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REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA)

Second Review Cycle (2016)

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REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA)

Second Review Cycle (2016)

IOMC
INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2017

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FOREWORD

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This document reports the learnings and lessons obtained from the review experience of the five case studies, listed below, submitted to the 2016 review cycle of the IATA Case Studies project. The topics discussed in this document include the strongest aspects and uncertainties of each case study, and the document identifies areas for developing further guidance on IATA.

1. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR THE REPEATED-DOSE TOXICITY OF PHENOLIC BENZOTRIAZOLES, ENV/JM/MONO(2017)23, Series on Testing & Assessment No. 271.

2. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR PESTICIDE CUMULATIVE RISK ASSESSMENT & ASSESSMENT OF LIFESTAGE SUSCEPTIBILITY, ENV/JM/MONO(2017)24, Series on Testing & Assessment No. 272.

3. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT OF 90-DAY RAT ORAL REPEATED-DOSE TOXICITY FOR SELECTED N-ALKANOLS: READ-ACROSS, ENV/JM/MONO(2017)25, Series on Testing & Assessment No. 273.

4. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT OF 90-DAY RAT ORAL REPEATED-DOSE TOXICITY FOR SELECTED 2-ALKYL-1-ALKANOLS: READ-ACROSS, ENV/JM/MONO(2017)26, Series on Testing & Assessment No.274.

5. CHEMICAL SAFETY ASSESSMENT WORKFLOW BASED ON EXPOSURE CONSIDERATIONS AND NON-ANIMAL METHODS, ENV/JM/MONO(2017)27, Series on Testing & Assessment No. 275.

This document has been prepared by a project team of the Working Party on Hazard Assessment and was endorsed at the 1st meeting of the Working Party on Hazard Assessment in June 2017.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

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1. INTRODUCTION

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The Cooperative Chemicals Assessment Programme (CoCAP)[1] was revised in 2014 to enhance the activity of the development and the application of IATA. This programme provides a forum for scientific exchange of approaches on how novel methods are applied to assess the hazard of chemicals, and establish common and best practices for the use of these methods for assessing different types of chemicals. The IATA Case Studies Project[2] was launched in 2015 under the revised CoCAP. The objective of the project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This project reviews case studies submitted from member countries every year. The review results are discussed in a project meeting. The discussion includes the topics of strongest aspects of case study, uncertainty of case study, areas for further developing guidance and possibility of the use of case study in a regulatory context. In every review cycle, the case studies approved will be published with a considerations document capturing the learnings and lessons stemming from case studies. The outcomes of the first review cycle of the project (2015), four case studies and a considerations document, were published (OECD, 2016a; 2016b; 2016c; 2016d; 2016e).

In the second review cycle (2016), the five case studies shown in Table 1 were reviewed. The final case studies are published [ENV/JM/MONO(2017)23-27, Series on Testing and Assessment no. 271-275]. These case studies are illustrative examples, and their publication as OECD monographs does not translate into direct acceptance of the methodologies for regulatory purposes across OECD jurisdictions. In addition, these cases studies should not be interpreted as official regulatory decisions made by the authoring member countries. This document describes the review results of each of the five case studies and summarises the learnings and lessons stemming from the case studies reviewed in the first and second review cycles.

[1] OECD, Cooperative Chemicals Assessment Programme (CoCAP). http://www.oecd.org/chemicalsafety/risk-assessment/oecdcooperativechemicalsassessmentprogramme.htm

[2] OECD, IATA Case Studies Project. http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm

Table 1. Case Studies Reviewed in the Second Review Cycle (2016)

No.	Title	Lead	Purpose of Use	References
1	Repeated-Dose Toxicity of Phenolic Benzotriazoles	Japan	To address how read-across can be applied to screening assessment under Japan's Chemical Substances Control Law	OECD, 2017a
2	Pesticide Cumulative Risk Assessment & Assessment of Lifestage Susceptibility	United States	Highlight how AOP knowledge can be used to design a testing strategy to focus on the potential for life susceptibility and how the associated data have been used to assess cumulative risk.	OECD, 2017b
3	90-Day Rat Oral Repeated-Dose Toxicity for Selected n-Alkanols: Read-Across	ICAPO[*1]	Illustrate specific issues associated with IATA, in particular read-across and to stimulate discussion on the topic. The proposed use of the data estimations resulting from this IATA is risk assessment.	OECD, 2017c
4	90-Day Rat Oral Repeated-Dose Toxicity for Selected 2-Alkyl-1-alkanols: Read-Across	ICAPO	Illustrate specific issues associated with IATA, in particular read-across and to stimulate discussion on the topic. The proposed use of the data estimations resulting from this IATA is risk assessment.	OECD, 2017d
5	Chemical Safety Assessment Workflow Based on Exposure Considerations and Non-Animal Methods	JRC[*2] BIAC[*3]	Demonstration of an exposure-based chemical safety assessment workflow not relying on animal testing	OECD, 2017e

*1: ICAPO: International Council for Animal Protection in OECD Programmes
*2: JRC: European Union / Joint Research Centre
*3: BIAC: Business and Industry Advisory Committee to the OECD

2. PROCESS FOR REVIEWING THE CASE STUDIES

The following 13 countries/organisations participated in the second review cycle: Australia, Canada, Denmark, Germany, Japan, the Netherlands, Sweden, the United States (US), European Union/European Commission (EU/EC), EU/Joint Research Centre (EU/JRC), EU/European Chemicals Agency (EU/ECHA), Business and Industry Advisory Committee to the OECD (BIAC) and International Council for Animal Protection in OECD Programmes (ICAPO). In addition, the United Kingdom and EU/European Food Safety Authority (EU/EFSA) participated in the review meeting of the case studies.

