► FKZ 3715 67 4180

“Tierversuchsfreie Bewertung unter REACH - Weiterentwicklung und Nutzung des Read-across Ansatzes”

Risk assessment under REACH without animal testing – development, application and acceptance of the read-across approach.

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► ON BEHALF OF THE

► FEDERAL ENVIRONMENT AGENCY (UMWELTBUNDESAMT)

► Hannover, March 2018

Abstrakt

Für die Abschätzung des Gefährdungspotentials von Stoffen auf die Umwelt und/oder auf die menschliche Gesundheit werden in der Regel Studien am Tier durchgeführt. Alternative Methoden wie z. B. Read-across, werden mit dem Ziel eingesetzt, Tierversuche möglichst zu vermeiden. Der Read-across Ansatz ist prinzipiell ein starkes Werkzeug, um relativ einfach (d.h. ohne den Einsatz von komplexen quantitativen Vorhersagemodellen) und mit geringem (ökonomischen) Aufwand Gefahrenpotenziale von Substanzen verlässlich vorherzusagen, und/oder in einem Weight-of-Evidence Ansatz miteinander zu vergleichen.

Die Zielsetzung dieses Projektes ist es, der Read-across Methodik mit einer „Best Practice“ Anleitung zu einer besseren gegenseitiger Akzeptanz zwischen Anwendern (Industrie) und Bewertern (Behörden) zu verhelfen. Im Fokus liegen umweltrelevante Endpunkte wie akute/chronische Fischtoxizität und Bioakkumulation im Fisch. Zur Erstellung der Best Practice Anleitung wurde zunächst der Status Quo mittels einer Literaturrecherche und einer Online-Befragung von Experten ermittelt (Interim Report, Abschnitt I). Anschließend wurden die hierbei identifizierten, kritischen Themen innerhalb eines Workshops (WS) mit Experten aus Wissenschaft/ Industrie und Behörden erarbeitet (Status Report). Die Resultate des WS sind in die „Best Pratice“ Guideline eingegangen, in welcher drei exemplarische Arbeitsabläufe für die Endpunkte akute und chronische Toxizität im Fisch und Bioakkumulation dargestellt sind (Synthesis Papier). Die vorgestellten Arbeitsschritte leiten den Nutzer durch die Erstellung des Read-across und geben Hinweise auf wichtige Punkte die bei der Erstellung der Datenmatrix und der Formulierung der Hypothese berücksichtigt werden sollten.

Abstract

Risk assessment traditionally use in-vivo animal data to evaluate the safe use of compounds with regard to human health and the environment. Alternative methods like read-across aim to reduce the number of in vivo experiments. Read-across is a technique used to predict endpoint information for one target chemical, that lacks relevant experimental in vivo data, by using data from the same endpoint from one to many similar chemicals, called source compound. Read-across is a relative simple tool, as it does not requires complex statistical methods and can predict the toxicity of an untested compound with relatively low economic effort.

This project aims to develop a best “practice guidance” for the read-across assessment of environmental endpoints such as acute/ chronic fish toxicity or accumulation in fish. This guidance may help to improve the mutual acceptance of read-across approaches between applicants and authorities. The best practice guidance is based on three different steps. First, the state of the art was evaluated by reviewing relevant literature and publications from recent projects. We further performed an online survey with stakeholders to document and identify experiences with read-across and its current limitations (Interim report). Critical topics were identified and discussed with stakeholders from industry, academia and authorities within a workshop (status report). The results of the WS contribute to the best practice guideline, which comprises a general workflow on read-across and three workflows for the endpoints acute and chronic fish toxicity and bioaccumulation and recommendations for improvements (synthesis paper, section III). The described workflow guides the user through the preparation of a read-across approach and addresses important points that have to be taken into account e.g. for setting up a data matrix or for the formulation of a the read-across hypothesis.

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ACF	atom-centered fragment
BCF	bioconcentration factor
Caleidos	EU Project: Chemical Assessment according to Legislation
DNEL	derived no effect concentration
ECHA	European Chemicals Agency
EPA	U.S. Environmental Protection Agency
ENV	environment
Koc	partition coefficient between organic carbon and water
Kow	octanol water partition coefficient
NOAEL/NOEC	no observed adverse effect level/concentration
MoA	mode of action
PBT	persistent bioaccumulative toxic
PNEC	predicted no effect concentration
(Q)SAR	quantitative structure activity relationship
RAAF	read-across assessment framework
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SC	source compound
TC	target compound
tpa	tonnes per annum (year)
UBA	Umweltbundesamt
vPvB	very persistent and very bioaccumulative
WoE	weight of evidence

Zusammenfassung

Zielsetzung und Vorgehen

Für die Abschätzung des Gefährdungspotentials von Stoffen in Hinblick auf die Umwelt und/oder auf die menschliche Gesundheit werden in der Regel Studien am Tier durchgeführt und deren Resultate auf den Organismus bzw. die Population in der Umwelt extrapoliert.

Die Anwendung alternativer Methoden wird in verschiedenen regulatorischen Kontexten in der Risikobewertung gefordert, mit dem Ziel, Tierversuche weitestgehend zu vermeiden und bestehendes Wissen optimal zu nutzen. Die REACH Verordnung z.B. fordert explizit die Verwendung von alternativen Methoden und hat die allgemeinen Bestimmungen zur Nutzung dieser Methoden in Anhang XI beschrieben. Für Inhaltsstoffe von Kosmetika geht die Entwicklung seit dem Inkrafttreten der Kosmetikdirektive Nr. 1223/2009[1] in 2013 noch weiter. Zur Beurteilung der Toxizität von Kosmetikinhaltstoffen ist das Testen von Tieren verboten, die gefährdungsfreie Anwendung der Stoffe für den Verbraucher muss jedoch sichergestellt werden. Alternative Methoden zur Vorhersage des Gefährdungspotentials einer Substanz umfassen in vitro oder in silico Modelle.

Aktuelle Auswertungen der Europäischen Chemikalienagentur (ECHA) zeigen, dass Read-across als eine der häufigsten alternativen Methoden in der Risikobewertung von Chemikalien unter REACH eingesetzt wird[2]. Die Vorhersage einer toxikologischen Endpunktes durch eine Read-across Betrachtung kann durch die ECHA und bewertende Mitgliedsstaaten akzeptiert werden, wenn die dadurch generierten Daten vergleichbar sind zu denen aus den geforderten experimentellen Tierversuchen, wie z.B. in der REACH Leitlinie R7 festgelegt[3].

Die prinzipielle Hypothese des Read-across ist es, für eine Substanz mit fehlendem experimentellem Datensatz für einen spezifischen Endpunkt (z.B. Mutagenität im Amestest oder akute Fischtoxizität) den Effektwert vorherzusagen. Die zu bewertende Chemikalie, genannt Zielsubstanz, wird hierbei mit einer oder mehreren ähnlichen Substanz(en), für die valide und relevante experimentelle Daten vorliegen, verglichen. Die ähnlichen Substanz(en) werden im Folgenden „Ausgangssubstanz(en)“ genannt. Je nach Anzahl der vorliegenden Ausgangssubstanzen unterscheidet man den Analog-Ansatz, eine Eins zu Eins Betrachtung, bzw. eine Gruppen- oder Kategorienbetrachtung, bei der mehr als eine Ausgangssubstanz vorliegen. Innerhalb einer Gruppe werden intrinsische Stoffeigenschaften durch eine Interpolation vorhergesagt.

Wichtig dabei ist, dass die Ähnlichkeit einer Stoffgruppe nicht nur auf der strukturellen Ähnlichkeit von Molekülen beruht, sondern die Stoffe auch gemeinsame relevante toxikologische Eigenschaften haben. Die Analyse der gemeinsamen toxikologischen Eigenschaften kann je nach abzuschätzendem Endpunkt verschiedene Parameter einbeziehen, wie z.B. strukturell ähnliche Metabolite des physikalischen oder biologischen Abbaus, sowie die Wirkungsstärke einer toxikologischen Eigenschaft.

Es können qualitative Endpunkte vorhergesagt werden wie z.B. eine „Ja/Nein“-Antwort bei Mutagenität. Es sind jedoch auch quantitative Vorhersagen möglich, wie z.B. die Ableitung einer Wirkstärke (DNEL oder PNEC). Die Wirkstärke der zu betrachtenden Eigenschaft über die Stoffgruppe hinweg,

[1] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (Text with EEA relevance, http://data.europa.eu/eli/reg/2009/1223/oj

[2] ECHA (2014a). The use of Alternatives to Testing on Animals for the REACH Regulation. Second report under Article 117(3) of the REACH Regulation. Helsinki, Finland, European Chemicals Agency: 131p.

[3] ECHA (2014b). Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 3.0. Helsinki, Finland, European Chemical Agency: 396p.

sollte innerhalb der Gruppe entweder zu ähnlichen Werten führen oder einem plausiblen und konsistentem Trend folgen. Ein plausibler Trend wäre z.B. ein Anstieg der Toxizität mit steigender aliphatischer Kettenlänge.

Die Analyse der strukturellen und toxikologischen Ähnlichkeit ist komplex und bei weitem nicht alle Read-across Beurteilungen werden durch die zuständigen Behörden akzeptiert[4]. Daher wurde kürzlich von der ECHA eine Richtlinie publiziert, die einem Workflow und die Datenanforderungen für humane Endpunkte mittels sechs verschiedener Szenarien definiert (RAAF-Read-across Assessment Framework[4]). Hierbei werden zunächst Ausgangsstoffe mit ähnlichen strukturellen Eigenschaften identifiziert, anschließend deren physiko-chemischen, toxikokinetische und schließlich toxikodynamische Eigenschaften verglichen. Durch diesen Prozess werden die relevanten Ausgangstoffe definiert und im Weiteren zur Vorhersage der Toxizität der Zielsubstanz herangezogen. Das RAAF soll zu einer systematischen und transparenten Beurteilung der eingereichten Dossiers und dadurch besseren Akzeptanz der Read-across Bewertungen führen. Zu Beginn des Projektes lag noch kein RAAF zur systematischen Bewertung von Umweltendpunkten vor. Dieses wurde in der Zwischenzeit publiziert und wesentliche Ergebnisse werden mit denen in diesem Projekt erzielten Resultaten in Abschnitt 1.2 gegenübergestellt[5].

Der Read-across Ansatz ist prinzipiell ein starkes Werkzeug, um relativ einfach (d.h. ohne den Einsatz von komplexen quantitativen Vorhersagemodellen) und mit geringem (ökonomischen) Aufwand Gefahrenpotenziale von Substanzen verlässlich vorherzusagen, und/oder in einem Weight-of-Evidence Ansatz miteinander zu vergleichen.

Die Zielsetzung dieses Projektes ist es, der Read-across Methodik mit einer „Best Practice“ Anleitung zu einer besseren gegenseitiger Akzeptanz sowohl auf Anwenderseite (Industrie) als auch auf Bewerterseite (Behörden) zu verhelfen. Im Fokus liegen umweltrelevante Endpunkte wie akute und chronische Fischtoxizität und Bioakkumulation im Fisch.

Diese Best Practice Anleitung wurde in drei Schritten entwickelt. Zunächst wurde der Status Quo mittels einer Literaturrecherche und eines Online-Fragebogens ermittelt. Die Ergebnisse sind im Zwischenbericht dokumentiert (Interim Report, Abschnitt I). Aufbauen auf den Ergebnissen des Zwischenberichtes wurden drei Fragestellungen identifiziert:

Topic 1- Similarity: a key requirement of read-across

Topic 2- Uncertainty in read-across approaches

Topic 3- Use of tools and databases for read across

Diese wurden innerhalb eines 1.5 tägigen Workshops mit Experten von Behörden und aus der Industrie, sowie Wissenschaftlern innerhalb von Knowledge Cafes erarbeitet und die dabei erhaltenen Ergebnisse von den Teilnehmern anschließend priorisiert. Die Ergebnisse dieses Workshops sind im Statusreport dokumentiert (Abschnitt II).

Ein verbindliches Vereinbarungspapier basierend auf den Resultaten des Workshops zwischen Industrie und Behörden wurde zu Beginn des Projektes aufgrund möglicher Interessenskonflikte als nicht realistisch eingeschätzt. Ein weiterer Grund hierfür ist, dass die Teilnehmer des Workshops zwar ihre jeweiligen Organisation und deren Erfahrungen vertreten, sie jedoch in der Regel nicht mit der Befugnis ausgestattet sind, verbindliche Vereinbarungen für ihr Unternehmen/Organisationen abzuschließen. Daher wurde anstelle des Vereinbarungspapiers eine „best practice guideline“ erarbeitet (Ab-

[4] Ball, N., M. T. Cronin, et al. (2016). "Toward Good Read-Across Practice (GRAP) guidance." ALTEX Online first: 18p.

[5] ECHA (2017) Read-Across Assessment Framework (RAAF). ECHA-17-R-01-EN

schnitt III). Die „best practise guideline“ entwickelt in übersichtlicher Weise drei exemplarische Arbeitsabläufe für die Endpunkte akute und chronische Toxizität im Fisch und Bioakkumulation. Zudem enthält sie eine Anleitung mit einem generellem Read-across Workflow und Anmerkungen zu weiterem Verbesserungsbedarf (Synthesis report, Abschnitt III).

Wesentliche Projekt-Ergebnisse

Ein wesentliches Ergebnis des Zwischenberichtes (Abschnitt I) ist, dass es einen großen Unterschied gibt zwischen der wissenschaftlichen Sichtweise und den tatsächlichen Gründen die zur Ablehnung von Read-across Dossiers z.B. durch die ECHA führen. Ball et al. (2016) untersuchte die Gründe für die Ablehnung von Read-across Dossiers (Compliance checks und testing proposals) für humane Endpunkte. Sehr häufig fehlten experimentelle Daten für den zu betrachtenden Endpunkt (für die Ausgangssubstanzen und/oder relevante Metabolite), war die wissenschaftlicher Plausibilität oder die Stoffidentität der Zielsubstanz z.B. im Fall von UVBB Stoffen, nicht klar beschrieben. Unter wissenschaftlicher Plausibilität wurden viele Fälle zusammengefasst, in denen z.B. Metabolismus nicht klar adressiert wurde. Die Analyse der chemische Ähnlichkeit als Startpunkt des Read-across, die in verschiedenen Publikationen als eine zentrale Herausforderung angesehen wird (Scholz et al. 2015, Blackburn et al. 2015), spielte keine zentrale Rolle für die Ablehnung von ECHA Dossiers. „Uncertainty“ ließ sich ebenfalls in den meisten Fällen auf fehlende Daten zurückführen.

Es ist weiterhin überraschend, dass mehr Dossiers mit Read-across für die Endpunkte Bioakkumulation, akute und chronische Fischtoxizität eingereicht wurden, als experimentelle Studien. Dies führt zu Gruppenbetrachtungen, in denen die Toxizität vieler Chemikalien einer Gruppe mittels weniger experimentellen Daten vorhergesagt wird. Aus dieser Analyse wurde geschlossen, dass für zukünftige Read-across Ansätze und deren Akzeptanz die Anzahl relevanter und qualitative hochwertiger experimenteller in vivo Studien eine entscheidende Rolle zukommt. Die Onlinebefragung und der Literaturreview zeigten weiterhin, dass der prinzipielle Workflow als auch die Anforderungen an die Dokumentation dieses Arbeitsablaufes gut etabliert sind, während die Kriterien und die damit verbundenen Qualitätsstandards, die zu einer Akzeptanz führen weiter entwickelt und durch Behörden wie z.B. ECHA kommuniziert werden müssen. Es wäre in diesem Zusammenhang sinnvoll Fallstudien und deren Akzeptanzkriterien z.B. mittels einer öffentlich verfügbaren Datenbank zu veröffentlichen. Weiter wurden Trainingskurse und Workshops als sinnvoll erachtet. Eine bessere Anleitung/Workflow für die Betrachtung verschiedener Endpunkte wurde als sinnvoll angesehen, wohingegen weitere Dokumentvorlagen als weniger nützlich eingeschätzt wurden, da sie die Flexibilität die bei der Betrachtung der Fallstudien notwendig sein sollte eventuell einschränken.

Die Ergebnisse des Workshops werden in Abschnitt II zusammengefasst. Da sehr viele Einzelaspekte von den Teilnehmern des Workshops genannt wurden, werden im Folgenden nur die wesentlichen Erkenntnisse wiedergegeben.

Read-across ist eine Stoff- und Endpunkt spezifische Betrachtung. Daher ist es schwierig für alle ökotoxikologischen Endpunkte oder die Vielzahl von möglichen Stoffgruppen „harte Kriterien“ bzw. Grenzwerte zu definieren, die zur Beurteilung akzeptabel bzw. inakzeptable führen. Die Teilnehmer des Workshops konnten sich jedoch auf einen generellen Workflow einigen, der in diesem Abschnitt dargestellt wird. Vergleichbar mit dem RAAF für humane Endpunkte, ist chemische Ähnlichkeit der Startpunkt um Ausgangsstoffe zu identifizieren. Da strukturelle Ähnlichkeit nicht ausreicht, müssen weitere endpunktrelevante Daten wie z.B. Mechanismus, Stabilität, Abbau etc. mit berücksichtigt werden. Da die Definition harter Ein- und Ausschlusskriterien als schwierig angesehen wurde, wurden vorgeschlagen, dass Behörden „minimale Datenanforderungen“ pro Endpunkt definieren. Diese minimalen Datenanforderungen sollten idealerweise mittels Fallstudien illustriert werden. Durch geeignete Fallstudien könnte das Verständnis von Kriterien die zur Akzeptanz bzw. Ablehnung geführt haben weiter ausgebaut werden. Dieses bessere Verständnis führt langfristig zu einer besseren Qualität der eingereichten Dossiers.

Die Teilnehmer des Workshops waren nicht in der Lage Grenzwerte bzw. Ein- und/oder Ausschlusskriterien zu definieren. Diese Kriterien werden jedoch gebraucht und sollten in Folgeprojekten mit Experten in diesem Gebiet evaluiert werden.

Im Synthesepapier wurden für die Endpunkte akute und chronische Fischtoxizität sowie Bioakkumulation Arbeitsschritte für eine Read-across-Vorhersage beschrieben. Es stellte sich heraus, dass einige Schritte für alle Endpunkte gleich sind, während andere wie die Art der zusätzlichen Informationen vom jeweiligen Endpunkt abhängen. Der Read-across-Ansatz ist ein flexibles Werkzeug. Es gibt keine klaren Kriterien zur Minimalanzahl von Stoffen, die benötigt werden. Read-across ist immer im Einzelfall zu bewerten. Die vorgestellten Arbeitsschritte leiten den Nutzer durch die Erstellung der Read-across und geben Hinweise auf wichtige Punkte die bei der Erstellung der Datenmatrix und der Formulierung der Hypothese berücksichtigt werden sollen. Neben Fallstudien, die von den Behörden als geeignet angesehen wurden, würde eine Sammlung der verfügbaren Informationsquellen in elektronischer Form die Qualität der Read-across Vorhersagen verbessern helfen.

Summary

Aim und Approach

Risk assessment traditionally use in-vivo animal data to evaluate the safe use of compounds with regard to human health and the environment. In different regulatory contexts, the use of alternative methods is demanded to replace, reduce or refine animal testing as far as possible, e.g. for registration of chemicals under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). REACH laid down the use of alternative methods and in vivo testing as last resort (ANNEX XI). For cosmetics risk assessment has to be based on alternative methods such as in vitro and in silico models, because animal testing is banned since 2013 with the cosmetic directive Nr. 1223/2009[1].

Under REACH[2], read-across is most frequently used as alternative approach.. The prediction of a toxicological property by read-across can be accepted by national authorities or ECHA, in case that the predicted value/property provide equal information as compared to the traditional in vivo experiment (REACH guidance R7[3]).

Read-across is a technique used to predict endpoint information for one target chemical, that lacks relevant experimental in vivo data, by using data from the same endpoint from another chemical, called source compound. A one to one prediction is called analogue approach, a many to one prediction is called category approach. Within a category the intrinsic property is predicted by interpolation. It is important to notice that the compounds within a category should have similar properties or follow a consistent trend e.g. the observed toxicity increases with increasing side chain length.

Source and target compounds have to be similar. Similarity comprises structural similarity and toxicological similarity, on the basis of similar toxicological properties and/or activities. Read-across can be qualitative or quantitative. In qualitative read-across, the presence (or absence) of a property/activity for the target chemical is inferred from the presence (or absence) of the same property/activity for one or more source chemicals. Qualitative reading across gives a 'yes/no' answer, whereasa quantitative read-across quantitative read-across is used to obtain values, such as PNECs or DNELs.

The evaluation of "similarity" is a complex process and therefore many read-across dossiers were not accepted in the past. ECHA recently published the read-across assessment framework (RAAF)[4] for human health endpoints to improve this situation. The RAAF provides a workflow for read-across assessments by using six different scenarios. The assessment starts with structurally similar compounds, thereafter toxikokinetic and toxicodynamic data are considered. Most similar compounds are then used to predict the toxicity of the target compound. The RAAF aims at a more systematic and transparent evaluation of read-across dossiers.

A RAAF for environmental endpoints was not available in the beginning of this project but has been recently published and is compared to the main findings from this project in Section 2.2.

Read-across is a relative simple tool, as it does not requires complex statistical methods and can predict the toxicity of an untested compound with relatively low economic effort.

This project aims to develop a best "practice guidance" for the read-across assessment of environmental endpoints such as acute and chronic fish toxicity or accumulation in fish. This guidance may help to improve the mutual acceptance of read-across approaches between applicants and authorities.

The best practice guidance is based on three different steps. First, the state of the art was evaluated by reviewing relevant literature and publications from recent projects. We further performed an online survey with stakeholders to document and identify experiences with read-across and its current limitations (Interim report, section I). Based on the results of the interim report three main questions were identified:

- Topic 1- Similarity: a key requirement of read-across

- Topic 2- Uncertainty in read-across approaches
- Topic 3- Use of tools and databases for read across

Stakeholders from industry, academia and authorities discussed these three topics within a 1.5 days workshop and prioritized the obtained results (section II, status report).

A mutual agreement on a read-across workflow was considered unrealistic at the beginning of this project. On the one hand because of potential conflicts of interests between authorities and companies but also because most stakeholders are allowed to present a scientific valuable position but are not authorized to present legal binding statements of their organisations'. Therefore, we developed a best practice guideline, which comprises a general workflow on read-across and three workflows for the endpoints acute and chronic fish toxicity and bioaccumulation and recommendations for improvements (synthesis paper, section III).

Results - summary

The most important finding of the literature review (interim report, section I) is a difference between scientific perception and practical ECHA decisions. Although evaluation of ecotoxicological endpoints was not the topic of Ball et al. 2016 the evaluation of currently available ECHA decisions indicate that the read across rejections are mainly based on a lack of sufficient or suitable endpoint study data (for source compounds or relevant metabolites), scientific plausibility and lack of identity data for the target compound e.g. in case of UVCBs. Scientific plausibility includes many cases in which data on toxicokinetics e.g. metabolites were missing. Chemical similarity concerns appear to be of no to minor relevance and uncertainty refers mainly to the lack of data but does not arise from a matrix of variables that can be classified by low to high as proposed by Schultz et al. (2015) or Blackburn et al. (2015).

Surprisingly, more read-across than experimental studies have been submitted for the endpoints bioaccumulation and long-term, toxicity to fish. This finding leads to categories with a number of chemicals that can be assessed by a few experimental studies. It can therefore be concluded that the future challenge for acceptance of read-across are neither the conceptual challenges of the workflow such as chemical similarity, quality of data, uncertainty and plausibility but the quality of submitted data in terms of sufficient experimental studies or suitable chemical identity data.

The questionnaire as well as evaluation of literature and ECHA decisions suggest that the principle workflow od a read-across assessment as well as its documentation requirements are rather well established. The acceptance criteria and subsequently the quality requirements towards a read across approach, however, need to be improved. It can be assumed that an increased communication of ECHA decisions may represent an expedient approach to increase the acceptance by transparently providing criteria for acceptance on a case by case basis. Communication in this context mainly comprise a generation of a database on case examples and ECHA decisions as well as the organization of training courses and workshops. Provision of additional guidance, for example, on specific endpoints as well as improvement of uncertainty assessment were considered as useful while provision of more templates were considered as less useful, as they may inappropriately restrict the flexibility of case by case assessments.

The main outcomes of the workshop are summarized in section II (status report). Many different aspect were noted by the participants of the workshop and only main findings are summarized in the following.

The participants noted that read-across is endpoint and case specific. Therefore, it is difficult to develop clear-cut, hard criteria that defines from which threshold on a certain decision is acceptable or not acceptable e.g. by taking into account different substance classes and different endpoints.

It was, however, possible to derive a general read-across workflow that includes the principle assessment elements for ecotoxicological endpoints. This workflow is depicted. Comparable to the human

RAAF, chemical similarity is a good starting point to define source compounds. But chemical similarity is not sufficient and toxicological data have to be considered to conclude on relevant source compounds e.g. mechanistic properties, stability, fate etc. . As a definition of hard acceptance criteria might not be possible, the participants asked for minimal data requirements per endpoint. These data requirements should be ideally provided by authorities and supported by illustrative case studies. Illustrative case studies were considered to be a very valuable source of guidance and would support the understanding of acceptance criteria and by this improve the quality of read-across dossiers. The participants were not able to define thresholds and in- and exclusion criteria per assessment elements. These criteria are nonetheless needed, and should be part of follow up evaluations with stakeholders in this area.

In the synthesis paper workflows have been described for the endpoints acute and chronic fish toxicity and bioaccumulation in fish workflows have been described. It turned out that some steps are identical, e.g. identification of source compounds while others like additional information on physico-chemical parameters strongly depend on the endpoint to be addressed. Read-across is regarded as a flexible tool. There are no general definition with regard to minimal number of source substances needed for a prediction. As read-across has to be assessed on a case-by-case basis, no clear criteria can be formulated for acceptance of such approaches. The described workflow guides the user through the preparation of a read-across approach and addresses important points that should be considered when setting up a data matrix or for the formulation of a hypothesis for the read-across. Besides the best-practice example, the use of read-across could be facilitated by making templates available on how to document the read-across cases.

I. Interim Report

FKZ 3715 67 4180

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Hannover, June 2016

1 Literature review

1.1 Preface

It might be possible to avoid or reduce testing for future registrations by encouraging registrants to use alternative approaches in the initial phase of dossier formation. Due to a lack of experience by industry and regulators, it is, however, difficult to quickly arrive at an agreement on the ultimate acceptance of results by scientists, industry and regulator (Scholz et al. 2013).

The aim of this project is to provide materials and background information in order to promote the use and acceptance of read-across approach under REACH. The project focuses on environmental and ecotoxicological endpoints, in particular the prediction of acute and chronic toxicity for algae, daphnia and fish, prediction of interspecies sensitivity and measurement of bioaccumulative and persistent groups of substances (PBT and vPvB) or substances with endocrine properties to be discussed on a workshop with stakeholder from industry, academia and authorities.

Since the project addresses the two aspects: 1) acceptance of read across and 2) read across for environmental/ ecotoxicological endpoints the report is conceptually subdivided in an overview of general character for read across that is relevant for acceptance of read across for environmental/ ecotoxicological endpoints and a discussion of specific requirements that are potentially needed for read across for environmental/ ecotoxicological endpoints

2 Background

2.1 OECD guidance

The use of alternative methods in the risk assessment with the aim to avoid animal testing by using existing knowledge is required in different regulatory contexts. The REACH Regulation calls explicitly the use of alternative methods and the general rules for the use of these methods is described in Annex XI. It is stated that ‘‘every effort must be made so that testing chemicals on animals is a last resort – when there is no other scientifically reliable way of showing the impact on humans or the environment.

Read across that is based on the hypothesis that highly similar chemical structures behave almost comparable. It is a procedural approach that requires an expert evaluation but it is not related an empiric method in overall conclusion.

In the read-across approach, endpoint information for one chemical (the source chemical) is used to predict the same endpoint for another chemical (the target chemical), which is considered to be "similar" in some way (usually on the basis of structural similarity or on the basis of the same mode or mechanisms of action). In principle, read-across can be used to assess physicochemical properties, toxicity, environmental fate and ecotoxicology. For any of these endpoints, it may be performed in a qualitative or quantitative manner (OECD 2014).

Substances whose physico-chemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or ‘category’ of substances. Structural similarity is a pre-requisite for any grouping and read-across approach under REACH. These similarities may be due to a number of factors:

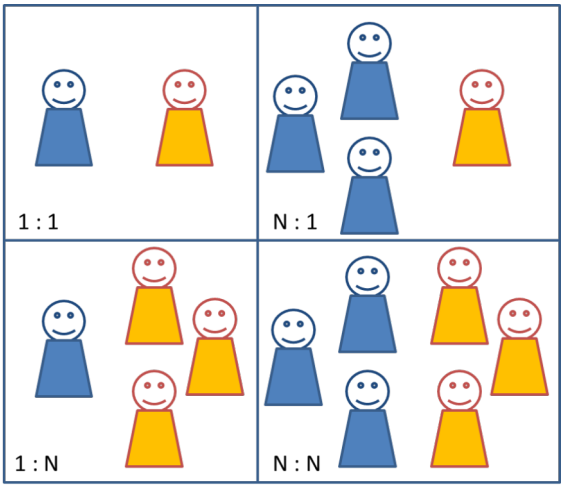
- Common functional group (i.e. chemical similarity within the group),
- Common precursors and/or likelihood of common breakdown products via physical and/or biological processes which result in structurally-similar degradation products (i.e. similarity through (bio)transformation), or

• A constant pattern in the changing of the potency of the properties across the group (i.e. of physico-chemical and/or biological properties).

According to the OECD and ECHA guidance read-across can be performed in the following ways to fill data gaps:

- One-to-one (one analogue used to make an estimation for a single chemical)
- One-to-many (one analogue used to make estimations for two or more chemicals)
- Many-to-one (two or more analogues used to make an estimation for a single chemical
- Many-to-many (two or more analogues used to make estimations for two or more chemicals).

Figure 1: Overview on four different types read-across approaches dependent on the amount of target and source compounds: data rich source compounds are indicated in blue, data poor target compounds in orange (source: Fraunhofer ITEM)



The term ‘analogue approach’ is used when read-across is employed between two structurally-similar substances. As a result of the structural similarity, a given (eco)toxicological property of one substance (the source) is used to predict the same property for another substance (the target). The term category approach is used when read-across is employed between several substances that have structural similarity. These substances are grouped together on the basis of defined structural similarity and differences between the substances. As a result of the structural similarity, the (eco)toxicological properties will either all be similar or follow a regular pattern.

To define "similar" source substances, substances are initially regarded with high chemical similarity to the target substance, for which good quality data for the endpoint under consideration is available. Chemical similarity means here, the source and target substance share relevant common structural properties or a consistent trend. Next the substances should have similar relevant physicochemical properties. A consistent trend could in this context, for example, be an increasing lipophilicity with increasing chain length. Often physicochemical parameters such as the log Kow is used to estimate the adsorption and bioaccumulation.

Moreover, the biological similarity of source materials is considered among themselves and with the target substance. Here, it is desirable that the substances under consideration have a common mechanism for the environmental and ecotoxicological processes. Metabolism or transformation is a critical aspect and it must be pointed out whether the critical metabolites formed are either identical, or do not represent a cause for concern for the considered endpoint. In principle, by the observation of the end point across the category away i) a qualitative statement can be made - such as the substance is rapidly degradable - or it can quantitatively the potency – e.g. a NOAEL / NOEC or BCF - are predicted.

Read-across can only be used on a case-by-case basis by providing a hypothesis, adequate justification, documentation and supporting data may be required for acceptance (OECD 2014, ECHA 2015).

2.2 REACH regulation

"Application of the group concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach)" (REACH Regulation Annex XI, Section 1.5).

Registrants are obligated to consider and, where they can, use appropriate alternative approaches to fulfil applicable REACH information requirements concerning vertebrate animal studies. If read-across which meets the information requirements is applied, unnecessary animal testing may be avoided as there will be no need to carry out one-by-one testing of all their substances to fulfil the information requirements. 'Read-across and grouping', or 'read-across', is one of the most commonly used alternative approaches for data gap filling in registrations submitted under the REACH Regulation. Read-across entails the use of relevant information from analogous substances (the 'source' information) to predict properties for the 'target' substance(s) under consideration.

2.3 Biocides regulation

In Annex IV the Biocidal Products Regulation 528/2012 (BPR) indicates grouping of substances and read-across for adaptation of data requirements. For example, a read-across between Alkyl (C12-16) dimethylbenzyl ammonium chloride (ADBAC) and didecyldimethylammonium chloride (DDAC) was accepted for a leaching study used in the calculation of Predicted Environmental Concentrations (PECs) at TM level (TMII 09). The study was conduct on the analogue DDAC. The RMS considered this study acceptable, without an assessment factor, because it resembles a worst-case taking into account the high water solubility for DDAC.

2.4 Cosmetics regulation

For cosmetic ingredients the development goes even further. Since the entry into force of the cosmetics regulation 1223/2009 in 2013, the testing of animals is prohibited for assessing the toxicity of cosmetic ingredients, but the hazard-free use of the substances for the consumer must be ensured. Alternative methods for predicting the hazard potential of a substance may be in vitro test systems or in silico methods.

2.5 Read-across in Canada

The aim of the Canadian categorisation approach is the evaluation of human and environmental endpoint. The Government of Canada has been planning to assess and manage, where appropriate, the potential health and ecological risks associated with nine groupings of substances.

- Aromatic Azo and Benzidine-based Substance Grouping
- Boron-Containing Substances
- Certain Organic Flame Retardants Substance Grouping
- Cobalt-Containing Substance Grouping
- Internationally Classified Substance Grouping
- Methylenediphenyl Diisocyanate and Diamine (MDI/MDA) Substance Grouping
- Phthalate Substance Grouping
- Selenium-containing Substance Grouping
- Substituted Diphenylamines Substance Grouping

The methodology of the read-across approach Environment Canada refers to the OECD guidance document. Categorization is defined as Sorting of chemical substances on the Domestic Substances List - a

list of substances used commercially in Canada between 1984 and 1986, before the original Canadian Environmental Protection Act was enacted. Most of these substances had not been examined by environmental and health scientists in government, and categorization was the first step to finding out which need further attention.

A workflow can be derived from the case examples of aromatic azo and benzidine- based substances as presented in the following table

Table 1: Read-across workflow as proposed by the government of Canada

Step			Example
1.		Hypothesis	Presence of a property that requires classification.
2.		Substance identification	The identities of the 22 substances are presented in the following table.
3.		Documentation of physicochemical data	Available data was documented.
4.		Documentation of endpoint data	Considering data of 7 different stubstances 6 studies on fish, 2 studies on invertebrates and 2 studies on algae were used to determine
5.		Evaluation of endpoint	A PNECwater for the group of 22 substances was derived

A category is established based on similarity of functional groups although several physicochemical endpoint (melting point, vapour pressure, Henry, water solubility) and in some cases also the log Kow was not available for single substance. In this case data gap filling was provided by QSAR estimations for these physicochemical endpoints. For a certain endpoint such as PNEC derivation the available studies are gathered to cover the complete group by using the lowest toxicity level as worst case while parameters such as the log Kow is not considered as endpoint descriptor. Based on these considerations it is assumed that the Canadian regulatory body focuses on the grouping of a category and accepts data gaps or data quality issues for single substances of the category to establish a group for priorization or classification.

2.6 Use of Read-across by the US EPA

According to US EPA 'Read across' is a technique of filling data gaps. To 'read across' is to apply data from a tested chemical for a particular property or effect (cancer, reproductive toxicity, etc.) to a similar untested chemical. The read-across technique is often applied within groups of similar chemicals assembled for assessment using either analog approach (grouping based on a very limited number of chemicals) or category approach (grouping based on a larger number of chemicals). In an analog/category approach, not every chemical needs to be tested for every endpoint." (EPA, Glossary of Terms, Methods of Toxicity Testing and Risk Assessment)

With regard to read-across a white paper was published by the US EPA and refers the OECD guidance document. The document further presents a case example of an analogue and a category read-across for pyrethroids. Data from chemicals were used to interpolate or extrapolate an LC50 value. Using simple regression techniques and log P as parameter, the toxicity is predicted. The authors conclude that an important aspect of this category is that it is mechanistically-based, since all component chemicals modulate the sodium ion channel resulting in neurotoxicity. Another consideration is that all the test data are based on a standard test method (e.g. flow-through exposure; 96 h duration, etc.). However, the examples are flawed by some scientific inconsistencies since the difference between the predicted and measured toxicity level of bifenthrin is not addressed. The uncertainty in general and the impact

of electronic charge characteristics is mentioned as a descriptor but not discussed. The document does further not present a workflow or evaluation criteria for acceptance of the read-across approach.

To support read-across approaches and data gap filling the Analog Identification Methodology (AIM) was designed to facilitate a data search on a chemical of interest in addition to identification of potential structural analogs and associated data. The Chemical Assessment Clustering Engine (ChemACE) instantly "clusters" chemicals in a large user defined chemical list based on structure. The tool is useful for identifying structural diversity in a chemical inventory and instantly highlighting analogous chemicals for potential read-across.

2.7 Experience with read-across under REACH

2.7.1 Key developments

- Guidance on Grouping of Chemicals (OECD 2007)
- Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals (ECHA 2008)
- How to report read-across and categories (ECHA 2012)
- Guidance on Grouping of Chemicals, second edition (OECD 2014)
- Read-Across Assessment Framework (RAAF) (ECHA 2015)

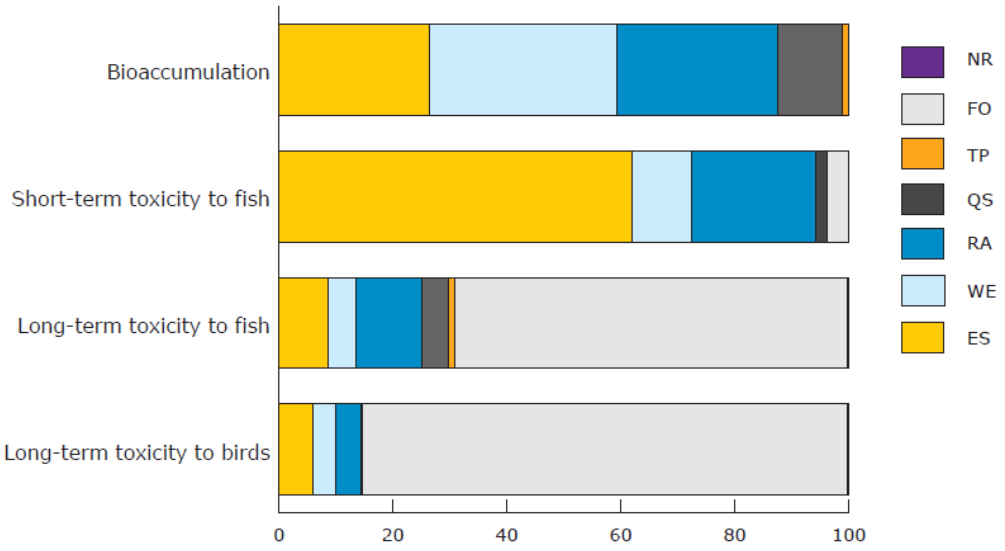
2.7.2 Use of read-across under REACH

The UBA report (Umweltbundesamt 2015) on the project "REACH Compliance: Data Availability of REACH Registrations" presents findings and results from the screening of 1932 dossiers of lead and individual registrants covering phase-in substances with a production volume of equal or above 1000 tpa. 1814 dossiers have been evaluated, including an evaluation of 9070 environmental endpoints. The results of the evaluation were classified as "conform", "non-conform" or complex if a classification as "conform" or "non-conform" was not possible or required an in-depth evaluation of the documented information. The main crosscutting reason why endpoints were considered "complex" was the justified waiving of standard information or the use of surrogate data ("adaptation"). Among this grouping/read-across approaches according to REACH, Annex XI 1.5 were a frequent reason for the endpoint conclusion "complex". According to the UBA report read-across was used in about 30% of ecotoxicological endpoints.

These data indicate that there is a preference to use alternative or non-testing approaches under REACH (see Box 1 on REACH regulation statistics). However, at present no data are available on the extent to which ECHA has required additional animal testing for situations that registration dossiers have made use of hazard assessment based on alternative methods.

The evaluations from 2014 (ECHA 2014a, b) show that in human toxicology and ecotoxicology read-across is used as alternative approach for predicting an endpoint under REACH (ECHA 2014b, Figure 2). With regard to short-term toxicity to fish, experimental studies were available for 62% of the substances. Registrants used various alternative options to cover the remaining 34.2% of the substances entries, mostly by read-across (21.7%). For long-term toxicity to fish, only for about 8.7 % of all compounds experimental data were available, read-across was used to predict this endpoint in 11.5% of substances. Most frequently the flag "omit study" was given (about 68.8% of all compounds). For bioaccumulation in fish read-across was used for 28.2% of the substances, with 26.5% of all compounds having experimental data

Figure 2: Relative proportions of the principal options to fulfil information requirements for environmental endpoints for the substances (phase-in, at or above 100 tonnes per year and at or above 1 000 tonnes per year, 3 662 substances), taken from the recent ECHA report on: The Use of Alternatives to Testing on Animals for the REACH Regulation (ECHA 2014). Abbreviations: ES – Experimental studies/WE – Weight of evidence/RA – Read-across/QS – (Q)SAR/TP – Testing proposal/FO – Flags to omit study/NR – Not reported. (source: ECHA Report)



The Endpoint Study Record (ESR) approach consists of the analysis of all endpoint study records submitted for the 3 813 dossiers for a given endpoint for the phase-in substances between 100 and 1 000 tonnes per year. Registrants have submitted a total of 1 741 ESRs related to the fish bioaccumulation study in the IUCLID database. Of these ESRs, 226 (13%) were filled by experimental data. For read-across, where the number of entries for phase-in substances between 100 and 1 000 tonnes per year was 298 (20% less than in 2010), for phase-in substances at or above 1 000 tonnes per year was 247 (11.4% less than in the previous data pool) and for non-phase-in substances was 13 (4.5% less than in the previous data pool). The opposite has occurred for the use of a weight of evidence approach and (Q)SARs. In fact, for substances between 100 and 1 000 tonnes per year, a weight of evidence approach was chosen in 992 cases (57% of ESRs) and (Q)SARs in 193 cases (11.1% of the ESRs).

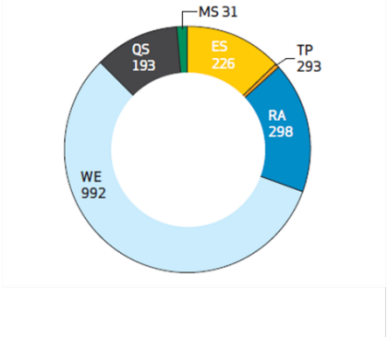
For the short-term toxicity to fish, registrants have submitted 6 104 ESR entries for phase-in substances between 100 and 1 000 tonnes per year. Experimental data were indicated in 2 368 ESRs (38.8% of the entries), which represents a 9.9% reduction compared to the previous submission. 2 154 entries (35.3%) used a read-across approach indicating a respective increase of 8% for read-across.

