye irritation and skin irritation and corrosion are also being developed by ICCS. A read-across-based strategy is often proposed for more complex (systemic) endpoints - such as repeated dose toxicity, carcinogenicity and reproductive and developmental toxicity - but it can also be used for endpoints such as skin sensitization and genetic toxicity.

Environmental safety: A read-across approach can be used to address ecotoxicological endpoints, like short-term and long-term toxicity to fish to avoid vertebrate testing. It can also be used to avoid unnecessary higher tier testing for environmental fate, such as simulation degradation studies in water, sediment or soil, long-term *Daphnia* studies or terrestrial toxicity testing.

Overall, a clear problem formulation ensures the read-across hypothesis is transparent, scientifically plausible, and ready for the evaluation in subsequent steps.

2.2.4 Step 3. Analogue Identification and Screening of Potential Analogues

Step 3 Objective: Search for all potential analogue candidates using a variety of approaches, which may include similarity indices, MMP analysis and comparison of functional groups and scaffolds, and other approaches.

Different approaches can be used to identify potential analogues. For example, the EFSA guidance proposes to consider structural similarity, MoA, toxicokinetic profile, metabolism or manufacturing process (EFSA, 2025). In addition, analogue identification can be performed to address the data gap for different endpoints, or it can focus on a specific endpoint, as outlined by Moustakas and colleagues (Moustakas *et al.*, 2022). As an example, if there is a data gap for genotoxicity, the search of analogues could focus on chemicals which present reactivity toward nucleic acids (Moustakas *et al.*, 2022). Similarly, if there is a data gap for carcinogenicity driven by DNA-reactive mechanisms, as is the case for nitrosamine impurities, the search of analogues could focus on compounds containing the N-nitroso group, which upon metabolic activation can react with DNA (EMA, 2020; 2025).

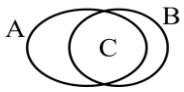
While endpoint-specific searches may be appropriate in certain cases, the present guidance focuses on a general analogue search applicable across different endpoints. The first step of the analogue identification process is a search using *in silico* tools and relevant toxicity databases. Two approaches are proposed to perform the search, which are both based on the structure of the target substance.

2.2.4.1 Similarity Index Threshold

Pragmatically, one parameter that can be used to identify potential analogues is the similarity index (**Figure 3**). The similarity index quantifies the structural resemblance between molecules based on shared features, with higher values indicating greater similarity[6]; this index calculates the overlap of structural features, often represented as binary fingerprints and is highly dependent on the fingerprint chosen. There are several different methods to calculate the similarity index (Leary *et al.*, 2025; Patlewicz *et al.*, 2017), including the Tanimoto coefficient[7] and the Dice coefficient[8]. The SCCS

[6] The similarity index can vary from 0 (no similarity) to 1 (high similarity).

[7] The Tanimoto coefficient measures the similarity between two molecules on the basis of structural fingerprints, by counting the number of bits 'on' in both molecules (C) and the number of bits 'on' in each molecule separately (A, B).



The Tanimoto coefficient is calculated with the following formula: C/A+B-C

[8] The Dice coefficient measures the similarity between two molecules on the basis of structural fingerprints, by counting the number of bits 'on' in both molecules (C) and the number of bits 'on' in each molecule separately (A, B).

recommends applying a similarity threshold of ≥ 0.7 when evaluating and documenting potential analogues (SCCS, 2023).

One approach to run the analogue search is to first set the threshold at a high similarity score (e.g., ≥ 0.9); if no candidates are found above this threshold, less stringent conditions can be applied in a stepwise approach (e.g., by lowering the similarity score by 0.1 at each step), then complement the results with expert judgement (**Figure 3**). In case a considerable number of candidates are identified, the analogues with the highest score and containing the core structure of the target substance can be selected for the second stage of the screening process.

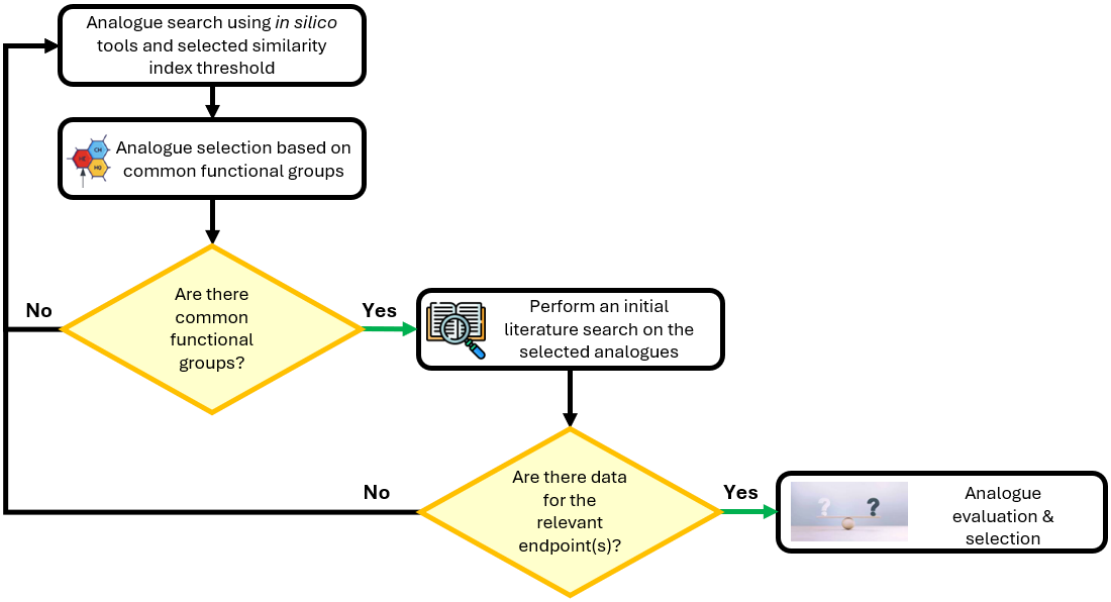
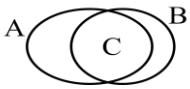


Figure 3: Stepwise Approach to Perform an Analogue Search Based on Similarity Index Threshold and an Initial Literature Search (Steps 3 and 4)

Several resources can be used to identify analogues that have a similarity index of a selected threshold. The following publicly available databases and *in silico* tools are examples of software that can be used to perform the analogue search (non-exhaustive list):

- **OECD QSAR Toolbox** is publicly available software developed by the Laboratory of Medicinal Chemistry (LMC, Bulgaria) (OECD, 2025a).
- **LRI AMBIT2,** which has been developed by IdeaConsult under the umbrella of Cefic-LRI (Jeliazkova *et al.*, 2016).
- **GenRA** (Generalized Read-Across), which has been established by the US EPA (Shah *et al.*, 2016).



The Dice coefficient is calculated with the following formula: 2C/A+B.

- **ToxRead**, which is part of the VEGA platform (Gini *et al.*, 2014).
- **ChemMine,** which is an online service for analyzing and clustering small molecules (Backman *et al.*, 2011).
- **Danish (Q)SAR database**, which is a web-based tool developed by the Danish Food National Institute (Danish (Q)SAR database, 2026).

Preferably, the analogue identification tool should have a large number of curated chemicals in the database to maximize the potential for identification of quality analogues. If one tool does not return a sufficient number of analogues, it may be helpful to expand the search with more than one tool (EFSA, 2025).

Once an initial pool of analogues is identified, analogue candidates and the target substance can subsequently be evaluated to determine whether they present common functional groups (**Figure 3**). The OECD QSAR Toolbox is commonly used to identify the functional groups of each substance using the right profiler; the profilers of this tool are rule-based systems that analyze the structure and properties of a given chemical to determine its characteristics. Among the different profilers, the ones specific to functional groups ('organic functional groups', 'organic functional groups (nested)', 'organic functional groups (US EPA)' and 'organic functional groups, Norbert Haider (checkmol)') can be selected for the analysis.

2.2.4.2 Matched Molecular Pair Analysis (MMP)

Another approach to search for analogues can incorporate structure-matching capabilities, including the ability to search by structural scaffold and functional groups (**Figure 4**). Matched molecular pair (MMP) analysis has highlighted specific small structural changes to consider when identifying analogues. These changes include variations in the length of alkyl chains, the addition of small alkyl groups to an aromatic ring (e.g., C1-C4), alterations in alkyl chain branching, modifications in bond order, and the number of repeat units or functional groups (Lester and Yan, 2021). These structural changes were derived from thousands of read-across predictions spanning systemic toxicity, developmental and reproductive toxicity, genotoxicity, and skin sensitization making the approach broadly applicable across human health endpoints. The OECD QSAR Toolbox can be used to perform this type of search. The automation of the MMP approach for analogue selection currently is in progress (ICCS (https://www.iccs-cosmetics.org/science/human-health/ci-read-across), aiming to provide open-source access to this highly effective search tool.

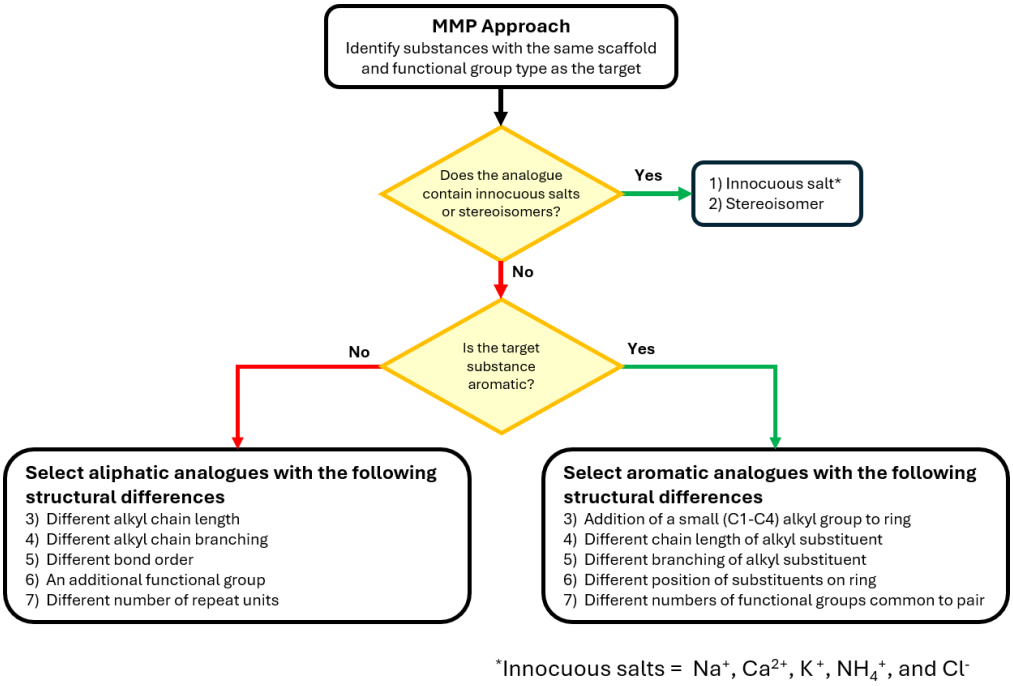


Figure 4: Analogue Search Based on MMP (adapted from (Lester and Yan, 2021))

Step 3 represents an initial screening, a preliminary selection of potential analogues, and the suitability of these candidate analogues ultimately depends on whether they present relevant data for the endpoint of interest (**Step 4**).

