Experts Workshop on Read-Across Assessment
Organised by ECHA with the active support from Cefic-LRI (October 3, 2012)
Use of "read-across" for Chemical Safety Assessment under REACH

Workshop Report

Abstract Summary

An Experts Workshop on Read-Across Assessment organised by ECHA with the active support from Cefic-LRI was held on the 3rd October 2012. The aims of the Workshop were threefold; to explore Industry experiences with "read-across approaches" to date, to try to reach common understanding to characterise scientifically valid "read-across" are and to provide insight in ECHA's rationale for assessing read-across proposals, the so-called RAAF.
Overall the workshop was successful in its aims. The forum provided an opportunity for direct interaction between different interested stakeholders and thus enabled a useful exchange to clarify many misconceptions and highlight existing opportunities. The RAAF was viewed as a useful construct not only for the evaluation of read-across proposals but also for their development. Experience from ECHA so far has been that many of the submitted REACH dossiers with read-across leave substantial room for improvement. The workshop helped to identify what improvements could be made. The discussions helped to clarify and address some of the difficulties raised in the use and acceptance of read-across.
Both ECHA and Cefic LRI expressed a willingness to continue cooperation on read-across. ECHA considered LRI as providing a good platform to address research and information needs for read-across. In the near future, illustrative examples for broad public access could be prepared.

Introduction

Read-across has garnered much attention, since it may be used as an alternative approach for addressing the information requirements under REACH. Annex XI, Section 1.5 of the REACH Regulation presents "the rules for adaptation of the standard testing regime set out in Annexes VII to X" based on "The grouping of substances and the read-across approach". Specifically, the results should be 'adequate' for classification and labelling and/or risk assessment, have 'adequate and reliable' coverage of the key parameters as in the corresponding test method, cover a comparable or longer exposure duration, and there must be 'adequate and reliable' documentation.

Analogue and category approaches

Per the ECHA technical guidance, the terms category approach and analogue approach

are used to describe techniques for grouping substances, whilst the term read-across refers to the process of data gap filling in either approach (ECHA, 2008).

In an analogue approach, comparisons are made between a very limited number of substances. Endpoint information on the source substance(s) is used to predict the same endpoint for the target substance, which is considered to be 'similar' in some way (usually on the basis of structural similarity and similar properties and/or activities). Potential source substances need to be reasonably data-rich from which comparisons can be made.

A chemical category is a group of substances whose physico-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity. In principle, more members are generally present in a chemical category, enabling the evaluation of trends within endpoints. As the number of possible substances being grouped into a category increases, the potential for developing hypotheses for specific endpoints and making generalisations about the trends within the category will also increase, thus the evaluation of the trend(s) would be expected to be more robust. Obviously the quality of the available experimental data contributes to the robustness of any read-across. Common behaviour, or consistent trends are generally associated with a common underlying mode of action. Read-across within a category is contingent on a convincing mechanistic explanation for why category membership goes with a certain predictive value for the endpoint under consideration and/or if trends are actually observed which clearly point to the possibility of prediction.

Background to the Workshop

Experience in the application of "read-across" has undoubtedly been gained within the context of the 2010 registrations (>1,000 tonnes/annum). There has been a need to share, evaluate and build upon the insights gained in anticipation of the greater opportunity or indeed necessity for application of "read-across" for 2013 registrations (>100 tonnes/annum) and beyond. Industry, ECHA and EU Member States all conceptually accept read-across approaches but difficulties still remain in applying them consistently in practice.

Efforts have been undertaken by both Industry and ECHA to identify and overcome some of the issues related to the acceptance of read-across approaches. Industry through ECETOC established a Task Force to prepare a Technical Report on read-across approaches (see http://www.ecetoc.org/publications). At the same time, ECHA were developing a systematic approach to facilitate their internal evaluation of read-across, the so-called Read-across Assessment Framework (RAAF). Cefic LRI and ECHA initiated a discussion to bring together Industry, ECHA and Member States (MS) in a workshop environment to exchange experiences in developing and evaluating read-across.

A workshop on the 'Use of Read-Across for Chemical Safety Assessment under REACH', organised by ECHA was held at ECHA's Conference Centre in Helsinki on 2nd and 3rd October 2012. The first day, a closed session, provided an opportunity for ECHA, the European Commission and MS to discuss and exchange views on ECHA's Read-Across Assessment Framework (RAAF), which is still under development. The RAAF is a tiered systematic approach, developed by ECHA to facilitate its internal evaluation of read-across. The second day, organised with the active support of Cefic LRI, aimed to bring together scientists representing a number of different stakeholders to gain insight on ECHA's RAAF, to share Industry experiences with read-across approaches in order to frame a discussion of what constitutes scientifically valid read-across. Over 100 participants representing amongst others ECHA, MS, Commission, OECD, Academia, NGO and Industry attended Day 2 of which some 40 participants were invited by Cefic LRI. This report authored by Cefic LRI aims to summarise the Workshop's main discussions.