3 563 ESR entries for long-term toxicity to fish for phase-in substances between 100 and 1 000 tonnes per year. A total of 420 ESRs were filled by experimental data (11.8% of the entries). The most used options were proposals to omit the study and the use of read-across approaches, which have been selected in 42.5% and 28.8% of the ESRs, respectively. Experimental data were indicated in 2 368 ESRs (38.8% of the entries), which represents a 9.9% reduction compared to the previous submission. 2 154entries (35.3%) using a read-across approach and 1 094 entries (17.9%) as weight of evidence, indicating a respective increase of 8% for read-across and 1.8% for weight of evidence approaches compared to the figures for the previous report published in 2011. A similar trend has been identified for non-phase-in substances.

Figure 3: Overview on Endpoint Study Record Analysis for three different ecotoxicological endpoints: A) Bioaccumulation in fish (1 882 dossiers covering phase–in substances 100-1 000 tpa, one or more ESRs may be present per dossier). B) Short-term toxicity to fish (1 882 dossiers covering phase-in substances 100-1 000 tonnes per year, one or more ESRs may be present per dossier). C) Long-term toxicity (fish) (1 882 dossiers covering phase-in substances 100-1 000 tonnes per year, one or more ESRs may be present per dossier). Legend: ES – Experimental studies/TP – Testing proposal/RA – Read-across/FO – IUCLID flags to omit the study/WE – Weight of Evidence approach/QS – (Q)SAR studies/MS – Miscellaneous. (source: ECHA Report)

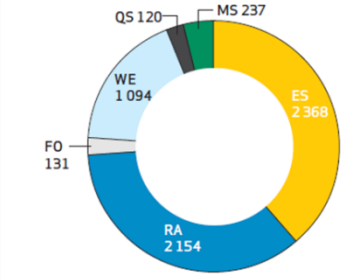
A

Bioaccumulation - fish (ENV)	No. ESR	% ESR
ES	226	13
TP	9	0.5
RA	298	17.1
FO	0	0
WE	992	57
QS	193	11.1
MS	23	1.3
Total	1 741	100



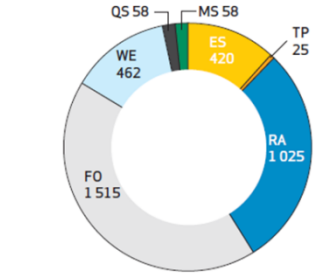
B

Short-term toxicity to fish (ENV)	No. ESR	% ESR
ES	2 368	38.8
TP	0	0.0
RA	2 154	35.3
FO	131	2.1
WE	1 094	17.9
QS	120	2.0
MS	237	3.9
Total	6 104	100



C

Long-term toxicity to fish (ENV)	No. ESR	% ESR
ES	420	11.8
TP	25	0.7
RA	1 025	28.8
FO	1 515	42.5
WE	462	13.0
QS	58	1.6
MS	58	1.6
Total	3 563	100



Surprisingly, more read-across than experimental studies have been submitted for the endpoints bioaccumulation and long-term, toxicity to fish (Figure 3). Considering WoE in addition about two-thirds of the endpoint studies refer to alternative approaches while testing proposals appears to be of no relevance. With regard to acute fish toxicity it should further be considered that the evaluated data refers substances between 100 and 1 000 tonnes per year. The acute data is, hence, mandatory and high quality of this data may also represents the basis of data and testing requirements for chronic endpoints. The evaluation by ECHA, however, did not indicate whether read-across is used as supporting or key information so that the interpretation on the relevance of the read-across for hazard assessment should be handled with care. The submission of read-across was further analysed by using the OECD eChemPortal. It should be noted that the data from the OECD eChemPortal is not consistent with the data of the ECHA report. Therefore, the data provides an overview and should be handled with care (Table 2).

Table 2: Use of read-across according to the OECD eChemPortal

	Entries	Substances
Bioaccumulation	435	232
Short term fish	1588	653
Long term fish	325	202

Read-across was used most often for the endpoint short term fish toxicity, for which 1588 entries for 653 substances were found. Table 3 lists the frequency of concomitant use of Read-across, OSAR and other approaches.

Table 3: Concomitant use of alternative methods

	READ-across	QSAR	Other	Read-across and QSAR	Read-across and Other	QSAR and Other
Bioaccumulation	232	359	114	41	19	52
Short term fish	653	194	109	46	18	4
Long term fish	202	111	21	16	5	1

The substance data set concerning read-across for the endpoint long-term fish was screened for potential categories. It should be noted that screening does not provide information on whether such a category was indeed formed by the registrants.

Table 4: Substance groups present in long-term fish

Group	N
Hydrocarbones	3
Benzenesulfonic acid, alkyl derivs.	4
Alcohols, ethoxylated	9
Amides	6
Fatty acids, Glycerides	9
N,N'-bis(alkyl)-p-phenylenediamine	3
Petrolium, coal tar, etc	11
Sulfuric acid, alkyl esters	9
Quaternary ammonium compounds	8

2.7.3 Practical guidance

In order to improve the common understanding of the application and documentation of read-across, ECHA published guidance documents and participated in different workshops.

This practical guidance 6 on “How to report read-across and categories” provides an overview of important practical aspects on read-across and/or a chemical category approach for substances to be registered under REACH and how to report these in IUCLID 5.

In line with the OECD guidance, incremental or constant changes across a category with a common functional group represent a rational to demonstrate a trend across a category. Consistency across endpoints may help to increase the confidence in a category approach and can be used to predict quantitative endpoint values. A substance should be characterized by chemical structures and purity profiles.

Moreover, it is noted that REACH Annex XI foresees the grouping of substances and read-across approach for data gap filling if the following conditions are met with regard to adequacy and reporting:

1. Results are adequate for the purpose of classification and labelling and/or risk assessment;
2. Results have adequate and reliable coverage of the key parameters addressed in the corresponding test methods;

3. An exposure duration comparable to or longer than the corresponding test method is covered, if this parameter is relevant;
4. Adequate and reliable documentation of the applied method is provided.

Hence, adequacy is the key for the acceptance, but how is adequate defined in the context. This can be derived from page 13 of the practical guidance 6: To be adequate, a category prediction should be adequate for the purpose of classification and labelling and/or risk assessment. The adequacy of the category prediction for the purpose of classification and labelling and/or risk assessment will be very much endpoint-dependent. Additional information might be needed to assess the generated prediction for adequacy in the context of a regulatory decision. Therefore, the validity, applicability and relevance can only be considered on a case-by-case basis.

The first point one can learn from this statement is that to be adequate, a prediction should be adequate. Second adequacy is endpoint-dependent and can only be considered on a case-by-case basis implicating that adequacy criteria are not set by the guidance.

The document does not address uncertainty.

2.7.4 Human Health RAAF

The RAAF has been developed by ECHA as an internal tool providing a framework for a consistent and structured assessment of grouping and read-across approaches under REACH. The RAAF establish standardization of assessment scenarios, assessment options and assessment elements in the regulatory context. A prerequisite for the acceptance of read-across prediction is an accurate documentation of the target and source substance with regard to their chemical and biological similarity. The validity of the prediction depends largely on the relevance of the parameters studied for the toxicological endpoint, the quality of the toxicological data and the final evaluation by the experts. For a traceable documentation and evaluation of read-across scenarios, it is therefore necessary to develop a uniform framework and standardized procedures. Based on these parameters, the accuracy of the forecasts or remaining uncertainty of the forecasts shall be assessed.

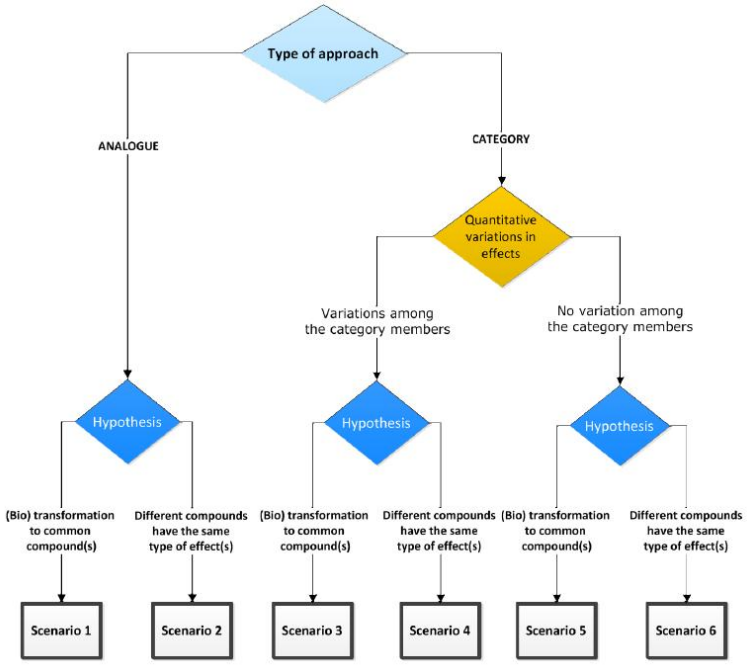
Past experience with REACH, however, shows that Enrolling Company read-across apply often incomprehensible i.e. the assumptions underlying the read-across example mechanistic and structural similarity not comprehensible and clear document. This leads to a low acceptance of the values derived therefrom, which suggests a targeted information and guidance required by the industry to really save with the help of these forecasts, animal experiments.

A guide to assessing read-across extrapolations by the ECHA, the so-called read-across Assessment Framework (RAAF) has just been published, in which the individual read-across scenarios can be distinguished in the field of human health and the evaluation steps are shown (ECHA 2015). The RAAF has the aim to standardize the evaluation of read-across cases and to make decisions of the Authority more transparent.

Various scenarios can be distinguished for read-across classified according to the number of available source and target substances such as 1: 1 (analog approach); 1: N; N: 1 (category- approach) and N: N (Figure 1).

In the human toxicology is in addition to the analog and categories approach nor the relevance of metabolites involved in the scenarios approach (Figure 4).

Figure 4: Overview of the scenario concept of ECHA RAAF for the assessment of endpoints in human toxicology. (source: ECHA RAAF)



In the context of read-across, a worst-case approach means that the strength of effect(s) in the target substance is actually expected to be lower than the strength of effect(s) observed for the source substance; hence using the value obtained from the source substance, the prediction constitutes a worst case that will not lead to an underestimation of the effect(s) that would be observed in a study with the target substance if it were to be conducted. Scientific explanations for such situations may be based on kinetic considerations (e.g. evidence for differences in bioavailability) or on potency considerations (e.g. evidence that structural features lead to a higher potency for the source substance) (RAAF).

Adequate and reliable documentation of the entire read-across methodology should be submitted. This documentation should contain the following elements:

- A detailed description of the study or studies on the source substance and their results (the source information) from which the property is read across.
- A scientifically-credible explanation (read-across 'hypothesis') as to why the property of the source substance can be read-across to the target substance. Any limitations in the hypothesis should be described by the registrant. See Guidance (R.6.2.6) on the "Reporting formats for analogue and category evaluations".
- The supporting evidence for the read-across hypothesis, such as scientific arguments, relevant information on other properties or other arguments.

The questionnaire address the penetration of the RAAF. More than half of the participants indicated either not to know the RAAF or only have noticed the document. Interesting, participants from authorities and consultancies know and apply the document while the penetration in particular by participants from industry appeared to be low

For environmental and ecotoxicological endpoints a guideline, hereinafter referred to as environmental RAAF (ENV-RAAF), is discusses at the moment by ECHA with the participation of scientists from the regulatory authorities of the Member States.

2.7.5 ECHA decisions

ECHA decisions on human heath endpoints have been analysed with respect to read-across by Ball et al. (2016). The analysis of read-across under REACH is based upon the compliance check (524) and testing proposal (388) final decisions that were publically available on the ECHA website. Approximately one fifth (107) of all disseminated compliance check decisions involved the use of read-across. Of these only one or two appear to have been accepted. The reasons for the rejection of the use of read-across summarized by four main categories by the authors (Table 5).

Table 5: Reasons for the rejection of the use of read-across in disseminated compliance check decisions adopted from Ball et al. 2016.

Reason for rejection	No. of cases
Unclear substance identity, not possible to ascertain structural similarity.	48
Lack of sufficient evidence to substantiate assumptions made within read-across justifications	43
Lack of scientific plausibility	20
Read-across to inappropriate data	5

The evaluation suggests that the rejection of read-across approaches is in the vast majority of compliance checks based on insufficient data on substance identity or the endpoint study provided by the applicant. Vice versa, it can be concluded that chemical similarity that is identified as one of the two mayor challenges for read-across the by Scholz et al. (2013) appears in practice on minor relevance as this concern was not raised by Ball et al. (2016) to be relevant for rejection. Chemical plausibility was depicted to be case specific and appeared to be based on conflicting supporting information such as metabolism. Further it has been noted that substance identity is an issue for UVCB e.g. the source chemical for read-across was a UVCB (composed of different isomers/branching w/ no details on proportion of isomers) whereas the target substance is a mono-constituent and vice versa.

With respect to testing proposals, 81 out of 388 testing proposals involved the use of read-across (either presented by the registrant or by a third party during consultation on the testing proposal). In proposals submitted by the registrant, a category (or analogue) testing plan was proposed where some members of the category would be tested and the data from these studies would then be used to read-across to the other category members. Test proposals appear to have been far more successful in the use of read-across, with 50 approved at least in some part.

The article by Ball et al. contributes to an improved understanding of read-across policy by giving a structured overview and new insights in the acceptance of read-across for toxicity prediction in the regulatory context of the EU. However, the publication has some restriction that should be considered for interpretation. A mayor short coming of this article is clearly that at no point read-across as part of the safety assessment is stated to have the demand to represent a safe, conservative or worst case evaluation if replacing standard data requirements as required by the REACH legislation or guidance documents (practical guide, RAAF). In this line the expectation towards read-across are only linked to the avoidance of animal test as it is indicated that the successful application and acceptance of read-across is critical to meeting the goal of characterizing hazards of substances subject to REACH while minimizing new animal testing. On the other hand it is criticized that only very low levels of uncertainty are currently accepted for a successful submission while this may be identified as the mayor demand towards the read-across approach by other stakeholders such as the general public and assessors in the evaluating agencies.

In preparation of the workshop, the underlying data extraction were reevaluated for ecotoxicological endpoints (data on 525 dossier evaluation decisions were kindly provided by Nicholas Ball). All read-

across decisions for ecotoxicological endpoints were reanalyzed to learn more about the criteria for which read-across approaches for ecotoxicological endpoints were rejected.

47 dossier evaluation decisions comprise reports on ecotoxicological endpoints like long and or short-term toxicity to aquatic and terrestrial invertebrates, plants and fish; effects on soil microorganisms; growth inhibition to aquatic plants. Dossiers with endpoints associated to fate, or biodegradation, or bioaccumulation were not included into this analysis.

In 8 dossiers evaluation decisions a read-across approach for ecotoxicological endpoints is described. In 3 studies the identity of the test compound was requested as well as the justification of the analogues. It has for example been stated that physico-chemical properties as well as trends in toxicological and ecotoxicological endpoints have not been described. Further as the identity of the target was not comprehensively shown in one case, ECHA was not able to judge on the relevance of the proposed source compounds. For the remaining 5 read-across studies, in 4 cases ECHA stated that the similarity or justification of analogues was not given (extrapolation instead of intrapolation based on variation of side chain length, N=1), or data on analogues did not meet data requirements (N=1), no read-across justification like similarity was given at all (N=3).

39 dossier evaluation decisions did not use read-across to assess the required ecotoxicological endpoint. 5 decision did not report the test substance identity, 17 reports failed because of insufficient data quality provided for the evaluated endpoint, 3 cases reported an incorrect classification, 5 reports were rejected because of exposure based waiving, 3 QSAR, 5 dossiers did not report adequately on the exposure assessment, 1 because of general incomprehensive waiving approach (Table 6).

Table 6: Reasons for rejections in dossiers evaluating ecotoxicity endpoints like long and short term toxicity to fish, algae and daphnia. In total 39 dossiers were evaluated

Approach	Reason for rejection	
Read-across reported (8 dossiers)	Unclear substance identity, not possible to ascertain structural similarity.	3
	Lack of sufficient evidence to substantiate assumptions made within read-across justifications/similarity not proven with regard to toxicity and/or PC properties	5
No read-across reported (39 dossiers)	Unclear substance identity	5
	Inappropriate data provided	17
	Incorrect classification	3
	Exposure based waiving not appropriate	5
	QSAR not appropriate	3
	Data on exposure assessment missing	5
	General waiving arguments not plausible	1

2.7.6 Experts Workshop on Read-Across Assessment with active support from Cefic-LRI

ECHA organized a workshop with support from Cefic Long-range Research Initiative (LRI) to develop the RAAF concept in June 2014. The workshop was divided into two parts: the objective of the first day was to exchange views between ECHA, the Commission and the Member States on the assessment of the read-across approach in a dossier. During the second day the discussion expanded to what constitutes a robust scientific justification for read-across. The second day was organized with the support of Cefic-LRI and was open to various stakeholders. The workshop build up on a workshop that took place

in 2012 to explore industry experiences with “read-across approaches” to date, to try to reach common understanding to characterize scientifically valid “read-across” are and to provide insight in ECHA’s rationale for assessing read-across proposals, the so-called RAAF.

Recently, in March 2016, Cefic organized together with American Chemistry Council, CAAT Europe, ASCCT, EU-Tox Risk, HTPC, Humane Society International, NIH, UL and John Hopkins University a follow up workshop on “good read-across practice”. This workshop focused on recent experiences and concepts being developed for read-across with regard to human health endpoints.

2.7.7 EUROECOTOX

EUROECOTOX is a network, initiated within a coordination action funded by the EC FP7 Environment Programme (2010-2012), and continued with coordination by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). The network was established to promote among others the validation and regulatory acceptance of new alternative ecotoxicity methods. A workshop report was published by Scholz et al. (2013) and gives an overview of alternatives to animal testing for environmental hazard identification and risk assessment.

2.7.8 Caleidos

The CALEIDOS LIFE project addresses the use of read-across (Benfenati et al. 2016). 40 participants of the study examined the use of read-across and QSAR for two ecotoxicological endpoints, namely BCF and acute fish toxicity. Nine substances without experimental data were predicted. The use of QSAR models is reported, therefore this publication is in a stricter term not a read-across exercise. 47 results for BCF and 41 results for acute fish toxicity were obtained from the participants and compared.

The estimation of BCF value by different QSAR models showed a quite good agreement between the participants for most of the substances. Most of the participants also provided information on the similar compounds they used for the assessment of the target compound. Further information on this validation of QSAR predictions is, however, not given in the publication. Also contradictory results were obtained with the same model (OECD QSAR Toolbox), as in some cases a specific substance was judged to be non-bioaccumulative by one participant, while it was judged to be very bioaccumulative by another.

In the case of fish acute toxicity the predictions showed more disagreement of results, again even when the same software tool was used. As an example for one substance the results varied from <1 mg/L to >100 mg/L (or non-toxic to toxic).

However, it is clear from the description above that the publication focus on the evaluation and comparison of QSAR predictions and not on the use of read-across.

3 Challenges of read-across

The challenge for a read-across can be summarized by the aspects: chemical similarity, quality of data, uncertainty and the plausibility. Schultz et al. (2015) acknowledge that the two major challenges of any read-across assessment represent chemical similarity and uncertainty.

3.1 Chemical similarity

Chemical similarity is considered as key issue, and may concern physico-chemical properties, governing bioavailability, structural features, modes or mechanisms of toxicological action, and routes of metabolic activation or detoxification (Blackburn et al. 2011).

The algorithm employing atom-centered fragments (ACFs) has been developed as a general-purpose measure of structural similarity. This algorithm allows to exploit data from a few analogues for predicting quantitative endpoints such as the acute toxicity towards fish (Schuurmann et al. 2011). This methodology is fully automated (Schuurmann et al. 1997).

Physical-chemical parameters are one critical determinant to the environmental and health properties of a substance affecting bioavailability, environmental fate, and thus the (eco)toxicity of a chemical. Consequently the similarity (or logical trend) among the physicochemical properties of category members is an important element in building a read-across approach. Nevertheless, chemicals with equal physicochemical properties may still have different interactions with enzymes that could result in a different metabolism and thereby distribution and elimination (OECD 2014). Furthermore, physicochemical data are considered as obligatory and should in this line provide supporting information.

Physicochemical properties are related to the chemical structure and can be modelled by a variety of accepted QSAR tools. For example, the US EPISuite estimates the parameters log Kow, vapour pressure, Henry's law constant, Koc (adsorption) or water solubility. It should be noted that the properties water solubility and Koc, for example, are related to the log Kow (EPISuite, EUSES, ECHA 2014b). Therefore, the log Kow represents a key parameter while other physicochemical properties may represent secondary parameters of the log Kow that are used in environmental risk assessment. These secondary parameters are considered to be useful in some cases or for specific endpoints.

An inventory of computer tools is given in Annex I and II (section I, interim report). These tools allow to evaluate similar compounds. The questionnaire revealed that computer tools are considered as important resource to establish a read-across case (Q10). However, only the OECD tool box appears to be of relevance while other tools are usually not used or not regarded as very useful. Toxmatch and AIM are regarded as useful by a few participants suggesting a principle status as alternative to the OECD tool box (Q11). It should be noted that tools such as ToxRead or Ambit (not explicitly considered in the questionnaire) are recently launched and can subsequently not be expected to have a high penetration within the community.

3.2 Data quality

Under REACH quality and standard data requirements are defined and can be evaluated by criteria independent of the read-across approach. Therefore, the assessment of data quality can be assessed in a standardized approach by using criteria such the application of guideline studies and GLP as well as scoring system such as the Klimisch Score (Klimisch et al. 1997) that is widely establish or the CRED method briefly proposed for environmental endpoints (Moermond et al. 2016). Although not part of the ECHA read-across guidance ECHA representatives state that if the correct test and measurement is carried out (according to the guidelines and under GLP), the information requirement is met for an read-across approach (de Raat & Netzeva 2012).

Therefore it can be concluded that the assessment of data quality may affect the outcome of a read-across assessment but does not pose procedural challenges for the read-across approach, in general.

3.3 Uncertainty

The uncertainty of the read-across approach can be related to the following aspects: chemical similarity, quality of data, uncertainty and the plausibility by Schultz et al. (2015). This view is confirmed by the results of questionnaire (Q9) indicating chemical similarity, quality of data, and uncertainty assessment as main challenges for read-across.

Moreover, uncertainty is related to the experimental study of the source substance. Uncertainty of experimental data may arise from inter- and intra-species variance as well as by acute to chronic extrapolation. For these variances a standardized approach is described in the guidance documents for human and environmental endpoints. However, these topics are in general relevant for risk assessment and do not present sources of uncertainty which are specific for read-across. Uncertainties directly related to the read-across approach are discussed in the following.

Blackburn and Stuard (2014) developed a systematic framework to describe potential areas of additional uncertainty that may arise in read-across (evaluated based on the number and suitability of analogues contributing data, severity of the critical effect, and effects and potency concordance). Further, they present a questionnaire for evaluating and documenting consideration of these potential additional sources of uncertainty by risk assessors. In the view of the authors the application of this framework represents a next step in standardizing the read-across process, both by providing a means to transparently assign a level of uncertainty to a SAR-based read-across assessment and by facilitating consistency in read-across conclusions drawn by different risk assessors.

The framework considers the several typical aspects in a read-across assessment including: number and structural similarity of analogues, the quality and quantity of the considered data, the nature and severity of the critical toxic effects as well as the potency of the analogues for those effects. Further it evaluates whether existing data on the target for other endpoints aligns well with corresponding existing data from the analogues. The authors support a semi-quantitative grading of uncertainty in the four categories: low, low to moderate, moderate and high. This grading is done with the help of a detailed questionnaire. Based on this assessment, additional default read-across uncertainty factors for the risk assessment are proposed e.g. 1 (overall low uncertainty); 3 (low to moderate uncertainty) and10 (moderate uncertainty). In case of an overall high uncertainty the read-across is not recommended. The authors conclude that the present framework offers the advantage of providing an explicit process to drive consistency and document the rationale for the level of confidence/ degree of uncertainty in the read-across. They highlight that the described experiences in the paper supports the need for a standardized, transparent framework to facilitate consistency and reproducibility in determining the confidence or uncertainty in a read-across. It is stated that they obtained, a better separation of uncertainty based on the use of surrogate data of structural analogues from any other data uncertainty e.g. attributed to test data on the target chemical directly.

The use of uncertainty factors represents a common approach in risk assessment. Blackburn and Stuard (2014) and Schultz et al (2015) suggest a quantitative uncertainty factor. The authors noted that numerical uncertainty factors provide another challenge and provide arbitrary examples of 1-2-3 to 1-10-100. It is recognized that the proposed default uncertainty factors (UF) for the various categories of uncertainty in the framework are somewhat arbitrary (1, 3, and 10) and that this framework should serves as a starting point for the read-across assessment uncertainty evaluation. The justification on arbitrary factors is based on the perception that it remains a pragmatic and usable solution but misses a scientific basis as acknowledged by the authors. Moreover, the uncertainty framework by Blackburn and Stuard uses nominal classification such as high, moderate or low but miss a practical instruction as well as a definition of adequate or suitable. Such an approach results in a transparent documentation but bypasses the objectivation of the uncertainty. Subsequently, the uncertainty assessment remains a subjective justification of the assessor with a proposed arbitrary factor.

As a rational for such a factor and its value is not provided and a major short coming is that such a factor and the value of an uncertainty factor is not based on empirical data the question arises whether uncertainty can be adequately covered by an arbitrary factor or whether the goal of an assessment should focus on the decision whether or not the approach is adequate. In such a case, an uncertainty factor is not required.

According to REACH guidance (R.8.4.3) the use of alternative data in a read-across approach in a quantitative way might be associated with some additional uncertainty in the dose descriptor derived. However, it should be noted that a default assessment factor is not indicated for alternative methods under REACH (Table R.8-6). The REACH guidance considers an assessment factor of 1. An additional factor regarding data quality concerns and consistency has scientifically not been established in a quantitative manner.

A multi-divisional scheme as proposed by Blackburn et al. (2014) and Schultz et al. (2015) has several advantages for documentation but should be critically discussed on the workshop as the goal of read-across used in the regulatory context is a regulatory decision and a decision scheme needs to bring it finally down to a dichotomous decision (i.e., accept or reject).

The uncertainty framework by Blackburn and Stuard (2014) further mixes up properties independent of uncertainty. For example, it considers the severity of an effect. However, the uncertainty of an approach is by definition expected to be independent of an intrinsic effect or effect level. For example, the validity of a read-across is independent on the study results (toxicity value) and its effects (growth for example). This interpretation is in line with the conclusion of the ECHA workshop "Use of ''read-across'' for chemical safety assessment under REACH" that the scientific principles and rigour should be identical regardless of whether the read-across predicted a presence or absence of toxicity (Patlewicz et al. 2013).

Schultz et al. (2015) also noted a procedural uncertainty. Unfortunately, this term was not revived in the paper by a definition but may be related to the uncertainty associated with the completeness and application of the read-across. In this context the authors state that the target chemical(s) is critical, as it is one of the focal points of the exercise. Again this statement was not defined or commented and it should be noted neither the RAAF nor the practical guide 6 state a term procedural uncertainty. A conceptual discussion of the assessment of uncertainty was conducted by a few publications that focused on uncertainty division schemes.

The above cited debates about uncertainty still miss to provide an empiric rational for the classification or assessment of uncertainty. Interestingly, several subcategories of uncertainty are defined and summarized without a discussion of its assess ability and the relationships between the different classes of uncertainty criteria. Chemical similarity represents by nature of the read-across approach an uncertainty as substances can only be similar but are not the same. The major challenge may represent the objectivation on conclusions on similarity. The transparency of data documentation as well as the use of software tools are expected to improve this process step. Interestingly, chemical similarity was not of mayor concern in the currently available compliance check decisions by so that its role for the acceptance of a read-across assessment needs to be discussed independent of its role for establishing a category (Ball et al. 2016). A decision on data adequacy is independent of other uncertainty criteria and should be performed previous to a plausibility assessment. The data quality can be assessed in a standardized approach as described above and should be assessed in a dichotomous decision scheme in acceptable or not accepted. Plausibility is not an independent criteria and can be assumed to be directly related to the similarity of substances and the quality of data that is provided. Thereby, plausibility may be supported by additional information such as in vitro test or data on metabolism and degradation.

Overall, an uncertainty framework has be proposed that characterize some aspects of uncertainty of read-across. However, the applicability of such an attempt needs to be critically addressed in future due to several conflicts which should be evaluated to in a targeted manner. First, the character of the different uncertainty aspects as well as their contribution to the overall conclusion have not be evaluated up to date and are based on personal perception. Furthermore, expected interdependency between the different uncertainty aspects noted in the publication are not evaluated. Second, the evaluation by Ball et al. (2016) showed that the aspect raised by the authors were usually not critical for acceptance of the read-across in currently available ECHA decisions as the rejection was primary based on data availability concerns (lack of or insufficient data). Hence, the discussed uncertainty aspects appear to have a procedural character but its overall relevance for acceptance of the read-across approach remains to be established. Third, the use of quantitative assessment factor is not established and its utility is questionable as qualitative and quantitative read-across assessment in used for data

requirement adoption that implicate that a worst case assessment is required according to the guidance documents. Though, the justification on the worst case character is expected to represent the critical aspect to deal with uncertainty. Here, the aspects characterized by Scholz et al. (2013) can be used as a starting point.

3.4 Plausibility

Even if read-across is carried out in a perfect way, it still has to be decided during the assessment whether the case is convincing enough to accept the prediction and, if so, under what conditions. The acceptance of read-across cases made according to the rules, still requires that the evaluator is convinced based on theory and supporting data. Ultimately, it is not possible to prove that the test with the target can be replaced. It can "only" be made scientifically credible on the basis of theory and supporting data (de Raat & Netzeva 2012). In this way plausibility can be characterized by the aspects of sufficient suitable data on the endpoint and the availability of supporting data on chemical or biological similarity that may be required for the confirmation of the hypothesis. Ball et al. (2016) showed that concern of plausibility that resulted in rejection of read-across by ECHA were based on a lack of or insufficient data on metabolism or supporting data, for example.

4 Read-across for Environmental and Ecotoxicological endpoints

Although not explicitly mentioned most concepts and initiatives for read-across have been introduced with a focus on human health. The human health RAAF or the EU SEURAT represent examples. A literature search revealed only a few scientific publications that explicitly deal with read-across for environmental endpoints. Besides this, several read-across approaches have been published by the OECD and Caley et al. (2007) summarized previous OECD Screening Information Assessment Meetings (SIAM) from 2004 to 2007. In the following an overview of relevant publications that explicitly deal with read-across for environmental endpoints is provided.

Employing a data set of 1365 organic compounds with experimental 48-h LC50 data for Daphnia magna and 692 organic compounds with experimental values for the 96 h fish toxicity toward the fathead minnow, a read-across approach has been developed that makes use of the atom-centered fragment (ACF) method as quantitative measure for structural similarity. Excess toxicity of the compounds was examined, that is characterized by the toxicity enhancement Te. Te is the ratio of the predicted baseline narcosis level LC50 over the experimental LC50. For a given compound, log Te was predicted as similarity weighted average of the log Te values of typically three most similar compounds. As a general trend in both studies, increasing the ACF minimum similarity increases the prediction quality at the cost of decreasing the application range. Furthermore, the performance of the linear solvation-energy relationships (LSER)-based read-across method is similar to the one of the Kow-based variant for Daphnia.

Rorije et al. (2013) conducted a read-across of musk fragrance cases (musk xylene, musk ketone and galaxolide) and compared the result to experimentally derived PNEC values. The read-across estimates were based on similarity in a hypothesised mechanism of action for (acute) toxicity of musk xylene.

A case example on read-across within a weight of evidence approach is presented by Brandt et al. (2016) on biodegradation of phenolic benzotriazoles in the environment. Phenolic benzotriazoles are identified to be very persistent in the environment.

ECHA has published decision on read-across (http://echa.europa.eu/information-on-chemicals/dossier-evaluation-decisions). As example, the group of perfluorinated compound and phenol-benzotriazole can be noted:

- Pentadecafluorooctanoic acid (PFOA)
- Henicosafluoroundecanoic acid

- Tricosafluorododecanoic acid
- Pentacosafluorotridecanoic acid
- Heptacosafluorotetradecanoic acid
- Bewertungsdokumente für Benzotriazole
- 2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)

4.1 Challenges for ecotoxicological and environmental endpoint

Within this project the question arised whether the concepts usually introduced with a focus on human endpoints can be transferred to ecotoxicological endpoints.

While the concepts to evaluate structural similarity or data quality are expected to be comparable to human endpoints the requirement for physicochemical data or additional data on environmental properties may be relevant (personal communication, ECHA 2017).

4.2 The ENV RAAF[6]

The ENV RAAF presents obvious synergies with the Human Health RAAF approach. However, it is recognized that the HH RAAF cannot be applied directly to the environmental read-across assessment due to the reasons explained in the following.

- Interrelated nature of environmental information
- Use of physicochemical properties
- Use of exposure/ risk consideration

A stepwise approach is provided. Specific requirements for environmental or ecotoxicological endpoints are discussed in Step 2 scientific evaluation.

4.3 Physicochemical data

Draft ENV RAAF indicated that identification of the most relevant physicochemical properties to evaluate is performed by use of a data matrix depending on the study for which a read-across has been proposed. Table 1 of the draft document presents the main requirements for the test material that need to be known before the testing and to be used for the evaluation of the test results (Draft ENV RAAF). Currently there is no evaluation or prove available that the properties in table 1 of the Draft ENV RAFF presents indeed a requirement that need to be known and stated in the read-across assessment documentation and a basic consideration shows that several of the physicochemical properties are of secondary value.

The OECD document concluded that for the environmental compartment, the type of supporting information that is appropriate to report will depend on the environmental endpoint intended to be read-across. However, basic physical-chemical properties that determine environmental distribution and fate (e.g., MW, water solubility, partition coefficients such as log Kow) will generally be useful (It should be noted that useful is distinguishable from required.). Moreover, a read-across approach is generally considered to be endpoint specific.

The log Kow represent the key descriptor that is either a descriptor of several physicochemical properties, and that is a direct descriptor for ecotoxicicity and environmental fate such as aquatic toxicology and the BCF. The log Kow is a parameter of the structure and can be predicted by generally accepted QSAR models (EPISuite). If functional groups of different substances are similar a low difference of the Kow between the different substances may indicate a high degree of similarity. However, a

[6] This section is based on a draft version of the RAAF for environmental endpoints. The final RAAF was not available when this paragraph was written.

consideration of the log Kow value without an analysis of functional groups is not appropriate to indicate a structural similarity in a read-across.

Read-across is endpoint specific and should focus of the relevant endpoint. In the context of a read-across for ecotoxicity it should be considered that the endpoint is the ecotoxicity value and not the description of the fate of a chemical. Several physical-chemical properties are required for fate modeling. But are these parameters indeed required for ecotoxicity testing and a read-across? Here, it should be assumed that fate parameters, especially, if these parameters are not of direct relation to the toxicity value, are not required for the read-across approach but may be useful to support the chemical similarity and to build a sound category.

Case example. Vapour pressure K_{oc} and K_{ow} as property for aquatic toxicity:

According to Table 1 of the Draft ENV RAAF (ECHA June 2015) vapour pressure and Koc are key parameters that are required for read-across assessment. For example, vapour pressure and Koc represents a parameter in environmental fate modeling and have to be considered as technical confinder in experimental testing either to consider evaporation of the test substance or to avoid adsorption to the test container under test condition, respectively. In experimental study this needs to be addressed and analytical monitoring determines the exposure of the test organism. Hence, the experimental test result should ideally be independent of the property vapour pressure and Koc. This consideration indicates that there is not a need for the documentation of the property vapour pressure or Koc as a critical parameter to derive aquatic toxicity in a read-across approach. However, it should be noted that a documentation of these parameters may support a read-across approach to indicate comparable properties of target and source substance.

It can be concluded that ecotoxicity tests including test on BCF are ideally conducted at steady state concentrations. Therefore, several physical-chemical properties may be abstracted in test and, subsequently, in the result of the test. Water solubility is related to the Kow value and may be modeled by a QSAR. Under test condition the vapour pressure may describe the dissipation of the substance from the test system. However, this is irrelevant under stable flow through conditions. Overall, only the log Kow represents the primary descriptor for ecotoxicological endpoint. On a case-by case abiotic degradation and additional parameters may represent supporting information to be relevant

- A proposed guidance document should therefore clear define which properties are required for an endpoint and which properties may be of use but are eventually represent supporting information or that are actually part of an environmental hazard and risk assessment that is beyond the scope of a single endpoint read-across assessment.
- The log Kow is a critical parameter for ecotoxicological endpoints.
- A table may indicate the relevant information that should be provided for an appropriate evaluation. Information gaps, however, should not result in principle in a rejection of the read-across and QSAR models may be appropriate for data gap filling as indicated by the OECD document. Accordingly, it is stated that the source of the information needs to be clear and whether the value is measured or calculated. Two common sources of models to predict physical-chemical properties are the OECD (Q)SAR Toolbox or EPISuite™ Epiwin are noted to be applied to establish PC data for a category.
- Considering that PC data represent obligatory or supporting information for chemical similarity assessment the use of validated or generally accepted QSAR prediction should be suitable to adequately address the information requirements for a read-across.

4.4 Uncertainty of environmental and toxicological endpoints

Schultz et al. (2015) concluded that molecular structure (functional groups) and chemical properties (PC data) alone are generally not sufficient to justify a read-across prediction. Further scientific justification is normally required to justify the chemical grouping, typically including considerations of bioavailability, metabolism and biological/mechanistic plausibility. The statement is related to the context of the paper regarding human chronic toxicity endpoints and based on work of Blackburn et al. (2011) and Wu et al. (2010), which defined the factors leading to category membership based on six case studies. A transmission of these factors to aquatic ecotoxicological endpoints need to be critically discussed within the project.

- Bioavailability and excretion are determined in bioaccumulation studies under steady-state conditions. These data on BCF may subsequently support a read-across for chronic endpoints in fish. However, bioavailability is often described by the log Kow as key descriptor, whereas metabolism and distribution is usually not available from standard data. In line the questionnaire with experts reveal that absorption, distribution, metabolism and excretion is of minor importance for ecotoxicological endpoints. First, this can be explained that kinetics such as absorption, distribution, metabolism and excretion in ecotoxicological relevant trophic levels such as fish are usually not available and an uncertainty exists if data form mammalian toxicity is used. Second, the exposure in aquatic tests is ideally given by steady state conditions from the water phase towards the organisms and usually not route specific such as in mammalian toxicity.
- Biological/mechanistic considerations may implicate the evaluation of the mode of action of a substance. The mode of action in aquatic ecotoxicology is usually related to the molecular structure and may be classified as polar narcotic, non-polar narcotic, or reactive according to Verhaar as well as based on other structural categorisation schemes such as ester, uncoupler, carbamate, phosphate ester (ECOSAR, Raimundo et al. 2007). These categorisation approaches for biological or mechanistic modes of action are related to common functional groups that are assumed to represent the deterministic feature of a substance in a category. Hence, it can be deduced that the mode of action with regard to environmental endpoints usually represent a subcategory of the molecular structure or of functional groups of a substance. This eventually leads to the conclusion that the molecular structure (functional groups) is the relevant model determinant that is accompanied with the mode of action and chemical properties (PC data) is usually sufficient to justify a read-across prediction of aquatic ecotoxicological endpoints.
- An evaluation of the structure, functional groups and a related MoA is supported by computational tools such as OECD tool box, EPISuite or Toxtree. The use of these tools does not supersede an expert evaluation but may contribute to an objectification of chemical similarity assessment and reduce uncertainty.

4.5 Workflow

The workflow for read-across has been established by the OECD guidance document and the HH RAAF by ECHA contributes to standardization of assessment scenarios and assessment elements. The questionnaire revealed that this workflow is at least in its principles well established. Consistently, the majority of participants noted that read-across can be conducted by a predominant standardized workflow. Nevertheless it should be noted that standardization in term of the provision of assessment templates is not useful due to the case by case character of a read-across and the need for flexibility and an additional guidance document is considered as useful (Q20).

4.6 In-vitro assays

Biological data, the use of in vitro assays or profiling techniques such as Omics has been raised as promising to support a read-across (Ball et al. 2016). The consideration of in vitro assays for screening of biological activity or toxicological key events is proposed by combining an in vitro battery of tests with existing in vivo data on the members of a category (Ball et al. 2016). For human heath endpoints such as mutagenicity, for example, several in vitro assay and integrated strategies are available to allow a comparison of data in a matrix. The penetration of in vitro assays for ecotoxicological endpoints is, however, limited in the current guidance documents and subsequently in registration dossiers. Therefore, the significance of in vitro assay in ecotoxicology and human health with regard to read-across assessment is considered to be comparable, but the expectations to be used for ecotoxicology endpoints and, subsequently, the penetration of in-vitro assays in read-across for ecotoxicological endpoints can be considered as low, at the moment. However, further effort may improve the application of in-vitro assays und subsequently its use in read-across in future.

5 Conclusion

Read-across is an expert opinion approach and represents a forecasting method to make predictions based on present data and analysis of trends in the case of the analogue and category approach. Subsequently, read-across assessment represents a subjective assessment and not a proof of a scientific outcome such as a confirmation of a scientific hypothesis by an experiment. To cope the gap between a subjective expert statement on read-across and an evidenced based conclusion the assessment procedure and documentation needs to be subdivided into pivotal milestones that can either be addressed by empiric methods or standardized approaches as well as documented in transparent way. In order to improve the common understanding of the application and documentation of read-across, OECD and ECHA published guidance documents and participated in different workshops. Major questions, however, on how the mentioned principles for a read-across should be fulfilled remain open. Moreover, several publications raised the topic of workflow steps and transparency in read-across. The conducted literature review gives an overview about the developments of the read-across approach. The expectation toward read-across for data gap filling and its applicability have been addressed by several proposals published in peer reviewed journals that mostly rely to two journals (ALTEX and Reg. Pharm. Tox.) and a scientific community that focuses on the key persons Hartung, Cronin and Patlewicz. The literature may be differentiated between proposals that intend to enhance the use read-across and publications that conceptually intend to improve the establishment categories by providing case reports or tools to evaluate chemical similarity. The scientific debate in journals focuses on the aspects chemicals similarity, establishment of categories and assessment of uncertainty, and provides proposals for uncertainty evaluation and process documentation.

The most important finding of the literature review is a difference between scientific perception and practical ECHA decisions. Although evaluation of ecotoxicological endpoints was not the topic of Ball et al. (2016) the evaluation of currently available ECHA decisions indicate that the read-across rejections are mainly based on a lack of sufficient or suitable endpoint study data or identity data. In this context it should be noted that a submission of more read- across than experimental data for endpoints such as bioaccumulation or chronic fish toxicity, for example, should be considered as somewhat surprising and would require categories with a number of chemicals that can be assessed by a few experimental studies. Chemical similarity concerns appear to be of no to minor relevance and uncertainty refers to the lack of data but does not arise from a matrix of variables that can be classified by low to high as proposed by Schultz et al. (2015) or Blackburn and Stuard (2014). Therefore, it can be concluded that the future challenge for acceptance are neither the conceptual challenges of the workflow such as chemical similarity, quality of data, uncertainty and plausibility but the quality of submitted data in terms of sufficient experimental studies or suitable chemical identity data.