2.2.5 Step 4. Initial Literature Search for Data Gap Filling

> **Step 4 Objective:** Conduct an initial literature search on the potential analogues identified in **Step 3** to select those for which there are relevant (eco)toxicological or environmental fate data suitable for filling the established data gaps.

For those analogues identified in **Step 3**, an **initial** literature search is conducted to determine whether they have (eco)toxicological or environmental fate data for the relevant endpoints, which could be used for read-across purposes (**Figure 3**). This initial literature search is intended for screening purposes only, to rapidly identify whether analogue candidates have relevant data for the endpoint(s) of interest and should be taken forward from **Step 4** to **Step 5**. It does not aim to fully evaluate data quality or consistency. A more comprehensive and critical evaluation of the available data, including comparison with the target substance, is conducted in **Step 7**.

This **Step 4** search can be conducted using several comprehensive databases such as (see also Section 2.2.2):

- **ECHA CHEM Database** (https://chem.echa.europa.eu/) is a public chemicals database maintained by ECHA. It contains the information submitted under the

REACH Regulation, including physico-chemical, (eco)toxicological and environmental fate data.

- **eChemPortal** (https://www.echemportal.org/echemportal/) is a public resource developed by OECD. It provides access to publicly available datasets of chemicals (including physico-chemical, (eco)toxicological and environmental fate data) from multiple national and international programs.
- **ChemEXPERT** (https://www.toxplanet.com/) is a commercial database providing a single search interface covering a simultaneous search in more than 500 global regulatory as well as scientific databases.
- **OECD QSAR Toolbox** is publicly available software developed by the Laboratory of Medicinal Chemistry (LMC, Bulgaria) (OECD, 2025a). It contains databases from different sources including the US HPV Challenge Program (OECD, 2025a).
- **SciFinder** (https://scifinder-n.cas.org/) is a comprehensive, curated scientific database, developed by Chemical Abstract Service (CAS).

The conclusion as to whether there should be further evaluation for each potential analogue should take into consideration three screening criteria:

1. Similarity with respect to the structure
2. Common (or different) functional groups
3. Availability of (eco)toxicological or environmental fate data for the relevant endpoints (**Figure 3**).

The selection process must be well-documented, explaining why a potential analogue has been selected or deselected. An example of how to report the selection/deselection process is illustrated in **Table 4**. The ultimate goal is to complete this process with one or more potential analogues with relevant (eco)toxicological or environmental fate data to be further evaluated.

Table 4: Template of a Table to Document the Selection/Deselection of Potential Analogues

	Target Substance	Analogue 1	Analogue 2	Analogue …
Step 4				
Chemical name				
2D Structure				
Similarity index value, if calculated*				
Common functional groups				
Different functional groups				
Availability of (eco)toxicological or environmental fate data for relevant endpoints (Yes/No)				
Conclusion on analogue selection (Yes/No)				

* Indicate the similarity index value including the fingerprint and metric used or 'Not Applicable' if MMP is used.

In cases where no analogues with relevant (eco)toxicological or environmental fate data are identified, **Step 3** should be repeated, e.g., by using additional *in silico* tools for the search and/or reducing the threshold of the similarity index. Overall, **Step 3** to **Step 6** is an **iterative process** (**Figure 3**). However, it should be recognized that this process can only be iterated a finite number of times. If, following reasonable refinement of the search strategy, no appropriate analogue can be identified, alternative approaches should be considered, such as exposure-based waiving. In some cases, data-poor analogues (those lacking experimental literature data) may still be valuable for the generation of targeted NAM-data. The resulting data, considered alongside data from the target substance and any data-rich analogues, can be used to fill key data gaps. For example, supporting a hypothesized mechanism of action for a given adverse effect and assessing whether the target substance and multiple analogues operate through the same mechanism. Integrating these complementary lines of evidence across the target substance and a set of relevant analogues can strengthen the biological rationale and increase confidence in the overall read-across assessment.

2.2.6 Step 5. Analogue Evaluation and Selection

Step 5 Objective: Evaluate the suitability of potential analogues for read-across purposes by comparing key attributes important for (eco)toxicity and environmental fate endpoints, including structural similarity, structural alerts, functional groups, physico-chemical properties, and (bio)transformation/biodegradation.

Step 5 is to determine whether the previously identified analogues are chemically and biologically similar to the target substance and therefore suitable for read-across purposes. In accordance with the OECD guidance (OECD, 2007; 2025b) and the ECHA RAAF framework (ECHA, 2017c), read-across of (eco)toxicological or environmental fate data from an analogue to the target substance should be justified on the basis of:

1. Structural similarity and common functional groups
2. Common structural alerts or reactivity
3. Common physico-chemical properties
4. Likelihood of common breakdown products via (bio)transformation/degradation processes

These four criteria must all be fulfilled, to have confidence in the final analogue selection. How each criterion is addressed may differ between human health and environmental safety, as outlined below.

2.2.6.1 Structural similarity and common functional groups

The first criterion of structural similarity and common functional groups has been partially addressed in **Step 3** using the selection screening criteria (see **Section 2.2.4**).

In this phase, if analogues were selected using a similarity index, the structural similarity of the analogues with the target substance is confirmed by ensuring the same molecular fingerprint and similarity algorithm (e.g., Tanimoto, Dice) is used for all target/analogue pairs. These pairs may have been selected using different (Q)SAR tools and, therefore, have different similarity indices. A similarity index can be determined by using publicly available *in silico* tools, like the OECD QSAR Toolbox or ChemMine (Backman *et al.*, 2011).

For the assessment of common functional groups, the profilers of the OECD QSAR Toolbox coupled with expert judgement are recommended (see **Section 2.2.4** for more information).

Analogues selected using the MMP approach are classified based on the MMP transformation that connects the target and analogue structures, as outlined by Lester and Yan (Lester and Yan, 2021) (see **Section 2.2.4** for more information). For aromatic compounds, the transformation categories include: the addition or removal of a small alkyl group on the ring, variations in alkyl substituent chain length, branching, and variations in the number of functional groups. For aliphatic compounds, the transformation categories encompass differences in alkyl chain length, branching or

bond order, the addition or removal of functional groups, and variations in the number of repeat units (**Figure 4**).

2.2.6.2 Common structural alerts or reactivity

The second criterion is related to common structural alerts or reactivity. Structural alerts are specific (sub)structural features within a molecule that have been associated with potential toxicity or adverse effects. Common structural alerts are indicative of similar reactivity between the target substance and the potential analogue(s). Importantly, the structural alerts should be evaluated at two levels:

- General similarity (i.e., whether the target substance and analogue share a comparable profile of alerts overall),
- Endpoint-specific similarity (i.e., whether alerts are relevant to the endpoint for which the read-across approach is proposed).

Structural alerts are typically identified using *in silico* knowledge-based expert systems for toxicity predictions.

For human health, examples of *in silico* tools include the following:

- The **OECD QSAR Toolbox** is publicly available software developed by the Laboratory of Medicinal Chemistry (LMC, Bulgaria) (OECD, 2025a). It contains several profilers, focusing on structural alerts for specific endpoints (e.g., skin sensitization, genotoxicity and carcinogenicity) (OECD, 2025a).
- **ToxTree** is publicly available software, which is based on a decision tree approach to identify structural alerts linked to toxic hazard (Patlewicz *et al.*, 2008).
- **OncoLogic™** is a publicly available expert system developed by the US EPA aimed at identifying structural alerts related to carcinogenicity (https://www.epa.gov/tsca-screening-tools/oncologictm-expert-system-evaluate-carcinogenic-potential-chemicals).
- **Derek Nexus** is a commercial knowledge base software (Barber *et al.*, 2015), which has been developed and marketed by Lhasa Limited; it comprises alerts, reasoning rules and examples.

For environmental safety, specific profilers of the **OECD QSAR Toolbox** (e.g., 'acute aquatic toxicity classification by Verhaar (Modified)', 'acute aquatic toxicity MOA by OASIS', 'Aquatic toxicity classification by ECOSAR' and 'iSafeRat Mechanisms of toxic action') can be used.

2.2.6.3 Common physico-chemical properties

The third criterion is common physico-chemical properties: similar physico-chemical properties are indicative of similar toxicokinetic behavior in the human body or in the environment.

- For human health, similar values in molecular weight, water solubility and partition coefficient (log Kow) suggest similar behavior of the target substance and the analogue regarding bioavailability and tissue distribution. More

specifically, molecular weight, water solubility, log Kow, vapor pressure and the ionization constant (pKa) collectively provide information on the likelihood and extent of systemic absorption, with molecular weight and lipophilicity influencing the ability of a substance to cross biological membranes, water solubility affecting dissolution at the site of absorption and pKa governing the ionized fraction at physiological pH thus affecting passive permeation. Molecular weight and lipophilicity are also the primary determinants of percutaneous absorption potential. Vapor pressure determines whether a substance is likely to reach the respiratory tract as a vapor: if vapor pressure is low, particle size distribution becomes more relevant for inhalation exposure via aerosol or dust.

- For the environment, physico-chemical property similarity provides information on exposure, ecotoxicological and environmental fate behavior. Comparable molecular weight, water solubility and partition coefficient suggest similar bioavailability and bioaccumulation potential. Vapor pressure provides information on the potential for volatilization from environmental compartments, while the pKa determines the degree of ionization at environmentally relevant pH values, which can influence partitioning, mobility and bioavailability.

In the absence of experimental data, *in silico* tools can be used to quantitatively predict a given physico-chemical parameter (Gadaleta *et al.*, 2024; Lester *et al.*, 2023). Some examples of tools are listed below:

- **Estimation Programs Interface (EPI Suite™)** is a publicly available platform developed by the US EPA; it consists of thirteen separate models, each estimating a physico-chemical parameter (including melting point, boiling point, water solubility, partition coefficient and vapor pressure) (US EPA, 2025).
- **ProtoPRED** is a commercial computational platform designed to predict a wide range of properties of a given chemical compound, including physico-chemical properties (ProtoPRED, 2025).
- **VEGA HUB** is open-access software which includes models to estimate partition coefficient, water solubility, vapor pressure and melting point (Benfenati *et al.*, 2013).
- **OPERA** is a publicly available and open-source data suite of QSAR models developed by US EPA; it provides predictions for different endpoints including physico-chemical properties (Mansouri *et al.*, 2018).

2.2.6.4 Likelihood of common breakdown products via (bio)transformation/degradation processes

The fourth criterion, which is critical to the evaluation of similarity of analogue and target substance, is related to the likelihood of common breakdown products via (bio)transformation for human health and environmentally relevant species, and degradation for environmental safety.

2.2.6.4.1 Human health ((bio)transformation)

A similar metabolic pathway of the target substance and the potential analogue suggests that the two substances do not give rise to metabolites with a different toxicological profile. If experimental data (either *in chemico*, *in vitro, ex vivo* or *in vivo*) are not available, *in silico* tools can be used to evaluate metabolites, although there are some limitations. For example, most *in silico* tools either do not provide a quantitative analysis of the corresponding metabolites, or they do not provide a complete metabolic pathway including Phase I and Phase II metabolic reactions.