Structure of the Workshop

The agenda for the second day of the workshop is available on the ECHA website see: http://echa.europa.eu/documents/10162/5649897/ws_raa_20121003_agenda_en.pdf

A set of background information was compiled and made available to participants 2-3 weeks before the workshop. This material comprised a thought-starter document extracted from the ECETOC Technical Report on read-across approaches (published 21 November see http://www.ecetoc.org/publications), a background paper from ECHA

summarising the read-across assessment framework (RAAF) in addition to the presentations and a set of Charge questions. The background information with exception to the charge questions is also available on the ECHA website http://echa.europa.eu/en/web/guest/view-article/-/journal_content/c6dd5b17-7079-433a-b57f-75da9bcb1de2.

The workshop started with an overview of the RAAF given by Karel de Raat, followed by a synopsis of the discussions from the previous day. Three case studies presented by Industry were then given which reflected the experiences from 3 different Industry sectors. After this, participants were convened into one of 4 break-out groups, to discuss a set of predefined questions ("Charge questions"). The background information coupled with the presentations given during the first part of the day was intended to help shape and frame these break-out discussions. The charge questions were largely drafted based on issues identified during the course of the development of the ECETOC Technical report on read-across approaches. These three questions were then refined in consultation with the workshop chairs and rapporteurs before being presented to ECHA for discussion and final agreement. Given the time limitations, all break-out groups discussed 2 of the 3 Questions. Elements of one of the questions were pre-assigned to respective break-out groups in advance of the workshop. After the break-out groups had reported back to the full session through their rapporteurs, with ensuing discussion, concluding remarks were made by ECHA (Wim de Coen and Mike Rasenberg) and by Cefic LRI OC (Bruno Hubesch and Grace Patlewicz).

The RAAF

ECHA is in the process of developing a framework for the assessment of read across cases to be used in dossier-evaluation work: 'The Read-Across Assessment Framework', or RAAF is meant to present a structured 'tool' for the assessment of read-across cases by ECHA evaluators. It is not meant to serve as guidance for Industry, although knowledge about how read-across cases are examined by ECHA could likely help a registrant improve the quality of their registration dossiers. In its current form, the RAAF covers only toxicity studies for human health endpoints.

The REACH guidance distinguishes two types of read-across: analogue-approach read-across and grouping/category-approach read-across. The first type is concerned with read-across between two or amongst a few analogues, the second type involves a larger group of substances and is supported by regular patterns in this group for the endpoint that has to be read-across. The RAAF is meant to cover both, analogue approach and grouping/category approach read-across. (The broader approach to chemical categories or grouping used in some other regulatory schemes or for other purposes should not be confused with the specific purpose for REACH information requirements as examined in the RAAF.)

The RAAF comprises a two-tiered assessment scheme. Tier I, which is relatively well

developed, leads the evaluator through a series of key questions about the nature and the occurrence of read-across cases in a registration dossier, the compliance of these cases with the legal text and the guidance, the presentation of the cases and their basic scientific quality. Depending on the answers to these questions, the outcome of Tier I can be that a read-across case is:

- set aside as not necessary to assess;
- rejected;
- accepted on grounds that are immediately evident and allow for the highest level of confidence (i.e. cases that are self-evident and obviously satisfactory);
- passed for further evaluation to the next tier (i.e. Tier II), when the answers from the Tier I questions fail to result in setting aside, rejecting or accepting the read-across case.

After the clear cases are 'filtered out' during Tier I, the remaining read-across cases are examined in Tier II of the RAAF. Tier II which is still in development, offers a structured framework to facilitate consistent, explicit and transparent expert judgment of read-across cases. Tier II also accounts for the fact that read-across may be associated with different levels of 'confidence' hence uncertainty from the read-across test result on the hazard assessment of the target substance may be a consideration. Tier II starts with establishing what basic type of read-across is being proposed, (i.e. the read-across hypothesis or rationale). There are a finite number of explanation types and each of these may be characterised by a set of specific aspects referred to as key aspects. These key aspects are examined in turn to determine the overall credibility and reliability of the read-across.

An example of a basic read-across type is "identical toxicants through biotransformation"; key aspects for this basic type may comprise considerations such as: Formation of common products that may cause toxic effects; Formation of different non-toxic compounds; Existence and influence of other (bio)transformation pathways; Influence of distribution and exposure and Toxicity of intermediates and parent compounds.

Industry case studies

Three case studies were presented from Industry highlighting some of the experiences from different sectors. These presentations are summarised in brief.