ECHA accepted read-across with respect to testing proposals. The procedure is different to the approaches that stop at the step data gathering and uncertainty evaluation. The decisions of ECHA indicate a more active approach where a category is built, available data gather and finally data gabs addressed within the predefined category by new experimental studies to decrease the uncertainty by covering the range of the category and by considering a worst case. From this ECHA perception the mayor step in a future workflow is the formation of a category with sufficient data. Furthermore, it can be derived that sufficient data has not been available for the evaluated ECHA decisions assuming that suitable experimental results are either not available or data is owned by third parties and not considered. In the second case the claim of a comprehensive category may subsequently requires the formation of groups of interest or task forces by the registrants involved in a category that promote the establishment of categories by sharing data and costs for additional data. This goes along with more effort but may finally result in a benefit to all parties, since the establishment of category safes costs while avoiding animal testing.

The questionnaire as well as evaluation of literature and ECHA decisions suggest that the principle workflow as well as the documentation requirements that can be derived from the workflow are rather well established while the acceptance and subsequently the quality requirements towards a read-across assessment need to be improved. In line with the regulatory requirement read-across is expected to represent a worst case estimation and should be based on sufficient and suitable data. It can be assumed that an increased communication of ECHA decisions may represent an expedient approach to increase the acceptance by transparently providing criteria for acceptance on a case by case basis. Communication in this context mainly comprise a generation of a data base on case examples and ECHA decisions, a database of submitted read-across categories that may allow the participation of different registrants in a category working group and the organization of training courses and workshops (Q17). Provision of additional guidance, for example, on specific endpoints as well as improvement of uncertainty assessment can be considered as useful while provision of templates were considered as less useful and may inappropriately restrict the flexibility needed for case by case assessment.

6 Development and results of an online survey

The project includes the development and execution of a questionnaire with the intention to establish reasons that prevent a more frequent use of read-across methods during the registration process.

The questionnaire was jointly developed between ITEM and the Hamburg office of EurA Consult AG early January 2016. For the establishment of a well-structured and comprehensible questionnaire it is very important to perform a so-called pretest. Such a pretest ensures higher response rates of interviewees and higher data-qualities due to accurate questions. The pretest of our questionnaire was conducted by four read-across experts and the questionnaire was modified due to their comments. Between the end of February 2015 and the middle of March 2015 the questionnaire was sent out via e-mail to a total of 160 potential interview partners. The majority of the potential interviewees came from Germany. However, they also included 4 parties from US and 22 parties from Europe. They all received an e-mail with the link to the anonymous online questionnaire (SoSci Survey) including a password. This online questionnaire is attached in the Appendix I.

From the 155 potential interview partners a total of 35 (22.6%) filled out the whole online questionnaire. Some respondents indicated that they were interested in the topic but felt that they lacked the experience necessary to provide meaningful feedback. It should be noted that some of the contacted potential interview partners are working at the same company/authority (like ECHA, Dow Chemical Company, BASF, etc.).

Figure 5: Q1: For which purpose do you use read-across?

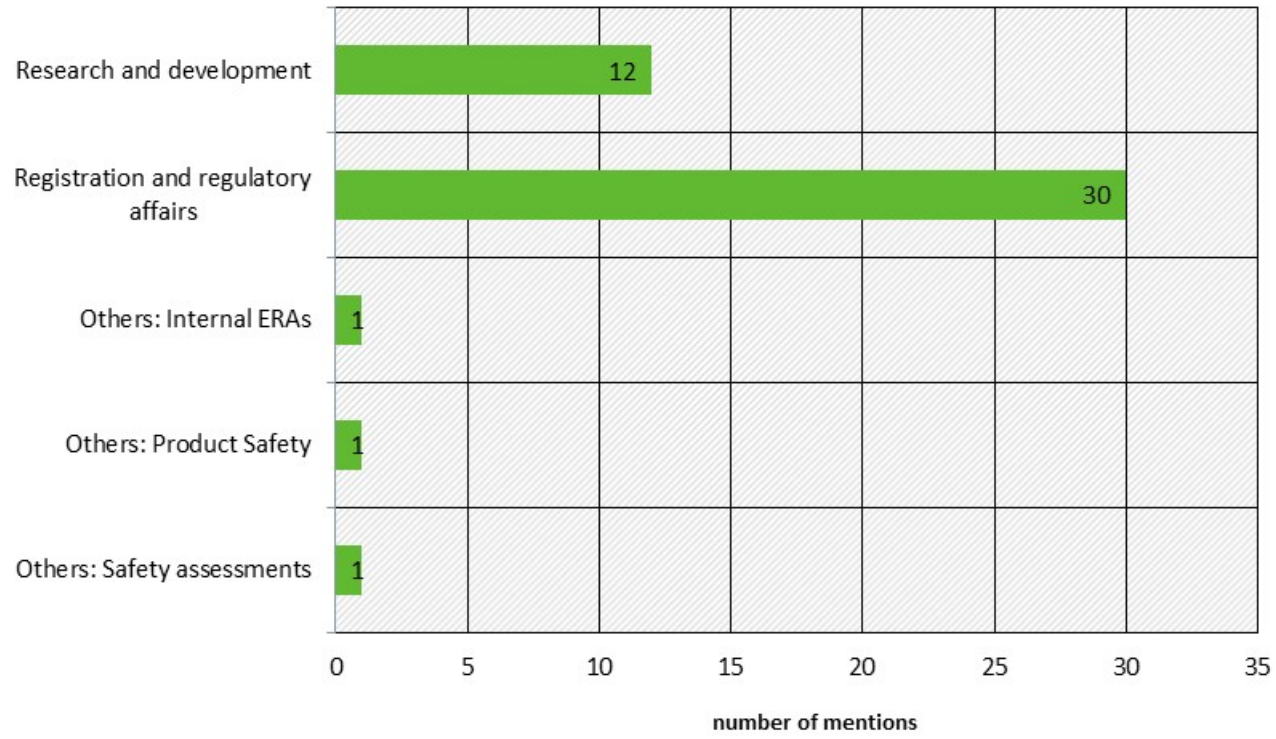


Figure 6: Q2: What is the regulatory context you are using read-across for?

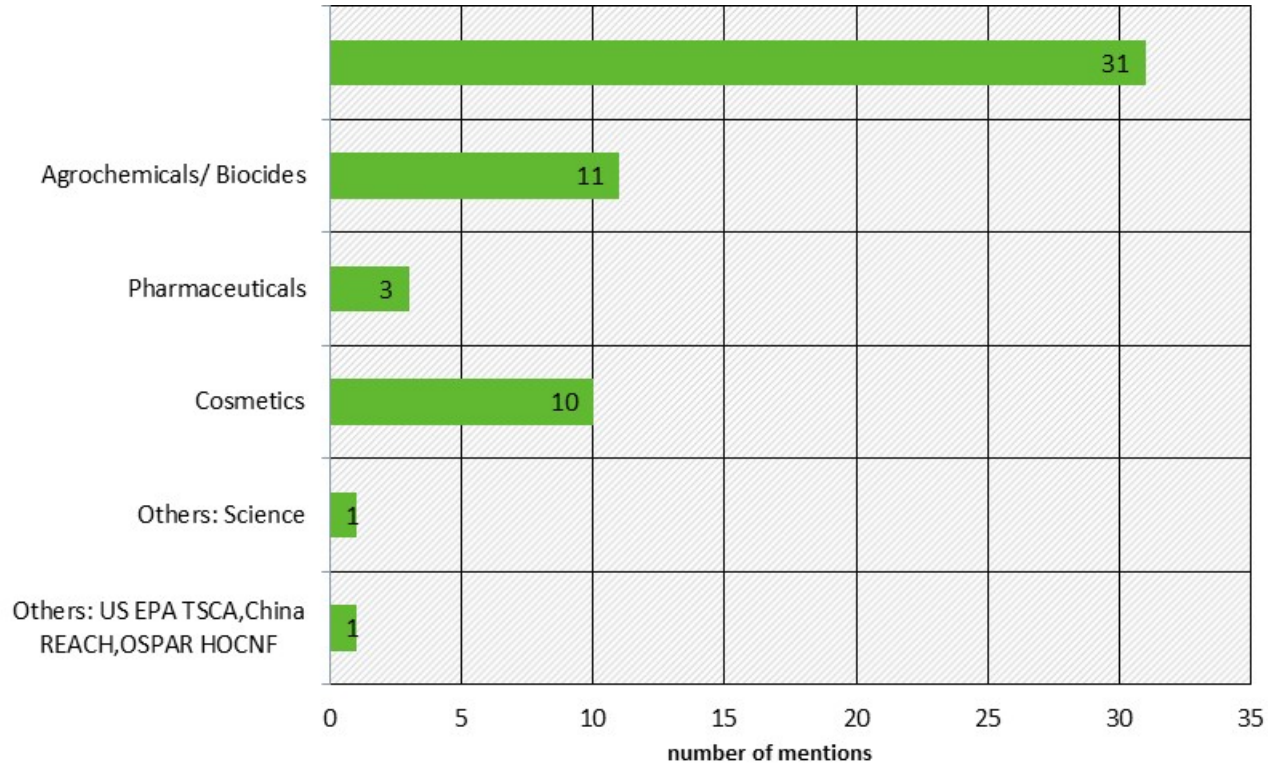


Figure 7: Q3: What are the endpoints you are using read-across approaches for?

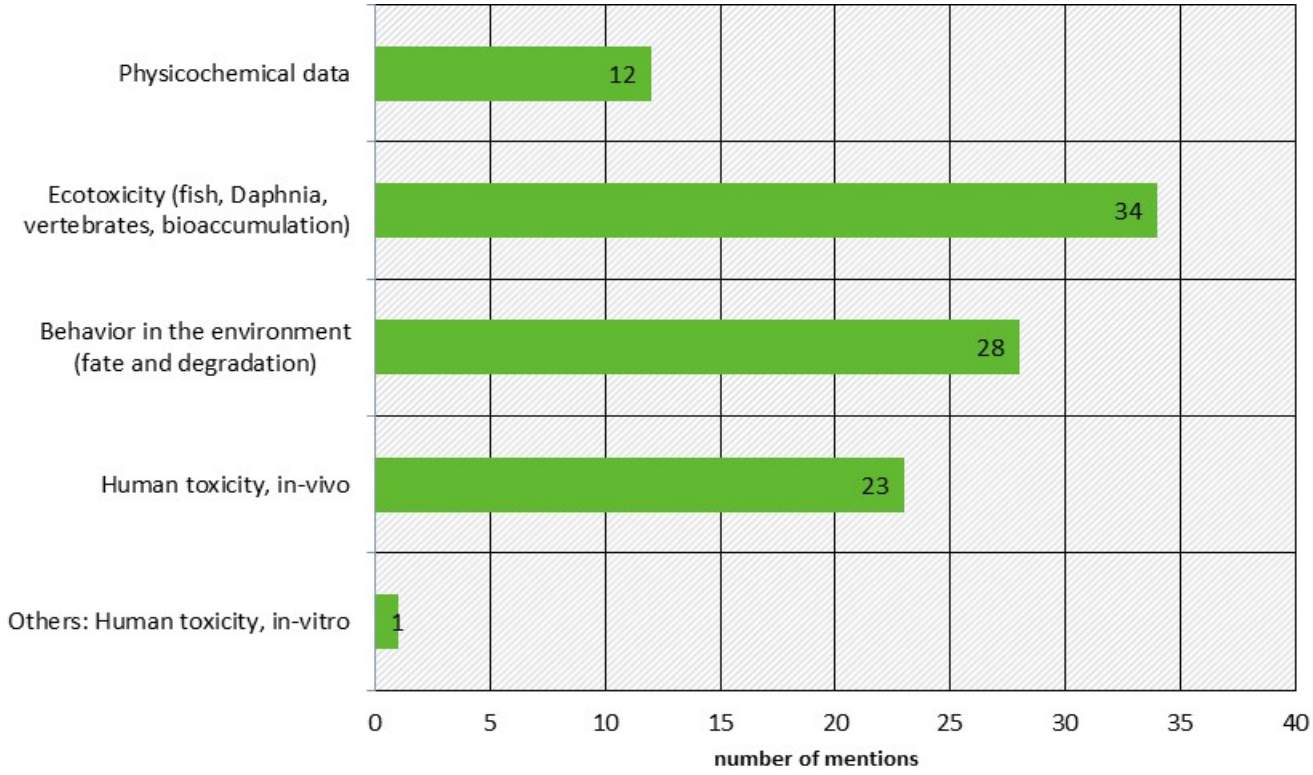


Figure 8: Q4: What is the aim of your company/institution for using read-across in ecotoxicology?

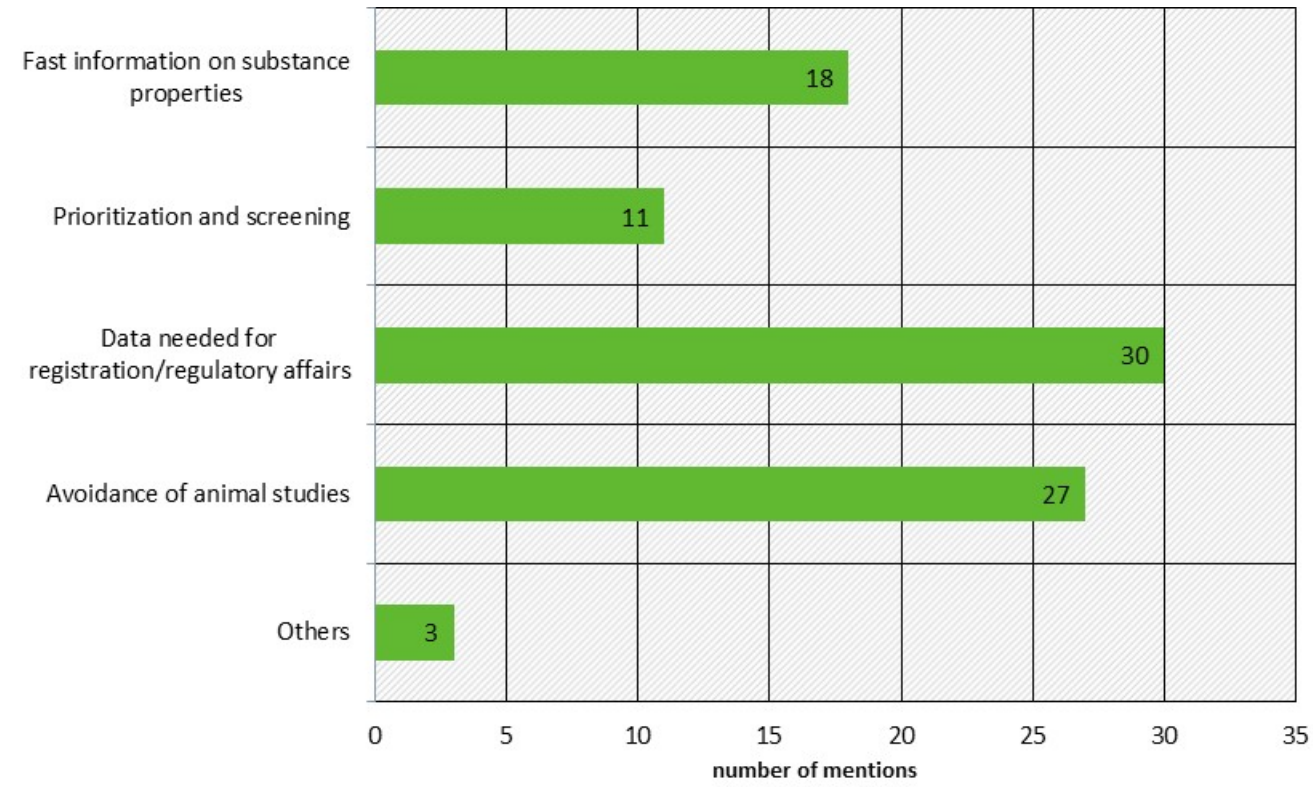
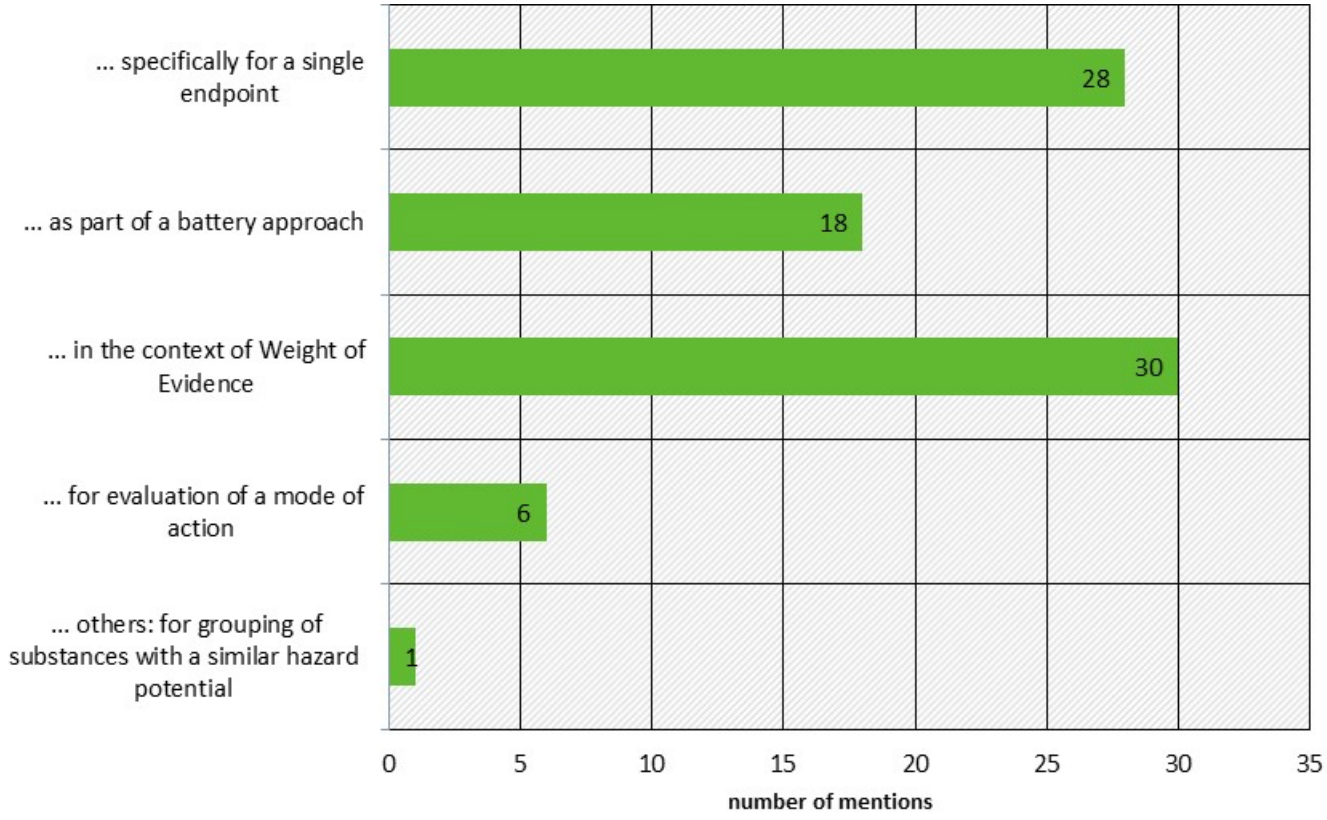


Figure 9: Q5: Which approach do you apply? Read-across is used ....



Endpoint	Step 1	Step 2	Step 3	Step 4	Step XY
acute toxicity estimate	get SMILES and available phys-chem data	use EPISuite			
acute toxicity to fish	category approach (e.g. discussed by consortia)	various online tools like AIM or DSSTox (US-EPA)	OECD-Toolbox	internal search-tools	OECD-Sids
acute fish toxicity LC50(96 h)	categorize the target molecule according to mode of action or presence of alert groups or functional groups	search for most structurally related chemicals within the identified category, for which data on the selected endpoint exist	refine selection of read-across chemicals considering key physico-chemical properties (e.g. water solubility and log K_{ow})		
LC50	gather data for class of cmpds	evaluate relationships of descriptors, e.g., phys-chem prop, and endpoint	identify sig. trends based on correlation b/n descriptors and endpoint, refine class dataset	if trend exists, accept read-across, if not evaluate other approaches, or drop read-across	
LC50 (fish)	data from same substance class available?	sufficiient similarity in chemical strcuture? Check with ChemID (Toxnet) if possible.	check for similarity of phys/chem properties		
fish	Search for similar chemical using relevant tools	Compare PC data and effect data			
acute fish toxicity	similar phys-chem properties	similar degradability and reactivity	mode of action, consider functional groups related to specific modes of action	similar ionisation	

fish toxicity	Gather available data for the substance of interest	Assess the substance in terms of its likely MOA based on alert profilers such as Verhaar, OASIS or just by virtue of chemical inspection to understand whether this substance is likely to act as a baseline narcotic or reactive chemical or something esle	Seach for related analogues based on structurally similar analogues or ones that are based on the same similarity rationale as identified in step 2	gather data on related analogues	assess the validity of the related analogues, their consistency in endpoint data for the fish toxicity endpoint and related endpoints as applicable
fish toxicity	identify structural analogues	exclude non-suitable analogues	identify valid data for source chemicals	interpolate	
could be any endpoint	assess available information on related compounds	extrapolate the available information to the chemicl of interest			
any ecotox endpoint	compare with other fata available	or just this value if nothing is available			
carcinogenicity	chemical input	structural alerts	search analogues	infering by similarity	
fish tox	Are data available from similar substance(s)?	compare substances regarding their properties (phychem, fate,...)	write read-across rationale	fill data gap	
acute fish tox	gather and evaluate existing data	if no relevant data exist, check for waiving or read-across	Do we or the manufacturer know similar chemicals	check in QSAR training data	e.g. OECD toolbox
fish chronic toxicity	strucuture similarity	chemical property similarity	toxicokinetic similarity	mode of action similarity	toxicodynamic similarity

acute fish toxicity	Define MoA based on structure	Find read-across candidate based on structural similarity	Compare experimental/predicted phys-chem properties between the substance and the read-across analogue	Apply read-across	
acute fish toxicity	identify structurally similar compounds	identify substance with potentially the same break-down products	potentially expand to substances less similar but with the same functional group		
fish toxicity	profile according to mechanism of action	select compounds according to mechanism of action e.g. Verhaar or similar	use profilers to identify other effects e.g. reactivity		
fish toxicity	OECD QSAR toolbox	Search for analogues	Building category	Data gap filling	plausibility check - uncertainty analysis - further literature and database searches etc
acute aquatic toxicity	Profiling of target molecule i. e. neutral organic, less inert, reactive unspecified, compounds acting by a specific mechanism	Search for data and if applicable identify the data gap	Category Definition i. e. define a category according to the outcome of the profiling results	Search for appropriate read-across substances within the defined category and fill data gap	
biodegradation	internal data	establish hypothesis	search dbs for structural similar substances	quality check	Hypothesis confirmed?
fish toxicity	Identification of the ecotoxicological moiety of concern	Identification of suitable read-across/QSAR substances (i.e., subsatnzes with the same hazard profile)	Conclude on substance-specific hazard		
acute toxicity	structural similarity	profiling including similar toxicity profiles	check of data availability	if applicable, integrated testing strategy	

acute Fish	Check structural similarity	Check PC data	Check other available ecotox data (e.g. acute Daphnia, Algae)	QSAR toolbox	
fish toxicity	structural similarity	consistency of the available experimental data (PC, ecotox and e-fate)	consistency of possible degradation products	data gap filling	
fish Toxicity	QSAR assessment of endpoint	Identification of Analogues	Compare QSAR with data on analogues	use all as a weight of evidence	
mutagenicity	run ToxRead	screen similar compounds related to the structural alerts	idnetify reasonable similar compounds	make conclusion	
fish toxicity	evaluation of existing data for the substance	evaluation of existing data for similar substances	comparison of existing data for the substance and for the similar substances	preparation of justification for read-across	
acute fish toxicity	empirical stuctural similarity (e.g. common functional group, common precursors or breakdown products, constant pattern in changing potency, similar carbon chain length, similar branching or linearity)	comparison of phys-chem properties: the phys.-chem. data should be similar or should have a clear trend	mode of action and ecotoxicology: the ecotoxicology should be similar or should have a clear trend based on some property of the substances (e.g. Ko/w, no acute environmental toxicity)	comparison of the ecotoxicological endpoints, weight of evidence approach	
acute fish toxicity	structure similarity	phys-chem. similarity	effects on aquatic organisms		
acute fish toxicity	QSAR Toolbox				

Figure 10: Q7: Please indicate your appraisal on the following statement: Read-across in ecotoxicology can be conducted by a standard procedure/workflow.

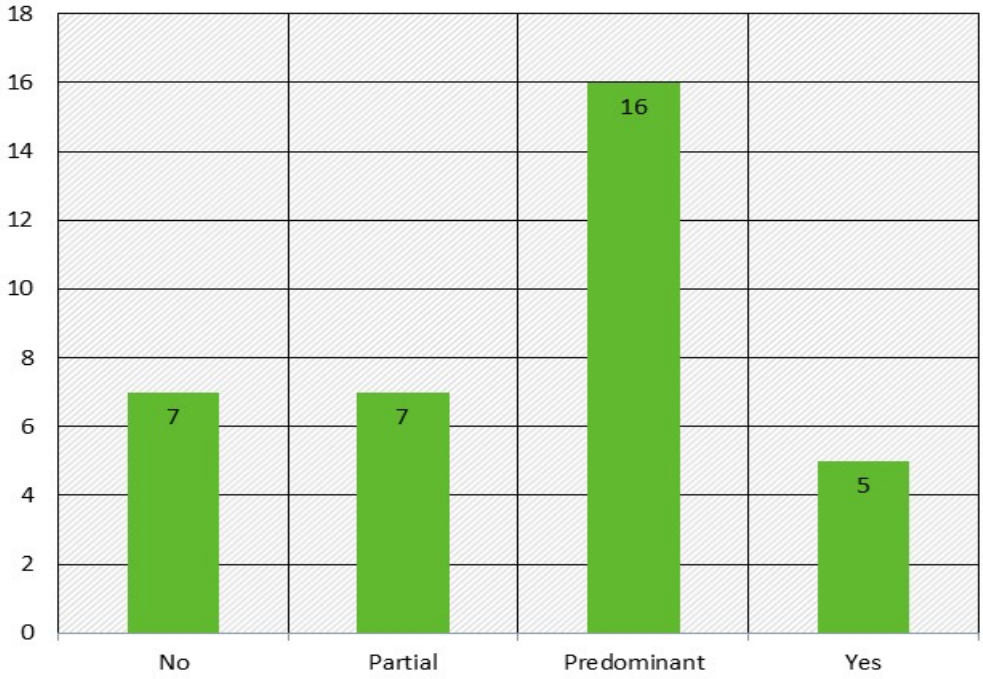


Figure 11: Q8: Are users sufficiently aware of the limitations and uncertainties of read-across methods?

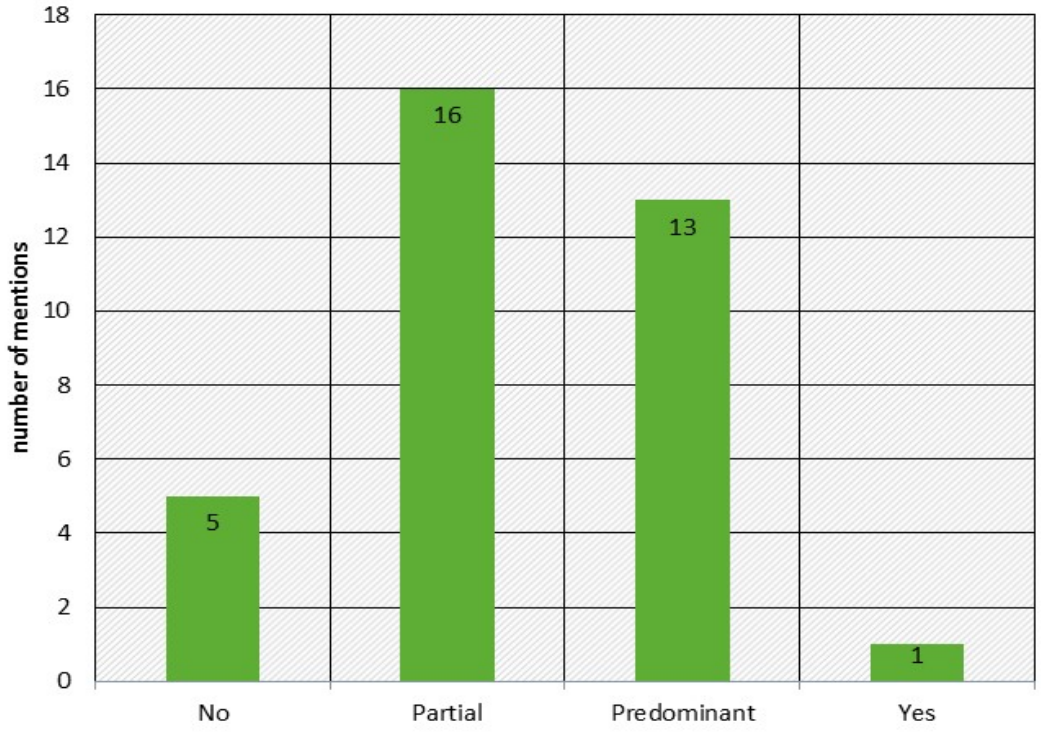


Figure 12: Q9: What are the main issues associated with the assessment of uncertainty in read-across approaches that are critical for regulatory acceptance?

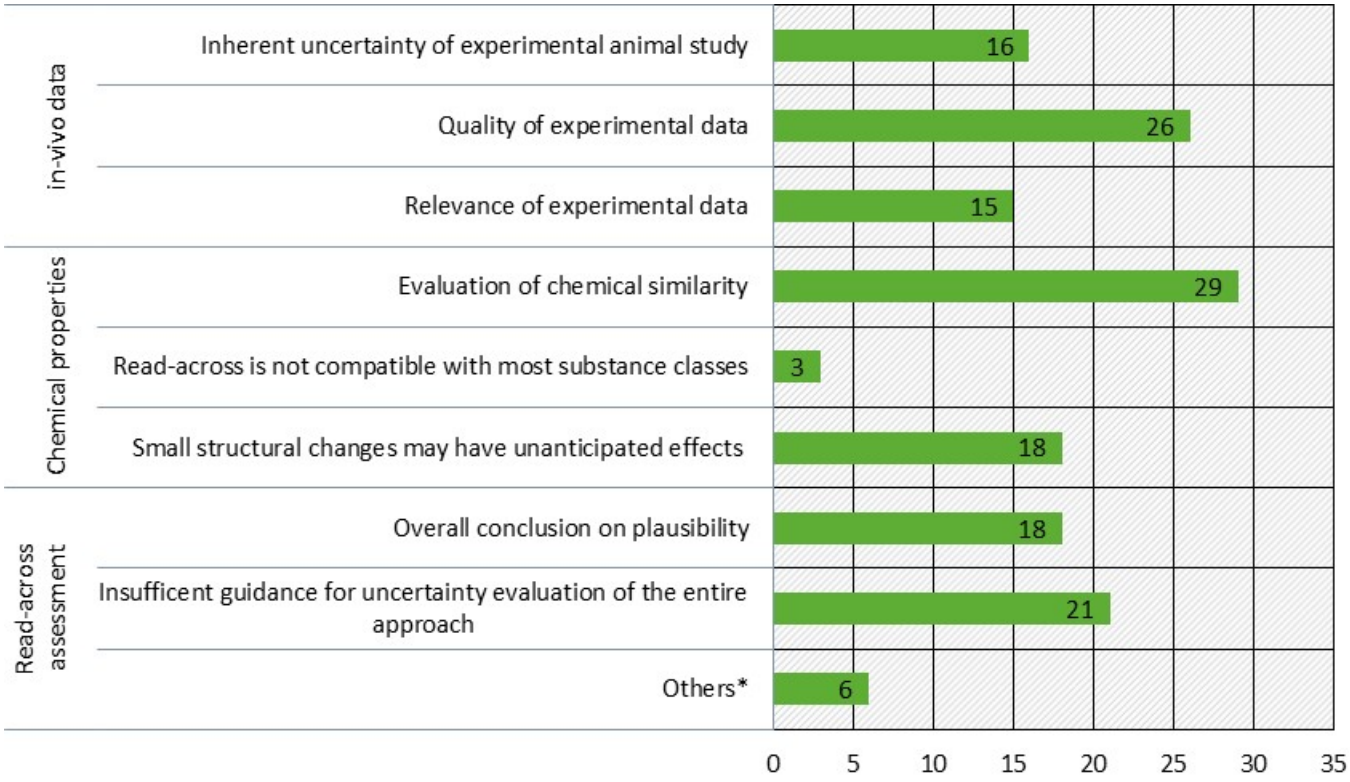
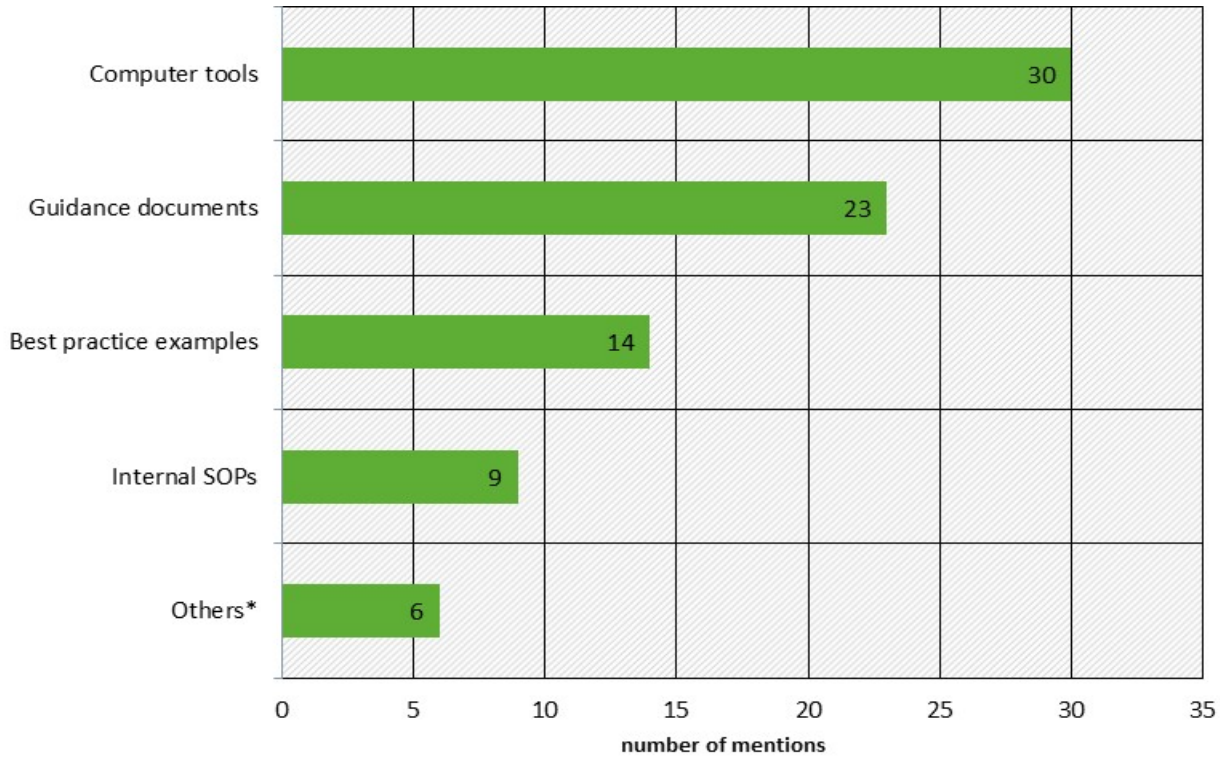
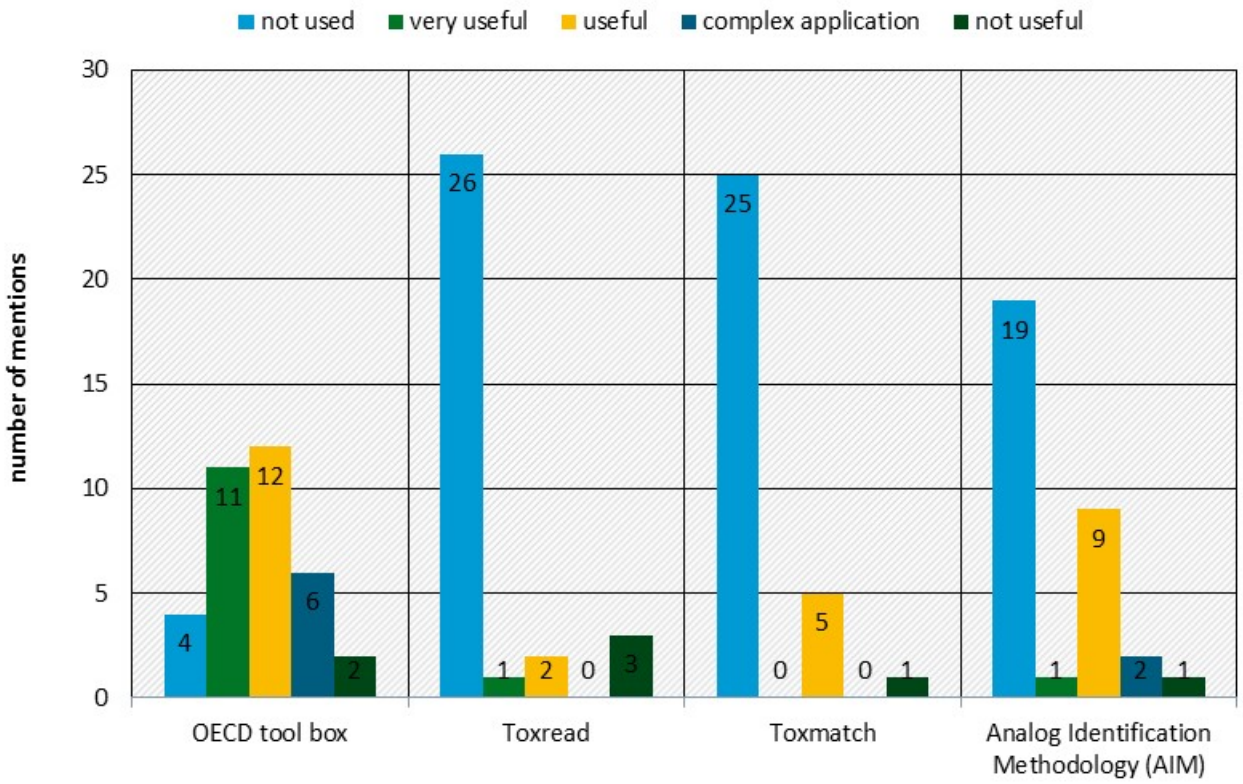


Figure 13: Q10: Which recources do you use to establish a read-across for ecotoxicology?



* Others: expert judgement, Guidance from other literature that isn't necessarily regulatory guidance (peer), expert's opinion, own and manufacturer´s experience, expert knowledge, scientific expertise

Figure 14: Q11: Are you using computer tools to establish read-across for ecotoxicology? Please give an indication of the usefulness of the tool for registration / in the regulatory context from users perspective.



Computer tool	Indication of usefulness
ChemIDPlus used for chemical similarity	very useful
MedChem Studio	
ToxNet (for chemical structure)	
AMBIT	useful (n = 3)
Automated read-across in ChemProp and Vega	useful
Epiwin reasonable	
Epiwin training set	
standard chemical databases on the web to check for structural analogues	
Derek	useful
Ecosar (to evaluate ecotox species sensitivities)	

Figure 15: Q12: Do you know the RAAF developed by ECHA for human endpoints?

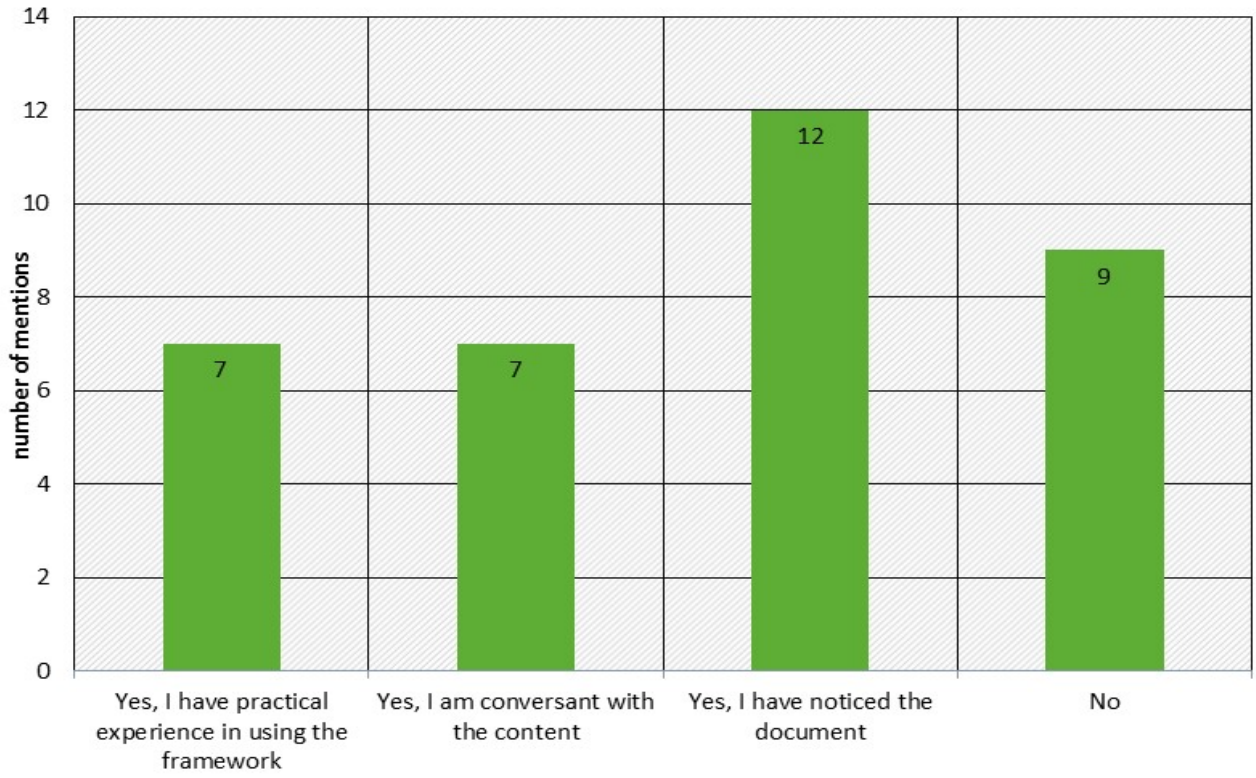
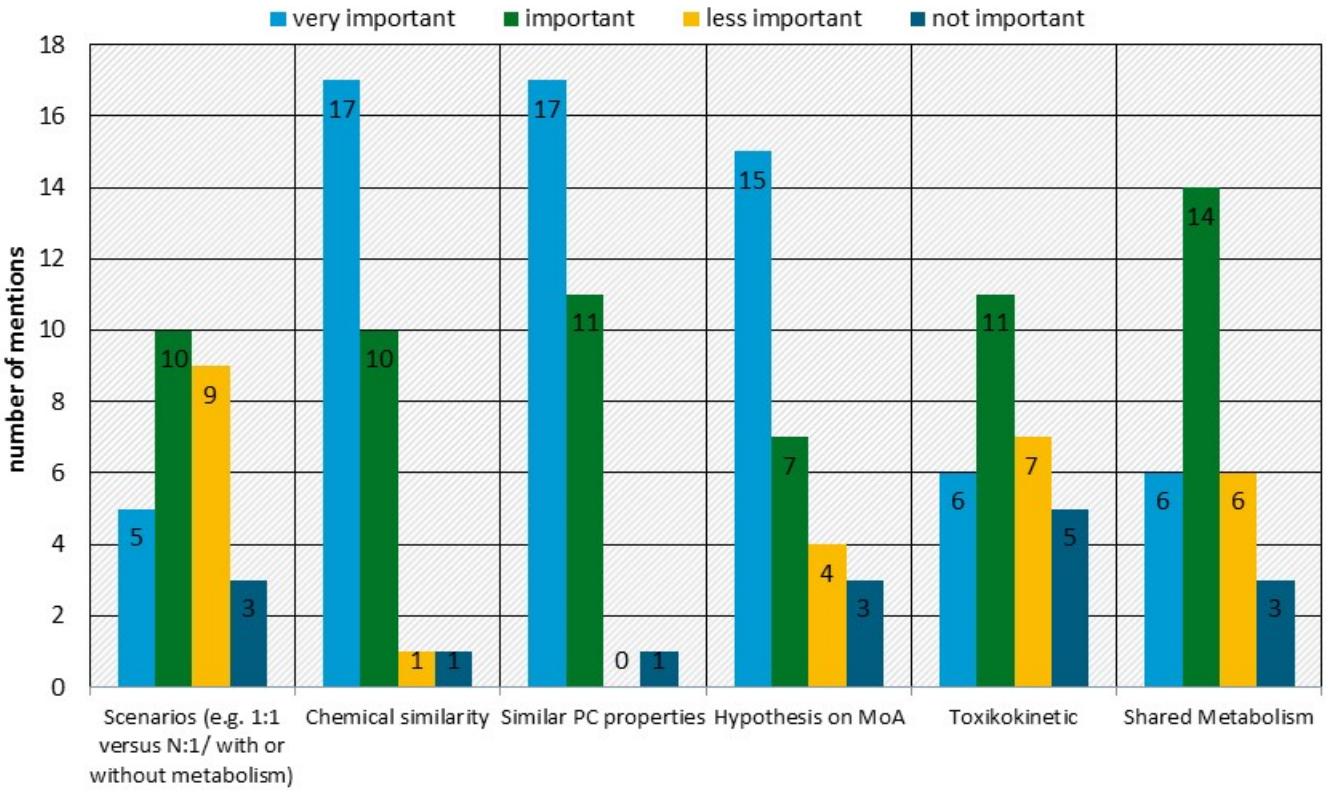


Figure 16: Q13: Which parameters that are also noted in the Human RAAF are useful for environmental endpoints?