Examples of *in silico* tools are provided below:

- **Meteor Nexus** is a commercial knowledge-based expert tool developed by Lhasa Limited. It predicts a complete metabolic pathway of a given substance, including Phase I and Phase II metabolic reactions, with a likelihood score[9] for each predicted metabolite (Judson *et al.*, 2015). However, this tool does not allow to quantify the predicted metabolites and may overpredict metabolite number and type.
- **TIMES (tissue metabolism simulator)** is a commercial tool developed by the Laboratory of Mathematical Chemistry (Dimitrov *et al.*, 2005). It uses a heuristic algorithm to generate plausible metabolic maps from a comprehensive library of biotransformations and abiotic reactions, with provides quantitative estimates of predicted metabolites based on their likelihood of formation.
- **GLORYx** is a publicly available tool, which uses reaction rule sets (Phase I and Phase II reactions) to generate structures of predicted metabolites combined with predicted sites of metabolism to score the predicted metabolites (de Bruyn Kops *et al.*, 2021). Similar to Meteor Nexus, this tool does not quantify the predicted metabolites.

The profiling of predicted metabolites with respect to structural similarities can help identify key similarities and ensure that the target substance does not produce additional or more toxic metabolites compared to the analogue.

In addition to utilizing *in silico* tools for comparing (bio)transformation pathways, literature data complemented with expert judgment can be employed to support the biotransformation of a functional group, backed by data from similar biotransformation reactions in analogous molecular environments.

In vitro metabolism data can be particularly valuable for comparing metabolic pathways between target and analogue candidates (see **Section 2.2.7**), as *in silico* predictions may not always capture structural effects that influence the rate and extent of biotransformation. Relative biotransformation pathways can be quantitatively compared by creating a fingerprint that includes the names of known or predicted (bio)transformations for both the target and analogue(s). The overlap of these biotransformation pathways can then be computed using a Tanimoto index or another preferred algorithm (Lester *et al.*, 2023). In addition, TIMES and Meteor Nexus both

[9] How likely the metabolite is to be formed.

include functionality to generate and compare maps of predicted metabolic transformations.

2.2.6.4.2 Environmental safety

A similar degradation (both abiotic and biotic) pathway of the target substance and the potential analogue(s) suggests that the two substances exhibit comparable environmental persistence, exposure profiles and ecotoxicological relevance. In addition, comparable degradation pathways reduce the likelihood that the target substance gives rise to degradation products with a markedly different persistence, bioaccumulation potential or ecotoxicological hazard as compared to the degradation products derived from the analogue(s).

Biotransformation in ecotoxicologically relevant species (e.g., fish) is an additional and important aspect of environmental similarity that should be considered alongside abiotic and microbial degradation. Differences in the biotransformation capacity of aquatic organisms between the target substance and analogue(s) can affect internal exposure, bioaccumulation potential and the nature of the transformation products formed *in vivo*. If relevant, the transformation potential in fish or other ecotoxicological test species can be assessed using *in vitro* fish metabolism assays (e.g., rainbow trout hepatocyte or S9 intrinsic clearance, OECD TG 319A and OECD TG 319B (OECD, 2018a; 2018b) and the results used as a comparison element between the target and the analogue(s).

In the absence of experimental data related to abiotic degradation (e.g., hydrolysis) and biodegradation, *in silico* tools can be used to support the evaluation of the degradation or biotransformation potential of both the target substance and the analogue(s). These tools can indicate whether the first transformation steps are similar; however, the information on the rate of degradation or the relative abundance of the degradation products, which are key elements for exposure-driven endpoints, is often limited.

Examples of *in silico* tools are provided below:

- **EPI Suite™** is a publicly available platform developed by the US EPA which includes a module to predict hydrolysis (Hydrowin) and a module to predict biodegradation (Biowin). However, the modules do not provide a degradation pathway with the description or quantification of potential degradation products (US EPA, 2025).
- **EAWAG-BBD (Biocatalysis/Biodegradation Database) Prediction** is a publicly available system, which predicts plausible pathways for microbial degradation of chemical compounds using biotransformation rules, based on reactions found in the EAWAG-BBD database or in the scientific literature (EAWAG, 2026). It allows the prediction of degradation, but it does not quantify these products.
- The **OECD QSAR Toolbox** is publicly available software developed by the Laboratory of Medicinal Chemistry (LMC, Bulgaria) (OECD, 2025a). It contains a hydrolysis simulator, which predicts hydrolysis products in acidic,

neutral and basic conditions. It also contains a 'microbial metabolism' simulator, that allows the simulation of aerobic degradation (OECD, 2025a).

- **Catalogic** is a commercial tool which has been developed by the Laboratory of Mathematical Chemistry (Dimitrova *et al.*, 2017). It predicts the biodegradation pathway of a given chemical including the quantification of the degradation products.

For predicted degradation products, their structural features and known or expected ecotoxicological or environmental fate properties should be evaluated to ensure that the target substance does not give rise to transformation products of higher concern than those arising from the analogue(s). If the uncertainty is not mitigated, experimental degradation studies (e.g., OECD 301, OECD 307 or OECD 309) may be required to confirm environmental fate similarity.

2.2.6.5 Summary of Analogue Evaluation and Selection

The results of the analogue evaluation can be summarized in a table format. An example is provided by **Table 5**.

Table 5: Template of a Table to Document the Analogue Evaluation and Selection (Step 5)

	Target Substance	Analogue 1	Analogue 2	Analogue …
Step 5				
Chemical name				
2D Structure				
Structural similarity and common functional groups and/or MMP category				
Common structural alerts or reactivity				
Different functional groups				
Common physico-chemical properties				
Likelihood of common breakdown products via biological/degradation processes/biotransformation				

Step 5 also serves as the decision point for determining whether the available analogues can support a category approach, which should only be considered when multiple analogues are available and show either similar properties and consistent endpoint patterns or show different but predictable properties and endpoint patterns that follow these differences; specific considerations for applying the category approach are described in Section 2.3.

2.2.7 Step 6. Analogue Suitability/Ranking

Step 6 Objective: Assess analogue quality based on the comparison of key attributes evaluated in **Step 5** and, finally, rank the analogues as Suitable, Suitable with Uncertainties, or Not Suitable.

The analysis of the results related to the four criteria in **Step 5** (1 - Structural similarity and common functional groups, 2 - Common structural alerts or reactivity, 3 - Common physico-chemical properties, and 4 - Likelihood of common breakdown products via (bio)transformation/degradation processes; **Table 5**) allows the selected analogues to be ranked as Suitable, Suitable with Uncertainties, or Not Suitable. Although the main principles of analogue ranking for human health and environmental safety endpoints are the same, there are some differences, therefore, the approaches are described separately below.

2.2.7.1 Human health

The ranking can be performed by using expert judgement or quantitively by computing a TotalScore.

Assessment of analogue suitability using expert judgment: The analogue(s) can be evaluated using expert judgement by following the decision tree illustrated in **Figure 5**, in close analogy to the process described by Wu and colleagues (Wu *et al.*, 2010).

In this decision tree, the starting point is represented by a potential analogue with an acceptable similarity index and common functional groups. The decision-making process consists of a number of questions, whose answer determines the path to follow, as explained below:

The first question is: does the analogue present common structural alerts?

- If the answer is yes, the next question is: does the analogue present a common or similar metabolic pathway?
- If the answer is yes, the following question is: does the analogue present values of the physico-chemical properties in the same range (ECHA, 2017b; Lester *et al.*, 2018), with a particular focus on water solubility, partition coefficient, pKa (charge) and vapor pressure (volatility) (see Section 2.2.6.3).
- In order to determine whether the physico-chemical parameters are in the same range, a rule of thumb can be applied, which can vary depending on the physico-chemical property. For example, analogues ranked as '**Suitable**' most likely differ in log Kow from the target by less than 2 units, have the same net charge, are both considered nonvolatile at room temperature with MW values within 200 atomic mass units of each other. Because toxicokinetics (TK) behavior depends on the combined influence of these properties, a quantitative assessment of physico-chemical similarity is particularly useful (Lester et al., 2023).
- If the answer is yes, the analogue can be ranked either '**Suitable**' or '**Suitable with Uncertainties**'. In the latter case, the uncertainties can be related to differences with

respect to functional groups and/or structural alerts: the analogue(s) should always represent the **worst-case,** with additional functional groups and/or structural alerts.

- If the answer is no, the next question is: does the analogue(s) represent the worst-case with respect to bioavailability/exposure? If the answer is yes, a confirmatory test must be conducted; if the outcome confirms the initial finding, the analogue can be ranked as '**Suitable with Uncertainties**' (see also Section 2.2.9).

If the answer to the first question is **no**, the following question is: is the analogue a potential metabolite or precursor of the target substance?

- If the answer is yes, there are two possible scenarios:
  - If experimental data show that the transformation rate is fast and sufficient to demonstrate the predominant exposure to the analogue, the analogue can be ranked as '**Suitable**' as demonstrated in Ellison et al., 2025.
  - If the assessment is based on *in silico* predictions, a confirmatory *in vitro* metabolism assay is recommended in order to determine the transformation rate and to quantify the metabolites.

In conclusion, the analogue(s) can be ranked according to the following categories:

- **Suitable:** if the analogue fulfils the four criteria and there are no uncertainties related to the bioavailability, structural alerts and metabolic pathway, it is ranked as '**Suitable**'. A potential analogue can also be classified as suitable, if the analogue is demonstrated to be either a precursor or a metabolite of the target substance by experimental studies, with the transformation rate being fast and the transformation efficiency being high. This would allow to show that exposure is mainly related to the metabolite/precursor (analogue) and not to the parent compound (target substance).
- **Suitable with uncertainties**: if the analogue partially fulfils the four criteria but there are uncertainties related to physico-chemical properties, which may impact the bioavailability/exposure, structural alerts or the metabolic pathway, it is ranked as '**Suitable with Uncertainties**'. The uncertainties must be described. The analogue should in any case represent the **worst-case** (i.e., it should be more bioavailable and/or with additional structural alerts), so that there would not be an underestimation of the effects that would be observed in a study with the target substance if it were to be conducted.
- **Not suitable**: if the analogue does not fulfil the four criteria and there are major uncertainties related to the read-across hypothesis, it is then ranked as '**Not Suitable**'.