Incorporation of metabolism into guideline studies – an opportunity to increase robustness of read across approaches? Use of metabolism 'Add-on' in practice: A Case study

This was presented by Nicholas Ball of Dow. He discussed the relative merits of utilising ADME information in read-across justifications. Read-across justifications for repeated dose toxicity endpoints are challenging to construct and subject to uncertainty. ADME information is well recognised as being useful in substantiating a read-across approach

but ADME studies themselves require animals, are expensive, technically challenging and lengthy to run. Furthermore ADME information is not a requirement per se under REACH hence only available ADME information needs to be submitted as part of a registration dossier.
Dr Ball put forward a proposal where ADME information could be conceivably generated as part of a range finding study i.e. a pragmatic means of generating supporting information that would be sufficient to substantiate a read-across hypothesis without the need for additional animals. As a case study to illustrate the approach, Dr Ball discussed a 100-1000 tpa substance, Di-EPh that is due for registration in 2013. Minimal data were available for Di-EPh itself but EPh, a structurally related analogue was associated with a complete dataset (REACH Annex VII-X and beyond – including ADME). An investigation to determine whether EPh was an appropriate analogue for read-across was hence undertaken.
Expert judgement hypothesised that Di-EPh would metabolise to an acid metabolite by one of two pathways; either via EPh, or directly from Di-EPh. Structurally similar substances were known to exhibit the following order of toxicity: Tri<Di<Mono. On this basis, the target substance Di-EPh was expected to be less toxic than the source substance, EPh.

Annexes VII and VIII studies were performed including OECD 422, the combined repeated dose toxicity and repro/developmental toxicity screening protocol. The latter was modified to include toxicokinetics. The results provided a measure of bioavailability, demonstrated no difference in target organs, substantiated the expected order of toxicity and confirmed the hypothesised metabolic pathway. The findings were used in support of a read-across for sub-chronic and developmental toxicity where the read-across probably represented a 'conservative' assessment.
The case study illustrated how ADME information could be pragmatically generated in support of a read-across without the need for additional animal testing. Dr Ball concluded with some summary remarks regarding the benefits and pitfalls of the read-across approach.

Read across strategy for molybdenum compounds. A case study
This study presented by Dr Silvia Jacobi, Albemarle was given on behalf of the Molybdenum REACH consortium under ECETOC. It concerned 11 substances based on molybdenum that were registered in 2010. Chemically molybdenum has a range of readily interconvertible oxidation states, able to form complexes with many inorganic and organic ligands including physiologically important compounds (Mitchell, 2003). The 11 substances of interest were investigated to determine whether read-across could be applied. Principles are already established for undertaking read-across for metal compounds.

Minimal data were collected as outlined by these principles. Water solubility with speciation of the dissolved species, *in vitro* bioaccessibility studies in various

physiological fluids were also carried out. It was found that upon dissolution in aqueous solutions at physiologically relevant concentrations and pH conditions, the only aqueous molybdenum species emerging from all the investigated molybdenum substances was the molybdate $[MoO_4]^{2-}$ anion. Therefore, for systemic toxicity, read-across between all of the molybdenum substances seemed generally justified, and highly soluble molybdates were proposed as appropriate source chemicals. For poorly soluble molybdates, read-across from the highly soluble/highly bioavailable molybdates was likely to constitute a conservative overestimate. Additional *in vivo* kinetic data and an *in vitro* dermal absorption study, confirmed the molybdate anion as the only relevant species. For the less soluble compounds, dissolution kinetics in the relevant media was considered a factor modifying the release of molybdate ions. For molybdenum compounds, a large database of published information on toxicokinetics both in humans and to a lesser extent in animals is available that was carefully evaluated for this read-across approach. These studies revealed that the molybdenum substances studied could be divided into two groups for read-across:

- Highly and moderately bioaccessible molybdenum compounds.

- Poorly available molybdenum compounds, having water solubility well below 10 mg/L, accessibility in physiological media: < 10%.

There was one exception; local inflammatory changes by molybdenum trioxide and species that could liberate H_3O^+. These formed a sub-group of the highly bioaccessible set, that were considered separately for their long term local effects (suspected carcinogenicity) by inhalation.

For those endpoints where no experimental data were available, experimental data from soluble compounds was used to read-across for the other substances. In this way, dossiers were produced for all 11 substances covering: acute toxicity (oral and dermal); irritation and sensitisation; genotoxicity and carcinogenicity, repeat dose and reproductive toxicity; environmental toxicity.

Building a category for the registration of higher methacrylate esters under REACH. A case study

Insights derived over a number of years of working on lower alkyl methacrylates (methyl to 2-ethylhexyl esters of methacrylic acid) lay the foundation for the approach devised for building categories for, and read-across between, some 50+ higher alkyl methacrylate esters. Dr Mark Pemberton of Systox Ltd showcased a stepwise approach that was devised to systematically yet pragmatically group these higher alkyl esters whilst taking into account their different tonnage bands, and datasets. The sequential approach began with a clustering of the esters based upon their chemical structure and properties. The initial clustering identified a number of sub-categories for which SAR

trends were anticipated. For each sub-category in turn, a documented rationale for the grouping was developed and populated with the available substance specific data. Obvious trends in data were described for each endpoint and the data fitted with the aid of the OECD Toolbox. Testing for sensitivity within the groups and for opportunities to broaden the groups by inclusion of other structurally related esters was performed. Physicochemical properties that appeared to be responsible, or at least contribute to, the observed trends were identified and tentative scientific rationale (Modes of Action) proposed. Toxicity alerts for known metabolites were recognised and addressed in the hazard evaluation leading to an overall assessment of data adequacy and confidence in the predictions.