Q14: Which parameters are not covered under point 14 but are key for read-across assessment in ecotoxicology? Please try to figure these out using the example fish toxicity.

Response 1: possibly pH/phys-chem dependence of fish media (soft vs hard freshwater, marine water)

Response 2: Rate of hydrolysis, abiotic degradation

Response 3: electronic properties (electronegativity, polar surface area, etc.), molecular properties (molecule length, width, shape, flexibility)

Response 4: inter species / inter phylum relationships

Response 5: bioavailability

Response 6: Cross species extrapolation

Response 7: Specific endpoints relevant for specific biota, assuming that in vitro-in vivo extrapolation is not really possible

Response 8: chemical similarity

Response 9: Different environmental fate properties (if toxicity depends on the duration of exposure)

Response 10: acute to chronic relationship for the category

Response 11: toxicodynamics - in vitro/in vivo extrapolation

Response 12: Compounds acting by a specific mechanism

Response 13: Uncertainty of the test: replicability and reproducibility

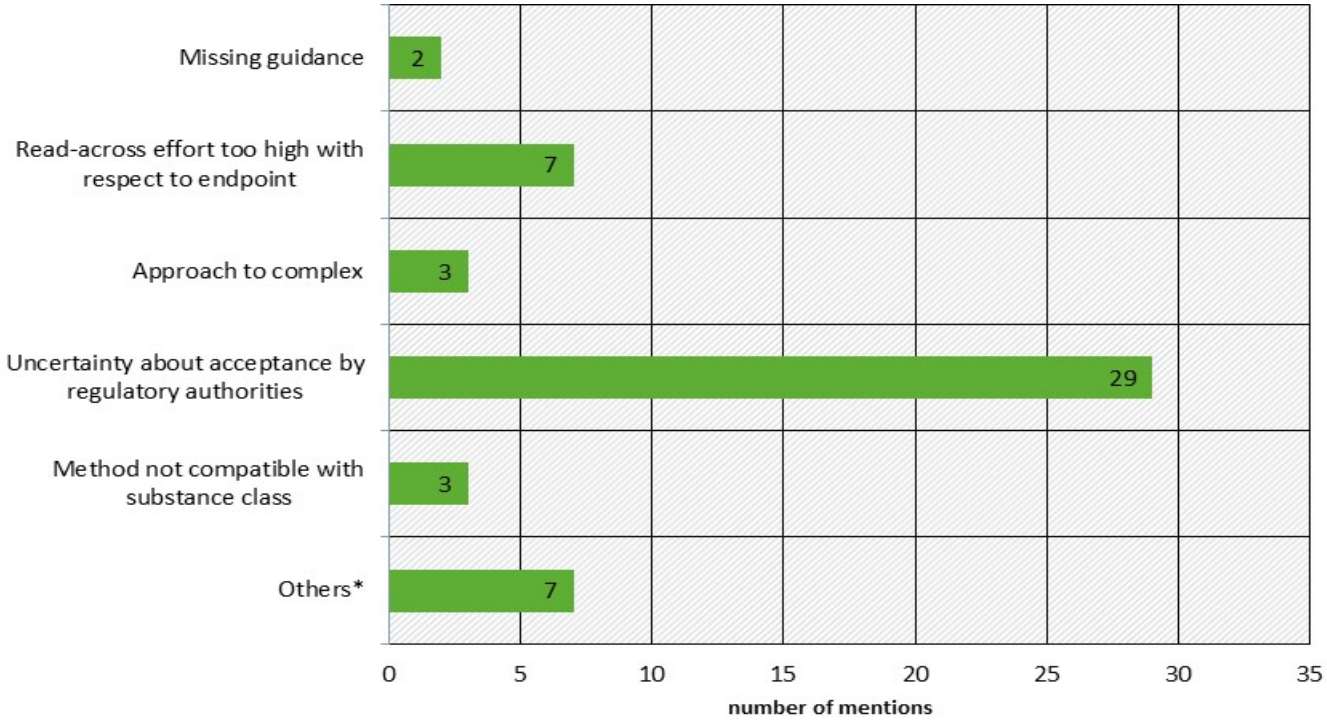
Response 14: Inorganic substances of a substance group may contain the same moiety of ecotoxicological concern. Thus, data from all substances in this group can be used in a read-across approach.

Response 15: Similar Fate properties (biodegradation, hydrolysis, Log Kow)

Response 16: In human tox these is more info about possible mechanism

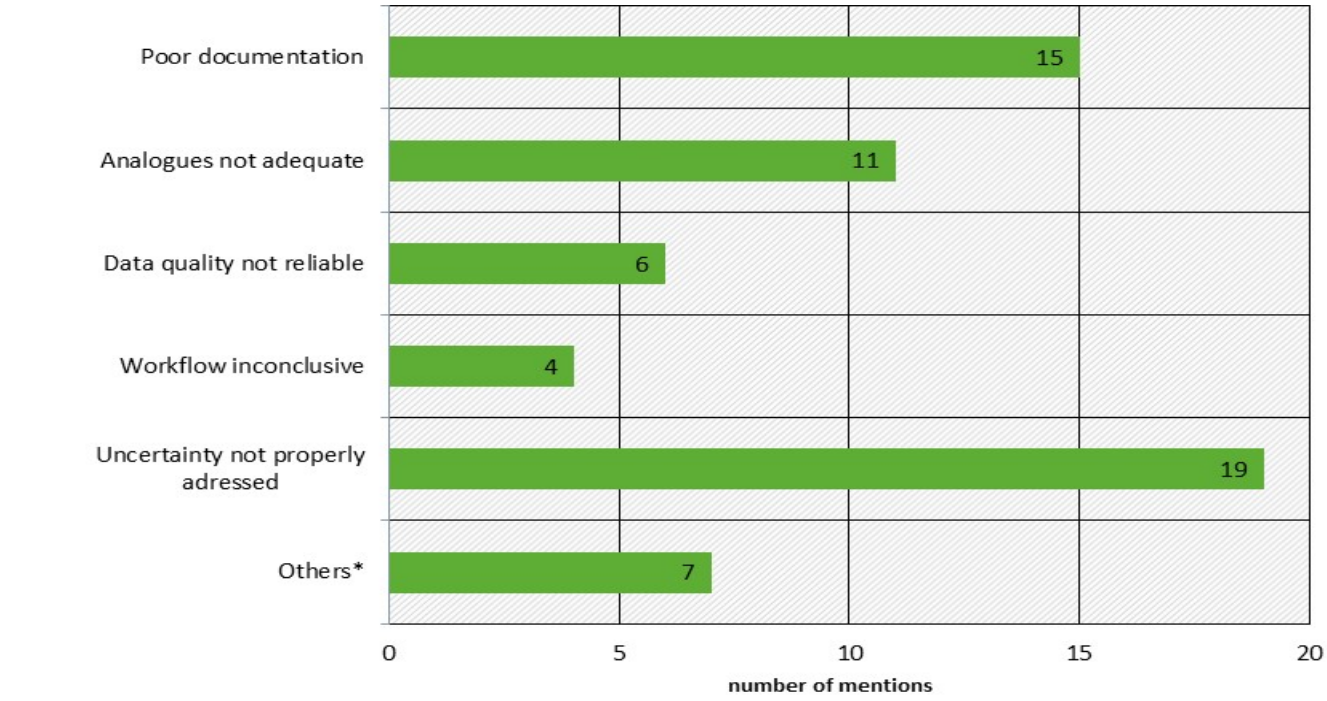
Response 17: In fish tox data on PK or metabolism are typically missing. Indications on acute vs chronic tox may be useful, but also difficult

Figure 17: Q15: What is the main reason why read-across methods are not used (more frequently) in your company?



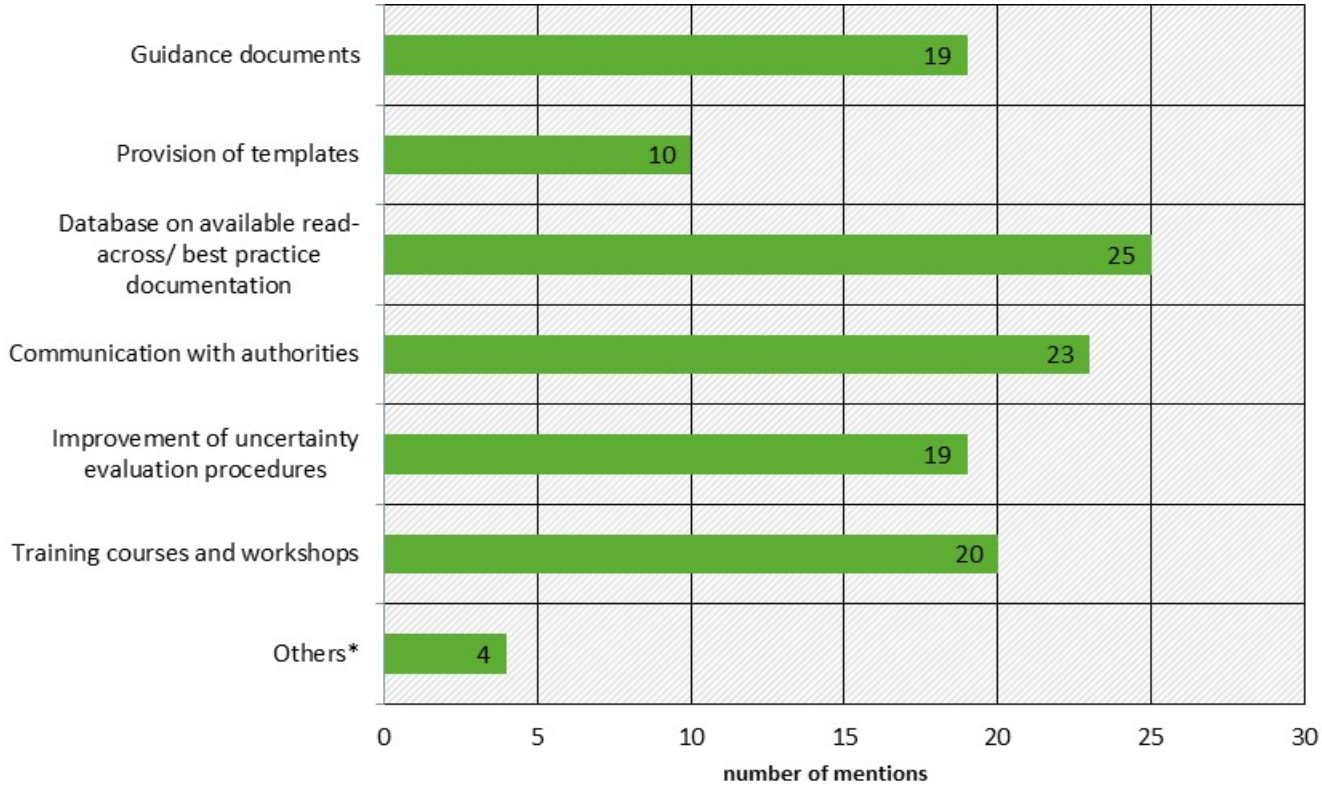
***Others:** 1. lack of data, 2. lack of reliable data for analogues, 3. difficult to evaluate uncertainties, 4. no source substances for category, 5. substance-specific data are available and read-across is not needed, 6. missing guidance for ecotoxicity, 7. I am not a company, and I use read-across

Figure 18: Q16: Which experience/ critical feedback do you have with read-across acceptance in the regulatory context?



***Others:** 1. no negative feedback yet, 2. impurity uncertainty (regards old study reports), 3. read-across is held to a higher standard than a test, 4. no data, 5. insufficient PC data to substantiate category, 6. some regulators may need training, 7. not clear how authorities assess the read-across

Figure 19: Q17: What are the main issues to be addressed to enhance the use of read-across in future?



***Others:** 1. Common screening tool; 2. less about the improvement of uncertainty more on how to identify the uncertainties and what would be seen as sufficient to address the uncertainities, addressing absence of toxicity, how to use new types of data omic, HTS, databases of examples that have worked or illustrative examples; 3. provide case studies of successful applications of read-across for various endpoints and for various compound classes; 4. include in curricula of (eco)toxicology education in silico methods, such as read-across

Figure 20: Q18: Which sector does your institution belong to?

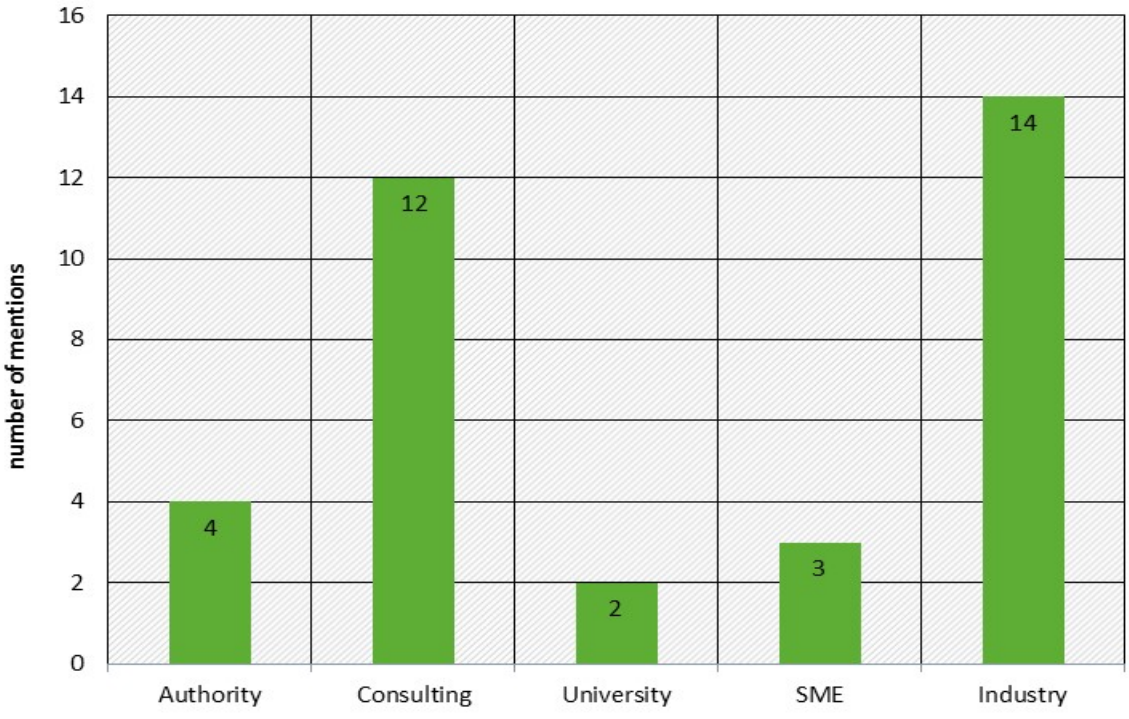


Figure 21: Q19: How do you assess your expertise in terms of read-across application?

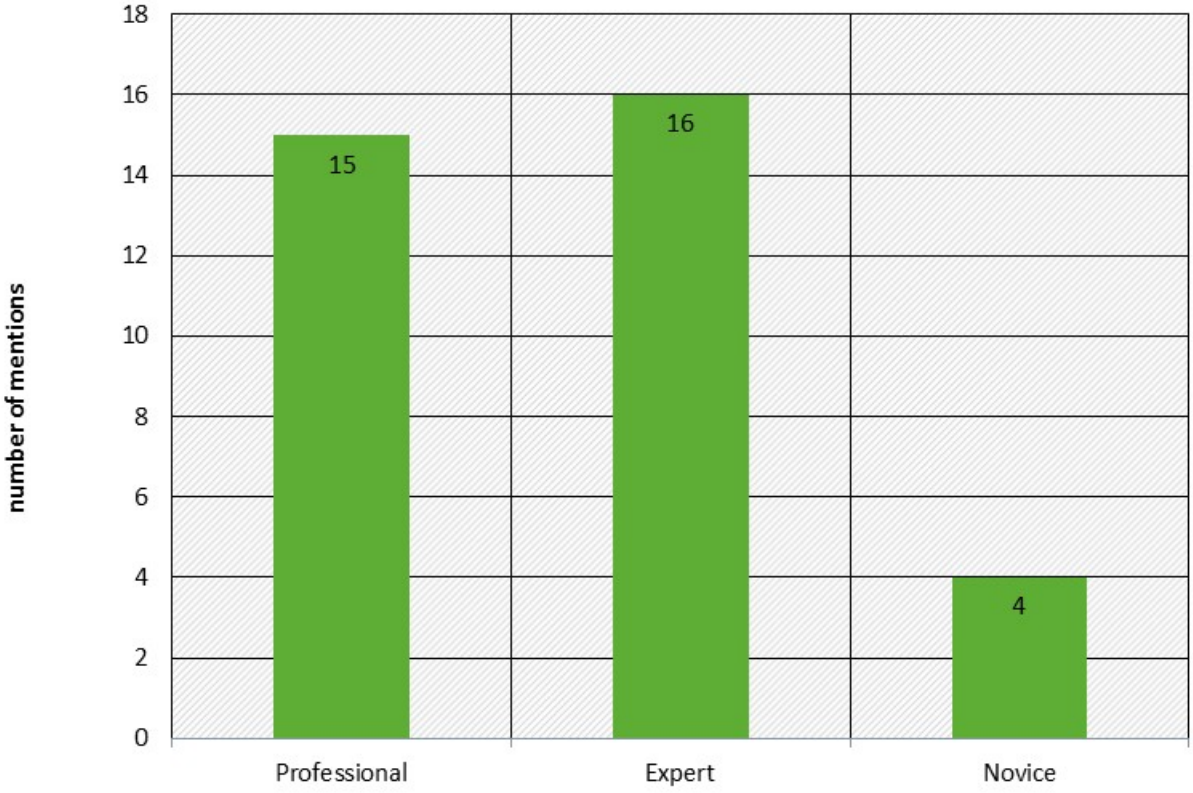


Figure 22: Q20: Is there a need for an improved RAAF guidance document for ecotoxicological end-points?

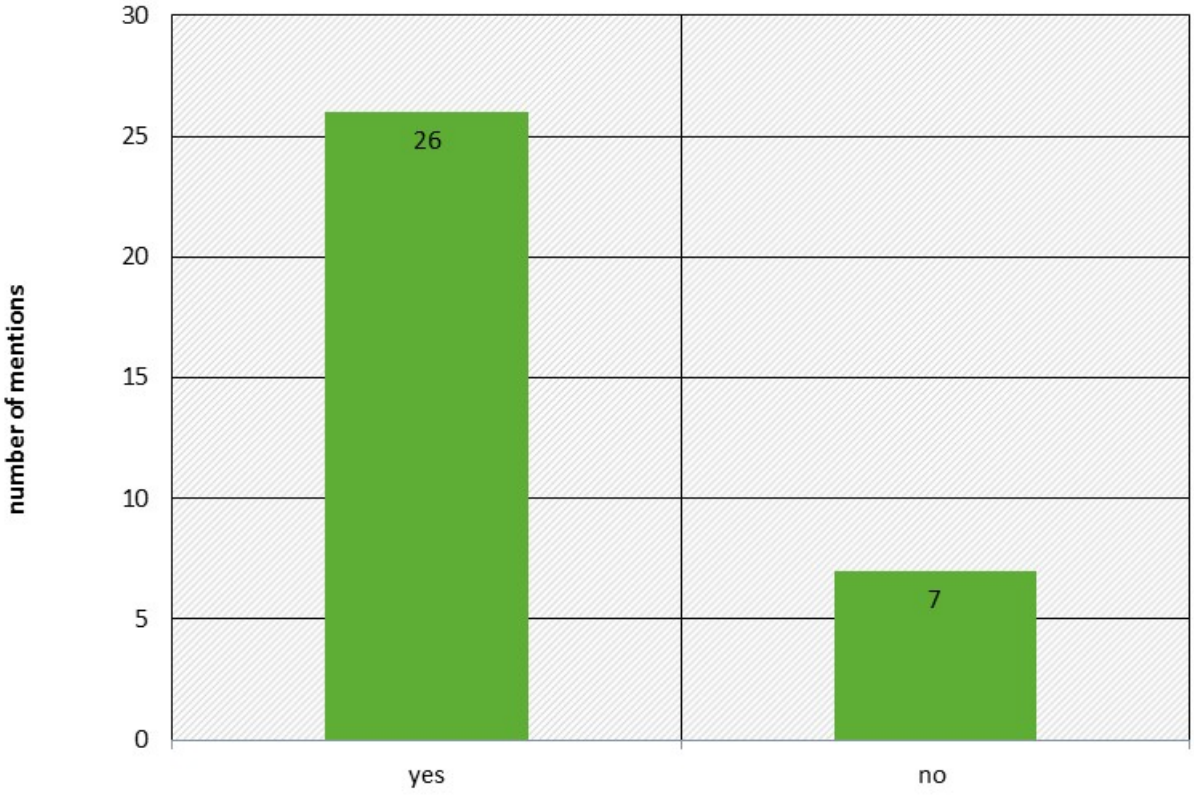


Figure 23: Q21: Would you or your institution participate in a workshop on improving the acceptance of read-across methods in June 2016?

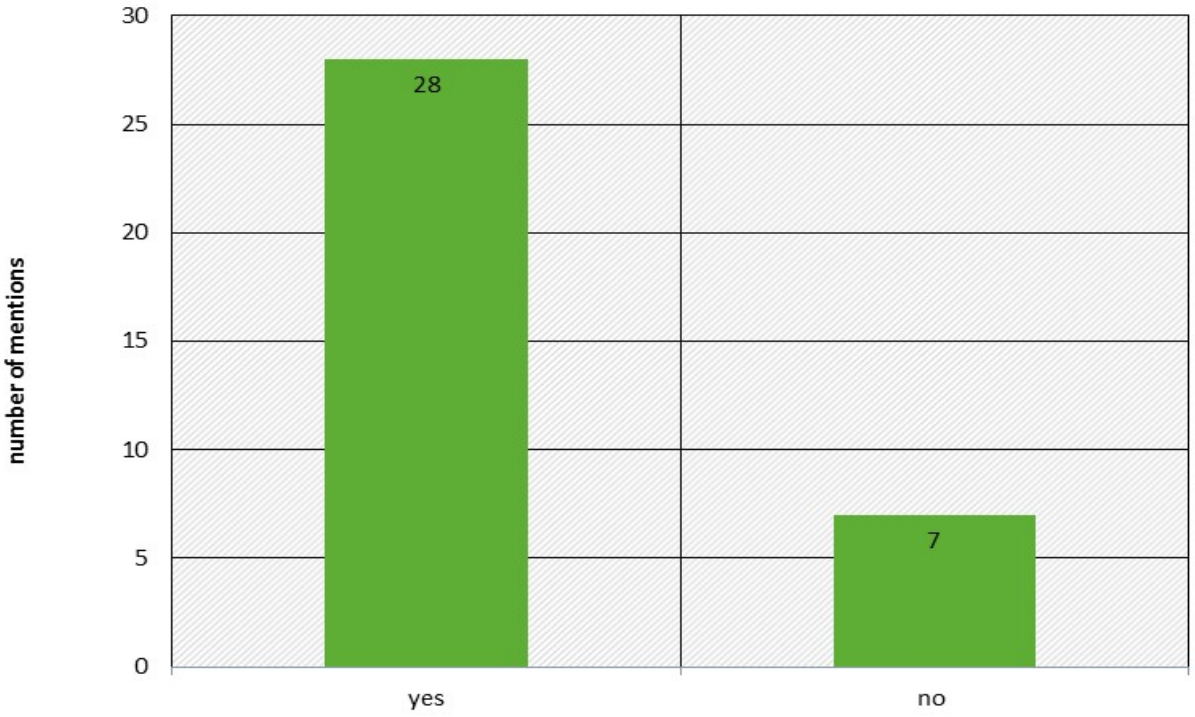


Figure 24: Q22: Would you be willing to participate in a telephone interview in advance of the workshop?

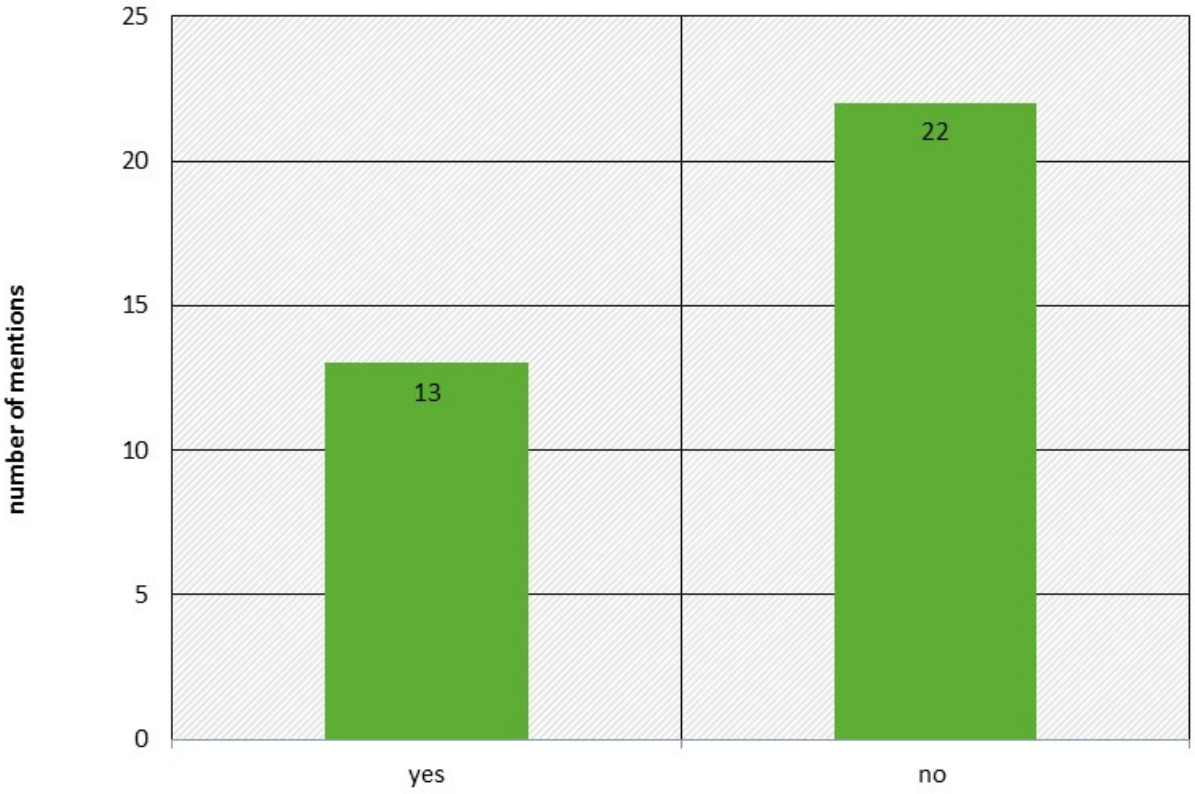
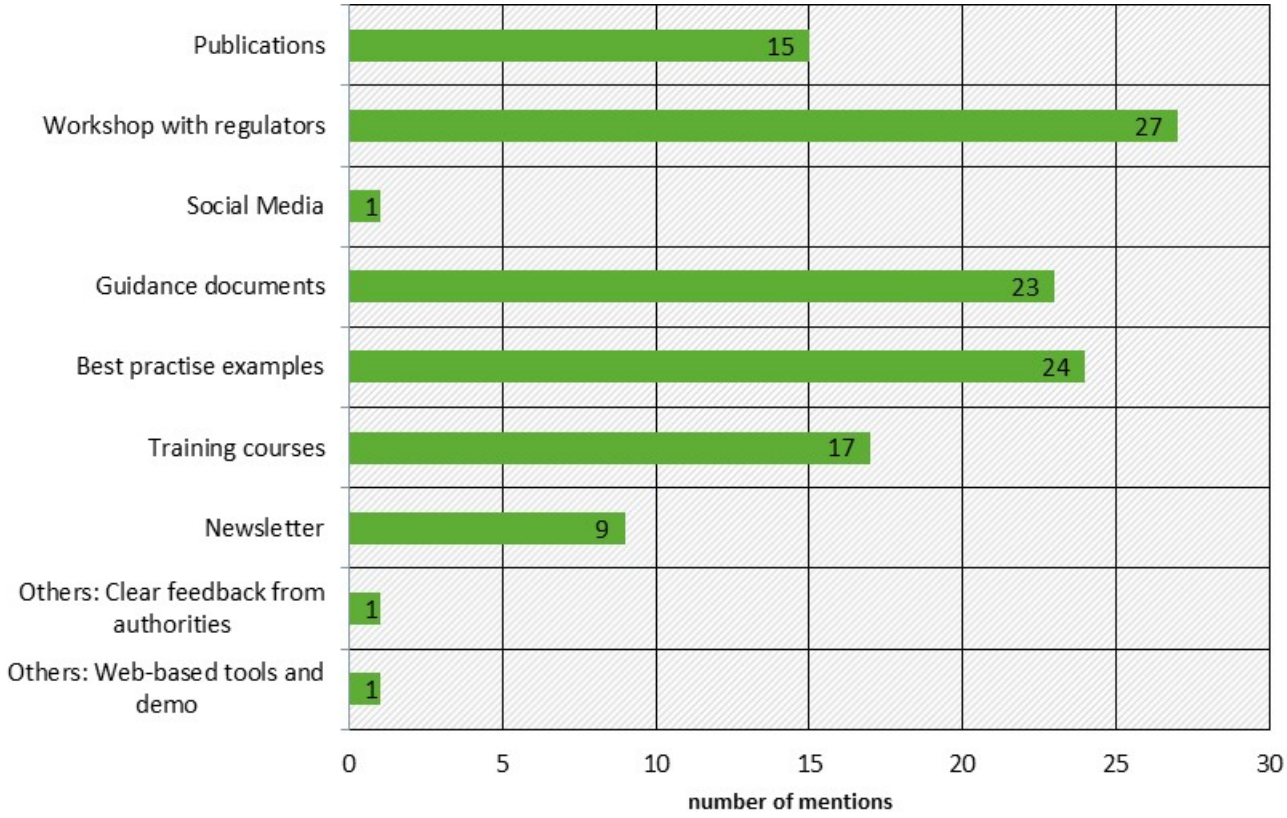


Figure 25: Q23: What would be the best channel to reach potential users and inform them about new approaches and concepts in read-across methods for ecotoxicology?



Q24: Do you have any suggestions for the workshop or general recommendations for the use of read-across?

Response 1: use experimental REACH data to verify/validate read-across concepts

Response 2: better coordination among regulatory agencies globally

Response 3: integrate possibly one example

Response 4: The key issue is similarity because similarity is context-dependent, i.e. two chemicals may be similar with regard to one endpoint (e.g. acute fish toxicity both with the same short-term mode of action, e.g. unspecific effects), but not with regard to another endpoint (e.g. chronic fish toxicity due to different modes of action, one with unspecific effect, the other may be an endocrine disruptor).

Response 5: Make it as practical as possible. There has been a slew of workshops, discussions etc. on this topic that have skimmed the surface in terms of what read-across is but not really how to do it. Also be mindful of the fact that whilst REACH might be a primary need right now for the 2018 registrations, a lot of registrants need to consider their registration strategy beyond the EU and pushing the dialogue to how the approaches can be harmonised in other sectors and regions is of critical importance. Of note, I have attempted to answer the questions mindful of the experiences over the past decade in Industry and within Government.

Response 6: Present case studies showing the successes and failures of read-across

Response 7: Discussion of real examples. The question always arises with borderline cases. Dummy examples like toluene and ethylbenzene are not useful.

Response 8: need to get better understand for human health RA and then adapt to Ecotox

Response 9: examples; use of Tools

Response 10: safe harbour policy until feedback from authorities of a read-across to avoid animal testing in the context of regulatory acceptance

Response 11: We recommend providing real examples for different organic and inorganic substance groups for application and limitation of read-across.

Response 12: stakeholders from industry and authorities should be present

Response 13: Address the reproducibility of read-across; once this is known, address accuracy of the assessment.

The matrix of the questionnaire is provided as Excel-Sheet:

RAX_questionnaire data.xlsx

ANNEX I Software tools

A detailed overview of available computational tools for human end environmental endpoints is presented by ECETOC (2012). In the following, it is focused on relevant tools for environmental endpoints.

Toxread

Toxread is a software application that will help the user to increase the reproducibility of read-across by Toxread showing structural similarities to chemical substances, "structural alerts" or other relevant features in common. Here, the software generates the structurally similar compounds (up to six), and shows the characteristics that are associated with the target compound. In terms of "structural alerts" the most similar compounds are generated. The current version includes read-across applications for the endpoints mutagenicity (Ames test) and BCF. The endpoint toxicity to fish is in development. To determine the status quo (3.1) are examples predictions are performed using Toxread i), ii) the evaluation criteria are analyzed from the reports and iii) be drawn up an assessment of their applicability for read-across.The software shows the most similar compounds (up to six), and the features associated with the target compound. For each structural alert it also indicates the most similar compounds which contain that structural alert.ToxRead contain libraries of chemicals with associated experimental values and libraries of structural alerts and algorithms of relevant features. It derives from the research within VEGA using the similarity index developed within VEGA, and integrating a series of libraries of structural alerts and relevant features from several sources. The libraries of chemicals have been checked and originate from the LIFE projects ANTARES, CALEIDOS and PROSIL.

Toxmatch

Toxmatch is a flexible and user-friendly open-source software application that encodes several chemical similarity indices to facilitate the grouping of chemicals into categories and read-across. The core functionalities include the ability to compare datasets based on various structural and descriptor-based similarity indices as well as the means to calculate pair wise similarity between compounds or aggregated similarity of a compound to a set.Key feautures are the implementations of a range of similarity indices, including distance-Like similarity indices and correlation-like similarity indices and the implementation of Verhaar scheme for modes of toxic action.

OECD Toolbox

The Toolbox is a software application intended to the use of governments, chemical industry and other stakeholders in filling gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The Toolbox incorporates information and tools from various sources into a logical workflow. Crucial to this workflow is grouping chemicals into chemical categories. The Toolbox allows a user to systematically group chemicals into categories according to the presence or potency of a particular effect for all members of the category. It allows a quick evaluation of chemicals for common mechanisms or modes of action as well as for common toxicological behaviour or consistent trends among results related to regulatory endpoints.

The seminal features of the Toolbox are:

1. Identification of relevant structural characteristics and potential mechanism or mode of action of a target chemical.
2. Identification of other chemicals that have the same structural characteristics and/or mechanism or mode of action.
3. Use of existing experimental data to fill the data gap(s).

Analog Identification Methodology (AIM) Tool

The Analog Identification Methodology (AIM) is a downloadable software program that facilitates analog analysis and data identification in support of chemical assessment or read-across approaches to help scientists and chemical managers predict potential hazards of untested chemicals. AIM software is available for free and is posted below as a downloadable software program without licensing requirements. Key characteristics of the program include:

- Ability to conduct comprehensive structural analysis of chemicals using over 700 individual atoms, groups and super fragments indexed in a predefined database
- Uses structural analysis to match potential analogs from an inventory of over 86,000 chemicals with publicly available measured data and links to the data sources
- Ability to recode defined substitutions or exclusion rules for the refinement of analog search strategies

The AIM approach comprises a large database of 31,031 compounds with publicly available toxicity data from a variety of sources. These compounds have been coded for the presence of 645 structural fragments and correction factors taken from the EPISuite KOWWIN program. Chemicals have also been coded with a ring index to enable faster retrieval.

Pass 1- Analogs are selected when an exact match for all fragments, corrections and ring types occurs. If seven or more analogs are located, the search is terminated and the list of analogs is provided.

Pass 2 - looks for additional analogs if less than seven analogs were located in pass 1. In this pass, analogs are selected based on two techniques. The first allows for different substitution patterns for alkyl substituents to be considered analogs. The second requires an exact match for only 262 structural fragments.

Pass 3 - looks for additional analogs if less than seven analogs were located in pass 2. This pass allows halogen (chlorine, bromine, or iodine) substitutions between the compound of interest and analogs.

Known Limitations

Rings - The current AIM methodology requires exact matching with respect to rings in the candidate compound. No substitutions are allowed (e.g. phenyl ring for a pyridine ring). The same number of rings is also required (e.g. dichlorodiphenylsilane will not be identified as an analog for trichlorodiphenylsilane). Methodology to remove this limitation is under investigation.

Number of analogs included in the analog list - If Pass 1 locates seven or more analogs, Pass 2 and Pass 3 are not currently implemented; therefore, some additional good analogs may not appear in the results.The Analog Identification Methodology (AIM) is a downloadable software program that facilitates analog analysis and data identification in support of chemical assessment or read-across approaches to help scientists and chemical managers predict potential hazards of untested chemicals. Key characteristics of the program include:

Ambit

The LRI AMBIT - IUCLID tool is loaded with non-confidential REACH data supplied by ECHA. The AMBIT database stores more than 450.000 chemical structures and their identifiers such as CAS, Einecs, Inchi numbers. Users can search and access a wide range of existing information about a chemical, as well as securely upload data generated by their own company. This process makes the tool both unique and powerful, particularly for data-poor small and medium sized enterprises (SMEs).

ANNEX II: Links: Guidance and Software tools

- Ambit
  - https://ambitlri.ideaconsult.net/tool
  - http://cefic-lri.org/lri_toolbox/ambit/
- Practical Guide 6: How to report read-across and categories
  - http://echa.europa.eu/documents/10162/13655/pg_report_readacross_en.pdf
- Read-Across Assessment Framework (RAAF) - ECHA
  - http://echa.europa.eu/documents/10162/13628/raaf_en.pdf
- The OECD QSAR Toolbox - OECD
  - http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm
- ToxRead
  - http://www.toxgate.eu/
- Analog Identification Methodology (AIM) Tool
  - http://www2.epa.gov/tsca-screening-tools/analog-identification-methodology-aim-tool
- Toxmatch
  - https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxmatch
- Practical Guide 6: How to report read-across and categories http://echa.europa.eu/documents/10162/13655/pg_report_readacross_en.pdf
- Information on the Experts Workshop on Read-Across Assessment with active support from Cefic-LRI held at ECHA on 03 October 2012 is available on the ECHA website:
  - http://echa.europa.eu/en/view-article/-/journal_content/c6dd5b17-7079-433a-b57f-75da9bcb1de2
- ECHA workshop on human Health RAAF:
  - https://echa.europa.eu/documents/10162/13628/workshop_summary_raaf_en.pdf
  - http://cefic-lri.org/wp-content/uploads/2014/03/ECHA-Cefic-LRI-Read-across-Workshop-Report_171211-FINAL.pdf
- Grouping of substances and read-across approach Part 1: Introductory note:
  - http://echa.europa.eu/en/view-article/-/journal_content/c6dd5b17-7079-433a-b57f-75da9bcb1de2
- Read-across illustrative example Part 2: Example 1 – Analogue approach: similarity based on breakdown products
  - http://echa.europa.eu/documents/10162/13628/read_across_example_1_en.pdf

7 References

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II. Statusreport

FKZ 3715 67 4180

“Tierversuchsfreie Bewertung unter REACH - Weiterentwicklung und Nutzung des Read-across Ansatzes”

Risk assessment under REACH without animal testing – development, application and acceptance of the read-across approach.

Sylvia E. Escher, Oliver Licht, Andrea Staudler

ON BEHALF OF THE

FEDERAL ENVIRONMENT AGENCY (UMWELTBUNDESAMT)

Hannover, November 2016

1 Introduction

This report summarizes the outcome of the workshop on read-across, held at the Fraunhofer Institute for Toxicology and Experimental Medicine ITEM on 13.-14.06.2016. In the workshop, stakeholders from industry, academia and authorities discussed the use and applicability of read-across for ecotoxicological endpoints like chronic fish toxicity. A schematic presentation of potential main building blocks in a read-across workflow is given in Figure 26 in no specific order. In a read-across approach, the (eco)toxicological property is "read across" from one to several data rich source compounds (SCs) to one or many data poor target chemicals (TCs). For this prediction of (eco)toxicity the impact of several aspects have to be taken into account to assure that source and target compounds show similar toxicological effects for the endpoint of evaluation. Similarity has to be evaluated with regard to structural and physico-chemical properties based on the assumption that compounds with common structural features show comparable (re)activity versus e.g. proteins/receptors and have common absorption, distribution, metabolism and excretion (ADME) properties. Other relevant data with impact on similarity might comprise data on fate and distribution in the environment, use of (eco)toxicological data from other endpoints e.g. longer or shorter duration, other species and differences in metabolism. In case of few experimental data, QSAR predictions might also provide valuable information e.g. on the mode of action or general reactivity of the read-across compounds.