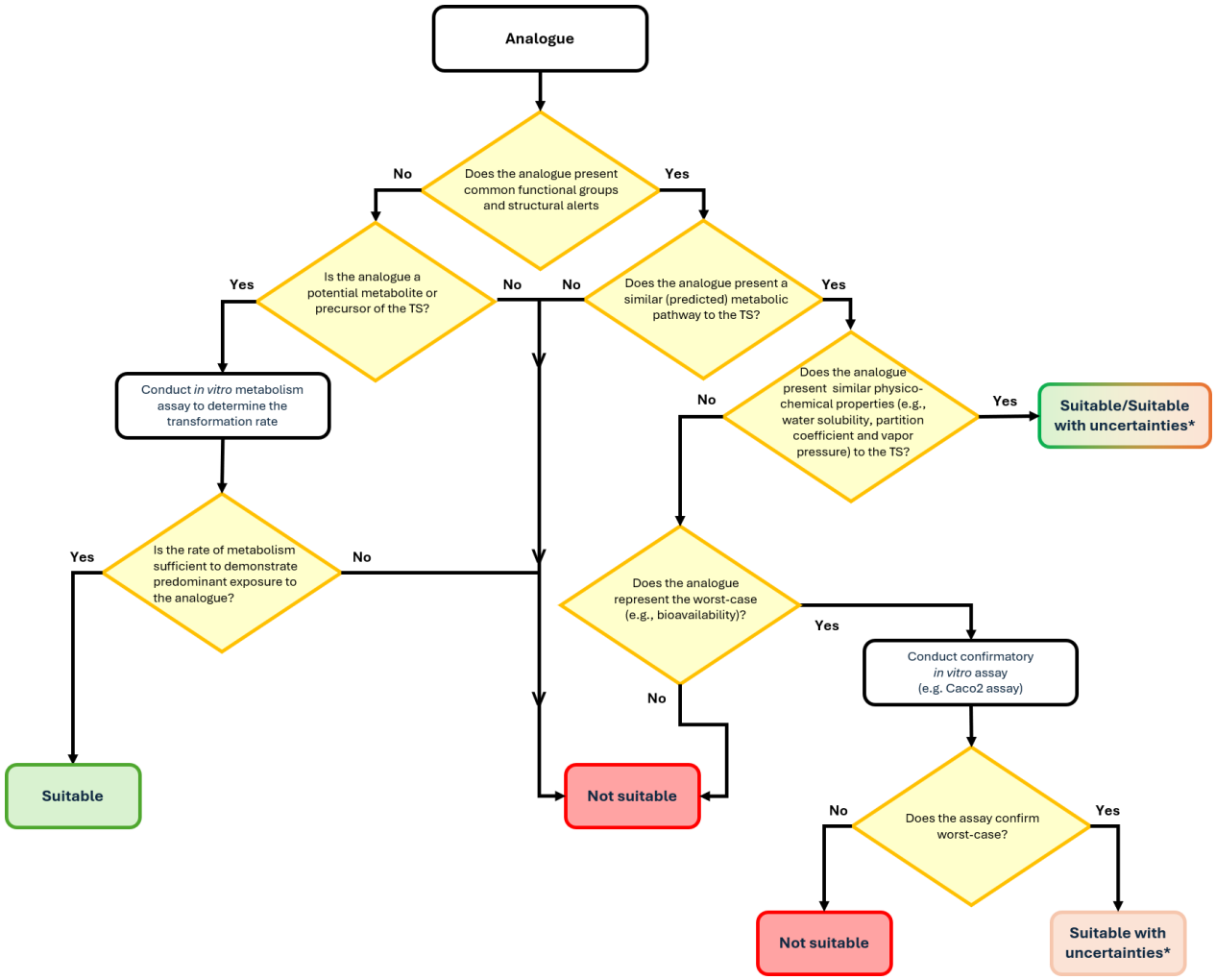


Figure 5: Decision Tree to Determine Analogue Suitability for Human Health Endpoints Using Expert Judgement

Note: * see text for additional information

Quantitative assessment of analogue suitability using TotalScore: The suitability of analogues can also be assessed quantitatively (Lester *et al.*, 2023), by comparing the relative reactivity between target substance and analogue pairs. A reactivity fingerprint that incorporates structural alerts present in either substance is defined, along with fingerprint keys that reflect bioactivity data (e.g., toxicogenomic profiling and pharmacological profiling). For the comparison of physico-chemical properties, fingerprint entries or keys may include log Kow, molecular weight charge, and volatility. For metabolism, relative biotransformation pathways can be quantitatively assessed by creating a fingerprint that lists known or predicted biotransformations for both the target substance and analogue. The overlap of these fingerprints can then be computed using a Tanimoto index or another preferred algorithm, yielding a similarity index between 0 and 1, which can be summed into a TotalScore for suitability ranging from 0 to 3, which reflects the quality of an analogue for predicting the toxicity of the target, as shown in **Figure 6**. When this scoring framework was applied across multiple chemical classes, the following score ranges were generally associated with suitability. Analogues with TotalScores approximately ≥2.5 were considered '**Suitable**', those with TotalScores typically between 2 and 2.5 were considered '**Suitable with Uncertainty/Interpretation**' and those with TotalScores of approximately ≤ 2 were considered '**Not Suitable**'. These ranges should be interpreted as empirically informed guidance rather than fixed cutoffs, allowing for limited case-specific variation. A publicly available tool to provide quantitative assessment of these toxicologically-relevant similarities is currently under development by ICCS (https://www.iccs-cosmetics.org/science/human-health/ci-read-across).

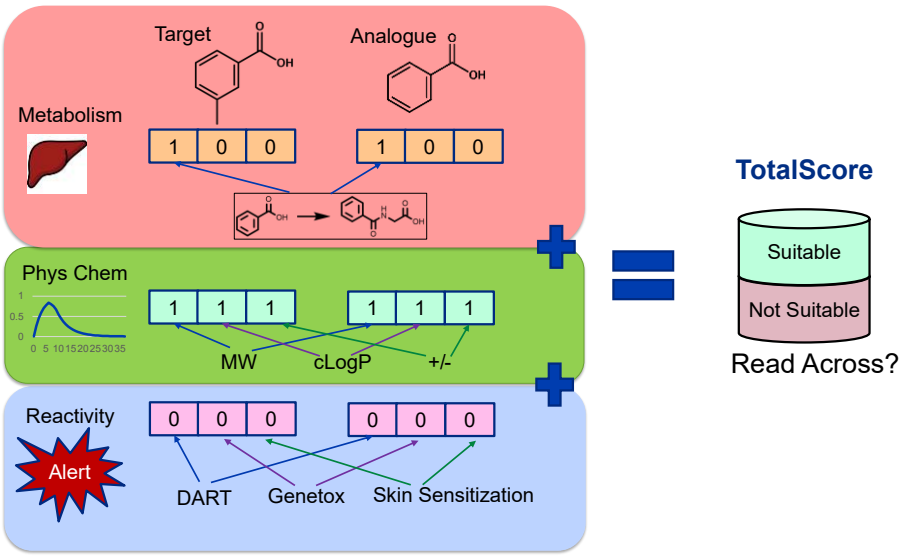


Figure 6: Quantitative Assessment of Analogue Suitability for Human Health Using TotalScore (from (Lester *et al.*, 2023))

2.2.7.2 Environmental safety

Expert judgement assessment of analogue suitability: For ecotoxicity and environmental fate endpoints, the analogue(s) can be evaluated using expert judgement following the decision tree illustrated in **Figure 7**. In this decision tree, the starting point is represented by a potential analogue. The decision-making process consists of a number of questions, whose answer determines the path to follow. These questions also address properties relevant to the PBT (Persistence – Bioaccumulation – Toxicity) assessment (ECHA, 2023), since comparable persistence, bioaccumulation potential and ecotoxicity profiles between the target substance and the analogue(s) are key elements to demonstrate a similar behavior.

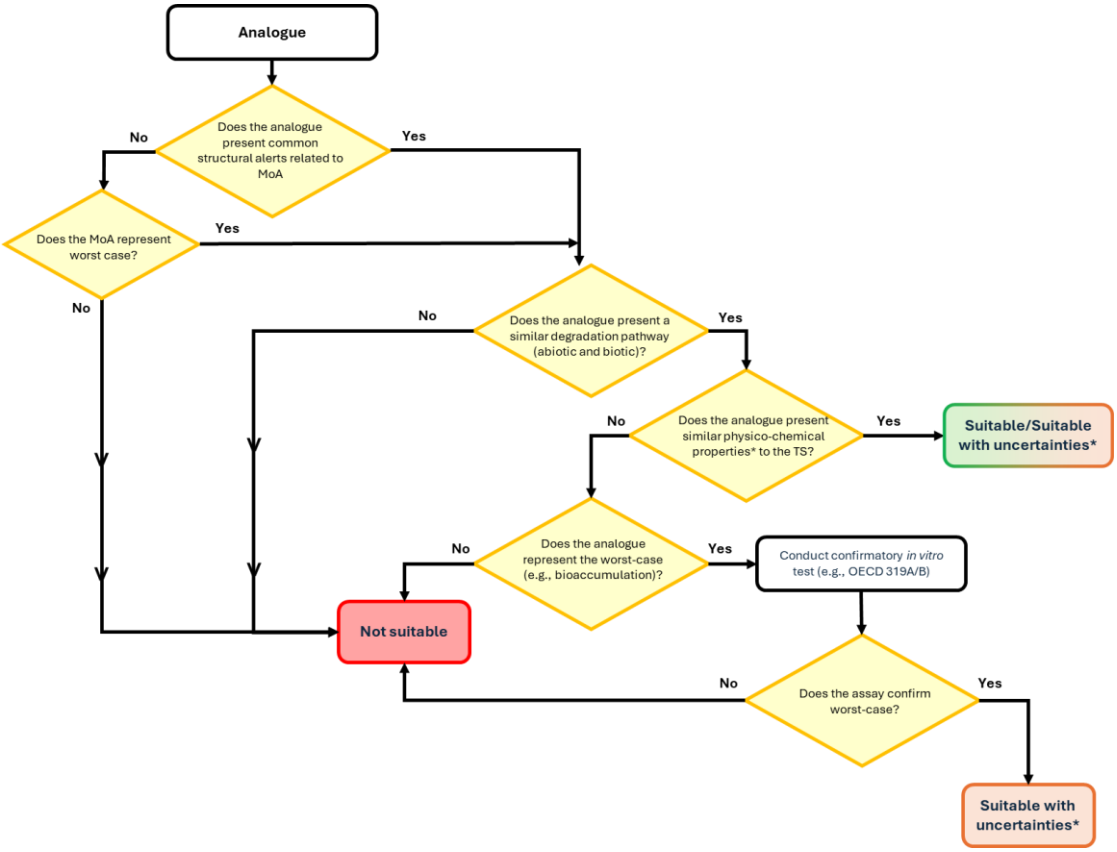


Figure 7: Decision Tree to Determine Analogue Suitability for Ecotoxicological and Environmental Fate Endpoints Using Expert Judgement

Note: * see text for additional information.

The first question is: does the analogue present common structural alerts related to the MoA (reflecting the toxicity component of the PBT assessment)?

If the answer is yes, the next question is: does the analogue present a common or similar degradation pathway (reflecting the persistence component of the PBT assessment)?