Break-out group discussion

The responses to the charge questions were taken from each of the break-out groups and synthesised together to remove redundancy. In general, questions that were common to all four groups were in good concordance. Discussions often overlapped between the different questions demonstrating the complexity and interconnectivity of the considerations around how to develop and justify read-across in practice.

Reponses to charge questions

Question 1 How does the RAAF practically impact the development of the read-across justification and guide the building of a read-across case in a new/different way?

The RAAF is a framework still in development, intended for internal use by ECHA to facilitate consistent assessment of read-across and currently concerned with mammalian toxicity endpoints. The RAAF was not started as guidance for Industry and is not ready to be published in its current form, however components of it could conceivably be taken up in future guidance as and if appropriate.
Notwithstanding these caveats, there was consensus amongst the four break-out groups that there was great value in exploiting the RAAF as a means of developing consistent and robust read-across justifications. The RAAF could serve to assist in framing the nature of the extent of the read-across rationale.

The basic types of read-across (as least those cited in the background paper) mimicked those outlined in Annex IX section 1.5 albeit these were described more explicitly for analogue and category approaches.

The 'key aspects' illustrated in the 2 examples in the background document had value in characterising the explicit principles that were essential in assessing validity of a read-across. There was agreement that whilst these key aspects are to an extent context dependent, it was recognised that understanding their identity would be of immediate

practical value to Industry registrants. Another consideration associated with the key aspects were confidence levels and if so how were these qualified/quantified.

There was also a recognition that some of the terminology surrounding the RAAF and its assumptions would need to be made explicit in the form of a glossary. For instance, whilst read-across is accepted to be endpoint specific, during the course of the discussions it was used in a sense of predicting study type, as in "prediction of the test result".

There was wide agreement that illustrative case studies would be invaluable to help Industry benchmark their own read-across justifications against the available guidance. Current guidance presents the theoretical framework of the workflow to follow and the template to complete to document the justification but there are no complete examples in the guidance to gauge sufficiency. Current feedback from ECHA typically focuses on weakness and deficiencies of read-across cases and is usually restricted to the draft decision letters sent for submissions undergoing a compliance check. There is no mechanism for communicating dossiers that pass such checks. There are good reasons for this; the regulation is structured to target "bad cases"; resources are limited and ECHA has to follow a set process. On the other hand communicating good practices would serve to promote consistency in the other 95% of dossiers that are not subject to a compliance check.

General feedback is communicated through ECHA's Annual Evaluations Reports. These reports aim to provide advice on the justifications for read-across though in practice they tend to focus more on the deficiencies observed. A suggestion was made to use elements of the RAAF to identify specific issues/areas for improvement and communicate those in the Evaluation Report. Further opportunities for collecting and exchanging experiences between ECHA, MS and Industry need to be exploited. One suggestion was to test out the RAAF against a selection of the case studies from the ECETOC report, another to use existing examples such as the OECD categories, or perhaps Industry cases that were appropriately anonymised – these could serve as the basis of illustrative case studies to help communicate good practices.

Several clarifications resulted as part of the discussion. The draft decision letter actually provides an opportunity for informal dialogue with ECHA and does not necessarily mandate conducting testing as prescribed in the Annexes which was an impression perceived by some of the Industry participants. A draft decision may be based on ECHA's conclusion that a read-across as proposed is not acceptable. This will often mean that the data requirement has been deemed as not (adequately) met. The default requirement is the performance of the standard test with the registered substance. However, the Registrant may find ways to strengthen their read-across proposal to a level of confidence acceptable to the assessing experts of ECHA or to fill the data gap in another acceptable alternative manner. Thus, a read-across could be conceivably substantiated by reference to modeled data such as that which is simulated within the

OECD Toolbox and need not be restricted to empirical experimental data alone although some experimental supporting information is typically required.

Elements of a successful read-across comprised a clear substantiated hypothesis coupled with as much explicit reasoning and evidence (using (Q)SAR models, experimental data) as possible even for aspects that appear to be obvious. A read-across justification that was systematically structured and presented in an easy to read format was more likely to be accepted as the reasoning could be readily followed.

Question 2a. Are there different read-across approaches needed for identifying toxicity versus its absence? What sort of evidence will be needed to be incorporated in the read-across justification if the test result to be read across is negative? What sort of evidence will be needed for interpolation versus extrapolation read-across?
This question was addressed by two of the break-out groups.

Identifying toxicity versus identifying its absence What sort of evidence will be needed to be incorporated in the read-across justification if the test result to be read across is negative?