Based on structural alerts models have been derived to distinguish between different modes of action. According to Russom et al. (1997)[7] seven mode of action can be established for fish: non-polar narcosis, polar narcosis, ester narcosis, oxidative phosphorylation un-couplers, reactive electrophiles/pro-electrophiles acetylcholinesterase inhibitors and central nervous system seizure agents. Substances, that does not contain structure related to these alerts, were placed in the group nonpolar narcosis.

Relevance and accuracy of the gathered data has to be evaluated with regard to the prediction goal. The gathered data build the ground to establish the read-across hypothesis, which outlines the rational for the prediction. Read-across is a complex and time consuming approach, which need a systematic and transparent documentation of the applied workflow and the integration of expert judgement. Databases and tools can be used to facilitate the gathering of the endpoint specific relevant data, evaluation of the read-across hypothesis, documentation of data and results and finally derivation of the prediction. Finally the uncertainty of the read-across prediction has to be addressed, ideally in a (semi)quantitative way. Uncertainty may arise from different steps in the read-across workflow, e.g.:

- chemical similarity: small differences in chemical structure might, however, be critical to the predicted ecotoxicological activity
- In vivo data:
  - inherent data variability of the experimental in vivo data
  - differences in experimental data because of differences in study protocols, dosing, strains, data quality etc.
- data gaps within the source compounds or only one source compound (analogue approach)
- adequacy and relevance: use of closely related data instead of the required guideline data to predict the expected outcome, e.g. short term data instead of long term data; interspecies differences etc.
- derivation of reference values – worst case versus regression approach

This non-comprehensive list of potential sources of uncertainty already illustrates, that uncertainty is a critical aspect in (eco)toxicological risk assessment and become even more complex by using experimental data from source compounds for the evaluation of a certain ecotoxicological property.

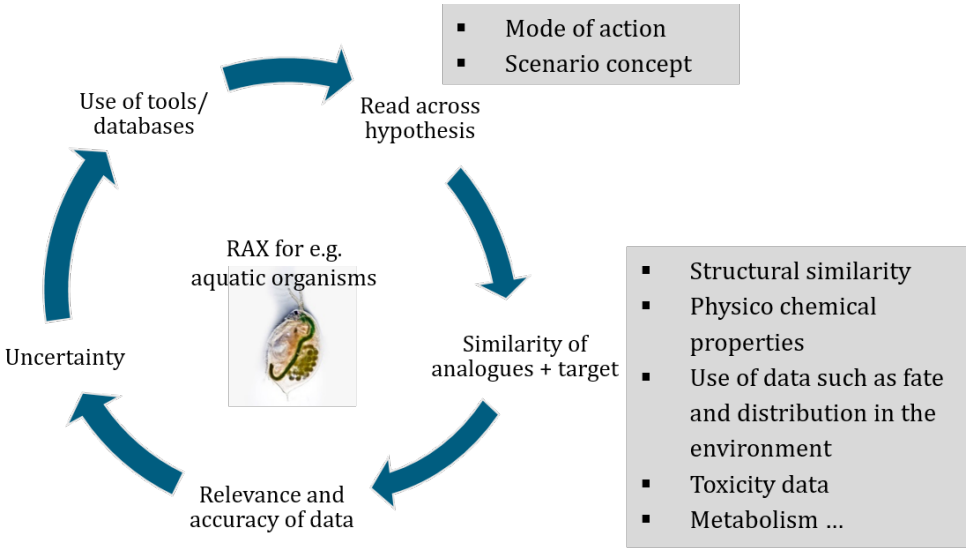
[7] Russom CL, Bradbury SP, Broderius SJ, Hammermeister DE, Drummond R A (1997), Predicting modes of toxic action from chemical structure: Acute toxicity in the fathead minnow (Pimephales promelas). Environmental Toxicology and Chemistry, 16: 948–967.

The workflow and use of data for read-across in ecotoxicology are still and area of intensive discussion. A read-across assessment framework (RAAF) was recently published for human health endpoints (ref). It aims to illustrate in a transparent manner the different types of data and evaluation steps, which are considered to be mandatory by the authorities and therefore give valuable guidance to the applicant in the preparation of the read-across dossier.

Three out of the main building blocks (Figure 26) were addressed in more details during the workshop, which are:

- Definition of similarity, a key requirement for the read-across prediction
- Uncertainty in read-across approaches
- Tools, databases and guidance

Figure 26: Schematic presentation of read-across building blocks. (source: Fraunhofer ITEM)



2 Project implementation

This UBA project proceeded in several steps. In preparation of the workshop in June 2016, an online questionnaire was submitted to stakeholders from industry, academia and authorities. In the questionnaire, stakeholders were asked to provide their experiences on the use of read-across predictions for ecotoxicological endpoints. In addition, a literature search and review of outcomes of recent, e.g. European projects related to read-across was conducted. The results of both activities are documented in the first status report. This information set the basis for the read-across workshop.

3 Read-across workshop in June 2016

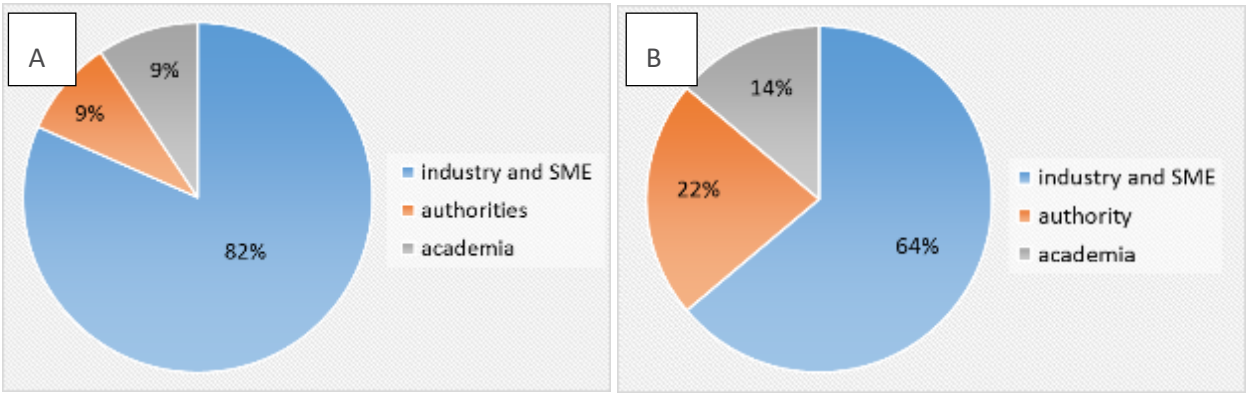
3.1 Invitations of participants and speakers

About 190 potential participants were identified and invited by sending out flyers in the middle of December 2015, and again in the middle of February 2016. 82% of these potential participants belonged to industry (including SMEs), 9% to authorities and 9% to academia and research institutes (Figure 27A).

The workshop was held in Hannover at the Fraunhofer ITEM on 13.-14.06.2016. 64% of the participants belonged to the industry sector. Of the remaining participants, 22% and 14% were working for authorities and academia, respectively (Figure 27B). A list of the final 37 participants is given in Annex I.

Stakeholders from industry, academia and authorities were invited to give an overview on their read-across experiences with a special focus on the applied read-across concepts. They were asked to illustrate drawbacks and advances. The invited lecturers were identified based on the review of relevant literature and projects (please refer to interim report).

Figure 27: Overview on participants into institutions: A) Invited potential participants; B) final workshop participants (source: Fraunhofer ITEM)



3.2 Workshop conception

The workshop was conceptually divided into four parts.

The workshop started with a presentation by Christoph Schulte, who introduced the general vision on the use of alternative methods with a special focus on the UBA perspective. Then an introduction was given by Sylvia Escher (Fraunhofer ITEM) outlining the workshop structure, its goals and the motivation of the UBA project "Tierversuchsfreie Bewertung unter REACH - Weiterentwicklung und Nutzung des Read-across Ansatzes". By this, we aimed to achieve a common understanding on the workshop conception and goals within the participants (the agenda of the workshop is enclosed in Annex II).

Thereafter key note lectures illustrated different read-across examples by also raising questions on "pros" and "cons" on read-across procedures. In the morning of day 1, five key note lectures on best-practise examples were given; two were presented by representatives of authorities (Bram Versonnen, ECHA and Annegret Biegel-Engler, UBA); two by scientists (Alistair Boxall, coordinator of the IMI iPie project and Ralph Kühne, senior scientist at UFZ Leipzig); and one by industry (Florian Schmidt, BASF). On day two, Robert Luttik introduced the topic "uncertainty". He has worked most of his life at RIVM, and after retirement is still working on the determination of uncertainty, e.g. for EFSA (EFSA draft[8]). His experience can therefore either be classified as "scientist" or "authority". Mardas Daneshian finally gave an overview on CAAT (Center for Alternative to Animal Testing) developments nicely illustrating the concepts and ideas of international science based read-across activities.

Thereafter a knowledge café[9] was performed with all participants of the workshop. Knowledge cafés as typical knowledge sharing techniques are simple and suitable tools for an open and creative conversation between different participants (Figure 28). The main aim is to develop a better collective understanding of a specific question or issue of mutual interest. Knowledge cafés do not guide participants to a pre-determined solution or outcome, rather they offer additional impulses for decision and issue-solving processes. It must be clear that the focus lies on the collective exploration of e.g. issues, challenges, opportunities, possibilities or risks. The knowledge gain in these cafés is more efficient than in

[8] EFSA draft: Guidance on Uncertainty in EFSA Scientific Assessment 1, EFSA Scientific Committee (https://www.efsa.europa.eu/sites/default/files/consultation/150618.pdf)

[9] http://assets.lwsite.com.br/uploads/widget_image/image/136/667/136667/the_world_cafe.png

individual conversations between single persons and can be documented and thereby be utilised for other interested people. There are many forms of knowledge cafes (e.g. the Gurteen knowledge café, world café or innovation café) which can be adapted to different purposes. Knowledge cafés are best convened where there are many stakeholders and opinions and there are no right or wrong answers. Knowledge cafés can be run in many ways - there is no definitive format, the way depends on the purpose. The most important value of such cafés is in the conversation itself. In many circumstances it makes sense to capture and analyse things from conservations to record the outcomes/discoveries. This enables the distribution of results to a broader range of interested people.

Figure 28: Schematic demonstration for the performance of the „world café“ (source: Eura AG, Andrea Staudler; https://informalmuseology.files.wordpress.com/2015/07/world-cafe.jpg)

Schematic demonstration for the performance of the „world café“ as an suitable example: (1) set the context that matters, (2) create hospitable space, (3) explore the asked questions, (4) encourage everyone’s participation, (5) cross pollinate and collect diverse perspectives, (6) listen together for patterns, insights and deeper questions, (7) harvest and share collective discoveries.

The following performance of the knowledge café was chosen to be best suitable of this workshop: After a short introduction of the knowledge café, speakers were asked to be hosts of the so-called “café tables”. Each topic was indicated at the café table and further broken down into a few sub-points to start/stimulate discussion. These sub-points served as starting points. Up front of the workshop, topics and sub-points were send to all participants, including the first status report of the project. The plenum was split up into six small conversation groups consisting of a host and 5-6 guests. Each group discussed a specific topic/question at the café table and captured their ideas and thoughts on boards. After 30 minutes, each guest had the chance to go to another café table with a different or the same topic/question. The hosts remained at the café table, welcomed the new guests with a short summary about the last round(s). Afterwards new groups shared their knowledge and ideas to the specific question. In total, three rounds for three questions were offered, so that each guest had the possibility to discuss on all topics.

Finally, a round table discussion was offered to all participants. As the topics and discussion evolved during the three rounds, this step aims to summarize all raised aspects. Therefore, the results of the six knowledge café sheets were presented by the respective hosts on day two. Then participants were asked to assess the outcomes by using a simple scoring system of colored stickers and or add missing points or give further recommendations. Each participant got one red (5 points – most important), one yellow (3 points), and one green (1 point – less important) sticker for each topic.

3.3 Analysis strategy

The different types of information gathered within the workshop are evaluated in the next section (section 4). The following approach was used to systematically address the different levels of evidences:

4.1 Summary on lectures: The content of the different lectures is briefly summarized. A focus is set on described read-across concepts and critically discussed aspects leading to certain read-across hypotheses. Advantages and drawbacks as seen by the speaker are outlined.

4.2. Knowledge café and Round Table Discussion: This section summarizes the results of the knowledge cafés and round table discussion.

First the topics and related questions as introduced to the know-ledge café participants are depicted. A short description of each topic as well as some questions were provided to the participants. The analysis of the results per topic starts with the pictures of the original flipcharts, on which participants illustrated their arguments and ideas verbally and graphically. The original figures are included into this report to illustrate relationships between different aspects. All mentioned aspects per topic were then summarized in tables including rating scores. As far as possible, the original description of the suggestions made by the participants was used in these tables. Abbreviations were wrote out. Many aspects were mentioned several times and/or are closely related, probably because two groups discussed one topic in parallel or participants felt that these aspects were of particular importance. These related aspects were grouped by using a “Content ID” to better illustrate the overarching “key aspect”. A group ID was used to discriminate between aspects being mentioned in both or only one group.

Some of the provided suggestions/terms served for clarification or interlink several aspects. In these cases the original term from the flipchart is given under the header “links to aspect”. Key aspects” per Content ID as derived from the Table 7, Table 8 and Table 9 are explained in more detail e.g. by giving the context in which these aspects were mentioned and interpreted.

4 Results

4.1 Summary on key note lectures

The workshop lectures are given in full detail in Appendix III. These slides do not all have a permission for data sharing, and are therefore only intended for internal use.

The UBA vision on the use of non-testing approaches was given by Christoph Schulte (UBA). He introduced the history and the basic concepts of the currently on-going paradigm shift in (eco)toxicology. This paradigm shift intends to replace the traditional in vivo methods by non-testing methods (non-animal studies) in risk assessment. Also, read-across was introduced (Figure 29) and the UBA vision on the use of alternative testing methods (Figure 30).

Figure 29: Background of the read-across workshop as introduced by Christoph Schulte (UBA).

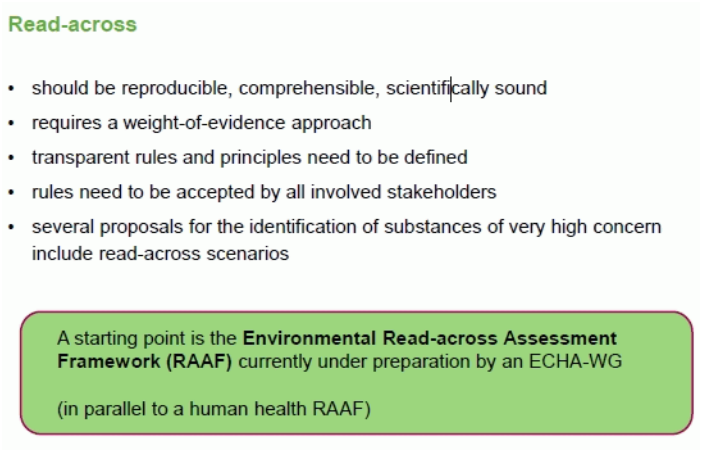
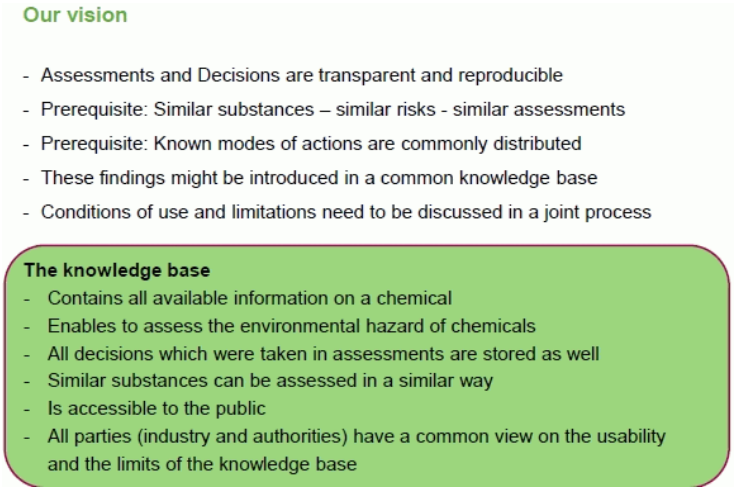


Figure 30: UBA vision on the use and applicability of non-animal test methods as introduced by Christoph Schulte (UBA).



In the introduction of the workshop, critical aspects from the first interim report of this project were presented to the participants by Sylvia Escher (Fraunhofer ITEM). It can be seen from the ECHA status report (ECHA 2014[10]) that read-across is widely used in ecotoxicity assessment. For short-term toxicity 60% of all dossiers used experimental data, about one third used read-across. For long-term fish toxicity only about 10% of the dossiers used experimental data; 20% used read-across, but the majority of studies were waived or omitted. The latter issue indicates that there are only few experimental chronic fish toxicity studies which can be used to build read-across approaches. Nick Ball recently analysed 524 compliance check final decisions that were publically available on the ECHA website (from July 31st 2015). The decisions were manually searched to identify those that included some reference to the use of read-across, either as proposed by a registrant, a third party or a member state during the course of the decision-making process. This dataset was kindly provided to Fraunhofer ITEM. Only 47 out of 524 decisions (9%) considered ecotoxicological endpoints. From these, 8 dossiers used read-across (Table 7), which were, however, rejected. Three out of them were about UVCBs, for which structurally related compounds could not be identified. 5 dossiers did not provide sufficient data to prove similarity with regard to the physico-chemical properties or toxicity values.

[10] ECHA (2014) The use of Alternatives to Testing on Animals for the REACH Regulation. Second report under Article 117(3) of the REACH Regulation. European Chemicals Agency.

Table 7: Overview on ECHA decision for ecotoxicological endpoints in which read-across was used. The following endpoints were considered: long- and or short-term toxicity to aquatic and terrestrial invertebrates, plants and fish, effects on soil micro-organisms, growth inhibition to aquatic plants. Not considered in this table were decisions on fate, biodegradation and bioaccumulation.

Approach	Reasons for rejection	
Read-across (8 dossiers)	Unclear substance identity, not possible to ascertain structural similarity. All UVCB.	3
	Lack of sufficient evidence to substantiate assumptions made within read-across justifications/similarity not proven with regard to toxicity and/or PC properties	5
No read-across applied (39 dossiers)	Unclear substance identity	5
	Inappropriate data provided	17
	Incorrect classification	3
	Exposure based waiving not appropriate	5
	QSAR not appropriate	3
	Data on exposure assessment missing	5
	General waiving arguments not plausible	1

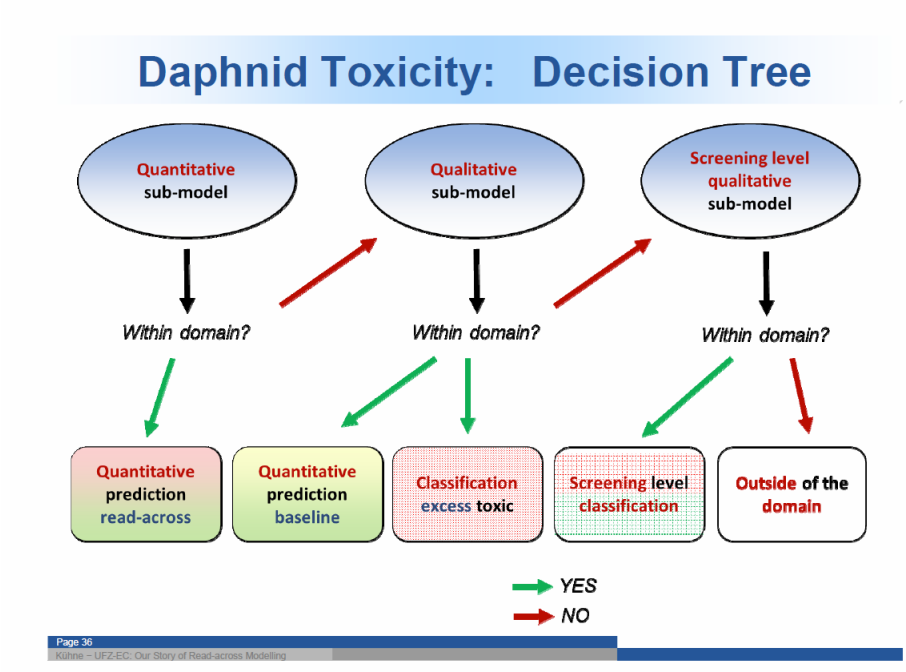
An example for a read-across case from Health Canada was also presented. A read-across group comprising 14 Phthalates was identified starting with chemical similarity. Based on acute toxicity data, they concluded on a common non-specific mode of action.

Bram Versonnen introduced the ECHA position on read-across. The grouping in read-across cases always starts with structural similarity of test and source compounds, but structural similarity alone is not sufficient to allow a prediction. A mechanistic explanation has to be provided, e.g. explaining how and why structural similarity is associated with similar biological fate and properties. The aim of the prediction is to provide sufficient information for classification and labelling and/or risk assessment. This includes that key parameters have to be covered (adequately and reliably) - but these key parameters are not listed per endpoint. Exposure duration of the provided tests have to be comparable or longer than the required tests. Documentation has to be adequate. An environmental Read-Across Assessment Framework (RAAF) is currently prepared, that will align to the RAAF for human health in content and structure. An example how the Environmental RAAF will explore “transformation of products” was presented.

Alistair Boxall (University of York) introduced the project on “Intelligent Assessment of Pharmaceuticals in the Environment” (iPie project) to the participants. The project is part of the IMI initiatives and develops a huge database on environmental endpoints for drugs. After a short overview on QSAR and screening models, he also pointed out the ongoing development for read-across. In the context of this talk, read-across was meant as reading across data from one species/endpoint to another species/endpoint. Therefore, he concluded that for human to environmental read-across, differences in bioaccessibility and uptake need to be considered. Further typical interspecies differences, like presence of absence of certain receptors and respective binding affinities, differences in metabolism and metabolic rate were outlined. This project is in the starting phase and might deliver suitable tools and a relevant data collection for read-across also for regulatory purposes. However, the classical compound to compound read-across is not yet part of their working plan. Regulatory objectives need to be better communicated so that projects with such high potential develop pragmatic and ready to use tools for risk assessment.

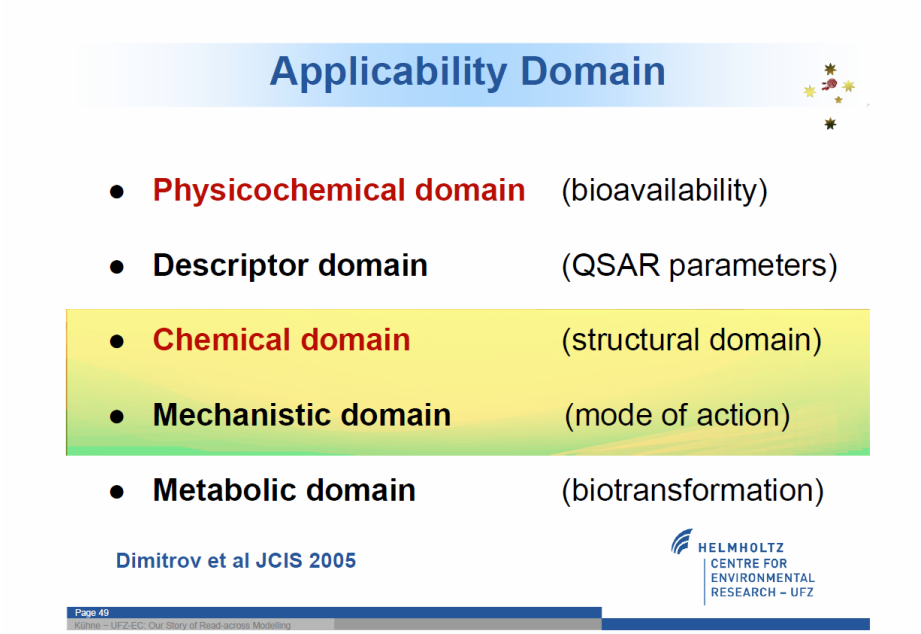
Under the topic "Experiences with read-across – best practise examples" Ralph Kühne (UFZ Leipzig) presented the results from the UFZ working group. In contrast to QSAR, computational read-across models hardly use fitting, but as QSAR they are predictive within the applicability domain. He presented several models for prediction of physico-chemical parameters, BCF values and ecotoxicity data. Atom-centered fragments was one approach to develop the models. Another important point for further consideration were decision trees that can be used for hierarchical selection of models for prediction of quantitative, qualitative and screening level results based on the fit to the specific applicability domain (Figure 31).

Figure 31: Decision tree for daphnid toxicity, where inclusion to different applicability domains trigger the model to be used to achieve a quantitative, qualitative or screening level result (slide 36 from presentation of R. Kühne, UFZ Leipzig)



He also stressed the point that the parallel use of different models can lead to a better outcome. For the development of a workflow, the points presented under the term “Applicability Domain” can be used for the definition of similarity (Figure 32).

Figure 32: Points to consider for the description of the applicability domain (slide 49 from presentation of R. Kühne, UFZ Leipzig)



Florian Schmidt from BASF presented a best-practice example on read-across, giving an industry perspective. He suggested key criteria for applying read-across in ecotoxicology: structural similarity (as starting point), followed by homogeneous physico-chemical values (with exceptions), biodegradability and bioaccumulative behaviour. He stressed that especially physico-chemical properties, e-fate and bioaccumulation cannot be evaluated separately, but have to be considered in the endpoint-specific context (slide 8). Further he stressed, that similar to QSAR models, a read-across prediction needs a defined applicability domain. The applicability domain set out the rules for the inclusion and exclusion of compounds into the read-across group, e.g. based on the above mentioned key criteria. The read-across assessment shall be done by weight-of-evidence, a worst-case approach is suggested depending on data availability, quality and reliability. Half-life and metabolism were illustrated as critical parameters for compounds being readily hydrolyzed, for example.

Annegret Biegel-Engler (UBA) presented three best-practice examples worked out by the UBA, the German federal environmental agency. Requirements for read-across and workflows were illustrated by camphore- and per- and polyfluorinated substances as well as phenolic benzotriazoles. In the case of the two camphor compounds (case 1) an analogue approach was presented. The “relevant source compound” was identified based on chemical similarity (structural + physico-chemical properties), second biological similarity (comparison of available in vitro and in vivo data, QSAR prediction). From the second step it was concluded that the source compound is likely to have a potential for endocrine disruption. This led to the read-across hypothesis, that the target compound is a potential endocrine disruptor, too. Third metabolites were predicted for both compounds, which turned out to be predicted as strong ER binder for source and target compounds. This example clearly shows a possible workflow for a read-across approach. The read-across prediction was however rejected by the Member State Committee, as experts believe that the anticipated mode-of-action was not sufficiently supported by experimental data from the source compound. Thus, the source compound was not identified as ED-substance, and Germany therefore withdrew the SVHC-proposal for the target compound.

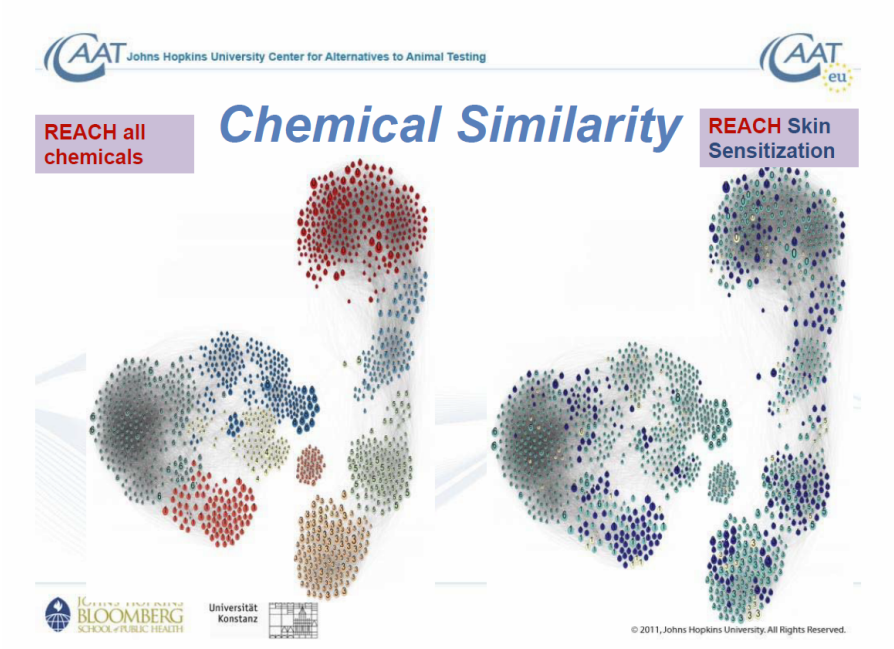
The persistency example of long-chain perfluorinated substances (PFCAs, case 2) also starts with structural similarity – all analogues only differ with regard to carbon chain length. BCF data were available for C8, C12, C14-PCFAs (source compounds), and were used to predict compounds of interim

chain length (C11 and C13). The analysis of experimental data on partition coefficient, BCF and bioaccumulation in humans shows a clear trend based on chain length, so that a vPvB assessment based on the source compounds was possible. This example illustrates nicely, which key parameters beside chemical similarity shall be used to assess the endpoint "persistence". The assessment of persistency of phenolbenzotriazoles also starts with chemical similarity (case 3). In this case study, other data types were used to judge on the persistency of the source and target compounds. Test of "ready biodegradability", evidence from a water sediment study and a field study. Finally, the argumentation was mainly based on a monitoring study which indicated that source compounds are present in concentration to comparable to the emission concentration the in sediment decades after the end of manufacturing. Although in case 2 (PFCAs) and 3 (phenolbenzotriazoles) the same endpoint was investigated (persistence), the selection of key parameters to assess and support the read-across hypothesis in a weight-of-evidence approach was completely different. This illustrates clearly the case specificity of read-across arguments, as well as its dependency on available data.

Under the topic "lectures and discussion" on day 2, Robert Luttik (EFSA consultant) talked about uncertainty of ecotoxicological endpoints and the possible consequences for read-across. He presented results from comparison of ecotoxicological endpoints for different species like rainbow trout and fathead minnow, or between different crustacean species and Daphnia at acute and long-term level. In addition, he also showed how good extrapolation factors used in pesticide regulation are, to cover the species tested. In conclusion, besides a more general comment on the assessment factors per se, Mr. Luttik is of the opinion that no additional assessment factor is necessary if the uncertainty in read-across is not larger than the uncertainty from experimental data.

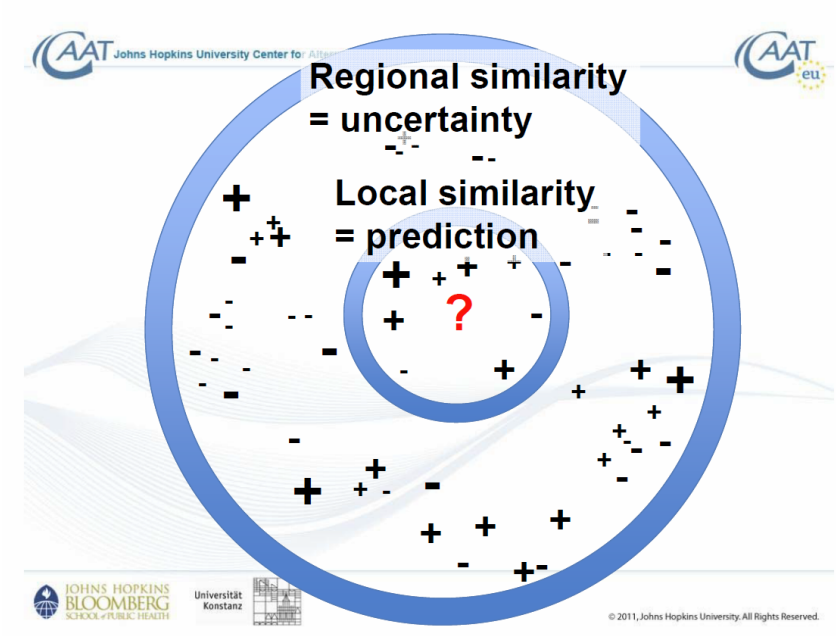
Also under this topic Mardas Daneshian presented an update on the activities of the Center for Alternatives to Animal Testing (CAAT) on read-across. He focussed on recent developments to use the available REACH data for read-across, e.g. identification of sensitising substances and new approaches on data mining using similarity maps based on Tanimoto distance. This similarity maps are one good visualisation tool to define similar compounds (Figure 33).

Figure 33: Visualization of chemical similarity derived from Tanimoto distance (slide 19 from M. Daneshian, CAAT).



In the presentation a discrimination between uncertainty and prediction was made. Local similarity was proposed to predict the desired endpoint and regional similarity to assess the uncertainty of the prediction (Figure 34).

Figure 34: Use of regional and local similarity for the discrimination of uncertainty and prediction (slide 30 from M. Daneshian, CAAT).



Future outcome is an automated tool for read-across based on REACH information and that is in line with the ECHA RAAF for Human Health.

4.2 Knowledge café and round table discussion

The use of alternative methods to assess the ecotoxicological and/or toxicological risks of chemicals is one option for data assessment in different regulatory contexts (e.g. REACH Annex XI). Comparative methods such as read-across aim to avoid animal testing and to reduce costs by an optimal consideration of existing knowledge. The read-across approach is based on the hypothesis that similar chemical structures with similar physico-chemical properties show similar biological behaviour. Thereby the toxicity of an untested substance (target substance) is extrapolated from the relevant information of "similar" substance(s) (source compound(s)). The endpoint can be either estimated by an analogue approach (one-to-one) or a category approach (many-to-one).

In the knowledge café, we aimed to evaluate three important aspects for read-across, namely the evaluation and definition of similarity (topic 1), documentation and assessment of uncertainty in read-across approaches (topic 2), as well as use and availability of tools and databases for read-across (topic 3).

The participants were asked to discuss these topics in the context:

- Can we develop guidance on a workflow for read-across (chemical-to-chemical extrapolation) for ecotoxicity? E.g. for chronic fish toxicity?
- How to establish a read-across hypothesis in general?
- Are there differences in guidance and workflow for analogue and category read-across approaches?

4.2.1 Topic 1- Similarity: a key requirement of read-across

The following sub-points were provided to the participants illustrate potential discussion points.

Subpoints for discussion:

- How to establish a read-across hypothesis?
- Which criteria can be used to define similar compounds? Which data are relevant, e.g. per endpoint?
    - Chemical similarity (structure and physico-chemical parameters)
    - Fate and distribution in the environment
    - Mode of action
- Plausibility of read-across hypotheses and results. Is it possible to define criteria, which help to judge transparently on the plausibility of the read-across approach? Several aspects might be discussed:
    - Within a read-across approach, a trend or worst-case scenarios can be used. Is guidance possible to objectify this decision? Which criteria have to be applied?
    - Are the selected data both on chemical similarity, as well as for read-across relevant with regard to the assessed endpoint?
    - Chemical similarity – it might well be that for some endpoints a high chemical similarity, e.g. compounds which do share all functional groups, is not mandatory, whereas other properties such as fate and distribution and mode-of-action are more relevant. Is guidance possible, how to illustrate this finding transparently in a read-across approach?
- Which data are key for environmental endpoints, which are key for human health endpoints?

Figure 35: Flipcharts for topic 1 “similarity: a key requirement of read-across” as developed by group 1 and group 2 within the workshop. (source: Fraunhofer ITEM)

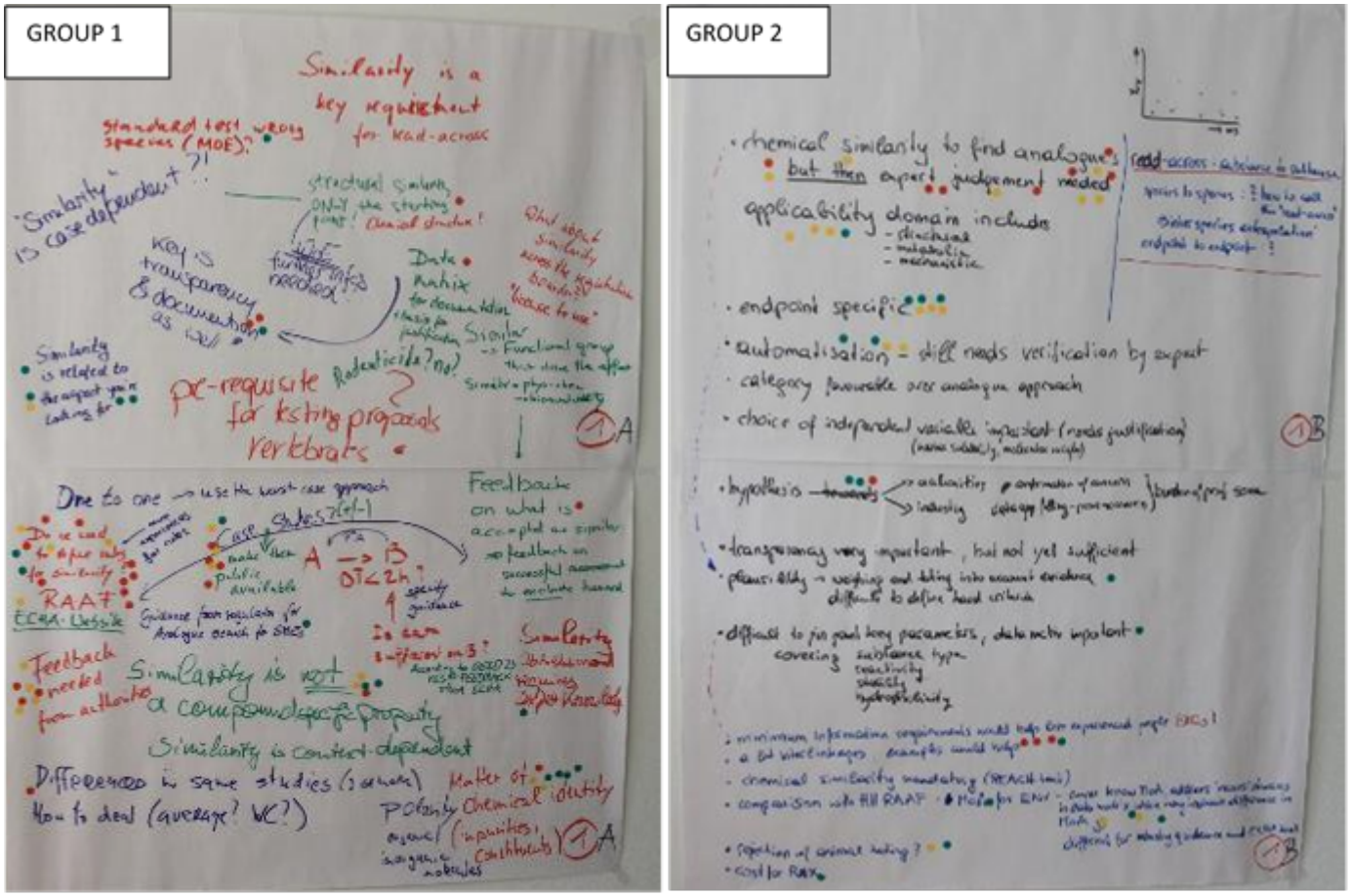


Table 8: Outcome of the discussion on topic 1 “similarity: a key requirement of read-across”. All aspects from Figure 35 are listed. A “content ID” groups related aspects, whereas the “group ID” indicates the group raising the aspect. The column “links to aspect” comprises clarifications or linking arguments. “P” indicates the sum of points allocated by the participants to prioritize the workshop outcome.

Group ID	Content ID	Aspect	P#	Links to Aspect	P#
2	0	Definition of read-across: substance to substance		Differentiate from: Species to species is defined as interspecies extrapolation/endpoint to endpoint is defined (no suggestion)	
1	1	Similarity is related to the aspect you are looking for			
2		Similarity is endpoint specific	12		
1		Similarity is case dependent	7		
1		Similarity is content dependent			
1		Similarity is not a compound specific property			
1		Similarity is a matter of chemical identity (impurities and constituents), other aspects might include polarity, organic, inorganic molecules	15		
1		Similarity assessment requires expert knowledge	1		
1	2	Structural similarity – only the chemical structure		WoE (weight of evidence) further infos needed	
2		Chemical similarity is mandatory (REACH text)			
2		Chemical similarity- plausibility is important- weighting and taking into account different types of evidence, difficult to define hard criteria	1		
2		Chemical similarity to find analogues as starting point ….		…but then expert judgement needed	43
				Applicability domain - similarity includes evaluation of structural/metabolic and mechanistic properties	10
				Automatisation is possible - still needs verification by an expert	8
				Choice of independent variable important (water solubility , MW) - needs verification by expert	
1		Similar functional groups that drives effect and similar phys. chemical properties			

1	3	Key is transparency and documentation	11		
2		Transparency is very important, but not yet sufficient			
1		Data matrix for documentation is basis for justification	5		
2		Data matrix important, but difficult to pin point key parameters e.g. substance type, reactivity, stability, hydrophobicity	1		
2		Minimum information requirements would help less experienced people e.g. SMEs		A lot of interlinkages and examples could help *(this aspect is repeated under content ID 4)*	16
1	4	Do we need to define rules for similarity? RAAF! On ECHA website	56	More experiences/Examples for rules	
1		Feedback required on what is accepted as similar e.g. feedback on successful assessment to exclude hazard (negative read-across)	5		
1		Feedback from authorities needed	27		
1		Case studies on read-across are needed. Acceptable and not acceptable read-across cases. Wish expressed to make them publically available.	30		
2		A lot of interlinkages and examples could help	16		
2	5	Comparison with human RAAF: MoA (mode of action) for ENV (environment) – cover known MoA, address inconsistencies in data matrix which may indicate differences in MoA.	9	Guidance might differ between ECHA and industry	
1	6	Analog approach- one to one prediction- use the worst case approach			
2		Category approach is favourable over analogue approach			
1	7	Example illustrated A->B; B is used to predict toxicity of A; half live of A is smaller than 2h. Guidance requested to illustrate, whether data from B is sufficient for read-across.		Comment was given by another participant that this problem is already addressed in OECD 23.	
1	8	Differences observed in similar studies. How to deal with results? Average? Worst Case?	5		

1		Similarity of test and their use in read-across standard test but wrong species- here MoE (margin of exposure*) is required	1		
2		Read-across hypothesis - burden of proof is identical but intention differs	12	Authorities use it to confirm hazard Industry uses it to fill data gaps and avoid testing	
2		Read-across leads to rejection of animal testing	4	Cost of read-across are critical compared to testing approaches, e.g. considering uncertainty of read-across acceptance	1
1		Read-across is prerequisite for testing proposal vertebrates			
1		What about similarity across the registration border “license to use?”			

* unclear from flipchart if margin of exposure is meant here; # p = points

Assessment of Topic 1 – Similarity

49 different aspects (individual statements/interlinked workflows/sketches) were mentioned on the flipcharts within the discussion on “similarity”. One group provided a definition of read-across, which is a substance-to-substance prediction. This “reading across” has to be discriminated from species to species (often called interspecies extrapolation) or endpoint to endpoint extrapolation (for which no other term was suggested). In environmental risk assessment the term “read-across” is often also used for the two other types of extrapolation. The workshop, however, focused on substance-to-substance read-across. According to the content of the 49 individual aspects, they can be grouped into eight different categories (Table 7):

ID 1- similarity is case dependent; endpoint-specific; content-dependent etc. (35 points). Participants in both groups mentioned this aspect several times. This is a common statement and reflects the difficulty to develop a standard procedure/workflow for the assessment of read-across predictions.