- If the answer is yes, the following question is: does the analogue present values of the physico-chemical properties in the same range (ECHA, 2017b; Lester *et al.*, 2018)?
    - In order to determine whether the physico-chemical parameters are in the same range can be judged using property-specific rules of thumb (see also Section 2.2.7.1). For environmental endpoints, the key parameters are water solubility, log Kow and vapor pressure. Log Kow is of particular importance in the environmental context, as it is the primary determinant of both bioaccumulation potential and partitioning behavior across environmental compartments.
- If the answer is yes, the analogue can be ranked either '**Suitable**' or '**Suitable with Uncertainties**'. In the latter case, the uncertainties can be related to differences with respect to functional groups and/or structural alerts, with the analogue always representing the **worst-case**.
- If the answer is no, the next question is: does the analogue(s) represent the worst-case with respect to exposure (e.g., BCF, reflecting the bioaccumulation component of the PBT assessment)? If the answer is yes, a confirmatory test (e.g., *in vitro* tests for bioaccumulation or biodegradation studies) has to be conducted: if the outcome confirms the initial finding, the analogue can be ranked as '**Suitable with Uncertainties**' (see also Section 2.2.9).
- If the answer to the first question is no, the following question: is the analogue represent a worst-case with respect to the MoA?
- If the answer is yes, the analogue can be further evaluated by addressing the second question of the decision tree.
- If the answer is no, the analogue is ranked as '**Not Suitable**'.

In conclusion, similar to human health, the analogue can be ranked according to the following categories:

- **Suitable:** if the analogue fulfils the relevant similarity criteria for the endpoint under consideration, with no critical uncertainties related to environmental fate, exposure or ecotoxicological behavior.
- **Suitable with uncertainties**: if the analogue partially fulfils the four similarity criteria but uncertainties remain with respect to environmental exposure, bioaccumulation potential, degradation behavior or ecotoxicological behavior, it should be ranked as '**Suitable with Uncertainties**'. These uncertainties should be clearly described and justified. The analogue should, in all cases, represent a **worst-case** with respect to exposure, persistence or bioaccumulation or toxicity, such that the read-across does not underestimate the effects that would be observed if a study on the target substance were to be conducted.

- **Not suitable**: if the analogue does not fulfil the four criteria and there are major uncertainties related to the read-across hypothesis, it is then ranked as '**Not Suitable**'.

2.2.7.3 Summary of Analogue Suitability/Ranking (Step 6)

The results of the analogue suitability/ranking can be summarized in a table format. An example is provided by **Table 6**.

Table 6: Template of a Table to Document the Analogue Suitability/Ranking

		Target Substance	Analogue 1	Analogue 2	Analogue …
Step 6					
Chemical name					
2D Structure					
Qualitative approach	*Suitable/Suitable with Uncertainties /Not Suitable*				
Quantitative Similarity Approach (qSIM)*	*TotalScore (0-3)*				
	Suitable/Suitable with Uncertainties /Not Suitable				

*qSIM currently only used for Human Health endpoints.

In case no suitable analogue with relevant (eco)toxicological or environmental fate data is identified, alternative assessment strategies should be explored, like exposure-based waiving or NGRA.

If applying the category approach, **Step 6** defines category suitability and boundaries; specific considerations are described in Section 2.3.

2.2.8 Step 7. Comparison of the (Eco)Toxicological or Environmental Fate Endpoint Data of the Target Substance and the Analogue(s)

Step 7 Objective: Perform an **in-depth** literature search and compare the available data of the target substance and its analogue(s) to determine whether the target substance and the analogue(s) present a similar (eco)toxicological and/or environmental fate profile.

Once one or more suitable analogue(s) have been identified, the next step is to perform an in-depth literature search to identify the (eco)toxicological data of the selected analogues for different endpoints in order to compare the (eco)toxicological or environmental fate data of the target substance and the analogue(s).

When available, NAM data can provide additional information for comparing the (eco)toxicological or environmental fate data of the target substance and the analogue(s). This data may encompass various types of analyses, including

transcriptional profiling that assesses the number and types of differentially expressed genes (DEGs) (De Abrew *et al.*, 2019; Shah *et al.*, 2022), pharmacological profiling that evaluates activities against a range of targets (Burbank *et al.*, 2024), and information derived from HTS or 'omics' technologies, such as *in vitro* bioactivity data from EPA's Toxicity Forecaster (ToxCast) and Tox21 assays (Richard *et al.*, 2016).

For both human health and environmental safety, the comparison can be performed using an expanded data matrix to that described in **Step 1**, Section 2.2.2 (**Table 7**)[10]. If applying the category approach**, Step 7** determines which is the best approach (e.g., interpolation, extrapolation) to fill data gaps for the different category members (specific considerations are described in Section 2.3).

Table 7: Template of a Data Matrix to Compare the (Eco)toxicological or Environmental Fate Behavior of the Target Substance *versus* the Analogue(s)

Property/Endpoint	Target substance	Analogue 1	Analogue 2
Chemical information			
2D structure			
Common name			
INCI name			
Synonyms			
CASRN			
Molecular formula			
SMILES			
Purity			
Known impurities			
Potential impurities			
Physico-chemical properties			
Molecular formula			
Molecular weight (or MW distribution, if applicable)			
Particulate size or size range (if applicable)			
Physical form			
Water solubility			
Partition coefficient			
Vapor pressure			
Pka (dissociation constant)			
Charge at physiological pH			

[10] Properties and endpoints can be added and removed, as required.

Property/Endpoint	Target substance	Analogue 1	Analogue 2
Mode of action			
Biological activity			
Human health endpoints			
Toxicokinetics (absorption, distribution, metabolism, and excretion)			
Acute toxicity (oral, dermal, inhalation)			
Skin irritation and corrosion			
Serious eye damage and eye irritation			
Skin sensitization			
Repeated dose toxicity (oral, dermal, inhalation)			
Genetic toxicity			
Carcinogenicity			
Reproductive toxicity			
Developmental toxicity			
Environmental fate endpoints			
Hydrolysis			
Biodegradation			
Adsorption/desorption			
Bioaccumulation			
Ecotoxicity endpoints			
Short-term toxicity to fish			
Long-term toxicity to fish			
Short-term toxicity to aquatic invertebrates			
Long-term toxicity to aquatic invertebrates			
Toxicity to aquatic algae and cyanobacteria			
Toxicity to microorganisms			
Sediment toxicity to benthic organisms			

Property/Endpoint	Target substance	Analogue 1	Analogue 2
(e.g., *Chironomus*, *Lumbriculus* or *Corophium* spp)			
Short-term toxicity to soil macro-organism (e.g., earthworm)			
Long-term toxicity to soil macro-organism (e.g., earthworm)			
Toxicity to soil microorganisms (nitrogen transformation test)			
Toxicity to terrestrial plants			
Toxicity to birds			

2.2.8.1 Human health

Data available for both the target substance and the analogue(s) represent 'bridging/anchor' data, which are a key element of a robust read-across approach and are explicitly recognized as an important AE within RAAF, as extensively described in (Roe *et al.*, 2025).

Endpoints such as irritation and genotoxicity would provide information on toxicological behavior from a qualitative point of view, while data on acute toxicity and other systemic endpoints would provide information from a quantitative perspective. If the target substance and the analogue(s) present comparable behavior with respect to qualitative and quantitative endpoints, this would support the read-across hypothesis for the endpoint being assessed. If they present differences in behavior with respect to specific endpoints, the potential causes should be identified to determine whether the read-across strategy is still valid. For example, if the target substance is not irritating to skin while the analogue is irritating, this difference may be due the fact that the analogue presents a higher dermal absorption. In this case, the read-across approach would still be valid. On the other hand, if the target substance and the analogue present differences in the adverse effects observed in repeated dose toxicity studies that are indicative of a different MoA, the analogue is then considered not suitable for read-across purposes. NAM data may provide additional information to determine whether the target substance and the analogue(s) share a similar MoA.

2.2.8.2 Environmental safety

Data available for both the target substance and the analogue(s) represent 'bridging/anchor' data, which are a key element of a robust read-across approach and are explicitly recognized as an important AE within RAAF, as extensively described in (Roe *et al.*, 2025). More specifically, comparable data on biodegradation, adsorption

and bioaccumulation would support the hypothesis that the target substance and the analogue present a similar environmental fate.

If available, comparable values with respect to short-term and long-term toxicity to aquatic organisms would further strengthen the read-across hypothesis and NAM data may provide additional information that would help determine whether the target substance and the analogue(s) share a similar MoA.

If differences between the data for the target substance and the analogue(s) are observed, they should be evaluated in the context of exposure, environmental fate or known species-specific sensitivity. For example, higher aquatic toxicity of an analogue may be attributable to greater bioavailability or slower degradation rather than an intrinsically different MoA, therefore the read-across approach would still be valid. If experimental data suggests differences in the MoA or the formation of degradation products of higher ecotoxicological concern, the analogue is then considered not suitable for read-across purposes. If experimental data suggest differences in the MoA between the target and the analogue, or that the target may form degradation products of higher ecotoxicological concern than the analogue, the analogue is not suitable for read-across. Conversely, if the analogue is expected to form more toxic degradation products than the target, the read-across is still valid as a conservative (worst-case) approach, but this will need to be evaluated closely and justified, with the assumption being documented.

2.2.9 Step 8. Characterization and Mitigation of Uncertainties

Step 8 Objective: Specify and characterize potential uncertainties related to the selected analogue(s) and if necessary, define a targeted NAM-based strategy to mitigate them.

When evaluating the potential analogue(s), a key step is the identification of uncertainties related to the proposed read-across approach. In fact, uncertainties represent an inherent feature of read-across, as conclusions are derived from indirect evidence rather than substance-specific test data. Therefore, the identification, characterization and transparent reporting of uncertainties are critical to ensuring a read-across approach is scientifically robust and acceptable to regulators. This aspect has been extensively described in the different guidance documents (ECHA, 2017c; EFSA, 2025; OECD, 2014; 2025a) as well in scientific frameworks, with Blackburn and Stuard providing structured questions and decision criteria to identify uncertainties in a quantitative manner (Blackburn and Stuard, 2014), and other groups providing qualitative or semi-quantitative characterization of uncertainties (Patlewicz *et al.*, 2015a; Schultz *et al.*, 2015; Schultz *et al.*, 2019) or endpoint-specific tolerable uncertainty levels (Cronin and Schultz, 2026).

Overall, there is a general consensus that the uncertainty assessment should:

- Be endpoint-specific

- Be linked to the intended scientific or regulatory purpose
- Focus on the assumptions underpinning the read-across hypothesis and
- Support a transparent decision on the acceptance of the analogue or the need for additional data

2.2.9.1 Identification of the Key Sources of Uncertainties

Uncertainties in read-across typically arise from different key sources. The identification of uncertainties should be performed systematically using explicit guiding questions, taking into account that not all sources of uncertainties carry equal weight for each (eco)toxicological or environmental fate endpoint, and assessors should prioritize and adapt their evaluation according to the endpoint and regulatory context under consideration, documenting any adaptation (Cronin and Schultz, 2026).

The main sources of uncertainties include:

Toxicokinetics (TK) and Environmental Fate: For human health, uncertainties may arise when 1) the internal exposure is inferred without adequate supporting evidence, 2) absorption, distribution, metabolism and excretion (ADME) are expected to differ between the target substance and analogue(s), or 3) no experimental evidence is available to demonstrate that the analogue is a metabolite or precursor of the target substance. For environmental safety, additional uncertainties may arise when 1) the degradation pathway - including both abiotic (e.g., hydrolysis and photolysis) and biotic (e.g., biodegradation and biotransformation) processes - is expected to be similar between the target substance and the analogue(s) without adequate supporting evidence, 2) differences in environmental persistence or degradation rates between the target substance and analogue(s) are not adequately characterized, or 3) the (bio)transformation potential in ecotoxicologically relevant species (e.g., fish) has not been considered, such that differences in internal exposure or the formation of transformation products can lead to different toxicological profiles.