The precautionary principle, implemented in the REACH regulation, creates the impression that the burden of proof is greater for the absence of toxicity versus its presence even though the same scientific principles and rigour should be applied, i.e. a robust hypothesis incorporating information regarding similar chemical structure in relation to MOA and metabolism. Thus there is no mandate for any new or different read-across approaches for identification of toxicity versus its absence as such though confidence in the outcome may differ depending on the endpoint of concern. Nonetheless predictions of toxicity using read-across are likely to be more readily accepted than predictions demonstrating an absence of toxicity. Category approaches where the target substance is clearly part of the structural domain that has been tested are likely to provide reasonable evidence supporting the read-across than the scenario where the read-across is derived within an analogue approach.
A relevant issue here is whether the argument for absence of toxicity is based on the chemical in question being part of the tested negative structural domain or simply it not being part of the tested positive structural domain. The former case is clearly much stronger than the latter. For acceptance, an absence of toxicity prediction requires proof of the chemical in question being part of the tested structural domain for a relevant alternative assay or method and being transparently classifiable, on the basis of its structure and/or physico-chemical properties, in the sub-domain populated by non-toxic chemicals. For long-term toxicity endpoints absence of toxicity could be strengthened by PBPK modelling.

What sort of evidence will be needed for interpolation versus extrapolation read across?

It is intuitive that confidence in the read-across prediction is enhanced when experimental data for structural analogues allows for interpolation rather than extrapolation. For analogue approaches the interpolation/extrapolation distinction is perhaps less meaningful. An analogue approach by default is an extrapolation since the target chemical compared to the source either possesses the toxicity-determining features to a lesser degree (target predicted less potent than source) or a greater degree (target predicted more potent than source). To date, analogue approaches have been structured using many lines of evidence ((Q)SAR, *in vitro* etc) to justify the robustness and validity of the read-across i.e. a weight of evidence basis.

A recommendation proposed by one of the break-out groups was to clarify the terminology presented in the REACH legal text against that which is described in the Technical Guidance. The legal text expressly stipulates interpolation ("Application of the group concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for source substance(s) within the group by interpolation to other substances in the group (read-across approach)" yet the Technical guidance describes both interpolation and extrapolation within categories as viable approaches to undertaking read-across.

Question 2b. Are 'category' or 'analogue' strategies different in terms of justification? What are the advantages and disadvantages of these approaches? What are the practical hurdles/solutions to optimal utilisation given the current REACH framework?

This was addressed by two of the break-out groups.

Are 'category' or 'analogue' strategies different in terms of justification? What are the advantages and disadvantages of these approaches?

Category and analogue strategies per se do not differ intrinsically in terms of scientific justification, but they can differ in weight of evidence and grounds for confidence. Usually there are more grounds for confidence in a negative prediction from the category approach than from analogue approach – this is because although the approaches are similar the read-across is based on toxicity data from more compounds. However, exceptions in a group are possible. This can depend on the read-across hypothesis, for the scenario where the read-across is due to the biotransformation of a parent to a metabolite, an analogue approach can work particularly well. Note that is not to say that for other read-across types a category is always required to become convinced that read-across is possible.

For the analogue approach, there is more confidence if both source and target substances are co-members of categories for other endpoints, as shown conceptually in

Table 1 i.e. where there is demonstrated consistency not only in the trend within an endpoint but across other endpoints.

Table 1. Analogue approach where source and target compound are co-members of categories for other endpoints.

	A	B	C	D	E
Physico-chemical properties	X	X	X	X	X
Toxicokinetics Bioavailability	X	X	X	X	X
28 day study	X	X	X	X	X
Screening study	X	X	X	X	X
Developmental toxicity	X->	read-across			X (boundaries)
2 generation study	X->	read-across			

A, B, C, D, E represent 5 substances in Table 1. They are all members of the same category for the first 4 endpoints. For the 5th endpoint, developmental toxicity, there are data only for A and E; for the 6th endpoint, the 2 generation study, there are only data for A. Since B is a co-member with A for the other endpoints, this suggests that a read-across between A and B may be potentially viable for these 2 endpoints. In other words,

since source A and target B appear to exhibit the same outcome for a broad range of endpoints, there is an expectation that they may behave similarly for the endpoints where data gaps remain for B. This conceptual illustration obviously belies the complexity of the read-across and depends very much on the read-across type, the endpoints involved as well as the substances themselves. It does not negate the need to provide a credible scientific explanation for relating A and B for these endpoints or the supporting evidence. Nonetheless the data matrix offers a useful construct to start to explore endpoint trends for the category members.

What are the practical hurdles/solutions to optimal utilisation given the current REACH framework?

There are both scientific and practical hurdles in undertaking analogue or category approaches. The practical hurdles are largely cost and procedural based - gaining legitimate access to good quality data that is required for the read-across approach, populating the dossier with the appropriate level of study information, and having the necessary information to characterise the target substance and source analogues as well as their respective impurity profiles.