ID 2- chemical similarity is a starting point but then needs further justification e.g. weight-of-evidence/expert judgement (62 points). Participants in both groups mentioned this aspect six times. In addition, four aspects specify criteria to assess chemical similarity, e.g. an evaluation of the applicability domain including structural/ metabolic and mechanistic properties. Similar functional groups, which drives the investigated type of toxicity and common physico-chemical properties were also suggested. The authors did not specify how the causal relationship between functional groups and the type of toxicity is derived. One criterion could be the consideration of independent variables. Water solubility and molecular weight were given as examples. In addition, under ID 3 also parameters such as substance type, reactivity, stability and hydrophobicity were mentioned. It was stated that automatisation might be possible. This aspect contrasts with the statements that plausibility is important, by weighting and taking into account different types of evidences. Expert judgement was seen as central point to finally conclude on similar compounds. This point also got the highest individual weighting with 43 points. It was therefore considered difficult to define hard criteria for similarity assessment.

The discussion reveals that participants on the one hand have a clear vision how chemical similarity is derived e.g. by taking into account structural (similar functional groups, substance type) and metabolic properties (reactivity, stability) as well as and common physico-chemical properties (hydrophobicity,

MW or water solubility). Probably, it is possible to generate automated workflows for this first prioritization of analogues. On the other hand, they also agree that this "chemical similarity" is only the starting point and similarity has to be further evaluated and weighted by taking into account endpoint specific parameters like "mechanistic properties" and expert knowledge.

ID 3- transparency of documentation (17 points). Participants in both groups mentioned this aspect five times. A transparent approach and documentation was seen as key aspect. A data matrix was mentioned twice as useful tool to fulfill such a requirement, but the choice and documentation of key parameters was considered difficult. The discussion of transparency and documentation also relates back to ID 2, in which the definition of hard criteria to select relevant and accurate parameters were considered necessary, but difficult.

ID 4- feedback, case studies and examples. This aspect summarizes five different suggestions/demands on feedback, guidance and case study examples, which might help the risk assessors to build reliable read-across scenarios (applicant) and also to assess their reliability (authority). Both groups mentioned this aspect and it got the highest scoring of 78 points.

A need for guidance on the assessment of similarity is expressed, which might be e.g. similar to the current human RAAF. It has been stated, that such guidance has to be ideally provided by ECHA. In this line also more feedback in terms of illustrative case study examples is demanded from authorities. Case studies, which demonstrate read-across for non-toxic compounds (negative read-across), accepted and non- accepted read-across approaches are mentioned.

A similar demand for case studies, feedback, guidance and examples e.g. integrated in a decision support system is expressed in ID 4 within the discussion of topic 3 "Use of Tools and Databases for Read-across" and under ID5 under this topic.

ID 5- how to consider MoA (Mode of Action). MoA plays a central role in the human RAAF. For environmental endpoints only few different MoAs are known e.g. narcotic compounds. It was suggested to consider as a first step all data on known MoA per compound. In case that a MoA is not identified, then it was suggested to address potential data inconsistencies to assess and identify differences in MoA. Which data have to be considered to assess "data inconsistencies" has not been pointed out.

ID 6 – analogue versus category approach. Two general comments on analogue approach were provided. A worst-case approach was suggested in case of analogue approach, and secondly, the participants felt that the category approach is favorable over the analogue approach.

ID 7- one example was discussed in which metabolism plays a central role. Substance A is rapidly converted into B (e.g. by hydrolysis). It was controversially discussed whether toxicological data of B (assuming a good to adequate data quality), are suitable to predict the toxicity of A. The comments illustrate that more than one participant considered this example as problematic, but at the same time another participant suggested to follow OECD guidance 23 (Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures). The example on the flipchart and its relevance for read-across is not clear. Probably, we can state, that metabolism increase the complexity of the read-across prediction and there is a need to cover/illustrate this aspect in the guidance document.

ID 8- other. Under this ID, we summarized a couple of different aspects, which consider read-across and typical general problems in environmental risk assessment. Three aspects address interspecies and endpoint extrapolation. Further participants mentioned, that read-across is done with different intentions in industry and authorities. In this context, it was stated that risk assessors from industry use read-across to avoid testing, whereas authorities use read-across to confirm safe use of compounds. It was also discussed that read-across on the one hand avoids animal testing, but on the other hand is a time and therefore cost intensive approach. For some tests/endpoints read-across might therefore not be an alternative, also given by the fact that most of the read-across cases are currently not accepted.

4.2.2 Topic 2- Uncertainty in read-across approaches

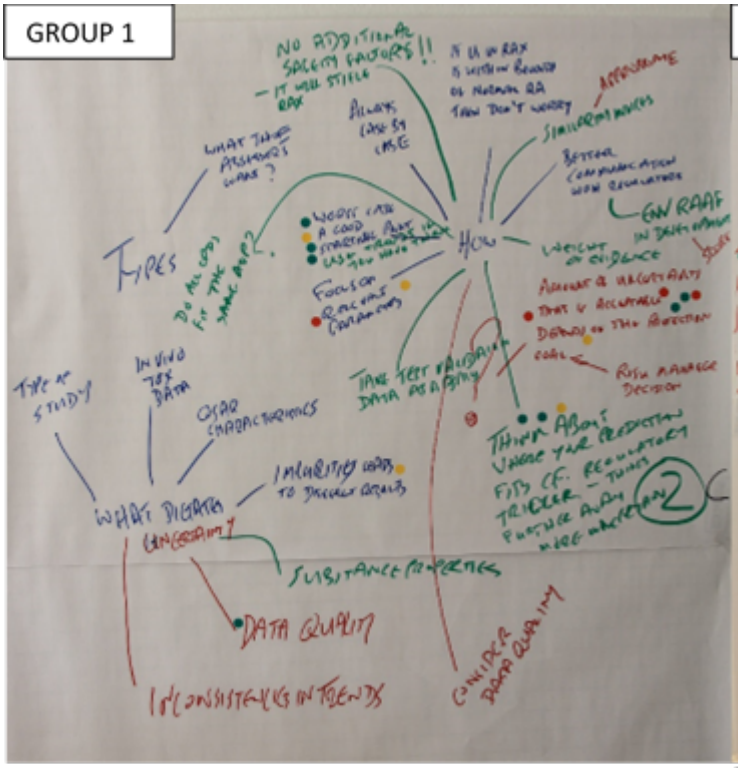
Read-across is hypothesis driven, therefore uncertainty, e.g. of the similarity of source and target compounds, is an important aspect and needs to be considered. In addition, uncertainty is one aspect to conclude on the acceptance of the read-across approach.

Subpoints for discussion:

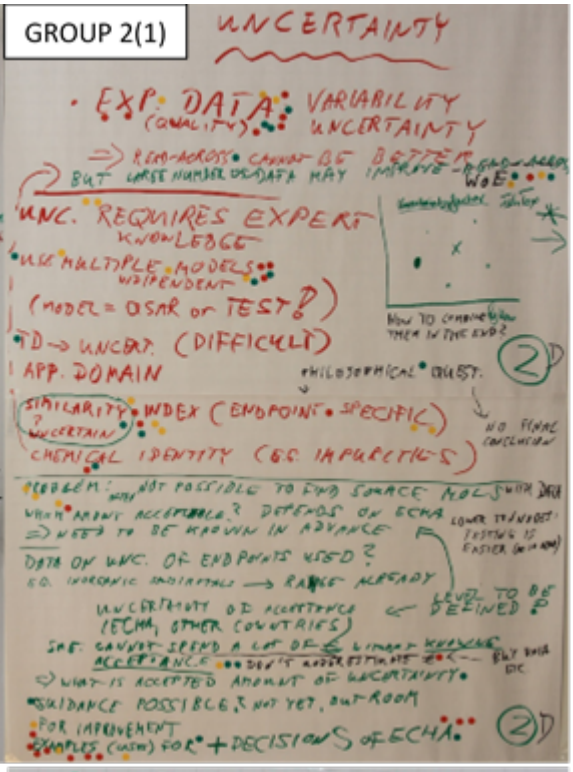
- Which types of uncertainty occur in read-across approaches? Please differentiate from general types of uncertainty in the assessment of ecotoxicological endpoints.
    - Similarity of analogues
    - Data quality of experimental data
    - Data gaps - one to many extrapolation versus many to one extrapolation
    - Relevance of used parameters or not used parameters per endpoint
    - ….
- How can uncertainty be addressed and described, e.g. qualitatively, quantitatively, semi-quantitatively?
- Do we need an additional uncertainty (safety) factor to address uncertainty for read-across e.g. proposed by Blackburn et al. 2014? If so, based on which data/criteria?
- Which amount of uncertainty is acceptable? Is guidance on this aspect possible?

Figure 36: Flipcharts for topic Uncertainty in read-across approaches (source Fraunhofer ITEM)

GROUP 1



GROUP 2(1)



GROUP 2 (2)

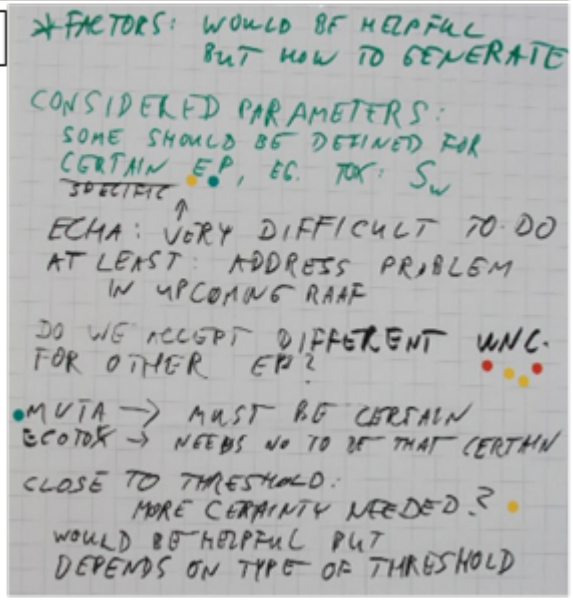


Table 9: Outcome of the discussion on topic 2 “uncertainty” by group 1 and 2. All aspects from Figure 36 are listed. A “content ID” groups related aspects, whereas the “group ID” indicates the group raising the aspect. The column “links to aspect” comprises clarifications or linking arguments. “P” indicates the sum of points allocated by the participants to prioritize the workshop outcome.

Group ID	Content ID	Aspect	P#	Links to Aspect X	P#
1	1	How? Uncertainty should be addressed (considered)?		Always case by case	
1				Worst-case a good starting point/ use TTT if you have them	6
1				Focus on relevant parameter	8
1				Appropriate similarity indices	
1				Weight of evidence	13
1				Take test validation data as a basis	
1				Uncertainty requires expert knowledge	6
1				Use multiple models independent (Model= QSAR or Test)	27
2	2	Experimental data (quality): variability, uncertainty	42	Read-across cannot be better	
2				But large number of data may improve read-across, WoE	
1	3	Uncertainty factor		No additional safety factor – it will stifle read-across	
2				Would be helpful but how to generate	
1	4	What dictates uncertainty?		type of study	
1				in vivo toxicity data	
1				QSAR characteristics	
1				impurity leads to different results	3
1				substance properties	
1				data quality	1
1				inconsistencies in trends	
1				chemical identity	
2				Chemical identity (e.g. impurities)	
2				TD* > uncertain (difficult)	1
2				Applicability domain	
2				Similarity index (endpoint specific): Uncertain	32
2				Philosophical quest. No final conclusion	
2				Considered parameters: should be defined for certain endpoints, e.g. water solubility for (aquatic) toxicity	4
2				According to ECHA very difficult to do, at least address problem in upcoming RAAF	
1	5	What is the accepted amount of uncertainty?	1	Amount of uncertainty that is acceptable depends on the protection goal (risk manager decision)	20

1				If uncertainty in read-across is within bounds of normal RA then don´t worry	
2				Do we accept different uncertainties for other endpoints?	16
2				Mutagenicity -> must be certain	1
2				EcoTox -> needs not to be that certain	
2				Problem: Which amount is acceptable? Depends on ECHA -> need to be known in advance	8
2				Uncertainty of acceptance (ECHA, other countries) -> level to be defined!	
2				Close to threshold: More certainty needed? Would be helpful but depends on type of threshold	3
2	6	Example (cases) for positive decisions of ECHA	61	better communication with regulators (ENV RAAF in development)	
2				Guidance possible? Not yet, room for improvement	1
2	7	other		SME cannot spend a lot of € without knowing acceptance;	
2				Do not underestimate € (buy data etc.)	10
2				For lower tonnages, testing is easier (accor. to ECHA?)	
2				Problem: Often not possible to find source molecules with data	6

P=points, * TD assumed to be test data or toxicity data

Assessment of Topic 2 – Uncertainty

50 different aspects (individual statements/interlinked workflows/sketches) were mentioned on the flipcharts within the discussion on “uncertainty”.

ID 1- How Uncertainty should be addressed (considered)? (total 60 points) the uncertainty assessment is considered to always be a case-by-case evaluation that requires expert knowledge and should be addressed in a weight-of-evidence approach. Worst-case assumption could be used as starting point and focus should be on relevant parameters. Uncertainty information from test validation can be used as a basis and multiple independent models should be used.

ID 2- Experimental data (quality): variability, uncertainty (total 60 points). Under this aspect, it was mentioned that the read-across prediction can, in principle, not be better than the experimental data. However, inclusion of large number of data may help to improve read-across and weight-of-evidence approaches.

ID 3- Uncertainty factor (total 0 points). Although no points were attributed to this ID, it was listed separately as the use of a specific safety factor was controversial discussed with one comment that such a factor would be helpful. However, establishment of such a factor is questionable. In contrast, another comment said that no additional safety factor should be applied as this will stifle the use of read-across.

ID 4- What dictates uncertainty? (total points 41). Under this aspect, several specific points were mentioned. The most import point was that similarity indexes are endpoint specific. For the e.g. physico-chemical parameters that should be considered for assessment of uncertainty of the read-across it

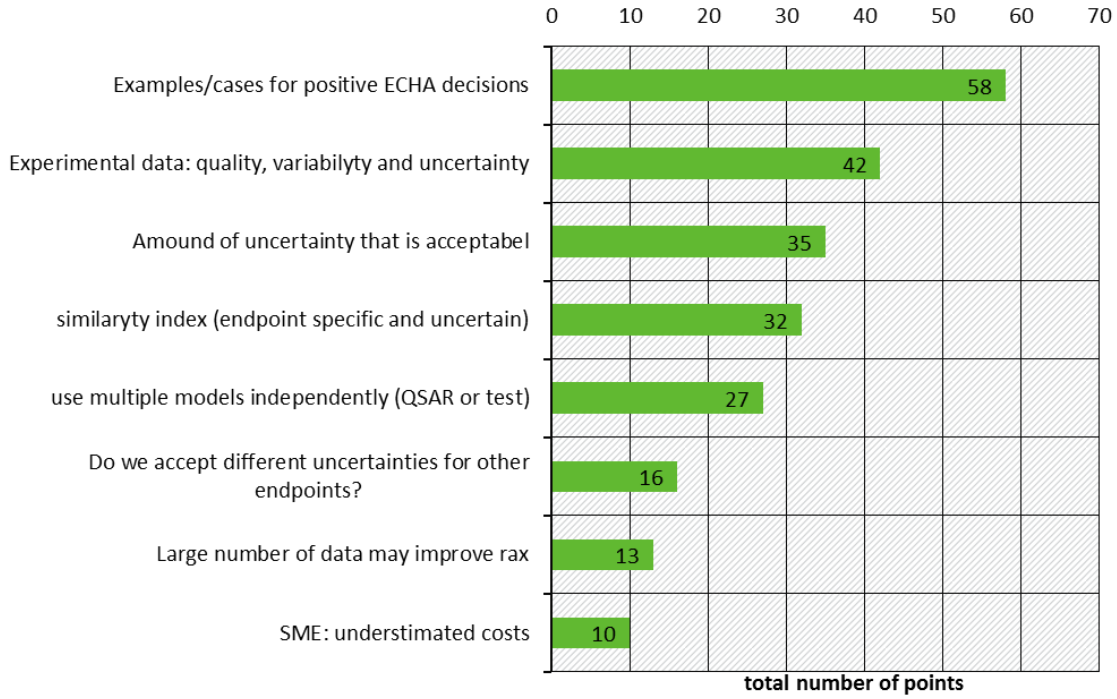
should be defined what range of parameters are acceptable (e.g. water solubility for aquatic toxicity). Also impurities should be taken into account.

ID 5- What is the accepted amount of uncertainty? (total points 41). The discussion focused on the acceptance of read-across approaches by authorities. It was also discussed that different levels of uncertainty should be considered for endpoints like mutagenicity, which has to have a higher certainty compared to other endpoints. Also existing trigger values e.g. for classification or identification as PBT should be taken into account. Values at the border of such trigger values need a higher certainty compared to values farer away from such values. The level of "certainty", which is needed for an acceptable read-across prediction should be known in advance. However, it depends on the protection goal and therefore is a risk manager decision.

ID 6- Example (cases) for positive decisions of ECHA (total points 62). The need for examples (case studies) for positive decisions was given the highest score. It was mentioned, that a better communication with regulators is needed and referred to the coming ENV RAAF. With this document the situation may improve.

ID 7-other (total points 16). Under this ID several aspects that may hinder SME in the use of read-across are listed. At first the uncertainty of acceptance will make it difficult to justify the costs. For lower tonnage band substances, testing may be the easier way to obtain the data. Information generated from adequate testing are accepted to fulfill the data requirement without further discussion e.g. on adequacy as may be expected for data derived from read-across. If you want to use read-across, it is often difficult to find and to obtain the required data.

Figure 37: Suggestions with ratings > 10 points for topic Uncertainty in read-across approaches.



From Figure 37 it is clear that the workshop participants would like to have case studies and examples for positive application of read-across in the registration dossiers, after such approach has been evaluated by ECHA. Such examples would help the future users of read-across approaches to have a better understanding of what kind of approaches are acceptable and in which cases experimental testing with the non-tested target substance should be considered primarily. A second major point is the fact that uncertainty is driven by the experimental data used for read-across. This includes the important

point on the quality of the data, such as the data from guideline studies with detailed description and how variable are such experimental data per se. The value derived from read-across approach cannot have lower uncertainty than the underlying experimental data. For example, data from guideline evaluation (round-robin test) e.g. a zebrafish embryo acute toxicity test, revealed an intra- and inter-laboratory variability of 30% (see Busquet et al. 2014[11]). For long-term endpoints, such as NOEC obtained in the 21-d Daphnia reproduction test, the variability is higher with up to fourfold, however a smaller data set was used here (Cooney 1995[12]).

Having this in mind, this leads to the next important topic: what kind of uncertainty is regarded acceptable and in conclusion, what would be points or values of uncertainty that would lead to a non-acceptance of the read-across approach. This has only been raised and discussed on the pictures. No final conclusion was drawn. So this might be an important point for guidance to set boundaries, within which read-across is regarded as acceptable and what are knock-out criteria for such approaches.

Looking at the results from group 1 (Figure 36) picture 2c, it is obvious that the question "How uncertainty should be addressed" is in the focus of the discussion. Several answers or statements were given. It was stated that if uncertainty in read-across is within the boundaries of uncertainty for risk assessment, then it should be no problem ("then don't worry"). The uncertainty should be assessed in a weight-of-evidence approach and is considered by the participants to be a case-by-case approach. Worst-case estimates can be a good starting point and a focus should be on relevant parameters. Test validity information can be used as a basis. For the assessment of uncertainty, appropriate similarity indices should be used. However, no specific example were given. The level of uncertainty can be difficult depending on the distance to certain regulatory trigger values. If the prediction is far away, more uncertainty can be regarded acceptable. The amount of uncertainty is depending on the protection goal and is a risk manager decision. However, it was also raised that an additional assessment factor for values from read-across approaches may stifle the use of read-across. A better communication with regulators was also mentioned as a need, and it was made reference to the ENV RAAF from ECHA that is currently under development.

In a second block it was discussed, what dictates uncertainty. The points raised here were type of study, impurities that leads to a different result, substance properties, data quality, inconsistencies in trends, in vivo toxicology data, QSAR characteristics. The last two points are difficult to interpret in this context.

On the pictures from group 2 some of the aspects mentioned above were also given, e.g. read-across data cannot be better than experimental data which have variability and uncertainty. However, large number of data may improve read-across. But in some cases, it is not possible to find source compounds with data, or it is difficult to obtain the data, or costs are unclear or too high. Here the question about acceptability was raised on the background of costs. Participants wanted to know in advance what level of uncertainty is acceptable. For the lower tonnage band substances, it can be much easier to perform the required testing then to spend a lot of money to buy access to data and to set up the read-across case. However, guidance is yet missing and again it was made reference to the upcoming ENV RAAF from ECHA.

Also on this picture it was pointed out that assessment of uncertainty requires expert knowledge and especially chemical identity (e.g. impurities) can lead to different results compared to pure substances.

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[11] Busquet F, Strecker R, Rawlings JM et al. (2014) OECD validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing. Regul Toxicol Pharmacol 69: 496–511

[12] Cooney (1995) Effects-Toxicity testing Chapter 2 Freshwater Tests. In: Rand GM, Fundamentals of aquatic toxicology: Effects, environmental fate and risk assessment, CRC Press, 71-102

It can be useful to take into account different (independent) models, here meant to be QSAR or test results. It was also discussed if similarity (or uncertainty) is endpoint specific. However, is was regarded as a philosophical question where no final conclusion can be drawn. For some endpoints, it would be helpful to have information or generate data on certain other endpoints, e.g. for aquatic toxicity endpoints information on water solubility should be available. General guidance is difficult but ECHA will address this point in the upcoming ENV RAAF. Again, it was discussed if different levels of uncertainty can be accepted for different endpoints. It was argued that e.g. a mutagenicity prediction hs to be certain, while less certainty can be accepted for ecotoxicity data. Further, a prediction close to a regulatory threshold has to be more certain compared to predictions clearly above or below such thresholds. However, according to the participants this depends on the type of threshold.

4.2.3 Topic 3- Use of tools and databases for read-across

In preparation of the workshop, experts indicated their awareness and use of databases and tools for read-across by using a questionnaire. Computational models are considered as important tool to establish a read-across case. Further, the analysis of REACH decisions on read-across shows, that chemical similarity needs a transparent and objective reasoning and tools may be helpful here as well.

However, only the OECD Toolbox is known and in use, whereas other tools like Toxmatch5 and AIM6 are usually not used. Only few participants regarded Toxmatch and AIM as useful.

Toxmatch[13] is a flexible and user-friendly open-source software application that encodes several chemical similarity indices to facilitate the grouping of chemicals into categories and read-across. The core functionalities include the ability to compare datasets based on various structural and descriptor-based similarity indices as well as the means to calculate pair wise similarity between compounds or aggregated similarity of a compound to a set. AIM, the Analog Identification Methodology, is a downloadable software program that facilitates analog analysis and data identification in support of chemical assessment or read-across approaches to help scientists and chemical managers predict potential hazards of untested chemicals[14]. ToxRead and Ambit are not considered in the questionnaire, as they have been launched recently. Therefore, a high publicity and penetration within the community is not expected.

Subpoints for discussion in knowledge cafe:

- Confidence and acceptance of read-across versus costs and needs for justification.
- How can we strengthen the use of tools to document in a more objective and transparent way the definition of compounds for read-across? The analysis of trends etc.?
- How can we use existing knowledge better, such as databases?
- Can we increase the acceptance of read-across approaches by using specific tools?
- Do we need guidance on available tools and/or databases?

[13] https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/

[14] https://www.epa.gov/tsca-screening-tools/analog-identification-methodology-aim-tool

Figure 38: Flipcharts for topic 3- Use of tools and databases for read-across

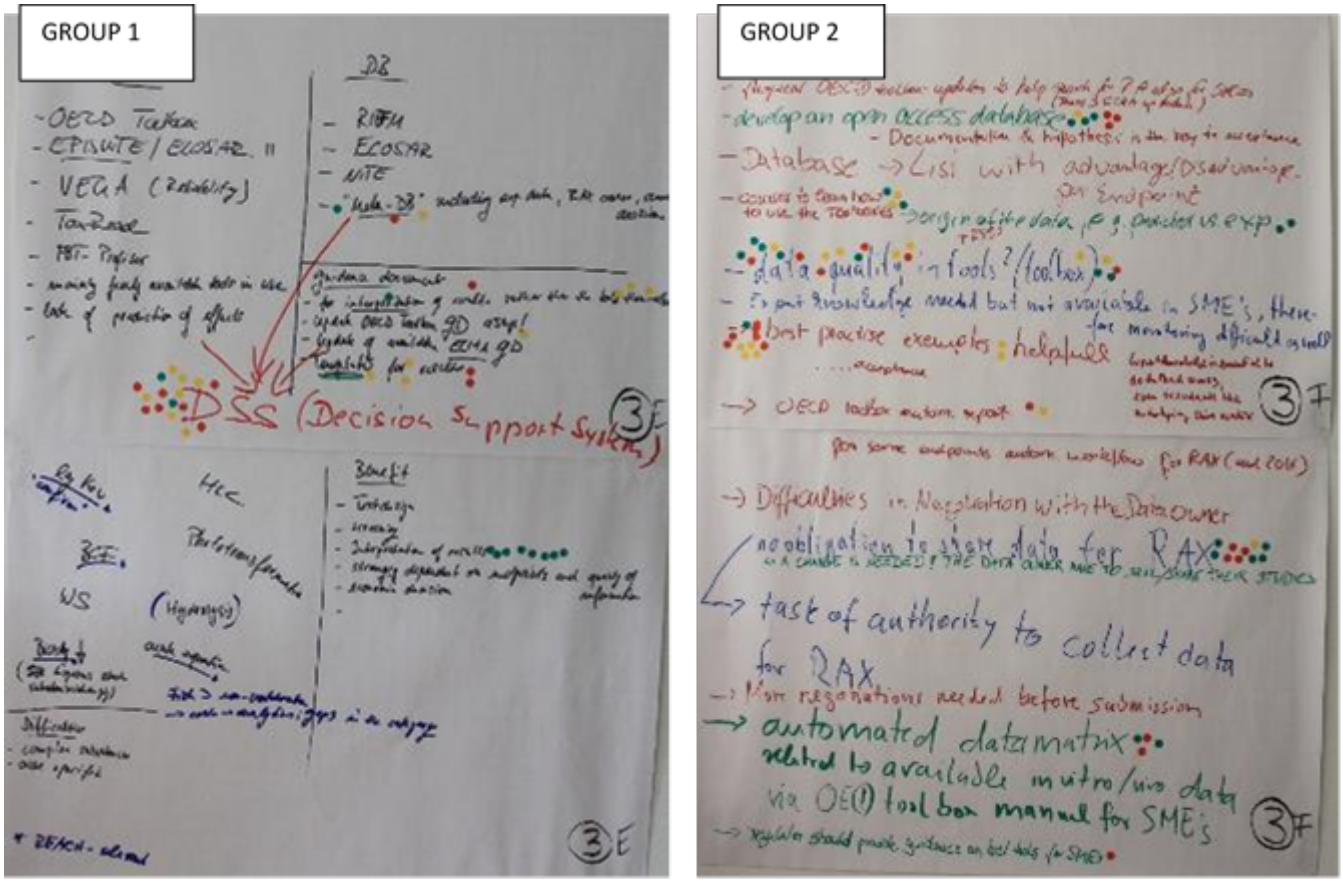


Table 10: Outcome of the discussion on topic 3 " Use of tools and databases for read-across" by group 1 and 2. All aspects from Figure 38 are listed. A "content ID" groups related aspects, whereas the "group ID" indicates the group raising the aspect. The column "links to aspect" comprises clarifications or linking arguments. "P" indicates the sum of points allocated by the participants to prioritize the workshop outcome.

Group ID	Content ID	Aspect	P#	Links to Aspect	P#
1	1	List of tools provided: OECD toolbox/EPISUITE/ECOSAR II/VEGA (reliability)/Tox-Read/PBT-Profiler		Mainly freely available tools are in use	
				Most tools can't predict values/effects by read-across (original term: lack of prediction of effects)	
2				Frequent OECD Toolbox updates needed to help search for read-across, also for SMEs (phase 3 ECHA update)	
2				Quality of data is often not specified e.g. in OECD Toolbox	58
2				Origin of the data is often not indicated e.g. predicted values versus experimental values	2
2				OECD Toolbox shall provide automated report, for some endpoints such automated workflows will be integrated in next OECD Toolbox version by end of 2016.	8

2				Automated data matrix related to available in vitro and in vivo data via OECD Toolbox needed	12
2				Regulator should provide guidance on best tools for SMEs	5
1	2	List of databases provided: RIFM / ECOSAR/NITE (Hess DB)		Meta database needed, including experimental data, read-across case studies and read-across decisions	8
2				Develop open access database	21
2				Develop database that includes list of advantages/disadvantages per endpoint	
1	3	Guidance documents (GD) for interpretation of results rather than tools themselves	14	Update of available ECHA guidance	
1/2				Update OECD Toolbox guidance documents (GD)	3
1				Templates for Read-across	19
2				Documentation & hypothesis is the key for acceptance	
2				Best practise examples helpful also indicating acceptance	43
1	4	Decision support system (need identified as result of ID 1, 2 and 3, group 1))	58	Beneficial for test design	
1				Beneficial for compound screening	
1				Beneficial for interpretation of results	8
				Beneficial for economic decisions	
1				Strongly dependent on endpoints and quality of information	
2	5	Data sharing: Difficulties in negotiation with data owner		No obligation to share data for read-across	28
2				A change is needed! Data owner have to share/sell their studies	
2				Task for authorities to collect data for read-across	
2				More negotiation needed before submission	
2	6	Training: courses required to learn how to use read-across tools e.g. OECD Toolbox	8	Expert knowledge needed but not available in SMEs, therefore monitoring difficult.	
2				Expert knowledge regarded to be essential, also to evaluate the underlying data matrix	
1	7	Sketch on the use of different types of parameters for read-across to analyse data gaps in the category: HLC, phototransformation, (hydrolysis), water solubility (WS), log K_{ow}* BCF (bio concentration factor)*		Difficulties mentioned are: complex substance and case specificity. Fish > non vertebrate (“>” probably meaning “is of higher relevance”)	

		biodegradation (strongly compound dependent)* acute aquatic toxicity*		

P=points

34 different aspects (individual statements/interlinked workflows/sketches) were mentioned on the two flipcharts within the discussion on “tools and databases”. According to the content of the 34 individual aspects, they can be grouped into 7 different categories

ID 1- read-across tools (85 points): Group 1 provided a list of tools, which are in use for read-across. The OECD Toolbox and ToxRead are tools which allow to build a read-across workflow. VEGA, PBT-Profiler, EPISUITE and ECOSAR are, however, tools/models which predict values for certain endpoints. VEGA also provides the experimental data on closest neighbors in the trainings set of the models, which might be a good starting point for read-across. This inventory indicates that there is a need for training on tools and their appropriate use for read-across. A list of specifications is provided which expresses e.g. current advantages and disadvantages of these tools. General remarks include that mainly freely available tools are in use; most tools cannot predict values/effects by read-across. The origin of the data is often not indicated, even predicted values are not differentiated from experimental values. Similar to this comment, the quality of data is often not specified (e.g. in the OECD Toolbox). The specification of data quality is of particular importance, as this individual aspect got 58 points. Both data type and data quality contribute to the assessment of accuracy and uncertainty. A couple of specifications address the OECD Toolbox: Participants felt that the OECD Toolbox needs more frequent updates and shall provide automated reports/workflows on read-across. It has been stated, that for some endpoints such automated workflows will be integrated in the next OECD Toolbox version (announced for end of 2016). A further need for automated data matrix was expressed related to available in vitro and in vivo data. Finally, a wish for guidance by regulators on best tools for SMEs was expressed (this aspect is also related to ID 3).

ID 2- databases (29 points): Group 1 provided a list of databases which are in use for read-across like RIFM[15] (database on fragrances, not open source), ECOSAR (QSARs models, which include acute and chronic toxicity endpoints for fish, aquatic invertebrates (Daphnia), and aquatic plants (green algae) and NITE (link, Hess DB, in vivo and mechanistic data, accessible via OECD toolbox). The two mentioned databases mainly contain toxicological data and their use for ecotoxicological risk assessment is therefore not evident. ECOSAR is to our knowledge a QSAR tool, and does not provide access to the underlying compound-specific data. Its use for read-across is therefore not evident as well. Three further specifications indicate a need for an open source “meta” database, which also includes best-practice examples.

ID 3- guidance documents (79 points): In this workshop several statements express the need for more guidance documents; these guidance documents (GD) shall be more focused on the interpretation of results rather than the use of tools themselves. In this context the point “best-practice examples helpful, also indicating acceptance” got the highest individual scoring of 43 points. This need has also already been identified under topic 1 “similarity a key requirement of read-across” in ID 4- feedback, case studies and examples. A need for better templates on read-across was expressed by also mentioning that documentation is key for acceptance. Further an update of the guidance how to use the OECD Toolbox was mentioned.

[15] http://www.rifm.org/rifm-science-database.php

ID 4- decision support system (66 points): As indicated graphically by group 1, all points mentioned under ID 1, 2 and 3 lead directly to the development of a decision support system. A number of benefits were indicated, e.g. test design, compound screening, interpretation of results, and economic decisions. It was also indicated that the applicability of this tool is strongly dependent on endpoints and quality of information. The "decision support system" is the only content within the workshop that is mentioned by only one group. It is likely that the second group did not come up with this solution/terminology because such a tool is not yet existing. However, group 2 mentioned a lot of aspects under ID1 to 3 similar to group 1, indicating that they were thinking along the same lines.

ID 5- data sharing (28 points): Difficulties in negotiation with data owners were mentioned. Participants felt that a paradigm change is needed. According to the workshop participants, data owners shall be forced to share or sell their data also for read-across. Participants further expressed that ECHA is in charge to collect data for read-across. A need for more negotiation before submission of the read-across dossier was mentioned.

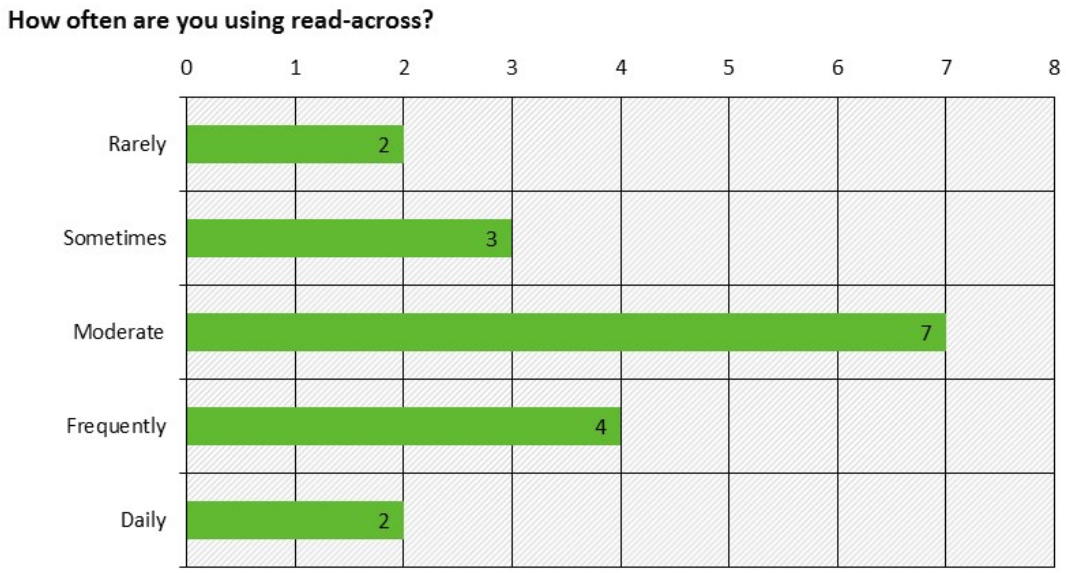
ID 6- training (8 points): Participants expressed their need for training on tools and read-across approaches, since in many SMEs this expertise is missing. Expert knowledge was regarded to be essential, e.g. to evaluate the underlying data matrix. Expert knowledge is also one key aspect under topic 1 (ID 2).

ID 7- sketch on read-across example (0 points): different parameters were mentioned in a sketch to illustrate their use in a read-across approach. Difficulties mentioned included complex substances and case specificity. This sketch probably served to illustrate aspects mentioned on the flipchart by looking on a concrete example. It is not possible to derive key aspects from this discussion as they were not mentioned more explicitly.

4.3 Workshop questionnaire

After the workshop an anonymous online questionnaire was mailed to participants to get feedback regarding their workshop evaluation. A total of 31 participants were invited by sending the corresponding link and password of the questionnaire. Participants belonging to UBA, Fraunhofer ITEM, and EurA (in total 6 participants) were not invited to the survey to avoid conflicts of interest. We got the feedback from 18 participants (58%) indicating that this survey is very representative. Their read-across expertise ranged from "novice" (28%) over "experienced user" (55%) to "professional" (17%). Among these, participants use read-across rarely (11%), sometimes (17%), moderately (39%), frequently (22%) or even on a daily basis (11%) (Figure 39). This rough classification indicates that one third of the participants providing feedback can be considered as less experienced, two third as experienced in read-across approaches.

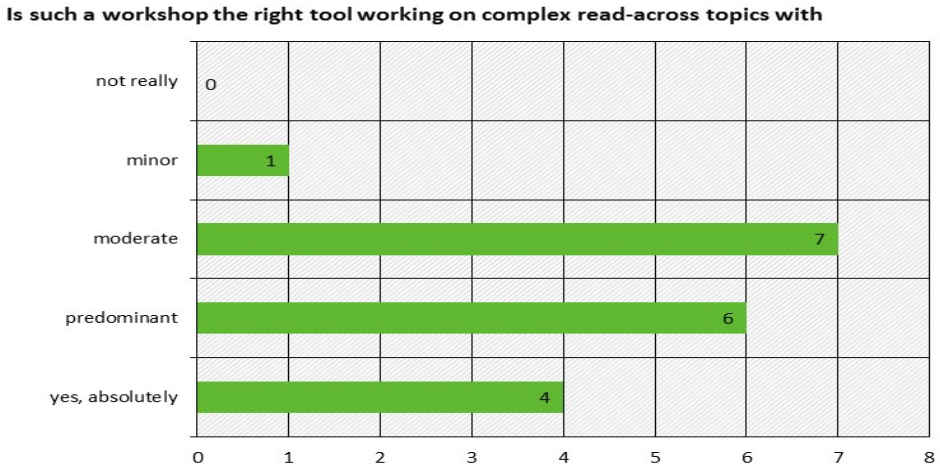
Figure 39: Results of the questionnaire regarding the frequency of using read-across. Bars indicate the number of mentions.



Overall, the feedback was positive and many participants appreciated the read-across workshop. 83% (15 out of 18) of the questionnaire participants would participant again in such a workshop (Figure 40) and almost all participants (17 out of 18) consider a workshop as an appropriate tool to work on complex read-across topics with stakeholders (**Fehler! Verweisquelle konnte nicht gefunden werden.**). One participant stated that "it was good to bring different parties together generating a common understanding of the current "read-across situation" across academia, authorities and industries and to learn from each other". "The workshop was important and helpful for the registration of chemical" was the feedback of another participant. Some comments for improvement were provided. Two participants suggested to provide more examples and case studies from industrial side and authority side with less academic and consultant involvement. One participant suggested more applied discussions/presentations and another one that it would be helpful hearing all presentations upfront the Knowledge Cafe, although difficult to organize. One organizational comment mentioned that there should be a social event at first day of the workshop with a dinner in a restaurant.

Figure 40. Results of the questionnaire regarding potential read-across workshop participation. Bars indicate the number of mentions.

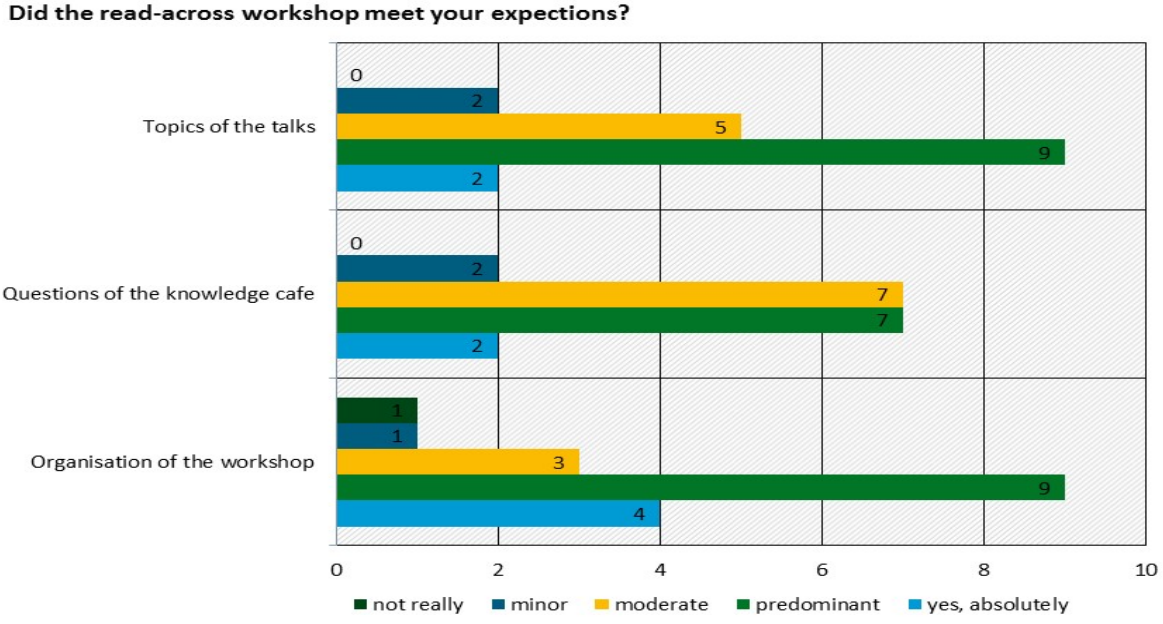
Figure 41: Results of the questionnaire regarding the suitable of such a workshop as a right tool. Bars indicate the number of mentions.



In general, the workshop "predominately to absolutely" met the expectations of 61% participants regarding topics, knowledge café, and organisation of the workshop (Figure 42, Figure 41). Only in 11% of the cases, expectations had only minor proven satisfactory (Figure 42). In more detail, the topics of the talks and the organisation of the workshop was exceptionally good to excellent. The survey results of the knowledge café questions showed a similar trend: 11% (2 out of 15) were not satisfied indicating a "minor" coverage, 39% indicated a moderate coverage, and for about 50% the addressed questions fully covered the expectations of the workshop participants. This is a critical point which needs more attention in follow-up projects and workshops.

Several factors might contribute to this discrepancy in expectation, e.g. difference in i) read-across experience or ii) in understanding of priority and relevance of the questions. Further, the used management tool "knowledge café" might have raised different expectations. A knowledge café event is a typical type of organisational workshop which aims to facilitate an open and creative conversation on a topic of mutual interest to surface their collective knowledge, share ideas and insights, and gain a deeper understanding of the subject and the issues involved.