Data Quality, Relevance and Consistency: Uncertainties may arise from limitations in the reliability, comparability or completeness of the studies used for read-across. These may include deviations from test guidelines, insufficient substance characterization, differences in study design (e.g. species, exposure route and duration), inconsistent findings across analogues, or poor reporting and quality assurance.

Study reliability should be evaluated in a reproducible manner. The Klimisch scoring system (Klimisch *et al.*, 1997), as recommended in the ECHA guidance ECHA R.4 (ECHA, 2011), classifies studies into four categories:

1 = reliable without restrictions (guideline-compliant, preferably GLP);
2 = reliable with restrictions (minor deviations but scientifically acceptable);
3 = not reliable (methodological deficiencies or poor documentation);
4 = not assignable (insufficient information for assessment).

Such scoring supports a transparent prioritization of data: studies rated Klimisch 1 or 2 are generally associated with lower uncertainty when used for read-across purposes,

while Klimisch 3 studies carry higher uncertainty and should be interpreted with caution. However, studies with lower reliability should not be automatically excluded, as expert judgement may still consider them informative for specific endpoints. If appropriate, additional tools (e.g. ToxRTool for toxicological studies (ECETOC, 2026; Schneider *et al.*, 2009) or CRED (Criteria for Reporting and Evaluating ecotoxicity Data) for ecotoxicity data (Kase *et al.*, 2016)) may be used to enhance consistency and transparency in evaluating data quality.

Biological Activity and MoA: Uncertainties may arise if a similar biological activity is assumed but not demonstrated, when mechanistic information is lacking, or when structural differences may result in the interaction with different biological targets.

To support a consistent and transparent application of the different types of uncertainties, a non-exhaustive set of guiding questions is provided below. These questions are intended to prompt critical evaluation of key assumptions and should be applied in a flexible, endpoint-specific manner.

Guiding Questions to Support the Identification Of Uncertainties

Toxicokinetics (TK) and Environmental Fate

- Is it reasonable to assume comparable internal exposure between the target substance and the analogue(s)?
- Could differences in ADME affect systemic or target-organ exposure?
- Is experimental evidence available to confirm that the analogue is either a precursor or a metabolite of the target substance?
- Is the source analogue at least as bioavailable and bioaccumulative as the target substance?
- If the read-across hypothesis is based on the fact that the analogue is either a precursor or a metabolite, is the transformation rate fast enough?
- For environmental endpoints, is the degradation pathway — including both abiotic and biotic processes — expected to be comparable between the target substance and the analogue(s), and is this supported by experimental or modelled evidence?
- Have differences in environmental persistence or partitioning behavior been adequately characterized?

Relevant parameters to address this type of uncertainty include qualitative or quantitative information on bioavailability, metabolic pathways, clearance, route-to-route relevance, and, if available, toxicokinetics or PBK (Physiologically Based Kinetic) modelling outputs (EFSA, 2025; OECD, 2025b).

Data Quality, Relevance, and Consistency

- Are the source studies reliable and fit-for-purpose for the intended read-across application?
- Are observed effects consistent across the selected analogue(s)?
- Are there unexplained inconsistencies in target organs, dose–response relationships or effect severity?

Relevant parameters to address this type of uncertainties include study reliability, comparability of study designs, consistency of outcomes and reliance on single studies or isolated data points (ECHA, 2017c; EFSA, 2025).

Biological Activity and MoA

- Is a shared biological activity plausible, even when a detailed MoA has not been fully elucidated?
- Do available NAMs data indicate concordant biological responses?
- Could structural differences plausibly lead to interaction with different biological targets?

Relevant parameters to address this type of uncertainty include the biological activity profiles, key events or stress responses, mechanistic plausibility, and available AOP-relevant information (EFSA, 2025; OECD, 2025b; Patlewicz *et al.*, 2015b).

2.2.9.2 Characterization of Uncertainties

Uncertainty characterization should be proportionate to the regulatory context and the available data. In line with EFSA (EFSA, 2025) and Blackburn and Stuard (Blackburn and Stuard, 2014), a **qualitative approach** is generally appropriate, supported when feasible by **semi-quantitative descriptors**.

At a minimum, the assessor should:

- Describe the **source and nature** of each uncertainty,
- Indicate the **direction of the uncertainty** (potential over- or under-estimation of hazard or potency), and
- Assign a **relative magnitude** (e.g., low, moderate and high).

As an alternative, semi-quantitative approaches (e.g., confidence ratings across similarity criteria or scoring of available evidence) may be used to support transparency and consistency (see **Section 2.2.9.4**).

2.2.9.3 Mitigation of Uncertainties

If the identified uncertainties are not acceptable for the intended regulatory purpose, targeted mitigation strategies should be considered. These may include:

- Selection of additional analogues,
- Generation of targeted NAM data to address specific uncertainties (e.g., metabolism, bioactivity and TK relevance),
- Application of TK or PBK modelling to support internal exposure assumptions,
- Use of additional supporting data.

Mitigation should be **problem-driven**, focusing on the uncertainties that have an impact on the read-across conclusion (EFSA, 2025; OECD, 2025b).

2.2.9.3.1 Human health

Based on the identification of uncertainties using a structured framework, the main sources of uncertainties relevant to human health read-across are summarized in **Table 8** together with examples on how to address or reduce these uncertainties.

Table 8: Key Types of Uncertainties for Human Health and Examples on How to Address or Reduce Uncertainty using NAM-based Strategies

Similarity Aspects	Potential Uncertainties	Possible Assessment/Mitigation Approach
TK	Bioavailability and exposure assumptions mainly based on physico-chemical properties or *in silico tools* without experimental confirmation	• Comparative *in vitro* permeability/penetration assays (e.g., Caco-2, Parallel artificial membrane permeability assay (PAMPA) and *in vitro* dermal penetration study (OECD TG 428)) • Integration with toxicokinetic considerations when relevant
	Insufficient evidence that the analogue is a precursor or a metabolite of the target substance	• Targeted *in vitro* metabolism studies using human-relevant systems (e.g., human liver S9 mix/microsomes) to characterize the biotransformation pathways and rates • Application of toxicokinetic or PBK modelling (e.g., PK-SIM®, a publicly available tool) to support internal exposure equivalence
Reactivity	Structural differences may alter the reactivity profiles relevant to toxicity	Targeted *in vitro* assays addressing differences in reactivity (e.g., protein binding or cellular stress responses)
Bioactivity	Absence or scarcity of bridging data addressing the biological activity relevant to the endpoint of interest Uncertainty regarding the concordance of biological responses	• Targeted NAMs to compare endpoint-relevant biological responses between the target substance and the analogue(s) • Use of high-throughput screening data with appropriate interpretation
Standard Toxicological Endpoints	Source studies used for read-across with limited reliability, poor documentation, or limited comparability (e.g. differences in guideline, species, exposure route or duration)	• Weight-of-evidence approach including additional suitable analogues • Targeted *in vitro* assays to support the observed *in vivo* effects. These may include (as appropriate): ○ *in vitro* 3T3 NRU (Neutral Red Uptake) assay in mouse fibroblasts (OECD TG 129) for acute toxicity ○ organ-specific toxicity assays, such as the HepG2 mitochondrial toxicity assay and bile salt export pump (BSEP) inhibition assay for liver toxicity, kidney transporter inhibition assays (OAT1/OAT3/OCT2/MATE1/MATE2-K), and 3D kidney microtissue nephrotoxicity models for kidney toxicity ○ genotoxicity assays (e.g., OECD TG 471, TG 473, TG 487 and ToxTracker®) ○ developmental toxicity screening assays (e.g., ReproTracker® and devTOX quickPredict™)

Similarity Aspects	Potential Uncertainties	Possible Assessment/Mitigation Approach
MoA	MoA not sufficiently supported by experimental data	• Use of available mechanistic data, including high-throughput screening data (e.g. ToxCast) • Targeted *in vitro* assays (e.g. cell stress response panels such as ToxProfiler® or cell stress panel, ToxTracker®), to support biological concordance between the target substance and analogue(s)

For a given endpoint, the assessor should consider which type of uncertainties are relevant, the extent to which available evidence supports the read-across hypothesis, and whether the identified uncertainties are acceptable for the intended scientific or regulatory purpose. Not all domains or mitigation approaches will be applicable in every case, and the selection of additional analyses or data generation should be driven by the specific uncertainties identified and their potential impact on the read-across conclusion.

When uncertainties can be adequately characterized and mitigated through the refinement of the similarity rationale, weight-of-evidence considerations, or targeted NAM testing, read-across may be applied with appropriate confidence. If a residual uncertainty remains after mitigation, this should be explicitly documented and considered in the final interpretation (see **Section 2.2.11**).

2.2.9.3.2 Environmental safety

Using the same framework as the one described for human health, the main sources of uncertainties relevant to environmental read-across are summarized in **Table 9,** together with examples on how to characterize or reduce these uncertainties.

Table 9: Key Types of Uncertainties for Environment and Examples on How to Address or Reduce Uncertainty using NAM-based Strategies

Similarity Aspects	Potential Uncertainties	Possible Assessment/Mitigation Approach
Environmental Fate	Exposure and bioaccumulation assumptions mainly based on predicted physico-chemical properties (e.g., log Kow and water solubility) without experimental support	• Inclusion of experimental fate and transport data (e.g., biodegradation and adsorption/desorption) and *in silico* modelling (QSAR, multimedia fate models) to support exposure similarity • *In vitro* fish metabolism assay (e.g., rainbow trout S9/hepatocyte intrinsic clearance (OECD TG 319A/B)) for bioaccumulation or internal exposure estimates
	Insufficient evidence that the target substance and the analogue(s) share similar persistence, transformation pathways, or distribution across different compartments	• Targeted environmental fate studies (e.g., biodegradation and adsorption/desorption testing), supported when appropriate by validated *in silico* tools for persistence, degradation and multimedia partitioning (e.g., CATALOGIC) in a weight-of-evidence framework to strengthen the confidence of the exposure similarity between the target substance and the analogue(s)

Bioactivity / Bridging Data on Invertebrates & Algae	Scarcity of bridging ecotoxicity data across key trophic levels
	Uncertainty in the sensitivity patterns across species and in the extrapolation from cellular responses to organism-level effects (particularly when fish testing has to be avoided or not available)
		• Use of NAMs to characterize biological responses across levels of biological organization, integrated within an ITS/IATA framework. This may include:
		  ○ fish cell line assays to support acute toxicity prediction and internal consistency of effects (e.g., RTgill-W1, OECD TG 249)
		  ○ effect-based NAM panels and invertebrate or algal assays to capture pathway-level perturbations and trophic-level sensitivity,-including endocrine-relevant pathways (e.g., Yeast reporter gene assay systems[11]; *Daphnia magna* reproduction test (OECD TG 211) and Chironomid life cycle toxicity test (OECD TG 233) - sensitive to juvenile hormone and insect growth regulators)
		  ○ Short-term, non-vertebrate toxicity tests (e.g., assays in *Daphnia* and algae) integrated in ITS/IATA frameworks

Standard Ecotoxicological Endpoints	Limited reliability of source ecotoxicity studies (e.g. differences in species, test design or exposure duration).	• Weight-of-evidence approach including additional suitable analogues • Targeted non-vertebrate tests (e.g., short-term assays in *Daphnia* or algae) to contextualize the observed effects

[11] https://pmc.ncbi.nlm.nih.gov/articles/PMC7251871/

Similarity Aspects	Potential Uncertainties	Possible Assessment/Mitigation Approach
MoA/Biological Response	Uncertainty regarding the conservation of the toxicity pathways across taxa, and whether biological targets are relevant across species	• Use of cross-species conservation tools, such as SeqAPASS (Sequence Alignment to Predict Across Species Susceptibility) to evaluate molecular target similarity • High throughput 'omics' or effect-based assay panels to compare key biological responses; weight-of-evidence integration of mechanistic data.