The scientific hurdles concern the knowledge of the presumed MOA driving the endpoint(s) under consideration. This informs the link between the chemical similarity and the grouping hypothesis in first place. Some of these MOA are better established for the simpler endpoints and encoded in (Q)SAR models or in tools such as the OECD Toolbox. For other endpoints, the lack of an adequate mechanistic understanding is the major hurdle. Toxicokinetic and ADME data will certainly help to substantiate a read-across where such information is not available or poorly understood since it will at least provide some insight about which tissues/organs are exposed as well as the kinetics of this exposure. Knowledge of the mechanism of action at the molecular level may provide greater confidence in a read-across estimate but this is not always available. The AOP (Adverse Outcome Pathway) concept, as it develops, may help in the future. Activities are in place for AOPs to be developed, evaluated and populated with *in vitro*, toxicogenomics, high throughput (HTP) assay data. Building a matrix as that shown in Table 1 is a useful solution where possible. Even if a MOA for an endpoint is poorly understood, demonstrating consistency in the hazard profile across and within endpoints can provide confidence in the validity of a read-across.

Question 2c. What considerations would help characterise applicability domains/boundaries of chemical groups/categories? Is the concept applicable in the "analogue" approach? How to define chemical (based on chemical structure alone) vs. biological (e.g. based on mechanism of action and other toxicological data) boundaries?
This was addressed by two of the break-out groups.

The same types of considerations for applicability domains and categories apply for

read-across as for QSAR. The topic was reviewed comprehensively in the QSAR context during the development of the original (Q)SAR validation principles as part of the ICCA-Cefic LRI workshop in 2002 as well as an ECVAM workshop held in 2005 (Netzeva et al, ATLA, 2005). Ideally, two or more chemicals could be assigned to the same domain if they have in common not only (a) functional group(s) but also the physico-chemical properties (e.g. chemical reactivity by a particular reaction mechanism, hydrophobicity in a particular range, a structural feature enabling binding to a particular receptor) required for activity in a particular endpoint. If this is possible, their relative potencies, at least in terms of qualitative ranking, may be estimated by consideration of the degree to which they possess the appropriate physico-chemical properties. When this ideal situation applies, mechanism-based QSARs or QMMs can be developed (e.g. for the S_NAr, Schiff base and Michael acceptor domains in skin sensitisation; see Aptula et al, 2005 for further discussion) or, where there are insufficient data, mechanism-based read-across can be applied. In this case similarity between target and source substance can be based on similarity in the physico-chemical parameters determining activity (Roberts et al, 2007). This concept is applicable to both the analogue and the category approach. If this is the case, potential non-linearities should also be considered.

However, the above ideal situation often does not apply, and other criteria for similarity have to be applied. Agreement on what is chemical similarity is lacking since it is not an absolute concept. The identity of a chemical is defined by a variety of factors and there are no simple similarity scale(s). In the extreme case, each chemical is its own category but that is not practical for read-across neither are simple organic chemical classes (e.g. alcohols, ketones, benzenes, etc.). What we do know is that similarity hypothesis can be based on a variety of chemistries including: 1) Common functional group, 2) Common bio-modification, 3) Constant pattern of changing a property across the group, 4) Common chemical reaction and 5) 2D molecular similarity. In some cases 3D similarity may be a consideration. Experience has showed that these methods of assessing similarity are not equal in attaining a chemical category for toxicological read-across. Under REACH of course, the starting point for the definition of a category has to be on the basis of chemical structure. Which structural elements the substances may have in common and what differences in structure are permitted will be characterised by integrating the variety of chemistries discussed above. By the same token, structural characteristics, which are not permitted e.g. known exclusions, are of similar importance.

Read-across based on purely statistical similarity (i.e. 2D similarity, derived as fingerprints and expressed statistically via different functions as a common part between two molecules) has a greater uncertainty because chemicals which are 'similar' in that respect can be dissimilar in terms of toxicity, including both the ability to elicit a particular hazard, as well as potency within that hazard. This can increase the uncertainty hence additional evidence is needed to increase the robustness of the read-across. A matrix of the type shown in Table 1 may be helpful in providing such

supporting evidence in addition to information on commonality in reaction chemistry and biotransformation (see earlier discussion).

Clearly stating and justifying the basis for the analogue/category formation (i.e. the similarity hypothesis) is an essential component to gaining acceptance for a read-across prediction. The analogue approach based on close structural similarity is likely to be most routinely applied for the data gap filling of long term effects.

Question 2d. How can read-across best be used in the context of test specific information requirements as specified in the Annexes VII to X of REACH? How should uncertainty inherent to read across approach be managed? How can one evaluate levels of uncertainty (qualitative/quantitative)? What levels are acceptable? Are additional assessment/uncertainty factors an appropriate option and if yes when and how?
This was addressed by two of the break-out groups.

Issues that Impact Uncertainty in Read-Across

There are a number of issues that impact the uncertainty and thereby the acceptance of a read-across prediction because they address adequacy and/or reliability.