Figure 42. Results of the questionnaire regarding the expectation of read-across workshop. Bars indicate the number of mentions.



5 Summary and Outlook

In this section we summarize the main outcomes of the workshop lectures and knowledge café. First, an overall summary on the key aspects on read-across is provided and discussed followed by an illustration of a read-across workflow for one ecotoxicological endpoint; which is “acute fish toxicity”. Afterwards, the results from the feedback of the participants is summarized.

Main outcomes of the workshop

Read-across is used for the prediction of a specific ecotoxicological endpoint. In this context a toxicological endpoint is predicted for a non-tested and therefore “unknown” target compound (TC) based on the toxicological data of “similar” source compounds (SC). As the predicted endpoint values will be used in risk assessment for the protection of human health and the environment, the read-across approach has to provide information similar to the omitted toxicological in vivo test. This means that the read-across outcome have to have an adequate coverage of key parameters. An inherent property of a prediction is uncertainty, which has to be outlined and compared to the uncertainty of the omitted toxicological in vivo tests.

Several key aspect can be extracted from the previous result section, which are listed as key words in the following:

1. Read-across is case and endpoint specific.
2. Chemical similarity is a good start point for similarity assessment and might even be supported by automatic workflows e.g. including visualization tools.
3. Chemical similarity alone is not “good enough”. Several other aspects need to be considered such as mechanistic properties, stability, fate etc. (see detailed discussion under topic 1: Content ID 2). The definition of hard criteria is difficult as these are endpoint specific and probably also compound specific. A well defined minimal set of criteria per would be helpful to build reliable read-across scenarios. These minimal data requirements shall ideally be provided by authorities.
4. Expert judgement is seen as key input in the evaluation of the different types of evidences to conclude on a read-across case study. This step can not be automated.
5. Tools and illustrative case study example are missing. A need for case study examples illustrating acceptable and non-acceptable read-across approaches was expressed in the discussion of all topics and groups in the workshop. These case studies will ideally be provided by authorities.
6. Read-across assessment needs to be transparent – e.g. always following the same evaluation criteria per endpoint (see key word 1)
7. Read-across needs to be supported by adequate and reliable documentation.
8. Uncertainty has to be addressed, which may arise from several steps of the read-across procedure e.g.:
    a) data gaps in the data matrix
    b) assessment of data quality (general risk assessment procedure, not read –across specific)
    c) assessment of relevance of the used ecotoxicological data for the predicted endpoint
    d) The applicability domain of the read-across prediction needs to be clarified with regard to the structural and ecotoxicological properties of source and target compounds.
9. An acceptable read-across has to be adequate for classification and labelling and or risk assessment” (citation Bram Versonnen slide 7).

A first general read-across workflow summarizing these steps is provided in Figure 43.

The read-across workflow generally starts with the definition of endpoint that needs to be predicted - the prediction goal. A central statement of the workshop is that the read-across assessment strategy is endpoint specific. Therefore, ideally endpoint specific workflows will have to be generated and can

then be selected for the development of the rea across approach and later on, also for the assessment of the submitted dossier by authorities.

Within the workshop the endpoint, it turned out that all participants believe that it will be very difficult to definition hard criteria, which need to be evaluated per endpoint. A guidance that is provided by authorities and illustrates minimal data requirements per endpoint would be beneficial. Some ideas on minimal data requirements were discussed (see topic 1) but a comprehensive evaluation per endpoint was not in the scope of this workshop. Minimal data requirements per endpoint may therefore be a subject for further stakeholder involvement.

As first step for all endpoints, chemical similarity was seen as good starting point. Chemical similarity includes structural properties, e.g. similar functional groups, reactivity of functional groups, stability of the compounds as well as similar physico-chemical properties such as water solubility, molecular weight and hydrophobicity).

Therefore, the read-across workflow continues with data gathering of the target compounds. Beside data on structural and physico-chemical properties, all available data on ecotoxicological endpoints (bioaccumulation + fate (experimental or predicted values)); longer or shorter term in vivo studies (other species)) will be gathered. As transparent data documentation is seen as key requirement, a standardized data matrix/template might be helpful to do this properly.

In the next step "similar" source compounds need to be identified. This has to be based on the characteristics and available data of the target compound (as gathered under step 2, Figure 39). In this context the following aspects need to be considered:

- Are groups of compounds with similar chemical properties known? For this step, it was mentioned several times that visualization tools or even automated workflows could be of help. Predefined example case studies are considered to be useful.
- Do any of the experimental or estimated data of the TC indicate an area of concern e.g. high bioaccumulation or persistence? If so, include these data into the assessment of similarity/ modify if needed the endpoint specific "minimal data requirements".
- Is the TC subject to degradation? Instable in the environment? Is metabolism an issue? If so, include parent and metabolites into similarity assessment.
- Do available toxicological data from related endpoints support the hypothesis on a certain mode of action?

Starting with chemical similarity, a preliminary inventory of source compounds is identified (Step 3, Figure 43). For these compounds, data as defined from the minimal data requirements and modification from step 2 will be gathered. The evaluation of the gathered with regard to relevance and accuracy will be done by using expert judgement.

The following aspects might help to structure the workflow:

- Assess data quality, accuracy and uncertainty of the source and target compounds.
- Address data gaps and their impact on uncertainty
- Is there any evidence form the gathered data that support a shared mode of action (MoA)? Are there any data, which conflicts with a certain mode of action? This assessment could be facilitated by illustrative examples or a list of relevant MoA per endpoint.
- Evidence that support a trend or group approach? Or is a worst case prediction adequate?

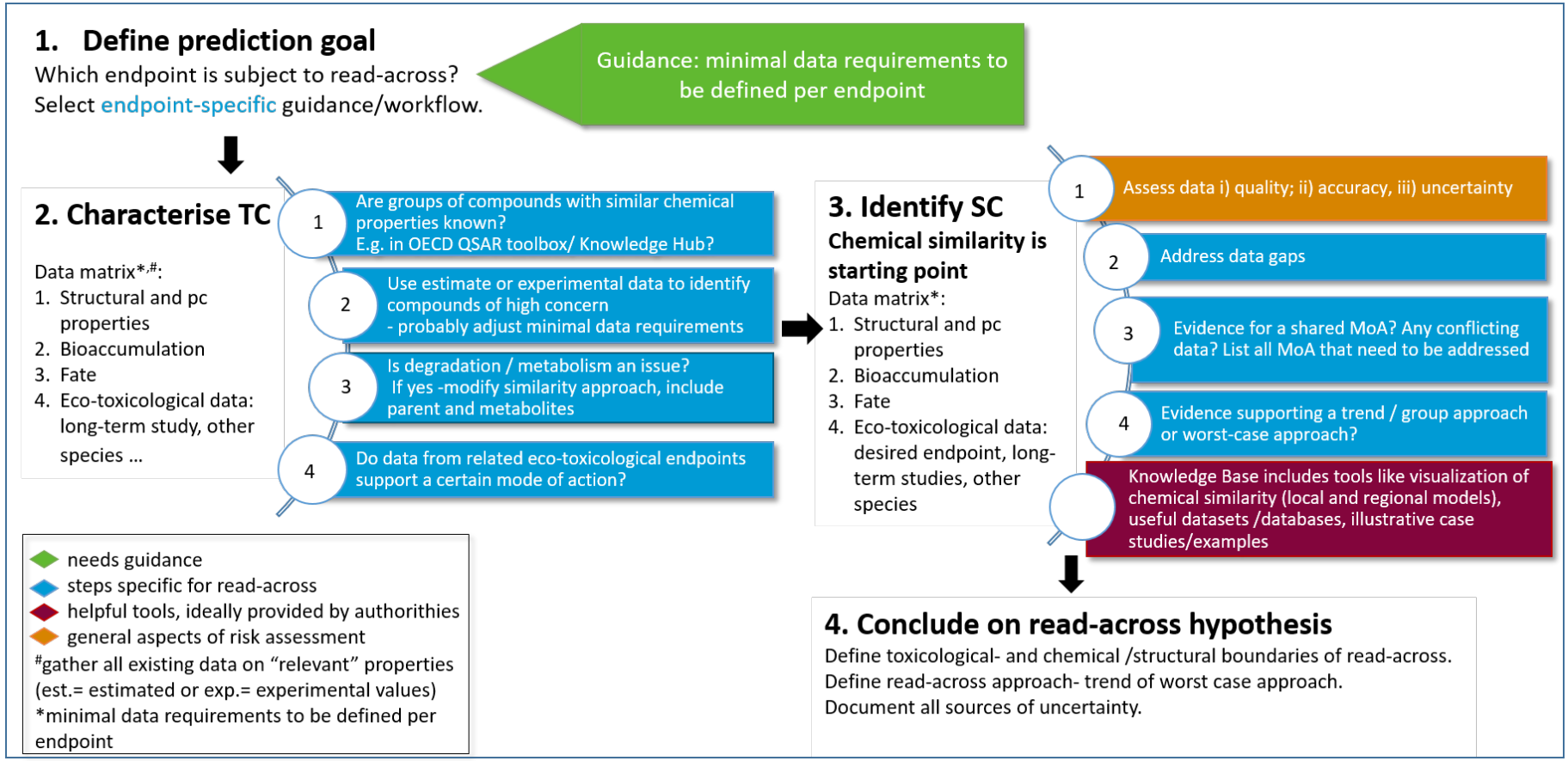
The evaluation under step 2 will help to define better the toxicological and chemical/structural boundaries of the read-across case. The inclusion and exclusion of source chemicals needs to be documented (Step 3, Figure 43) and a read-across hypothesis will be outlined e.g. based on common mode of action

which is this or that or based on one similar break down product etc.. The best fitting prediction approach has to be described (worst case versus trend) and sources of uncertainty to be outlined. As expert judgement is central part of the conclusion, is might be difficult to structure the workflow of step 3 further. This aspect was not in full detail discussed in this workshop, and may be another subject for further stakeholder involvement.

The workshop participants expressed several times the need of guidance and illustrative examples, which help to better understand the read-across workflow, including reasons for acceptance or rejection of the read-across. These examples, as well as links to suitable databases and tools could be integrated into a decision support system (topic 3, ID 4) or, as we called it in Figure 43, a “Knowledge Base”.

Further, better databases are needed, which e.g. indicate the quality of the gathered data, differentiate estimated from experimental parameters and offer tools to automatically extract selected data into the required data matrices

Figure 43: General read-across workflow; TC = target compound; SC = source compound (source: Fraunhofer ITEM)



6 Exemplary workflows

6.1 Exemplary workflow: acute fish toxicity

The next step in this project is the development of a guidance/workflow, which shall help applicants and authorities to assess read-across cases in a transparent and systematic way. Read-across is endpoint-specific. Therefore, an initial workflow is depicted exemplarily for the endpoint acute fish toxicity. This workflow is intended as starting point for discussion on the set-up of the different guidance documents. It has to be noted, that for REACH with its tonnage dependent data requirements, not all information listed in the workflow may be available. However, model prediction may be used instead and/or this has to be addressed in the discussion about uncertainty of the outcome of the read-across approach.

Workflow for acute fish toxicity (draft for discussion)

Characterization of target compound(s) (TCs)

1. identity of TCs: molecular structure, impurities
2. determine physico-chemical data, like water solubility, log Kow, vapour pressure
3. gather data on fate, persistence and bioaccumulation or use estimates (e.g. based on log Kow)
4. gather data on other ecotoxicological endpoints, e.g. data on algae and invertebrates
5. Does metabolism/degradation play a role? Is the parent compound stable in water? Is there evidence for metabolites and what is the kinetic? Differentiate between read-across for parent compound or data on degradation products.

Define source compounds (use a data matrix to transparently document the outcome of the following data evaluation):

1. Use structural similarity (threshold to discriminate between similar and non-similar is case dependent) as starting point
2. Gather physico-chemical data
3. Gather data on fate, persistence and bioaccumulation or use estimates (e.g. based on log Kow)
4. Gather available toxicity data on acute fish: evaluate reliability of toxicity data e.g. data quality, accuracy (e.g. interspecies differences), comparability between SCs (e.g. data gaps)
5. Identify a specific MoA based on experimental data or, for example, by using an ECOSAR prediction (or other suitable tools) – in case of a prediction, analyse the applicability domain
6. For a better understanding of the mode of action gather data on “other“ relevant ecotoxicological endpoints, in this case data on algae and invertebrates
7. Include expert knowledge - e.g. provide an opportunity to applicants to submit other supporting data (check document data reliability, relevance and accuracy).
8. Generate a data matrix incl. reliability
9. Analyse similarity/ trends for SCs and TCs using the data from (1) to (6); ask read-across specific questions
    a) Is there supporting evidence for common fate and persistence in the environment? Or do the SCs follow a consistent trend? Based on the evaluated properties: where is the TCs located in the trend compared to the SCs? Is worst-case or regression analysis an appropriate approach to predict TC’s properties? E.g. as a first step selection of data based on a worst-case estimation. If possible refinement should be considered where experimental data e.g. show a clear trend and target compound is clearly located at the lower boundary of results based on the comparison of physico-chemical data.

b) Is there supporting evidence for a common specific or unspecific MoA within the SCs? Define this MoA. Is there any conflicting evidence for SCs or TCs with regard to this MoA?
c) Conclude on most similar SCs – describe their toxicological and structural properties. Document also those compounds, which were not included into the set of SCs.

10. Formulate a read-across hypothesis based on above evaluation
    a) Address data gaps in the data matrix and their impact on the overall prediction. Is uncertainty of the prediction increased?
11. Generate a report including all information from above (e.g. use the ECHA template)

Besides this general read-across work-flow, a by legal definition animal-free test system has been developed for this standard data requirement on fish acute toxicity, i.e. the fish embryo toxicity test (OECD 236). Fish embryos in the stages tested are not considered as protected animal according to the current European legislation (Directive 2010/63/EC). Therefore, with this test system the test of vertebrates is for ecotoxicity endpoints up to a tonnage band of 100 t/a is not required. If the read-across workflow is not feasible (e.g. due to lack of data for SCs, or because of a TC of undefined composition – UVCB), this fish embryo test will be an alternative to cover the endpoint without animal testing. However, ECHA[16] recently concluded that according to their opinion, the results of the fish embryo toxicity test are usually not sufficient alone data requirement on fish acute toxicity.

A workflow for chronic fish toxicity will be set up accordingly in the synthesis paper. In this context, another aspect is of interest. The Technical Guidance Document on Risk Assessment (TGD) for risk assessment of new and existing chemicals and biocidal products (precursor of REACH and BPR) provides an option to omit the third aquatic long-term toxicity study (given as “Note d” in Table 16; see box below). In this note, it is stated that for non-bioaccumulative substances, the third chronic NOEC is not needed to reduce the assessment factor to 10 for PNEC derivation, if it is possible to determine with high probability, that the most sensitive species has been examined. No concrete guidance is given when "high probability" is reached and it should be noted that due to the new legislation, this guidance document is no longer applicable. However, the general concept of focusing on the most sensitive species is used also in recent discussions and publications (e.g. presentation of Luttik in this workshop and May et al. 2016). So, these might also be points (most sensitive species and bioaccumulation potential) to be considered for read-across in case of chronic fish toxicity and not only for setting the assessment factor for PNEC derivation.

Auszug aus dem “Technical Guidance Document on Risk Assessment Part II” Seite 101 Table 16 Note d)

An assessment factor of 10 will normally only be applied when long-term toxicity NOECs are available from at least three species across three trophic levels (e.g. fish, Daphnia, and algae or a non-standard organism instead of a standard organism).
When examining the results of long-term toxicity studies, the PNECwater should be calculated from the lowest available NOEC. Extrapolation to the ecosystem effects can be made with much greater confidence, and thus a reduction of the assessment factor to 10 is possible. This is only sufficient, however, if the species tested can be considered to represent one of the more sensitive groups. This would normally only be possible to determine if data were available on at least three species across three trophic levels.

[16] ECHA (2016) Ecotoxicity Aquatic: Update of the test guidelines. 2p, https://echa.europa.eu/documents/10162/21650280/oecd_test_guidelines_aquatic_en.pdf

It may sometimes be possible to determine with high probability that the most sensitive species has been examined, i.e. that a further long-term NOEC from a different taxonomic group would not be low-er than the data already available. In those circumstances, a factor of 10 applied to the lowest NOEC from only two species would also be appropriate. This is particularly important if the substance does not have a potential to bioaccumulate. If it is not possible to make this judgement, then an assessment factor of 50 should be applied to take into account any interspecies variation in sensitivity. A factor of 10 cannot be decreased on the basis of laboratory studies.

6.2 Feedback from participants

The workshop participants further provided a feedback on their "most important conclusion" after the read-across workshop:

Positive:

- Read-across is feasible but needs reliable guidance
- Read-across is a nice tool to get information on chemicals, but should not be used without any expert knowledge. Read-across should be used in a weight-of-evidence approach with other information used to confirm the conclusion on the endpoint.
- Good read-across on a case-by-case basis is well accepted
- It was good to learn that every party is struggling with read-across.

Critical:

- Read-across will most probably play a minor role in future due to the uncertainty in the regulatory acceptance.
- Read-across is a complex attempt with uncertain outcome
- Some stakeholders have the unrealistic expectation that read-across can be automatized fool proof for dummies at no costs. Instead, expertise is required, as in any other scientific work.

Needs:

- Read-across has to be done by an expert (consultant).
- There is a general need for more information; case study examples, good read-across practise, guidance on workflow, high quality data inventories
- Workflows are needed to be sure that the read-across approach is right: Feedback from ECHA is absolutely necessary (negative cases, where these read cross was rejected; positive cases; read-across for non-toxic compounds).
- More communication/examples are needed.
- Read-across is a very complex way to fill data gaps and requires case-by-case evaluation and even more requires further guidance and standard procedures, in order to be comparable and to be evaluated.

The overall evaluation of the workshop by the participants (see section 8) was very positive. In principle, a workshop seems to be a suitable event to address and discuss complex topics, such as read-across with stakeholders. Some aspects might need further consideration with regard to follow-up activities. An active discussion within stakeholders was achieved in the knowledge café groups. This was a suitable method to collect expert input. The discussion was guided by concrete questions but there was also room for new aspects. It has, however, to be noticed that the plenary discussion after the lectures was less active. Probably, participants were overwhelmed with too many different topics, examples and information; there was also no room for the repetition of most critical aspects. It might have been better to evaluate one topic per day; e.g. one day to work out concepts for similarity, a second for uncertainty, and a third for tools and databases. Further, a concrete case study, which has to be worked out by groups of stakeholders, would be a nice tool to facilitate the discussion, as it helps to raise questions or suggestions for improvements. Some topics are probably a bit too complex to be worked out in parallel to other topics. In the discussion of the topic "uncertainty" for example, a lot of

valuable aspects were mentioned during the knowledge cafes. Nevertheless, most of them are of critical importance for ecotoxicological risk assessment, but in this way not explicit for a read-across approach.

We therefore recommend a series of follow-up workshops as soon as a concept for ecotoxicological read-across is agreed. Such workshops will address the need of the participants for specific training, the need to define the minimal data requirements per endpoints, but also will provide feedback to authorities about the feasibility and pitfalls of the recommended approach.

Annex I: List of participants

Nachmane	Vorname	Firma	Speaker
Belz-Lumbeck	Daniela	ERM GmbH	
Biegel-Engler	Annegret	UBA	x
Boxall	Alistair	University of York	x
Brandecker	Julia	UMCO Umwelt Consult GmbH	
Bressling	Jana	Symrise AG	
Daneshian	Mardas	CAAT-Europe	x
Dilhac	Benoit	ECHA, Helsinki	
Escher	Sylvia	Fraunhofer ITEM	
Eschrich	Dietmar	Evonik Industries AG	
Fay	Reinhold	CHT R. BEITLICH GMBH	
Fioravanzo	Elena	S-IN Soluzioni Informatiche	
Gerloff-Elias	Antje	Dr. Knoell Consult GmbH	
Olmo-Gil	Eva	SCC GmbH	
Goehrig	Jutta	Jungbunzlauer Ladenburg GmbH	
Heinig	Simone	EBRC Consulting GmbH	
Hirmann	Doris	ECHA, Finnland	
Jensen	Imke	Jensen Consulting	
Kötter	Denise	Sasol Germany GmbH	
Kuehne	Ralph	UFZ Leipzig	x
Kuehntopp	Bärbel	Sasol Germany GmbH	
Laacks	Petra	Heubach GmbH	
Licht	Oliver	Fraunhofer ITEM	
Luttik	Robert	RIVM, Netherlands	x
Moltmann	Johann	UBA	
Mueller	Martin	Fraunhofer IME	
Nendza	Monika	AL-Luhnstedt	
Nyman	Anna-Maija	ECHA Helsinki	
Raschka	Andreas	EBRC Consulting GmbH	
Schmidt	Florian	BASF Schweiz AG	x
Schulte	Christoph	UBA	x
Schwartz	Dirk	Brunobock	
Staudler	Andrea	EurA Consult	
Straub	Jürg	F.Hoffmann-La Roche Ltd	
Su	Yi	Symrise AG	
Switalla	Simone	Symrise AG	
Vallotton	Nathalie	Dow Europe GmbH	
Versonnen	Bram	ECHA Helsinki	x

Annex II: Agenda of the Workshop

READ-ACROSS WORKSHOP

13. - 14. June 2016 in Hannover

Venue: Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)
Nikolai-Fuchs-Str. 1 in 30625 Hannover
Seminar Room 1A

Monday, 13.06.16

09:30- 10:00	Registration of participants
10:00 – 10:20	**Christoph Schulte**, UBA: Non-testing approaches under REACH – The UBA vision.
10:20 – 10:50	**Sylvia Escher**, ITEM: Project introduction
10:50 – 11:00	Introduction of participants (read-across expertise & workshop expectation)
Experiences with read-across – best practise examples	
11:00 – 11:45	**Bram Versonnen**, ECHA: The ENV RAAF - a framework for the assessment of read-across and grouping in environmental endpoints.
11:45 – 12:30	**Alistair Boxhall**, University of York: Prioritisation of Pharmaceuticals in the Environment and the iPiE Project.
12:30 – 13:15	**Lunch break**
13:15 – 14:00	**Ralph Kuehne**, UFZ Leipzig: UFZ Department of Ecological Chemistry: Our Story of Read-across Modelling.
14:00 – 14:45	**Florian Schmidt**, BASF: The use of read-across in ecotoxicology at BASF.
14:45 – 15:15	**Annegret Biegel-Engler**, UBA: best practise example on read-across for ecotox, from authority.
15:15 – 15:30	**Coffee break**
15:30 – 17:30	**Oliver Licht**, ITEM & **Andrea Staudler**, EurA Consult**:** *Knowledge-Café:* How to increase the acceptance of read-across? (Interactive forum with specific discussion in small groups)
17:30 – 18:00	Final discussion
~ 18:00 – 19:00	**Get-Together in the ITEM foyer (stand-up snacks)**

Tuesday, 14.06.16

Lectures and discussions	
08:30 – 09:15	**Robert Luttik**, ESFA: Uncertainty of ecotoxicological endpoints and the possible consequences for read-across.
09:15 – 10:00	**Mardas Daneshian**, CAAT: Overview on recent CAAT developments.
10:00 – 10:30	**Coffee break**
10:30 – 11:30	Round table discussion (Amendments to the outcome of the Knowledge-Café)
11:30 – 12:00	Summary and outlook
~ 12:00	**End of Workshop**

III. Synthesispaper

FKZ 3715 67 4180

“Tierversuchsfreie Bewertung unter REACH - Weiterentwicklung und Nutzung des read-across Ansatzes”

Risk assessment under REACH without animal testing – development, application and acceptance of the read-across approach

Sylvia E. Escher, Oliver Licht, Andrea Staudler

ON BEHALF OF THE

FEDERAL ENVIRONMENT AGENCY (UMWELTBUNDESAMT)

Hannover, January 2018

1 Introduction

The synthesis paper is part of the overall project "Risk assessment under REACH without testing on animals - development and application and acceptance of the read-across approach" funded by the German Environmental Agency. This project aims to develop a best practice guidance that will illustrate critical steps within the development of a read-across assessment. The project focuses on environmental and ecotoxicological endpoints like the prediction of acute and chronic toxicity for fish and bioaccumulation.

Risk assessment for the environment uses experimental data from studies with aquatic or terrestrial animals. This data will be needed for classification and labelling as well as for derivation of guidance values like PNECs. For industrial chemicals the data requirements are laid down in the REACH regulation and increases with tonnage. Above an annual tonnage of 10 t/a, the applicant has to provide vertebrate testing with fish according to OECD guidelines. The REACH Regulation calls explicitly for the use of alternative methods and the general rules for the use of these methods are described in Annex XI. It is stated that "every effort must be made so that testing chemicals on animals is a last resort – when there is no other scientifically reliable way of showing the impact on humans or the environment" (ECHA 2011). These approaches will reduce the need for generation of new experimental studies with animals.

Alternative approaches may comprise in vitro or in silico methods like QSAR models and read-across. Read-across is a grouping approach, which assumes that similar compounds will also cause similar toxicological effects. Similarity considers chemical similarity, the evaluation of structural and physico-chemical properties as well as similar toxicokinetics/biotransformation e.g. fate and distribution in the environment. Ideally the group of compounds do also show a similar mode of action. Physico-chemical data are often used as surrogate to estimate similar toxicokinetic/biotransformation properties.

In principle, read-across can be used to assess physico-chemical properties, toxicity, environmental fate and ecotoxicology. For any of these endpoints, it may be performed in a qualitative or quantitative manner (OECD 2014).Usually, we distinguish an analogue versus category approach according to the number of available source and target substances. In an analogue approach, endpoint information for one chemical (the source compound) is used to predict the same endpoint for another "similar" chemical (the target compound), to many similar target compounds. A category approach requires the evaluation of the chemical and toxicological properties of several "source compounds", which are used to predict one or several target compounds. In a category evaluation the read-across prediction might be based on a consistent trend/regular pattern or a worst case estimate.

Analogue or category approaches typically start with structural similarity between target and source compound(s). In the following the analysis of metabolism/degradation data and mechanistic properties guides the selection of "most" similar source compounds. The selection of source compounds is an iterative process and endpoint specific.

Minimal data requirements per endpoints might help to select relevant data, as illustrated in more detail in the endpoint-specific workflows (see section 2 - workflows for read-across). The predicted endpoint value will be used in risk assessment for the protection of human health and the environment; therefore, the read-across approach has to provide information similar to the omitted in-vivo test with e.g. vertebrates.

As explained above, the definition of similarity is the key element of the read-across approach. Often read-across cases are not accepted by authorities because a similar ecotoxicological behavior *in-vivo* cannot be proven. Several aspects contribute to this lack of evidence (Ball et al. 2016), e.g.

i) a clear read-across hypothesis is not provided;
ii) similarity of source compounds to the target compound is not provided and/or the rational/trend is not described;
iii) the target compound is an UVCB and thus definition of structural similarity is a challenge;
iv) data of the source compounds are not adequate or relevant for the assessed endpoint or
v) documentation and evaluation of the input data is not sufficiently described or done in a systematic way.

Therefore, there is a need for guidance to facilitate a systematic and transparent evaluation of the read-across approach and by this improve the use of read-across approaches and their acceptance by authorities.

The European Chemicals Agency (ECHA) recently published a guidance termed "read-across assessment framework (RAAF)" for human health (ECHA 2015) and for environmental endpoints (ECHA 2017). The ECHA RAAF has been developed as an internal tool providing a framework for a consistent and structured assessment of grouping and read-across approaches under REACH. The RAAF aims to standardize the evaluation of read-across cases for mono-constituent substances and to enhance transparency of ECHA decisions. It can, however, also guide registrants through a read-across evaluation by standardization of assessment scenarios, assessment options and assessment elements in the regulatory context.

A prerequisite for the acceptance of read-across prediction is an accurate documentation of the target and source substance with regard to their chemical and biological similarity. The validity of the prediction depends largely on the relevance of the parameters studied for the eco-toxicological endpoint, the quality of the eco-toxicological data and the final evaluation by the experts. The documentation should be adequate and reliable documentation (ECHA 2013a). Adequacy and reliability of experimental information is e.g. dependent on data quality, whereas the relevance of the experimental data is endpoint-specific. According to ECHA's Read-Across Assessment Framework (RAAF) the adequacy and reliability takes into account the design of the study for the source compound and if it fulfils the information requirement. With regard to the test material used it is investigated whether it fits to source compound, e.g. purity and impurities. Furthermore, it is important if the study result can be used for classification and labelling and/or risk assessment. Reliability was defined by Klimisch et al. (1997) as ''evaluating the inherent quality of a test report or publication relating to preferably standardised methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings.'', whereas relevance was defined as ''covering the extent to which data and/or tests are appropriate for particular hazard identification or risk characterisation.'' For a traceable documentation and evaluation of read-across scenarios, it is therefore necessary to develop a uniform framework and standardized procedures. Based on these parameters, the accuracy of the predictions/forecasts or remaining uncertainty of the forecasts shall be assessed.

The ECHA RAAF, however, does not describe endpoint specific workflows. This synthesis paper describe endpoint specific workflows that e.g. illustrate minimal data requirements and discusses potential threshold values and assessment criteria. It is based on the outcome of a systematic literature review and an online questionnaire (summarized in the "interim report"[17]) as well as a 1.5-day workshop with stakeholders from industry, academia and authorities (summarized in the "status report"[18]).

The most important finding of the literature review is a difference between scientific perception and practical ECHA decisions. Although evaluation of ecotoxicological endpoints was not the topic of Ball et al. (2016), the evaluation of available ECHA decisions indicates that the read-across rejections are mainly based on a lack of sufficient or suitable endpoint study data (for source compounds or relevant metabolites), scientific plausibility and lack of identity data for the target compound e.g. in case of UVCBs. Rejection based on scientific plausibility includes many cases, in which data on toxicokinetics e.g. metabolites were missing. Chemical similarity appear to be of no to minor relevance and uncertainty refers mainly to the lack of data. The assessment of uncertainty is not based on a matrix of variables that can be classified by low to high as proposed by Schultz et al. (2015) or Blackburn and Stuard (2014).

The systematic framework developed by Blackburn and Stuard (2014) describes potential areas of additional uncertainty that may arise in read-across (evaluated based on the number and suitability of analogues contributing data, severity of the critical effect, and effects and potency concordance). Further, they present a questionnaire for evaluating and documenting consideration of these potential additional sources of uncertainty by risk assessors. In the view of the authors the application of this framework represents a next step in standardizing the read-across process, both by providing a means to transparently assign a level of uncertainty to a SAR-based read-across assessment and by facilitating consistency in read-across conclusions drawn by different risk assessors. The framework considers the several typical aspects in a read-across assessment including: number and structural similarity of analogues, the quality and quantity of the considered data, the nature and severity of the critical toxic effects as well as the potency of the analogues for those effects. Further it evaluates whether existing data on the target for other endpoints aligns well with corresponding existing data from the analogues. The authors support a semi-quantitative grading of uncertainty in the four categories: low, low to moderate, moderate and high. However, no clear criteria are gives to discriminate between the different grades. Only qualitative description are given as "highly similar" or "similar" and "similar, having a consistent trend" or "minor differences in values" (see e.g. Schultz et al. 2015). It is recognized that the proposed default uncertainty factors (UF) for the various categories of uncertainty in the framework are arbitrary (1, 3, and 10) and that this framework may serves as a starting point for the uncertainty evaluation. The justification on arbitrary factors is based on the perception that it remains a pragmatic and usable solution but misses a scientific basis as acknowledged by the authors. Also, a practical instruction as well as a definition of adequate or suitable is not presented. Such an approach results in a transparent documentation but bypasses the objectivation of the uncertainty. Subsequently, the uncertainty assessment remains a subjective justification of the assessor with a proposed arbitrary factor.

Surprisingly, more read-across than experimental studies have been submitted for the endpoints bioaccumulation and long-term, toxicity to fish (Ball et al. 2016). This finding leads to categories with a number of chemicals that can be assessed by a few experimental studies. It can therefore be concluded that the future challenge for acceptance of read-across are neither the conceptual challenges of the

[17] Escher SE, May M, Staudler A (2016) Risk assessment under REACH without animal testing – development, application and acceptance of the read-across approach. I. Interim Report, FKZ 3715 67 4180, Hannover, June 2016

[18] Escher SE, Licht O, Staudler A (2016) Risk assessment under REACH without animal testing – development, application and acceptance of the read-across approach. II. status report, FKZ 3715 67 4180, Hannover, November 2016

workflow such as chemical similarity, quality of data, uncertainty and plausibility but the quality of submitted data in terms of sufficient experimental studies or suitable chemical identity data.

Within a 1.5-day workshop, stakeholders from industry, academia and authorities discussed their experiences as well as best practice examples on read-across (details in "status report"[19]). The following key aspects and assessment elements were identified:

1. Read-across is case- and endpoint-specific – this statement is complex and summarizes several aspects. The identification of source compound requires chemical, as well as biological similarity. Biological similarity might comprise same toxicological outcomes or similar mode of actions as well as environmental fate. In this context, chemical similarity includes same structural elements as well as physico-chemical properties or they follow a consistent trend, e.g. increasing lipophilicity with increasing chain length. A definition of minimal data requirements to guide this assessment was regarded as helpful. The minimal data requirements are endpoint-specific. For a certain endpoint, chemical reactivity has to be considered for the identification of source compounds. For another endpoint, bioaccumulation might be a key aspect. A guidance that illustrates minimal data requirements per endpoint and is ideally provided by authorities was believed to be beneficial. Within the workshop, some ideas on minimal data requirements were discussed but not a comprehensive evaluation per endpoint. Minimal data requirements per endpoint may therefore be a subject for further stakeholder involvement. The workflow has, however, to be flexible enough to include further evidence from non-standard assays that might contribute to a case-specific decision.
2. Chemical similarity is a good starting point to define source compounds and might even be supported by automatic workflows, e.g. including tools that help to visualize the chemical similarity.
3. Chemical similarity alone is not "good enough". Several other aspects need to be considered for a valid read-across, such as mechanistic properties, stability, fate etc. of the compounds under consideration. For example, substances that show chemical similarity may have different metabolism or different mode of action. The definition of hard criteria or thresholds that help to conclude on sufficient evidence for similarity was considered to be difficult. These minimal data requirements and guidance on hard criteria shall ideally be agreed upon by authorities and illustrated with the help of case studies.
4. Expert judgement is crucial in the evaluation of the available data to conclude on a read-across case study. This step cannot be automated. It is also difficult to define precise hard criteria or thresholds per data type and endpoint that needs to be fulfilled to conclude on the reliability of read-across group.
5. At present, endpoint specific workflows comprising minimal data requirements as well as illustrative case study examples illustrating thresholds are not available. Ideally, authorities may provide these workflows and examples as this will help to better understand acceptance criteria[20].
6. Read-across assessment has to be transparent – e.g. always following the same evaluation steps per endpoint. Templates/data matrices are helpful for the documentation.

[19] Escher SE, Licht O, Staudler A (2016) Risk assessment under REACH without animal testing – development, application and acceptance of the read-across approach. II. status report, FKZ 3715 67 4180, Hannover, November 2016

[20] A need for case study examples illustrating acceptable and non-acceptable read-across approaches was expressed in the discussion of all topics and groups in the workshop (see status report; Table 2 - content ID 3+4; Table 3 - content ID5+6; Table 4 - content ID 3+4).

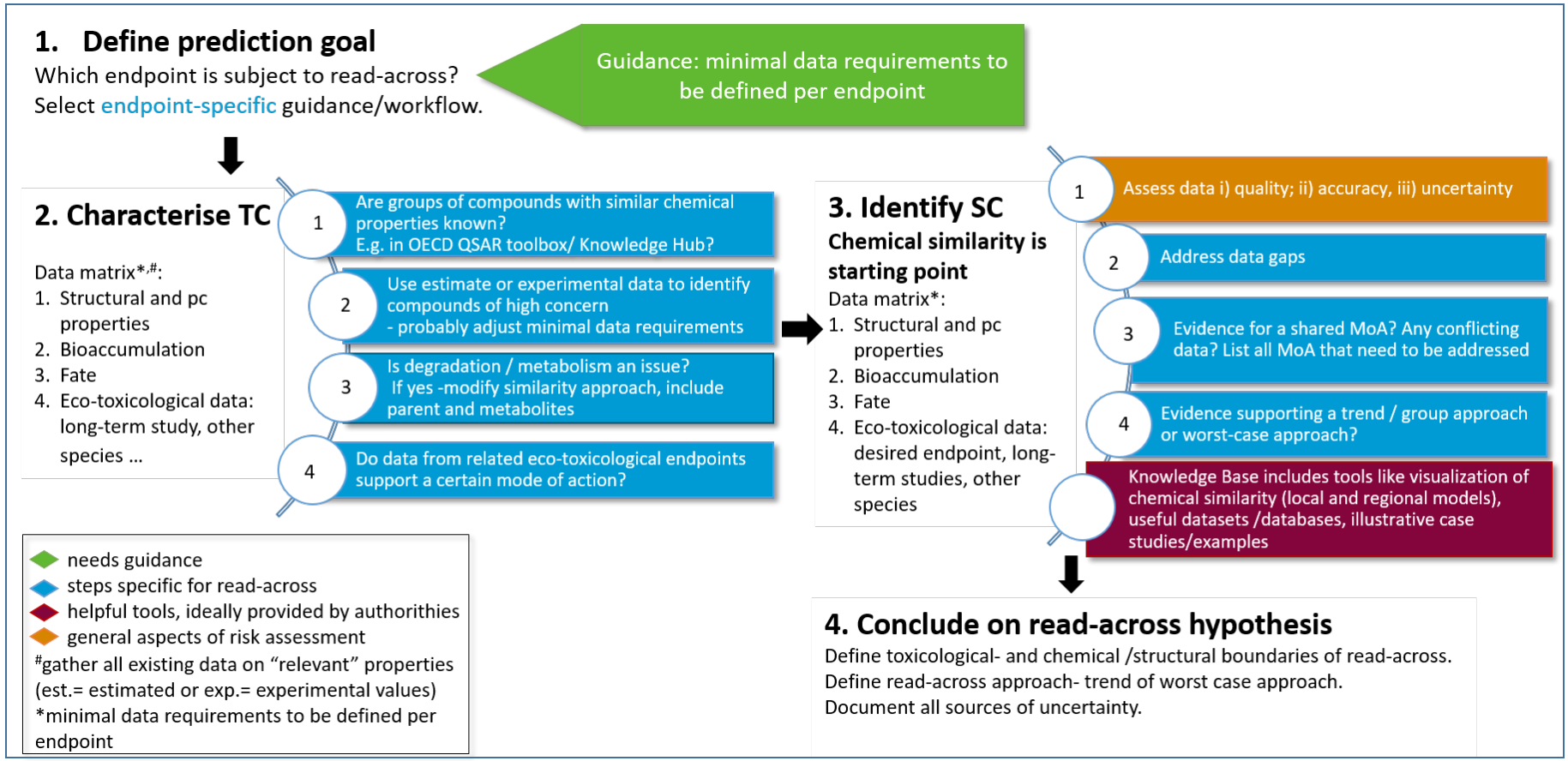
7. Read-across needs to be supported by adequate and reliable documentation.
8. Uncertainty has to be addressed and may arise from several assessment elements of the read-across procedure e.g.:
    a. Data gaps in the data matrix
    b. Assessment of data quality (general risk assessment procedure, not read –across specific)
    c. Assessment of relevance of the used ecotoxicological data for the predicted endpoint
    d. Variability and uncertainty of experimental data - test validation data shall be considered to account for the assessment of uncertainties attributed to the specific assay.
    e. Inclusion of large number of data may help to improve read-across and weight-of-evidence approaches.
    f. Decisions taken for compounds close to a certain thresholds might need more justification, than those far below a certain threshold.
    g. Similarity is endpoint specific - the chemical as well as toxicological boundaries of the read-across group need to be clarified. Impurities have to be taken into account.
    h. Uncertainty of acceptance by authorities – read-across will gain higher attention for applicants, in case that illustrative case studies about acceptable as well as not acceptable approaches are provided by authorities.

2 General read-across workflow

A first general read-across workflow summarizing the above-mentioned key aspects is provided in Figure 43. The read-across workflow generally starts with the definition of the endpoint that needs to be estimated - the prediction goal. As the read-across assessment strategy is endpoint-specific, ideally endpoint-specific workflows will have to be generated to assure a systematic and transparent evaluation of the data matrix. The minimal data requirements per endpoint shall be outlined and ideally be illustrated with case studies.

The read-across workflow continues with characterization and data gathering for the target compounds. Besides data on structural and physico-chemical properties, all available data on ecotoxicological endpoints [bioaccumulation + fate (experimental or predicted values)); acute or chronic toxicity studies (for species from other trophic level)] will be gathered. Transparent data documentation is seen as key requirement; a standardized data matrix/template might be helpful to do this properly.

Figure 44: General read-across workflow; TC = target compound; SC = source compound (source: Fraunhofer ITEM)



In the next step, "similar" source compounds need to be identified. This has to be based on the characteristics and available data of the target compound (as gathered under step 2, Figure 44). Chemical similarity was seen as good starting point[21]. Furthermore, chemical similarity is mandatory according to the current REACH legislation[22]. Chemical similarity includes structural properties, e.g. similar functional groups, reactivity of functional groups, stability of the compounds as well as similar physicochemical properties such as water solubility, molecular weight and hydrophobicity. In this context, the following aspects need to be considered:

- Are groups of compounds with similar chemical properties known? Possible candidates can be generated by using e.g. the OECD QSAR toolbox (Dimitrov et al. 2016) or other sources (see Patlewitz et al. 2017 for more information). For this step, visualization tools or even automated workflows could be helpful as well as predefined case studies ideally provided by authorities.
- Do any of the experimental or estimated data of the target compound indicate an area of concern e.g. high bioaccumulation or persistence? Such areas of concern should be included into the assessment of similarity and may modify the endpoint specific "minimal data requirements".
- The stability of the target compound is important to assess if the parent compound or metabolites have to be included into the similarity assessment. It should consider biotic and abiotic degradation in the compartment of concern as well as internal metabolism in the species of interest, e.g. fish.
- Do available toxicological data from related endpoints support the hypothesis on a certain mode of action?

Starting with chemical similarity, a preliminary inventory of source compounds is identified (Step 3, Figure 44). For these compounds, data as defined from the minimal data requirements and modification from step 2 will be collected. The evaluation of the data with regard to relevance and accuracy will be done by using expert judgement. This limits the use of the approach by registrants without specific knowledge in ecotoxicology and environmental risk assessment.

The following aspects might help to structure the workflow:

- Assess data quality, accuracy and uncertainty of the experimental data
- Address data gaps and their impact on uncertainty
- Is there any evidence from the gathered data that supports a shared mode of action? Are there any data, which conflict with a certain mode of action? This assessment could be facilitated by illustrative examples or a list of relevant modes of action per endpoint.
- Is there any evidence that support a trend or group approach? Or is a worst-case prediction adequate? A guidance on the applicability of a trend/linear regression can be given based on descriptive statistics. It is difficult to provide a general guidance when to follow the worst-case approach and when read-across to the "most similar" compound is more adequate. This will need expert judgement and a detailed explanation.