Note: Some stakeholders consider assays conducted with embryos (e.g., FET (OECD TG 236, EASZY (OECD TG 250), REACTIV (OECD TG 252), XETA (OECD TG 248) and RADAR (OECD TG 251)), as non-animal methods (NAMs) (Mitchell et al., 2023) or alternative methods (NTP, 2026).

For each environmental endpoint, the assessor should identify the relevant uncertainties, evaluate whether the available evidence sufficiently supports the read-across hypothesis, and determine acceptability in light of the intended regulatory purpose.

When mechanistic or high-throughput screening data are used to support biological plausibility, particular attention should be paid, as such data may not be species-specific and may not uniquely define a MoA. They should therefore be interpreted in conjunction with environmental fate and ecotoxicity information, rather than being considered in isolation.

Similar to human health, mitigation should be **problem-driven**, focusing on the uncertainties that affect the read-across conclusion (EFSA, 2025; OECD, 2025b).

2.2.9.4 Overall Uncertainty Conclusion

Potential uncertainties should be documented in a structured and transparent manner to enable regulatory review of read-across and to support traceability of scientific judgement. Reporting should focus on the uncertainties that are endpoint-specific and should reflect both the nature and the magnitude of the residual uncertainty after any mitigation measures have been applied.

A tabular format can be used to ensure consistency and completeness. **Table 10** provides a possible template on how to report and address uncertainties in agreement with the EFSA and OECD guidance documents (EFSA, 2025; OECD, 2025b).

Table 10: Template of an Uncertainty Assessment

Similarity Aspects	Supporting Evidence	Data Quality (low/moderate/ high)	Strength of Evidence (low/moderate/ high)	Key Uncertainties	Comments
TK/ Environmental Fate					
Chemical Reactivity					
Bioactivity					
Conventional toxicology					
MoA					
Overall Uncertainty*					

**Rank of the overall uncertainty of the read-across hypothesis based on the evaluation of the analogue data set characteristics (low, moderate, high) with the description of the reason*

Based on all the aspects related to the uncertainty characterization and mitigation, the assessor should determine whether the level of the residual uncertainty is acceptable without generating additional data, or whether additional targeted (NAM) testing is warranted to strengthen the read-across hypothesis. The outcome of this assessment may therefore fall into one of the following scenarios, with an optional **very low** category, which is applicable when the similarity is particularly high (e.g., a salt form or a one-carbon difference):

- **Residual uncertainty is low**, and the available evidence is considered sufficient to proceed directly to final read-across conclusions (see **Step 10**), without additional testing.
- **Residual uncertainty is moderate**, and targeted additional (NAM) data generation (see **Step 9**) is expected to meaningfully reduce the uncertainty.
- **Residual uncertainty is high or critical**, such that read-across cannot be supported without substantial additional data generation or may not be appropriate for the endpoint under consideration.

To facilitate traceability and cross-framework comparability, these uncertainty categories can be mapped onto EFSA's approximate probability scale (EFSA, 2018) and the Assessment Outcome (AO) scores of the ECHA Read-Across Assessment Framework (ECHA, 2017c), as shown in **Table 11**. This mapping is intended as a reference tool and does not replace expert judgement in assigning uncertainty levels (Cronin and Schultz, 2026).

Table 11: Mapping of Uncertainty Categories to EFSA Probability Ranges and ECHA RAAF Assessment Outcomes (from (Cronin and Schultz, 2026))

Uncertainty level	EFSA approximate probability	ECHA RAAF AO score
Very low	>95%	5
Low	90–95%	4–5
Moderate	66–90%	2–3
High	<66%	1–2

2.2.10 Step 9. Generation of Additional NAM Data to Strengthen Read-Across or Reduce Uncertainties (optional)

Step 9 Objective: Conduct the NAM-based strategy to address the potential uncertainties with the overall aim to strengthen the read-across hypothesis.

Once the uncertainties have been identified and characterized (**Step 8**), the assessor should determine whether additional targeted testing is warranted. This step is optional and is only undertaken when such testing can reduce key uncertainties and strengthen confidence in the read-across hypothesis. In such cases, a NAM-based strategy should be developed, and conducted to confirm the target substance and the potential analogue(s) present similar MoA or (eco)toxicological or environmental fate behavior (see also Sections 2.2.9.1 and 2.2.9.2).

The execution of this step is beyond the scope of the present document, but it has been included in the read-across workflow, as the testing results will provide support to the overall read-across approach.

2.2.11 Step 10. Conclusion and Documentation

Step 10 Objective: Document the entire process in a transparent manner, including the similarity between the target substance and the analogue(s) in all attributes considered, the reliability of the source study(ies) and any potential bias that could affect the read-across strategy.

The final step is represented by the conclusion on the read-across approach as well as by the development of a transparent documentation of the read-across justification (RAJ).

Based on all the available information, the assessor can finally perform the read-across for the relevant endpoint(s).

Analogue approach: In case of data gaps for qualitative endpoints (e.g., genetic toxicity for human health and biodegradation for environment), the presence or absence of this property is inferred from the established properties of the analogue(s). For quantitative

endpoints (e.g., repeated dose toxicity for human health and short-term toxicity to fish for environmental safety), the following approaches can be proposed, also considering other guidance documents (OECD, 2025b):

- To use the endpoint value of the analogue if the analogue represents overall the worst case with respect to the (eco)toxicological or environmental fate profile;
- To use the most conservative endpoint value among the different analogues, in case more than one analogue has been selected;
- To adjust the endpoint value based on molecular weight normalization or other scientifically justified scaling approaches, when appropriate.

If applying the category approach, the data gap filling for category members is described in Section 2.3.

The RAJ report is recommended to consist of two main parts:

1. The **first part**, ultimately leading to the formulation of the read-across hypothesis, describes in detail the steps, tools, databases and criteria that have been used and applied to identify and justify the suitability of analogues with available toxicological data for filling endpoint data gaps of the target substance. Following the flow described in **Step 3**, all analogues identified to meet the minimum similarity criteria should be presented, along with a justification why certain analogues were taken on to **Step 4-5** for evaluation, and why others were excluded. Likewise, the steps, tools and conclusions derived from the more thorough analogue suitability/ranking regarding similarity as described in **Step 6**, must be transparently documented, either in the form of the output by a tool (e.g., structural alerts, functional group, physico-chemical properties and metabolic path/breakdown products) or by expert judgement (e.g., prediction of TK/environmental fate behavior-based physico-chemical properties).

For human health, the suitability of the identified analogues can be assessed using expert judgement (**Figure 5**) or a quantitative approach using TotalScore (**Figure 6**), as described in Section 2.2.7. For environmental safety, the suitability of the identified analogues can be assessed using expert judgement (**Figure 7**).

For each suitability ranking of '**Suitable**', '**Suitable with Uncertainties**', or '**Not Suitable**', a summary justification covering all key criteria should be provided and the read-across hypothesis should be provided, underlining whether the analogue is similar or represents a worst case.

This will be complemented with a description of the uncertainties and the identification of potential testing/assessment strategies, aimed at addressing or reducing the remaining uncertainties related to an analogue for the assessment of a (eco)toxicological or environmental fate endpoint of the target substance by means of read-across, as discussed in Sections 2.2.8 and 2.2.9. This first part of the RAJ can be supported in the form of an Excel template (or in a different template form) including the comparison against the four criteria (**Table 5** and **Table 6**)

2. The **second and final part** of the RAJ documentation should provide a detailed endpoint-based comparison of the (eco)toxicological or environmental fate profile of the target substance and the analogue(s). This comparison should be supported with an endpoint-related data matrix containing existing physico-chemical properties, toxicological data or ecotoxicological data as well as newly generated NAM data relevant for endpoint assessment or MoA understanding (e.g., **Table 7**). It is also recommended to identify directly in the data matrix the actual read-across being applied and which data is used as the basis to fill the data gap.

Subsequent to the presentation of the available (eco)toxicological or environmental fate information, the proposed read-across approach should be discussed with regard to

- The strength of concordance of bridging data, qualitatively and quantitatively;
- Commonalities of MoAs between the target substance and analogue(s);
- The degree of conservatism with regard to the severity of effects and bioavailability/environmental fate;
- The overall quality of the database (e.g., availability of guideline-compliant studies);
- Any remaining uncertainties relevant to the endpoint under consideration.

Taking into consideration the uncertainty identification and characterization performed in **Step 8** and the outcome of any additional targeted testing conducted in **Step 9** (when applicable), all lines of evidence should be integrated to derive an overall, endpoint-specific conclusion on the acceptability of the read-across.

Based on this integrated evaluation, the acceptance of the read-across by the assessor could be assigned to one of the following categories:

1. **Accepted with very low/low uncertainty**
   The read-across approach is supported by consistent and concordant evidence across relevant similarity domains and no additional testing to address residual uncertainty is required.

2. **Accepted with moderate uncertainty**
   Residual uncertainties may remain, but they are not considered critical to the read-across approach. The nature, direction and magnitude of the remaining uncertainties should be explicitly documented, and their potential impact on the endpoint of concern considered within the relevant regulatory context. If required by the relevant regulatory program, additional testing to address residual uncertainty should be applied in accordance with applicable guidance.

In conclusion, the primary objectives of the RAJ is to 1) provide a scientifically well documented and transparent description of the read-across process, ensuring that the analogue identification and selection are conducted without bias, and 2) demonstrate that the prediction of a(n) (eco)toxicological and/or environmental fate property for the target substance is scientifically justified, based on the data available for the selected analogue(s). This justification is further supported by a well-documented assessment of

similarity, along with a clear evaluation of uncertainties and the scientific rationale for how these have been addressed or minimized.

2.3 Category Approach

As mentioned in Section 2.1, **Background and Key Elements of Analogue *versus* Category Approach**, a category approach for read-across can be used when chemicals can be grouped, if their physico-chemical, (eco)toxicological and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity (ECETOC, 2012; ECHA, 2017c; OECD, 2025b).

An advantage of the category approach is that the identification of patterns of effects across the different members may increase the confidence in predicting a given endpoint of one of the category members.