The first issue is the *in vivo* data used in the read-across. Read-across typically requires good quality experimental *in vivo* data for the regulatory endpoint of concern. There are different sources of data/information (legacy data, standard test guideline data, GLP data, etc.). The amount and quality of the data used in the read-across is likely to impact uncertainty and acceptance. It is intuitive that a read-across based on many data generated with a standard test guideline and GLP leads to less uncertainty than a read-across based on one or two legacy data points. Therefore, a clear and detailed explanation of the origin of the data will aid in assessing the uncertainty associated with the *in vivo* data.

Read-across predictions are likely to be developed for long-term health effects. Since these endpoints are data-poor and the mode of action not necessarily well understood, the analogue approach might be the preferable one. Moreover, since the tests are long term (28-days or longer), the metabolism is likely to be of consideration. The current state of metabolism predictions is such that information on transformation products and the rate of formation of these products is likely to be a key factor in accepting the read-across. Thus, information derived from experimental studies is likely to be required to justify the read-across. Similarly, toxicokinetic information and ADME information will aid in supporting the read-across.

The second issue is the chemical similarity of which the analogue or category is based, as discussed in Question 2c.

The third issue is the weight of evidence supporting the categorisation scheme employed. The goal is to use a categorisation schemes for read-across that clearly group chemicals into a toxicologically meaningful category supported by ancillary evidence,

often in the form of *in vitro* test results or other lower tier tests. Experience has shown that such categories to be truly useful in read-across must be endpoint specific. The most acceptable categories for read-across are those, which are data rich, and are based on integrating knowledge on how chemicals interact with biological systems with knowledge of the biological response(s) once compensatory systems are overcome (i.e. mechanistic information). Key elements to such a high quality chemical category include: 1) Showing in a scientifically convincing manner why the chemical category is a good one and 2) Providing the necessary information which underpins the explanation (i.e. mechanistic transparency).

These three issues all are aimed at reducing the uncertainty or increasing the confidence in the read-across prediction. Confidence in the read-across prediction is enhanced when:

a) there is mechanistic transparency, b) experimental data of good quality for structural analogues allows for interpolation rather than extrapolation, c) the number of analogues within the chemical category increase (i.e. read-across from many to one), d) supplemented by toxicokinetic and ADME information and e) supplemented by data from relevant *in vitro* and *in chemico* endpoints, (i.e. increased weight of evidence).

Uncertainty in a read-across should be handled inversely to the weight of evidence presented in the mechanistic and scientific justification (i.e. the greater the mechanistic understanding presented and the greater the weight of evidence with data from relevant alternative assays and methods provided, the more tolerance of the uncertain should be accepted). Read-across predictions should not be scrutinised to a greater extent than the *in vivo* tests upon which they are based on. There can be a tendency to set too high a bar for the performance of alternative approaches relative to *in vivo* data.

Extra generic assessment factors for derived no effect levels (DNEL(s)) or derived minimum effect levels (DMEL(s)) based on read-across data are not always required, because depending on the specific case, the read-across can be equally good as experimental data upon which it is based. However, by default, read-across is associated with additional uncertainty due to the fact that information on a target substance is being inferred from that available on a source substance(s). Whilst assessment factors can be a route by which uncertainty is addressed in deriving effect levels (note for some dichotomous endpoints, uncertainty factors are not appropriate), these should be used on a case-by-case basis and driven by the confidence associated with the underlying similarity hypothesis as well as the quality and type of study data forming part of the supporting weight of evidence information.

REACH requires registrants to deal with any additional uncertainties associated with the use of alternative approaches. To date, uncertainty has been dealt with on a case by case basis by Industry registrants and typically in a qualitative manner with verbal descriptors. From ECHA's perspective, uncertainty related to read-across has not been

considered in a systematic or transparent manner by registrants, nor was there an agreed practice how to do so in the read-across assessment.

Question 3. What type of case studies or other next steps (including tools) could be proposed that would be practically feasible and expected to provide most relevant data to inform the above questions? How can mechanistic data on key events in the framework of an AOP/MOA be used in the construction of a read-across case? How should PK/ADME information best be integrated into read-across strategies? What is the status of the known high through-put methodologies for utilisation in a read-across justification?
All four break-out groups addressed this question.

In the immediate future, a clear next step that was agreed as desirable during the course of the plenary discussion and mirrored in all four break-out groups was for more information on the RAAF to clarify the identity of the basic read-across types and to have an appreciation of the "key aspects" used in the evaluation of each read-across type. Illustrative case studies highlighting both acceptable read-across justifications and those that failed would help characterise what constitutes scientifically credible and robust. For the cases that failed, an indication of what additional information might have helped address the key aspects required would be of particular value. The goal of such case studies should be to develop a series of guiding principles that would help Industry as developers of read-across predictions and ECHA and MS as evaluators of those read-across predictions. The case studies could illustrate the key aspects necessary and demonstrate the level and nature of documentation required. One suggestion proposed was to exploit the insights and cases documented in the ECETOC TF report, other ideas include examples from OECD, or anonymised examples from Industry. .