[21] Chemical similarity as starting point was expressed in the discussion of the workshop (see status report; –Table 2 Content ID 1 +2).

[22] See REACH regulation as well as ECHA RAAF: „Structural similarity is a pre-requisite for any prediction based on read-across under REACH."

The evaluation under Step 2 will help to better define the ecotoxicological and chemical/structural boundaries of the read-across case. The selection of source chemicals needs to be documented (Step 3, Figure 44) and a read-across hypothesis will be outlined e.g. based on common mode of action or based on one similar breakdown product etc.. The best fitting prediction approach has to be described (worst case versus trend) and sources of uncertainty need to be outlined. As expert judgement is a central part of the conclusion, it might be difficult to predefine the structure the workflow of step 4.

The workshop participants expressed the need of guidance and illustrative examples, which help to better understand the read-across workflow, including reasons for acceptance or rejection of the read-across. These examples, as well as links to suitable databases and tools could be integrated into a decision support system, termed "Knowledge Base" (Figure 44).

Further, better databases are needed, which e.g. indicate the quality of the gathered data, differentiate estimated from experimental parameters and offer tools to automatically extract selected data into the required data matrices.

3 Endpoint specific read-across workflows

This chapter describes three examples read-across workflows (WFs) for the endpoints acute and chronic fish toxicity as well as bioaccumulation in fish. The WFs comprise specific assessment elements as well as minimal data requirements and shall help applicants to develop and assess read-across cases in a transparent and systematic way. All three WFs illustrate the evaluation process and guide through a concise and systematic documentation of a read-across case.

3.1 WF acute and chronic fish toxicity

The workflow "acute and chronic fish toxicity" starts with the characterization of the target compound. It is necessary to collect information on substance identity e.g. name, molecular weight and structural properties. Known impurities and their identity have to be documented as well. REACH allows the registration of different compositions of a chemical. In these cases the identity of all constituents present in the registered composition has to be documented.

Physico-chemical parameters are collected that are considered to be relevant for acute toxicity. The minimal data requirements include all parameters, which are important for distribution into the environment and for the exposure situation in ecotoxicity tests as recommended in the specific OECD guideline 203 for acute toxicity and respective guidelines for chronic toxicity. Therefore, water solubility, log K_{OW} and vapour pressure are mandatory.

In a next step, the information on fate, persistence and bioaccumulation have to be taken into account. For substances with low annual tonnages (<10 t/a or <100 t/a) only limited information from experimental studies will be available, as e.g. experimental data on bioaccumulation are only required at higher tonnages. In such cases, predicted values from relevant QSAR models can be used to fill the data gap. The reliability of the predicted values with regard to applicability domain, sensitivity and specificity of the model has to be outlined for the source and (later on) target compounds in the overall assessment. We recommended to use, if possible, different QSAR models e.g. rule based versus statistical models. In case that more than one statistical model is available, consider all models that differ with regard to the applied training and test sets. The results of the different models should be described and differences between the results should be assessed.

For information on specific mode of action of the target substance, acute toxicity data on other trophic levels like algae and *Daphnia* have to be taken into account. These experimental data have to be derived according to standard guideline requirements or equivalent with sufficient details on test design and raw data. Legal access to the study (incl. full study report and/or robust study summary) requires a letter of access time and may lead to additional costs. Robust study summaries provided on the ECHA homepage are often not sufficient to evaluate the quality of the study and data access for registration

purposes has to be clarified with the data owner. In general, studies with Klimisch Code 1 or 2 have to be considered. Other studies with lower reliability (Klimisch Code 3 or 4) can be listed and considered as supporting information. The use of "low quality" studies might increase the uncertainty of the read-across approach, e.g. if used as single source of information.

Several QSAR models are available that predict acute aquatic toxicity i) classification (Verhaar Scheme, Verhaar et al. 1992) or ii) MoA (e.g. "acute aquatic toxicity MoA by OASIS from the OECD toolbox). These predictions can also be used to characterize the TC if the compound falls in the applicability domain of the QSAR model.

In cases, where the target compound is not stable in water or there is evidence for rapid metabolism, the read-across has to consider all relevant degradation products and/or metabolites. Minimal data requirements as laid down for the target compound will have to be compiled for these compounds as well.

The information retrieved for the target compound (and if needed metabolites) feeds into a data matrix (Table 11) and will be evaluated. The data matrix may be adapted if for a specific case additional data are considered relevant. The full data matrix is generated after selection of source compounds (see text below).

In a first step species-sensitivity consideration can be applied (e.g. Kienzler et al. 2016). In the REACH guidance on information requirements and chemical safety assessment (R.7b.) it is mentioned "If there is compelling evidence , …, to suggest that the fish value is likely to be at least a factor of about 10 less sensitive than invertebrates or algae there are no further requirements for fish testing (ECHA 2016b). In case that this type of assessment is successful, the read-across assessment will stop at this stage.

Otherwise, the gathered PC properties listed in Table 11 will help to i) identify similar chemicals and ii) assess the reliability of the reported toxicity data. The reliability is lower if the experimental LC_{50} value is above the water solubility of the substance or in cases of volatile substances, if no information on analytical verification of test solution is available.

Table 11: Data compilation for the target compound and if needed degradation products and metabolites.

	Target compound		Degradation products or metabolites	
Compound identity: Name, molecular weight, structural formula, impurities				
	Add here		Add if needed	
Properties	Experimental	Predicted	Experimental	Predicted
Physico-chemical properties	Water solubility, log Kow, Vapour pressure			
	Exp. data list here	Pred. data list here	Add if needed	
Fate and accumulation	Biodegradation, BCF			
	Exp. data list here	Pred. data list here	Add if needed	
QSAR predictions for acute toxicity classification/MoA	Results list here		Add if needed	

Endpoint of interest (data gap)	Acute toxicity fish			
	Data gap			
Short-term toxicity for other species	Acute toxicity Daphnia, acute toxicity algae			
	Exp. data list here	Pred. data list here	Add if needed	
Additional information for chronic toxicity	Chronic toxicity fish, Chronic toxicity Daphnia, Chronic toxicity algae			
	Exp. data list here	Pred. data list here	Add if needed	
Supporting information				

In a second step, relevant source compounds have to be identified. Structure similarity is the starting point to identify an initial list of potential source compounds.

For, identification several options are available and the appropriate one has to be selected by the applicant. In case that the applicant has a very good idea about the structural element that drives the toxicity, it may be appropriate to include all compounds with this substructure.

In case that no prior knowledge about the impact of structural features is known, or many structural properties are present, different algorithms can be applied to identify analogues based on structural similarity. Algorithms are e.g. available in the OECD QSAR toolbox (http://www.oecdsaatoolbox.org) or can be set up with user-friendly tools like KNIME (http://www.knime.org). In principle, the chemical structure of the compound is encoded in a fingerprint, e.g. the presence or absence of specific molecular features is encoded by using 0 and 1, respectively. Based on these fingerprints well established algorithms like Tanimoto or Dice calculate a relative similarity between compounds (Cronin et al., 2013).

A threshold for structural similarity has to be defined by the user to discriminate between structurally similar and dissimilar compounds. However, a general threshold value for discrimination between similar and dissimilar compounds cannot be defined. Overall, it can be recommended to start with a low similarity threshold like 0.6, to assure that all potentially analogues will be included into the first list of potential source compounds.

An expert may also decide to include only compounds, which differ with regard to one property e.g. the carbon side chain length. This will e.g. make sense for target compounds that do not comprise very specific structural properties. One example is valproic acid (VPA), a short chain branched carboxylic acid. The calculation of structurally similar analogues with different algorithms of the OECD toolbox does not result in a list of structurally related carboxylic acids, e.g. differing in side chain length, but compounds that comprise other functional groups such as thiols or amines.

The same information as laid down in the minimal data requirements will be gathered for the source compounds and all data on acute fish toxicity (Table 11). In case of data gaps different QSAR models can be used to predict the acute toxicity, e.g. baseline toxicity models like ECOSAR, CHEM PROP or US EPA T.E.S.T (see Nendza et al. 2017). As described above the QSAR predictions need to be assessed with regard to their applicability domain, sensitivity and specificity.

Evaluation of source compounds

The data on physico-chemical properties, biodegradation and acute fish toxicity guide the selection of source compounds beyond structural similarity. This may lead to exclusion of structural similar compounds because physico-chemical and/or biological parameters indicate differences in mode of action or fate among others. This step results in a final selection of most relevant source compounds.

Special emphasis has to be given to data quality. According to ECHA guidance robust study summaries should be provided for all studies used in the read-across for the specific endpoint (ECHA 2013b) and sufficient data must be provided allow the assessment of their reliability.

In a next step, the accuracy of the gathered data on "acute fish toxicity" need to be addressed. Data from different fish species might limit the predictivity of the read-across prediction, as some fish species are considered more sensitive compared to others, e.g. salmonids are in most cases more sensitive compared to carp:

Case 1: Several species tested
The read-across prediction will be less reliable, if the data set is heterogeneous e.g. each source compound has one reliable experimental study but different species are tested. On the other hand, if data for several species are available for a single source compound this will give an indication of in vivo variability and will help to assess the variability of toxicological value also for the target compound.

Case 2: One species tested
The prediction for the target compound will be reliable if relevant experimental data are available for one species from different source compounds. On the other hand, the read-across will then be limited to the one specific species from which all data are obtained.

Case 3: Most sensitive species not tested
In this case the reliability of the read-across hypothesis would be high if the results from the tested species follow a specific pattern. However, the result would inherit some uncertainty regarding the predictability for e.g. sensitive species that need to be addressed in the reporting.

Furthermore, differences in exposure conditions e.g. semi-static or flow –through, may be another source of variation and could have an impact on predictivity. The same holds true if different exposure duration, e.g. 72 h, 96 h or longer periods, have been tested. As supporting information additional data and expert knowledge can be included. This may include e.g. in vitro assays, mechanistic studies to support the prediction and/or the trend of the read-across.

The generated data have to be evaluated, to conclude on a final list of source compounds and a read-across hypothesis. Several questions will guide the read-across approach:

1) Is there supporting evidence for similar fate and persistence in the environment? If yes, the confidence in the read-across approach will increase. Or do the source compounds follow a consistent trend, e.g. substance properties change in a specific direction with increasing chain length? Such trend also would increase confidence in read-across. Based on the evaluated properties: where is the target compound located in the trend compared to the source compounds? If the substance is located at the end of the category, the confidence will be lower compared a situation where the target substance is surrounded by neighbors. Is worst-case or regression analysis an appropriate approach to predict target compound`s properties? Or is a prediction based on the most similar source compound appropriate? As a first assumption, a worst-case approach should be followed using the lowest value from the source compounds. In case that the experimental data show a clear trend and interpolation to the target compound is possible, it is recommended to use a regression analysis instead of a worst case approach.

2) Is there supporting evidence for a common specific or unspecific mode of action within the source compounds? If all substances have the same mode of action, the confidence in read-across approach will increase. Are there any conflicts in the data within the groups of source compounds or target compound that indicate differences in mode of action? Any conflicting data needs to be addressed in the documentation and decreases the confidence in the read-across approach.

3) Conclude on most similar source compounds and describe their structural and toxicological properties. Document also those compounds, which were not included into the set of source compounds.

The evaluation of the data leads to a read-across hypothesis. Based on this read-across hypothesis potential analogues may be excluded, e.g. by demonstrating clear differences to the other source compounds and or the target compound. A clear documentation of inclusion and exclusion criteria, all gathered data for the selected and unselected source compounds will have to be provided by the applicant.

In case that a trend is observed a regression analysis can be used to interpolate the "acute toxicity value" from the data of the source compounds. In case that a specific trend is not observed, the property of the target compounds can be extrapolated from the most toxic compound within the source compounds. This is called a "worst case" approach. In case that there is evidence that "one" source compound is closer related than the other source compounds, the value of this "most similar" analogue can be used to predict the toxicity of the target compound.

Other experimental data can be considered in case that reliable and accurate experimental data on acute toxicity data are not available for all source compounds or too many data gaps prevent a conclusion on a trend or worst case approach. We recommend to use the fish embryo toxicity test (OECD guideline 236). Fish embryos tested up to 120 h are not considered as protected animals according to the current European legislation (Directive 2010/63/EC). The results from the FET show a high correlation (strong agreement, e.g. relationship with a slope near one and intercept near zero) to the results of the standard acute fish test (e.g. Belanger et al. 2013, Scholz et al. 2016). However, it is not yet fully accepted by ECHA, which recommended using results from this test only in a weight-of-evidence approach (ECHA 2016a). With this test system it is not necessary to test vertebrates for ecotoxicity endpoints up to a tonnage band of 100 t/a.

Again species-sensitivity consideration can be applied (e.g. Kienzler et al. 2016). In case that the applicant can show that the fish values of one to all source compounds are at least a factor of about 10 less sensitive than the gathered invertebrates or algae data.

The read-across hypothesis for chronic toxicity has to be formulated in a similar way. Minimal data requirements for chronic fish toxicity include nearly all endpoints that where listed for acute toxicity. However, for this endpoint e.g. the waster criteria like pH and hardness should be suitable long-term survival and growth. In addition, small residues of impurities might have a larger impact compared to acute toxicity.

4 Bioaccumulation in fish

The workflow for bioaccumulation in fish also starts with the characterization of the target compound. To characterize the target compound it is necessary to have information on molecular structure and on the impurities present in the registered substance. According to the requirements of OECD guideline 305 the physico-chemical parameters are needed which have an impact on the distribution in the environment and on exposure situations in ecotoxicity tests. These include water solubility, log K_{ow} and vapour pressure. In addition, the guideline requires results on acute toxicity towards fish, like a LC50 value, for the same species as to be tested in the bioaccumulation study.

The information on the target compound feeds in a data matrix and a possible structure is listed in Table 12 below.

Table 12: Data compilation for the target compound and if needed degradation products and metabolites.

	Target compound		Degradation products or metabolites	
Compound identity: Name, molecular weight, structural formula, impurities				
	Add here		Add if needed	
Properties	Experimental	Predicted	Experimental	Predicted
Physico-chemical properties	Water solubility, log K_{OW}, vapour pressure, surface tension (when no log K_{ow} is determinable), stability in water, pKa for ionisable compounds (pH value of solution)			
	Exp. data list here	Pred. data list here	Add if needed	
Fate and accumulation	Biodegradation, metabolism			
Endpoint of interest (data gap)	BCF			
	Data gap			
QSAR predictions for acute toxicity classification/MoA	Results list here		Add if needed	
Acute toxicity fish	Exp. data list here	Pred. data list here	Add if needed	
Additional information for other species, incl. chronic toxicity fish	Exp. data list here	Pred. data list here	Add if needed	
Supporting information				

In cases, in which the target compound is not stable in water or there is evidence for rapid metabolism the read-across can be based on the degradation product/metabolites.

In a second step, possible source compounds have to be identified (see section on fish toxicity for details). After identification of a number of potential source compounds the same steps for data gathering as for the target compound have to be performed (Table 12).

Evaluation of the source compounds

To assess the bioaccumulation in fish the user will consider the different BCF values of the source compounds and the accuracy of exposure and analytical conditions. Conditions that have an impact on BCF values are i) flow-through or semi-static exposure, ii) stable exposure of the test compound e.g. by analytical monitoring of its concentration in water, iii) steady state reached in the organism iv) normalization to lipid content of the tested species.

In case of inaccurate conditions the BCF values are probably of limited reliability and less comparable within the group of source compounds.

Alternative methods such as QSAR models can be used, as described above in the workflow acute and chronic fish toxicity. As BCF is one criteria that may trigger directly further limitations or authorizations to the substance resulting from the identification as SVHC (meeting the PBT criteria), special emphasis should be laid on BCF near the cut off value for the B criterion is 2000 (for vB 5000) and extrapolation procedure or worst-case assumption should be selected in a conservative way. Therefore, the prediction must be precise in that specific range.

5 General workflow

This section describes a general workflow applicable to all three endpoints (see Figure 44).

This workflow starts with the compilation of data for the target compound. Then a decision has to be made, whether or not metabolism/degradation play an important role in the toxicity of the target compound. If so relevant metabolites or degradation products should be the basis for the read-across assessment, if not the target compounds is the basis for the read-across.

In a second step, source compounds will be identified starting with chemical similarity and data will be generated for the given preliminary set of source compounds. As described above the gathered biological data will be evaluated for trends. With this data a first idea for a read-across hypothesis will be formulate. Potential source compounds will be excluded in case that differences in fate, distribution or mode of action provide sufficient evidence that these compounds are dissimilar to the target compound.

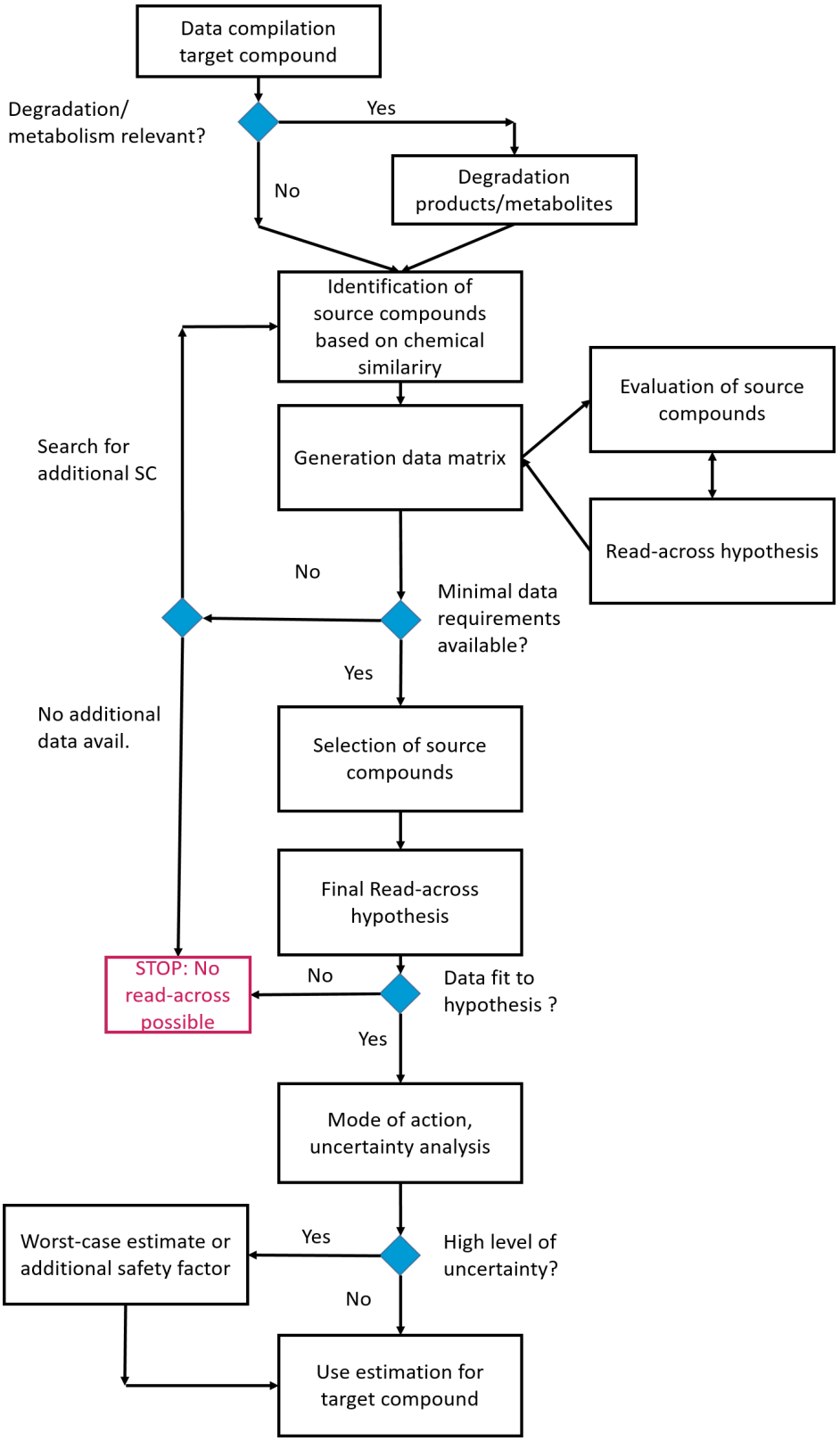
In a feedback loop this procedure can be repeated with the modified set of source compounds, if the minimal data requirements are not met.

Once the most similar source compounds are selected, the data retrieved for these selected source compounds will be used to develop the final read-across hypothesis.

Finally, uncertainty on read-across approach is assessed and a value for the specific endpoint (e.g. LC_{50}) is derived for further use in risk assessment and for classification and labelling.

For the assessment of uncertainty hard criteria can not be formulated as it is case specific for each read-across approach. The evaluation of four different read-across approaches for human health endpoint repeated dose toxicity as summarised by Schultz & Cronin (2017) characterises uncertainty with qualitative descriptions like “sufficiently similar” or “differences in similarity”. However, also for these endpoints no quantitative determinations are provided.

Figure 45: Overview of workflow with decision steps for termination and/or use of read-across approach (sc: source compounds, source: Fraunhofer ITEM)



The workflow is tested in two hypothetical examples. The first example will deal with a category approach and the second with a one-to-one read-across for the endpoint acute toxicity.

Example 1: Read-across for compound with different carbon chain length

In this first example the target compound is characterised by a carbon chain length of C5 and another structural element R. First of all, all target compound specific data will be gathered and included into the data matrix (Table 13). As described above in the minimal data requirement these data include physico-chemical data, relevant experimental data as well as available QSAR models.

In this hypothetical example the first read-across hypothesis is that the structural part R is characteristic for the toxicity of the target compound and therefore needs to be part of all analogues. This finding is based e.g. on knowledge on a specific MoA for "R-compounds". This knowledge may arise from the prediction of a certain MoA based on a reliable QSAR models, or prior knowledge on a certain mode of action from literature. "R" could for example be a certain functional group like "α,β-unsaturated aldehyde", which are characterized by a specific reactivity that triggers toxicity. Or "R" might be a certain structural element for which a specific mode of action is known e.g. "organophosphates" which decrease the activity of cholesterine esterase in vivo or phthalates, which are of concern to be endocrine disruptors.

For this hypothetical example there is no indication for metabolism based on the chemical structure, so metabolites have not to be considered and the identification of source compounds starts from the structure of the target compound.

From initial list of source compounds to final list of source compounds

The user has several options to identify the initial list of structurally similar compounds. In this example the user may either directly hypothesize that analogues with different length of the aliphatic chain will determine the structural domain of the category. If so, it will be appropriate to search directly for all possible analogues with longer or smaller side chain. In principle all compounds with side chain from C1 to C_∞ have to be considered as starting point. In this example the compilation ends at C14.

Alternatively, the user might use well established algorithms like e.g. Tanimoto or Dice e.g. provided by the OECD toolbox to compile an initial list of potential analogues. We recommend to use a relatively low similarity threshold of 0.6 to identify all potential analogues.

After an initial list of the source compounds have been identified, the data matrix will be filled with the available experimental and QSAR data (according to the minimal data requirements).

A first inspection of the physico-chemical data indicates that compounds C1 to C3 are volatile, whereas C4 to C14 are not volatile. In addition to the minimal data requirement, the parameter Henry's law constants and boiling point are added and support the assumption that C1 to C3 are volatile (boiling point below 100°C) and with a Henry's law constants of 10^{-3} to 10^{-4} atm m^3/mol volatilization is significant. C4 to C14 are essentially not volatile. With increasing chain length the log K_{OW} of the compounds increase. A cliff is seen from compound C10 on, indicating that these compounds are potentially bioaccumulating.

Generation of data matrix

As additional information on aquatic toxicity towards fish, structural alerts such as Verhaar classifications, ECOSAR classifications, and MOA by OASIS are used for identification of a specific mode of action. In this specific example, the target and source compounds have similar predictions.

As shown in Table 13, acute fish toxicity data are not available for all analogues, but increases with longer chain length. There is no indication for another specific mode of action as derived from comparison of toxicity data for different trophic levels (data not shown for sake of clarity).

Also predictions of acute toxicity values from QSAR models are included. In this example only one QSAR model is listed, but to use more than one models can help. However, the applicability domain of the models has to be checked.

Setting up the read-across hypothesis

The read-across hypothesis is that the acute fish toxicity follows a non-specific mode of action and that the LC50 value is decreasing with increasing chain-length.

Uncertainty analysis

In this example, QSAR prediction by ECOSAR supports the general trend as well as the quantitative value of the measured LC_{50} value. Although the LC_{50} data for C7 show some variation. However, the data have been obtained for two other species compared to the *P. promelas* used for C4 and C6. The results from C8 for *O. mykiss*, even if the reliability can not be assessed (Rel. 4) as it might be a hand-book data without detailed testing information, gives further support for the read-across hypothesis.

Derive acute aquatic toxicity value for target compound

In conclusion, the data indicate that the compounds C4 to C9 are the most relevant for reading across the toxicity values. As there is a consistent trend within the physico-chemical and toxicity values, a re-gression analysis may be applicable. The overall confidence in the read-across is high, as no indication for different mode of actions has been identified, only experimental data with high to god reliability contribute to the decision and no conflicting data are available in this idealised example. Therefore, an LC_{50} value of 17 mg/L as derived from regression analysis can be used to fill the data gap for the target substance, C5.

Table 13: Hypothetical example for acute fish toxicity in relation to carbon chain length and comparison with QSAR predictions and Mode of action.

		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14
Molecular structure		R-C1	R-C2	R-C3	R-C4	R-C5	R-C6	R-C7	R-C8	R-C9	R-C10	R-C11	R-C12	R-C13	R-C14
Struct. similarity to TC		75%	75%	80%	95%	100%	95%	90%	85%	80%	75%	68%	68%	65%	60%
physico-chemical properties (exp. values)	Water solubility (mg/l)	low	low	low	moderate	moderate	moderate	moderate	moderate	moderate	low	low	low	low	low
	log K_{ow}	1.0	1.5	1.8	2.6	2.8	3.0	3.2	3.4	3.8	>4.5	>4.5	>4.5	>4.5	>4.5
	Vapour pressure (hPa)	1100	1000	1000	750	700	700	680	650	650	600	600	500	450	200
	HLC (atm m3 /mol)	10^{-3}	10^{-3}	10^{-4}	10^{-5}	10^{-5}	10^{-6}	10^{-6}	10^{-7}	10^{-7}	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
	Boiling point (°C)	70	80	80	120	130	140	150	160	170	250	250	250	300	300
Fate and accumulation (exp. values)	Biodegradation	ready	--	--	ready	ready	ready	not ready	--	not ready	--	not ready	--	not ready	not ready
	BCF	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}	See log K_{ow}
MOA	Verhaar	class 1	class 1	class 1	class 1	class 1	class 1	class 1	class 1	class 1	class 1	class 1	class 1	class 1	class 1
	ECOSAR	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.	Neut. Org.
	OASIS	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.	Base. Narc.

		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14
Experimental data on acute fish	LC50 [mg/L]	Data gap	Data gap	20	20	Data gap	15	7; 25	3	Data gap	2	Data gap	Data gap	Data gap	
Source				LC50 (96 h) P. promelas; Rel. 2	LC50 (96 h) P. promelas; Rel. 2		LC50 (96 h) P. promelas, Rel. 1	LC50 (72 h) O. mykiss, Rel. 2; LC50 (72 h) O. latipes, Rel. 2	LC_{50} (72h) *O. mykiss*, Rel. 4		LC50 (96 h) P. promelas; Rel. 2				
ECOSAR QSAR 96h fish LC50 [mg/L]		Outside domain	Outside domain	35	31	27	20	17	6	1	1	0.5	0.5	Outside domain	Outside domain

HLC: Henry's law constant, Neut. Org.: Neutral Organics, Base. Narc. : Base-surface narcotics

Example 2: One to one read-across

In the second example, the target compound is an unsaturated alcohol and only one source compound is identified. For the target substance the available data is filled to the table, as done in the first example.

Identification of source compounds

In this example, there is no indication for metabolism based on the chemical structure, so no metabolites have to be considered and the identification of source compounds starts from the target compounds, the C6 structure. Only one substance, a linear alcohol is found as source compound. The data on physico-chemical properties confirm the similarity derived from the chemical structure.

Generation of data matrix

In total, four LC50 values from 10 to 100 mg/L are available for the source compound. In addition, structural alerts such as Verhaar classifications, ECOSAR classifications, and MOA by OASIS are used for identification of a specific mode of action. For the source compound no specific mode of action was indicated by the classification schemes. However, for the target compound a specific mode of action is derived. In this specific example, the predictions for target and source compounds are different. The LC50 value derived by QSAR is 8 mg/L.

Uncertainty analysis

The LC50 values (10 -100 mg/L) was obtained for different species and different duration of time. Also, the two extreme value came from one species a different time-points (96 h and 48 h) but where determined in different laboratories. Comparing the LC50 data obtained for the recommended exposure period of 72-96 h an LC50 of 10 to 60 mg/l will be considered. These values are in the same order of magnitude as the values derived from QSAR model. The QSAR model supports the experimental values for the source compound.

The low LC50 value derived by QSAR would lead to a different classification and labelling of the substance. Moreover, for the target compound a specific mode of action is derived (Vinyl/Allyl Alcohols in ECOSAR) compared to the non specific mode of action predicted for the source compound. Overall, the differences between the two chemicals are high for the endpoint of interest, acute fish toxicity.

Setting up the read-across hypothesis

Due to the differences listed above, a robust read-across hypothesis can not be established. One might consider to go back to identify additional source compounds to set up a category of substance or find a new partner compound for a one-to-one read-across. But if these substances can not be identified, the final conclusion of this example would be that a read-across is not possible and an experimental study should be performed. In this case, it could be checked if the FET-assay could be used as a “non-animal testing” alternative (see discussion above).

Table 14: Hypothetical example for a one to one read-across for acute fish toxicity.

		Target	Source
Molecular structure		R-CCC(C=C)O	OCCCCC-R
Struct. similarity to TC			>90%
Physico-chemical properties (exp. values)	Water solubility (mg/l)	high	high
	log K_{ow}	1.9	2.0

		Target	Source
	Vapour pressure (hPa)	1000	1100
	HLC (atm m3 /mol)	10^{-3}	10^{-3}
	Boiling point (°C)	75	80
Fate and accumulation (exp. values)	Biodegradation	Ready biodeg.	Ready biodeg.
	BCF	See log K_{OW}	See log K_{OW}
MOA	Verhaar	class 3	class 1
	ECOSAR	Vinyl/Allyl Alcohols	Neutral Organics
	OASIS	alpha, beta-unsaturated alcohols	Base-surface narcotics
Experimental data on acute fish	LC50 [mg/L]	Data gap	20/ LC50 (96 h) P. promelas; Rel. 2
			10/ LC50 (96 h) O. mykiss; Rel. 2
			100/ LC50 (48 h) O. mykiss; Rel. 4
			60/ LC50 (72 h) O. latipes; Rel. 2
ECOSAR QSAR 96h fish LC50 [mg/L]		8	40

Data specific cut offs and thresholds

For the sake of clarity, data-specific decisions/thresholds are not included in the general workflow (see above). Such decision would be necessary on e.g. the log K_{OW} value distribution and the definition of upper and lower boundaries for log K_{OW} values that need to be considered for inclusion and exclusion of compounds. In the following table, possible criteria for the decision steps are formulated. However, no concrete threshold or definitions can be given. It is also not possible to define discrete criteria or cut-off values for the different steps in the read-across development. Definition of such cut-off values is expected to be subject for further stakeholder involvement or by regulatory decision based on scientific evidence.

Table 15: Overview of criteria for target and soure compounds to be considered in read-across development

Criteria	Value/Option	Comment
Target compound		
Degradation relevant?	Yes/no, if yes select degr. product as target	E.g. information on hydrolysis half life
Metabolism relevant?	Yes/no, if yes select metabolite as target	E.g. if rapid metabolism in fish is expected. Information from in vitro or existing toxicological studies can be used as additional data source.
Source compound		
Minimal data requirements		

Criteria	Value/Option	Comment
Data on physico-chemical properties available?		Information from handbook data can be included
e.g. vapour pressure	Range of values or trend	No specific cut off possible. All source compounds should have a comparable tendency to evaporate or to stay in solution.
e.g. log K_{ow}	Range of values or trend	No specific cut off possible. Depending on hypothesis change in log K_{ow} in line with structural changes or all compounds within comparable range of values that would indicate a comparable behavior.
Data on fate and distribution	Same target compartment and stability	Is just a qualitative comparison possible or are specific thresholds needed to judge on similarity? E.g. all compounds are ready biodegradable fulfilling 10d window.
Data on endpoint	Experimental versus predicted values	Experimental data for all source compounds would be the best case. The more predicted values are used to fill data gaps or real data gaps are available the higher the uncertainty of the read-across case.
	All values for same species	Interspecies variability would be another source of uncertainty for the read-across.

The use of read-across is case specific and only qualitative descriptions like highly similar can be provided. Uncertainty is difficult to define with discrete values or cut-off criteria, as different sources of uncertainty has to be considered.

First, definition of chemical similarity and selection of source chemicals is one source of uncertainty. In one case, it might be useful to include more “chemically” different substances that share similarities to the target compound with regard to metabolism or mode of action. In other cases a more narrow definition might be needed.

Second, data availability and quality for the source compounds leads to an additional source of uncertainty. In this case, reliability scoring and weight of evidence should be considered. Some flexibility is needed in order to make more use of read-across. However, high level of protection for man and the environment should be assured. This has to be taken into account when using the read-across approach. If the read-across approach fails to generate reliable results, the final option is to test the required endpoint in a guideline study with vertebrates.

Once defined, these criteria with underlying ranges or discrete values can be integrated in the knowledge hub to facilitate to use of read-across by non-experts.

6 Relations of read-across and the weight-of-evidence approach

The read-across approach is defined in the REACH Regulation, Annex XI under Chapter 1.5 Grouping of substances and read-across approach as follows “Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or "category "of substances. Application of the group concept

requires that physic-chemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach)."

According to Annex XI 1.2 of the REACH regulation, weight-of-evidence is the use of "several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property, while information from each single source alone is regarded insufficient to support this notion".

As described above, the two concepts weight-of-evidence and read-across are separated in the REACH regulation. However, in the guidance on how to avoid unnecessary testing of animals[23], read-across is regarded as one piece of information in the weight-of-evidence approach. While information from the other sources (published literature, (Q)SAR predictions, data from existing studies, and in-vitro studies) are related to the specific target compound and also includes less reliable information for this substance, the read-across approach makes information on other substances for evaluation of the target compound available. Therefore, the read-across approach, although considered to be a part of the weight-of-evidence approach, has specific advantages as it takes more information into account and tries to derive the required value based on a hypothesis using available data for several source compounds that share chemical as well activity related similarities to the target compound.

The differences between weight-of-evidence and read-across are discussed in the following. In a weight-of-evidence approach for acute fish toxicity, several LC_{50} data all with restrictions for the target compound were used to conclude on a specific LC_{50} value and though become equivalent to the result of a guideline study. In contrast, the read-across approach is ideally based on at least some reliable data from the source compounds, takes into account model predictions and has a good hypothesis to conclude on the possible effects from the target compound. Therefore, this data can be considered more reliable compared the one derived in the former case. However, as ECHA guidance defines the weight of evidence approach broader including read-across as one piece of information, data from read-across can be considered as one reliable part for the building of a weight-of-evidence case. If weight-of-evidence cases do not take into account read-across information, this valuable source of information is lacking. For these cases, there may be a need for additional documentation and assessment of the information used.

7 Summary and conclusion

This project aims to develop a best "practice guidance" for the read-across assessment of environmental endpoints such as acute and chronic fish toxicity or accumulation in fish. This guidance may help to improve the mutual acceptance of read-across approaches between applicants and authorities. The development of the best practice guidance is based on three different steps. First, the state of the art was evaluated by reviewing relevant literature and publications from recent projects. Within an online survey with stakeholders experiences with read-across were documented and its current limitations were identify. Based on the results of the interim report three main questions were identified:

- Topic 1- Similarity: a key requirement of read-across
- Topic 2- Uncertainty in read-across approaches
- Topic 3- Use of tools and databases for read-across

Stakeholders from industry, academia and authorities discussed these three topics within a 1.5 day workshop and prioritized the obtained results (section II, status report).

[23] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/weight-of-evidence

The most important finding of the literature review is a difference between scientific perception and practical ECHA decisions. Evaluation of currently available ECHA decisions indicate that the read-across rejections are mainly based on a lack of sufficient or suitable endpoint study data (for source compounds or relevant metabolites), scientific plausibility and lack of identity data for the tar-get compound e.g. in case of UVCBs (Ball et al. 2016). Rejection based on scientific plausibility includes many cases in which data on toxicokinetics e.g. metabolites were missing. Chemical similarity con-cerns appear to be of no to minor relevance and uncertainty refers mainly to the lack of data but does not arise from a matrix of variables that can be classified by low to high as proposed by Schultz et al. (2015) or Blackburn et al. (2015).

Surprisingly, more read-across than experimental studies have been submitted for the endpoints bio-accumulation and long-term, toxicity to fish. This finding leads to categories with a number of chemi-cals that can be assessed by a few experimental studies. It can therefore be concluded that the future challenge for acceptance of read-across are neither the conceptual challenges of the workflow such as chemical similarity, quality of data, uncertainty and plausibility but the quality of submitted data in terms of sufficient experimental studies or suitable chemical identity data.

The questionnaire as well as evaluation of literature and ECHA decisions suggest that the principle workflow od a read-across assessment as well as its documentation requirements are rather well es-tablished. The acceptance criteria and subsequently the quality requirements towards a read-across approach, however, need to be improved. It can be assumed that an increased communication of ECHA decisions may represent an expedient approach to increase the acceptance by transparently providing criteria for acceptance on a case by case basis. Communication in this context mainly comprise a gen-eration of a database on case examples and ECHA decisions as well as the organization of training courses and workshops. Provision of additional guidance, for example, on specific endpoints as well as improvement of uncertainty assessment were considered as useful while provision of more templates were considered as less useful, as they may inappropriately restrict the flexibility of case by case as-sessments.

The main outcomes of the workshop are summarized in section II (status report). Many different as-pects were noted by the participants of the workshop and only main findings are summarized in the following.

Read-across is case and endpoint specific

Chemical similarity is a good start point for similarity assessment. Automatic workflows e.g. including visualization tools possible.

Chemical similarity alone is not “good enough”. Several other aspects need to be considered such as mechanistic properties, stability, fate etc. (see detailed discussion under topic 1: Content ID 2). The definition of hard criteria is difficult as these are endpoint specific and probably also

A well defined minimal set of criteria would be helpful to build reliable read-across scenarios. These minimal data requirements shall ideally be provided by authorities.

Expert judgement is seen as key input in the evaluation of the different types of evidences to conclude on a read-across case study. This step can not be automated.

Tools and illustrative case study example are missing. Case studies will ideally be provided by authori-ties.

Read-across assessment needs to be transparent – e.g. always following the same evaluation criteria per endpoint

Read-across needs to be supported by adequate and reliable documentation.

Uncertainty has to be addressed, which may arise from several steps of the read-across procedure e.g.:

- data gaps in the data matrix
- assessment of data quality (general risk assessment procedure, not read –across specific)
- assessment of relevance of the used ecotoxicological data for the predicted endpoint
- applicability domain of the read-across prediction - needs to be clarified with regard to the structural and ecotoxicological properties of source and target compounds.

An acceptable read-across has to be adequate for classification and labelling and or risk assessment"

The participants noted that read-across is endpoint and case specific. Therefore, it is difficult to develop clear-cut, hard criteria that defines from which threshold on a certain decision is acceptable or not acceptable e.g. by taking into account different substance classes and different endpoints.

It was, however, possible to derive a general read-across workflow that includes the principle assessment elements for ecotoxicological endpoints. This workflow is depicted. Comparable to the human RAAF, chemical similarity is a good starting point to define source compounds. But chemical similarity is not sufficient and toxicological data have to be considered to conclude on relevant source compounds e.g. mechanistic properties, stability, fate etc. . As a definition of hard acceptance criteria might not be possible, the participants asked for minimal data requirements per endpoint. These data requirements should be ideally provided by authorities and supported by illustrative case studies. Illustrative case studies were considered to be a very valuable source of guidance and would support the understanding of acceptance criteria and by this improve the quality of read-across dossiers. The participants were not able to define thresholds and in- and exclusion criteria per assessment elements. These criteria are nonetheless needed, and should be part of follow up evaluations with stakeholders in this area.

For the endpoints acute and chronic fish toxicity and bioaccumulation in fish workflows have been described. In a first step the target compound has to characterized, followed the identification of similar source compounds. Besides chemical similarity also similarities with regard to physico-chemical properties or mode of action should be considered. For the source compounds experimental data for the specific endpoint are collected as well as information on other endpoints and model predictions. Finally a read-across hypothesis is formulated and a specific value like LC_{50} is defined to be used to fill the data gap.

It turned out that some steps are identical, e.g. identification of source compounds while others like additional information on physico-chemical parameters depend on the endpoint to be addressed.

Read-across is regarded as a flexible tool. There are no general definition on minimal number of source substances needed for a prediction. As read-across has to be assessed on a case-by-case basis, no clear criteria can be formulated for acceptance of such approaches. Regulatory practice will give indication on which cases are accepted and which do not. To increase transparency, it is recommended that non-acceptance of read-across approaches should be justified and such cases should also be made public. The uncertainty has to be considered individually for each read-across case. For read-across case where nearly all information is available and the information is in line with the predicted hypothesis, the uncertainty might be regarded as low.

Where only few source compounds are available, more information on each source compound should be available and/or information should be of higher quality. What differences should be allowed to regard the data has been acceptable to be used for read-across, is another point of discussion with stakeholder. However, even in the OECD testing guideline the hard criteria for these parameters are often not defined. Therefore, it is difficult to define minimal data requirements that should be available to consider data on source substances as suitable. Here more guidance or best-practice examples are needed to get a better understanding on the boundaries of the read-across concept. This need has also been addressed by Schultz & Cronin (2017) as well as OECD (2016). Read-across approaches have

however advantages over pure weight-of-evidence approaches that focus only on information available for the target compound as it takes into account more information from source compounds and uses it in a structured manner.

On the other hand, too strict definitions and guidance might limit the use of read-across approaches and registrants will not invest the needed time and resources if the outcome is questionable. Under these conditions registrants might chose the option to perform the required experimental study or, in cases where a test proposal is required, wait for ECHAs decision to perform the test.

Besides the best-practice example, the use of read-across could be facilitated by making templates available on how to document the read-across cases. Ideally, an electronic tool might become available that guides the user to the different steps of a read-across and makes uses of all the different tools that are available for specific steps.

Two hypothetical examples illustrate the use of the proposed general workflow for acute aquatic toxicity, one for a category approach and the other for an on-to-on read-across. In the category approach emphasize is laid on the refinement of the list of source compounds based on the evaluation of physico-chemical properties. For the one-to-one read across, although chemical similarity is high a read-across hypothesis can not be established because for specific mode of action considerations for the target compound that is not the case for the source compound. These two example may help users to set up their own read-across case and draw their attention to different critical step when setting up their own hypothesis. However, as addressed several times in the report read-across is endpoint specific and has to be discussed on a case-by case basis these two examples can not addressed all critical point. After getting more information on accepted and non-accepted read-across approaches e.g. by ECHA, additional point may be incorporated in the specific guidance.

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