The workflow described in Section 2.2.1, **Overview of the Workflow**, can be applied to the category approach, with some key differences, which are indicated by the grey text (**Steps 5-7**) in **Figure 2**.

More specifically, Step 1. Target Substance Characterization, Step 2. Problem Formulation & Scoping, Step 9. Generation of Additional NAM Data to Strengthen Read-Across or Reduce Uncertainties **and** Step 10. Conclusion and Documentation are the same for both approaches, with no significant differences, other than terminology (analogue(s) = category members). **Steps 3-8** are described in the sections below to provide details on the differences between the analogue and category approach, so that the assessor can proceed in a systematic manner to conduct a read-across assessment using the category approach.

2.3.1 Specificities of the Category Approach

Step 3. Analogue Identification and Screening of Potential Analogues corresponds to the identification of the members of the potential category. These members can be established using a supervised approach or an unsupervised approach. The **supervised approach** uses pre-existing knowledge. For example, substances that are members of established categories from regulatory agencies (e.g., OECD HPV programme[12]) or those that present a similar manufacturing process with the same or similar starting material can be analyzed to determine whether they can be grouped as a category. Alternatively, the different members could be identified based on the results of a search for analogues performed as described in Section 2.2.4. This can be considered as an **unsupervised approach**, as it is not based on pre-existing knowledge.

In Step 4. Initial Literature Search for Data Gap Filling, if a supervised approach is used, the category members have already been identified and an initial literature search is not required. Therefore, an **in-depth** literature search will be conducted to identify available (eco)toxicological and/or environmental fate data as well as all the information related to the MoA of the different substances (see Section 2.2.2 for the data sources that can be used).

[12] https://hpvchemicals.oecd.org/ui/search.aspx

In Step 5. Analogue Evaluation and Selection, if multiple analogues have been identified, the key question is whether the analogues and the target substance can be part of a category. This is addressed by 1) evaluating the different substances on the basis of the four criteria described in Section 2.2.6 (1 - Structural similarity and common functional groups, 2 - Common structural alerts or reactivity, 3 - Common physico-chemical properties, 4 - Likelihood of common breakdown products via (bio)transformation/degradation processes), and 2) determining whether the physico-chemical, (eco)toxicological and/or (bio)transformation/environmental fate properties, together with the MoA are similar or follow a trend across the different substances. The observed trends should be biologically plausible and supported by consistency across chemical structures, MoA, and (eco)toxicological or environmental fate responses. As an example, substances that have similar alerts indicating a common biological activity or MoA or result in formation of common moiety following (bio)transformation *in vivo* can be considered as members of a given category.

A data matrix can be used to compare the behavior of the potential category members and to formulate the category hypothesis (**Table 12**). For example, data for a specific endpoint should be consistent across the category members, either by demonstrating a uniform hazard or following a qualitative/quantitative trend.

Table 12[13]: Template of a Data Matrix to Compare the (Eco)toxicological or Environmental Fate Behavior of the Category Members

Endpoint	Member 1	Member 2	Member 3	Member ….
Chemical information				
2D Structure				
Common name				
INCI name				
Synonyms				
CASRN				
Molecular formula				
SMILES				
Purity				
Known impurities				
Potential impurities				
Physico-chemical properties				
Molecular weight				
Vapor pressure				
Water solubility				
Log Kow/Log D				
Boiling point				

[13] This table is identical to **Table 7**, with Analogue' being replaced by 'Member'.

Endpoint	Member 1	Member 2	Member 3	Member ….
Melting point				
Pka				
Charge at physiological pH				
Mode of action				
Biological activity				
Human health endpoints				
Toxicokinetics (absorption, distribution, metabolism, and excretion)				
Acute toxicity (oral, dermal, inhalation)				
Skin irritation/corrosion				
Serious eye damage and eye irritation				
Skin sensitization				
Repeated dose toxicity (oral, dermal, inhalation)				
Genetic toxicity				
Carcinogenicity				
Toxicity to reproduction				
Developmental toxicity				
Environmental fate endpoints				
Hydrolysis				
Biodegradation				
Adsorption/desorption				
Bioaccumulation				
Ecotoxicity endpoints				
Short-term toxicity to fish				
Long-term toxicity to fish				
Short-term toxicity to aquatic invertebrates				
Long-term toxicity to aquatic invertebrates				
Toxicity to aquatic algae and				

Endpoint	Member 1	Member 2	Member 3	Member ….
cyanobacteria				
Toxicity to microorganisms				
Sediment toxicity to benthic organisms (e.g., *Chironomus*, *Lumbriculus* or *Corophium* spp)				
Short-term toxicity to soil macro-organism (e.g., earthworm)				
Long-term toxicity to soil macro-organism (e.g., earthworm)				
Toxicity to soil microorganisms (nitrogen transformation test)				
Toxicity to terrestrial plants				
Toxicity to birds				

In **Step 6. Analogue Suitability/Ranking,** the category is established, by defining its boundaries with clear inclusion and exclusion criteria, which specify the range of values within which reliable estimations can be made for the different category members with respect to the endpoint of interest. This would also allow the exclusion of substances that do not present sufficient similarities for the different aspects. Overall, these boundaries define the limits within which interpolation or extrapolation can be scientifically justified. For example, if there is regular quantitative trend for a given physico-chemical, the property-specific rules of thumb described in **Section 2.2.7.1** can be applied to define the boundaries of the category. As another example, the category members may differ in a structural feature like the position of a key functional group on a core structure: if this position affects the metabolic pathway, the potential member has to be removed from the category; such a case has been described by Lester and colleagues (Lester *et al.*, 2026).

Step 7. Comparison of the (Eco)Toxicological or Environmental Fate Endpoint Data of the Target Substance and the Analogue(s) corresponds to the data gap filling for the relevant endpoint(s). Several approaches can be used, including interpolation, extrapolation, and the previously described analogue approach (**Section 2.2**) (**Figure *8***).

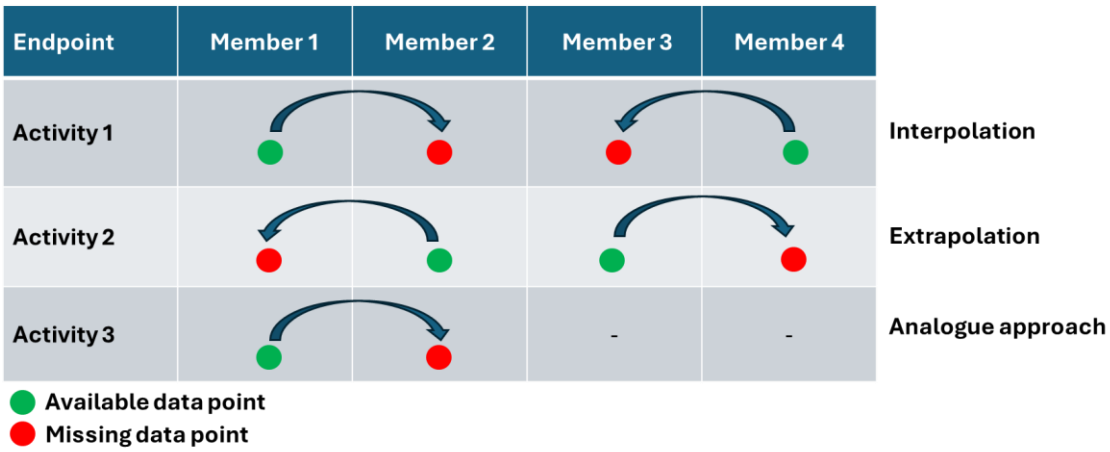


Figure 8: Data Gap Filling Using the Interpolation, Extrapolation, and Analogue Approach

- *Interpolation*: The category members can be arranged in line according to the (eco)toxicological or environmental fate trend. Interpolation uses data from the category members on either side of the trend line to predict the hazard of the data-poor category members (ECETOC, 2012; OECD, 2025b). For example, if two category members with shorter and longer chain lengths have measured toxicity values, the toxicity of an intermediate-chain compound can be estimated from those surrounding data (**Figure 8**).
- *Extrapolation:* The category members can be arranged in line with the (eco)toxicological or environmental fate trend. Extrapolation uses data from the category members on one side of the line to predict the hazard of the data-poor category member, which are at the other side of the line (ECETOC, 2012; OECD, 2025b). For example, if toxicity increases consistently with chain length across the tested category members, the toxicity of a longer-chain data-poor compound at the end of that trend line can be estimated by using the available data (**Figure 8**).

Interpolation is more widely accepted as it reduces the uncertainty of the prediction, while extrapolation may represent a worst-case or may underestimate the toxicity of the data-poor category member. The robustness of a category-based read-across depends on the size of the category and the quantity and quality of data available for each member. Where trends are poorly defined or absent, interpolation and extrapolation approaches are associated with increased uncertainty.

To minimize potential uncertainties, a weight-of-evidence approach can be applied, which would include information from (Q)SAR models, NAM-based assays, or other mechanistic or bridging studies (OECD, 2014). When there is sufficient data for a specific endpoint, a trend analysis may be applied, and internal local (Q)SAR models (e.g., (Q)SAR OECD Toolbox) can be developed using data from the category members. In case data is available only for a single category member for a given endpoint, then the canonical analogue-based read-across approach that has been described in Section 2.2 can be used (**Figure 8**).

As per the analogue approach, **Step 8. Characterization and Mitigation of Uncertainties** would characterize uncertainties related to the category and assess whether there is a need to strengthen the read-across hypothesis. Different scenarios can be envisaged; two common examples with possible solutions are described below.

- One category member may present inadequate data for the required endpoint, while this substance would fulfill all other parameters to be part of the category. In this case, a NAMt could be conducted for the required endpoint using the different category members to reduce the uncertainty (see also **Section 2.2.9** for possible NAMs to be used).
- No data is available for the different category members for a specific endpoint. In case this endpoint can be addressed using NAMs (e.g., skin sensitization for human health or RTgill-W1 cell line assay (OECD TG 249) for environment), testing should be conducted on representative category members selected to cover the defined category boundaries (e.g. short-, medium- and longer-chain alkyl substances). This ensures that the data generated are informative for the full category range. In case of systemic endpoints for human health or endpoints related to the use of vertebrates for environment, additional substances with relevant (eco)toxicological or environmental fate data that fulfil the category inclusion criteria may be incorporated to support the read-across hypothesis.

3 Conclusion

By following the 10-step workflow outlined in this ICCS RAX BPG, safety assessors can confidently apply read-across using either the analogue or category approach to support hazard and risk assessment. The document emphasizes the importance of selecting analogues or category members based on scientifically justified similarities in physico-chemical properties and biological activity, and of evaluating and transparently documenting all decisions and assumptions throughout the workflow. Robustness in read-across is strengthened by the integration of NAMs such (Q)SAR models, *in vitro* assays, and mechanistic studies.

The field of read-across is rapidly evolving, with advances in computational methods, increased availability of NAMs, and growing regulatory expectations for transparency and scientific rigor. As methodologies and best practices continue to develop, this guidance will be updated as appropriate by the ICCS to ensure that safety assessments remain robust, credible, and aligned with the latest scientific and regulatory requirements.

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