The RAAF as it is now targets health endpoints but future development could address other domains such as ecotoxicity, qualifying and systemising uncertainty etc. There was keen interest from participants to be able to contribute to those future developments and have the opportunity to continue similar discussions with ECHA and MS.

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The AOP/MOA provides the documented mechanistic plausibility or, better still, probability, that key events are linked to the *in vivo* outcome of regulatory interest. By using hypotheses based on the AOP/MOA and relevant alternative methods, the data

from these alternative methods can be used to test the hypothesis (see OECD 2011; 2012). Such data especially when available for multiple key events increases the weight of evidence that the read-across is acceptable or provides a justification for rejection. The more consistent these data are, the less the uncertainty in the prediction. This approach is well documented for skin sensitisation and the approach is mapped in the OECD QSAR Toolbox in v 3.0. This is foreseen as a way forward as new information and knowledge becomes available. A weight of evidence approach using HTP data, omics etc. ought to help substantiate future read-across justifications although tools and approaches will need to be developed to help interpret such information in the appropriate biological context i.e. the AOP. In the future, if the understanding and data are available, conceivably larger categories could be developed.

PK/ADME information form part of the mechanistic transparency and need to be assessed in terms of their relevance to the endpoint under consideration.

The best examples of HTP methods in assisting in read-across perhaps come from endocrine disruption where some of the pathways are reasonably well documented, key events have been outlined, and assays for those endpoints have been developed and used to create databases of good depth and breadth.

Overall concluding comments

Overall the workshop was useful. Experience so far has been that many of the submitted REACH dossiers leave substantial room for improvement. The workshop has helped to identify how such improvement can be achieved.

Both ECHA and Cefic LRI expressed a willingness to continue cooperation on read-across. ECHA considered LRI as providing a good platform to address research and information needs for read-across. The RAAF was noted to be a valuable approach in guiding Industry towards a structured way of formulating their read-across cases, with more scientifically grounded justification.

The workshop provided an opportunity for direct interaction between different interested stakeholders and thus enabled a useful exchange to clarify many misconceptions and highlight existing opportunities.

Taking it forward

It was agreed that it was desirable to maintain momentum by timely exchanges on various aspects of read-across. The importance of how to disseminate up-to-date progress to SMEs needing to submit registration dossiers was also emphasised.

As an immediate outcome of the workshop, a briefing document for SMEs would be provided to communicate the outcomes (this document). A summary report by way of a peer reviewed manuscript would also be pursued.

Assessment of workshop objectives

The workshop outline described a number of goals and proposed specific deliverables. The progress and delivery against these goals are summarised below.

Stated goals

- Present information on the read-across assessment framework (RAAF)

ECHA presented a draft perspective of the RAAF (the framework being under construction) and provided a background document that explained the idea of the RAAF in more detail. Both of these have since been posted on the ECHA website.

- Review practical case-studies (or realistic scenarios) of the application of read-across approaches

Three case studies from Industry were showcased to illustrate the experiences and challenges to date. These are also available from the ECHA website. More case studies including some of these are available as part of the ECETOC Technical report (published as TR116, see http://www.ecetoc.org/publications). An abridged thoughtstarter document was provided as background information ahead of this workshop.

- Identify the potential difficulties for the read-across acceptance (is the quality of the read-across proposals an issue?)

As identified in the break-out group discussion, quality of the dossiers is a major issue for ECHA and strategies to overcome such issues were discussed.

- Attempt to reach common understanding of what scientifically valid read-across represents and how to characterise it

The discussion gave rise to a much better understanding of what valid read-across means but clarity on how to practically address this will come from on-going dialogue and illustrative case studies. The work of the ECETOC TF could well be leveraged to help build consensus in what scientifically valid read-across represents.

Planned Workshop Outcomes

1) Presentation of the current thinking of ECHA on evaluation of read-across and review of the current state of the science on the scope and applicability of read-across approaches from an Industry perspective;

Presentations are now accessible from the ECHA website see http://echa.europa.eu/en/view-article/-/journal_content/c6dd5b17-7079-433a-b57f-75da9bcb1de2.

2) Characterisation of the uncertainty associated with the use of read-across approaches and associated policy criteria relating to acceptability (i.e., levels of documentation to justify and support decision making and dealing with uncertainty);

These were discussed in the context of the break-out discussions.

3) Better understanding of the barriers that can impede further adoption of read-across approaches as an integral component of chemical management under REACH;

These were discussed in the context of the break-out discussions.

4) Recommending initiatives to overcome these barriers (i.e., efforts on harmonisation) and;

These were discussed in the context of the break-out discussions.

5) Establishing a community of interest as appropriate, to further advance the debate.

These were discussed in the context of the final plenary report out session and will be followed up offline. General agreement and a willingness to continue a dialogue was reached.

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