For the case studies on grouping methods (Case Studies 1-4), the authors were requested to consider the template provided in the Annex. The template was developed based on the reporting format in the OECD Guidance on Grouping of Chemicals (OECD 2014a) and a case study document (OECD 2014b); and was used for the case studies of the first review cycle.

Reviewers were requested to answer the following guided questions when reviewing the case studies:

1. Is the purpose of the case study clear?

2. Are the justifications presented in the different sections sound? (e.g. hypothesis; analogue selection; justification for data gap filling; integrated conclusion; uncertainty discussion; other). If not, suggest how to improve it.

3. Are there specific topic areas in the case study that could benefit from the development of further guidance for application or interpretation? (e.g. building the hypothesis; identifying important IATA elements for the endpoint; selecting analogues; deriving integrated conclusion; uncertainty communication. etc.)

4. What are the strongest aspects of the case study?

5. What are the dominant and most relevant areas of uncertainty and how do you think they could be reduced? Could their reduction lead to a different conclusion of the case study?

6. Would you use the results of such a case study in your regulatory context? If no, why not (legislative/policy/scientific reasons)?

7. Does the template work well?

8. Other?

In addition, case study authors were requested to also answer the following guided questions:

1. Which areas of the case study was the most difficult to justify and why?

2. What information would have helped you in developing the case study?

3. Would the availability of guidance or tools in a particular area have helped you in developing the case study?

4. Would you use the results of such a case study in your regulatory context? If no, why not (legislative/policy/scientific reasons)?

5. Does the template work well?

6. Other?

The reviewer's comments and the revised case studies were discussed at the second meeting of the IATA Case Studies Project (28-29 November 2016) in order to finalise the case studies and summarise the learnings and lessons.

3. SUMMARY OF REVIEW RESULTS

3.1. Case Study 1: Repeated-Dose Toxicity of Phenolic Benzotriazoles [Japan]

This case study illustrates data gap filling by read-across for repeated-dose toxicity. A category for oral repeat dose toxicity consisting of 12 members of phenolic benzotriazoles was formed. Transcriptomic profiles were generated for five category members to be integrated into the assessment. Using read-across based on nine category members with experimental data, NO(A)ELs and targets effects of 3 other category members were determined. This case study is intended to address how read across can be applied to a screening assessment under Japan's Chemical Substances Control Law (CSCL)[3].

Please refer to ENV/JM/MONO(2017)23 Series on Testing & Assessment No. 271 for the case study to put the following points into context.

The strongest aspects of the case study were identified as follows:

- Clear explanation of the purpose of the study
- Incorporating transcriptomic information into the category justification
- Integrating all available information from open literature, QSAR and transcriptomics and providing predictions on the targeted substances based on repeated dose toxicity and metabolism data
- Provides a description of uncertainty of the prediction in a transparent way

The main points discussed for revising the case study were as follows:

- In the original version of the case study, the effect level of member 12 was estimated from member 7, which is the nearest structural analogue, but has only non-GLP data. It was pointed out that the estimated effect level could be underestimated because the non-GLP data might not cover all the effects to be considered, as reporting was unclear. It was suggested that the effect level of member 12 should be estimated from member 6, which had GLP data, to derive a more conservative estimation for screening. The authors revised this point according to the comment.
- An explanation was requested on why only hepatotoxicity was considered and not nephrotoxicity. The authors explained that hepatotoxic effects are more critical for hazard assessment of phenolic benzotriazole category for the reason that nephrotoxic effects were observed only in three category members and all these nephrotoxic changesappeared at the same or higher dose at which hepatotoxicity appeared.
- It was requested to expand the descriptions on the transcriptome tests (e.g. relationship between mechanism of hepatotoxicity and each test, reason for choosing the particular substances for the transcriptome study). The authors expanded the description on this point in the revised case study.

[3] Japan, Chemical Substances Control Law. http://www.meti.go.jp/policy/chemical_management/english/cscl/

The main uncertainties identified for the case study were as follows:

- For two of the three read-across assessments in the case study, the uncertainties on the similarity between the target and the source chemicals were assigned as high. For the category it was difficult to define the structural boundary of the subcategories due to the observation that small structural changes in source chemicals result in different toxicity levels. In addition, the data to support the mechanistic similarity (e.g. absorption, distribution, metabolism and excretion (ADME) data, transcriptomic data) were insufficient for the two read-across assessments.

- The uncertainty of mode of action (MOA)/AOP for forming subcategories was assigned as medium for the reason that the MOA/AOP was not well described, although the results demonstrated that transcriptomic data could be supportive for subcategory formation of phenolic benzotriazoles based on possible mechanism.

The main comments on the use of the case study in other member countries' regulatory context are as follow:

- ***Australia:*** This study provides clear insights into developing grouping approach, using phenolic benzotriazole, and focusing on repeated-dose toxicity. Therefore, the results of this study would be very useful in our regulatory context. Similarly, it is also noted that elements of the case study are also intended to support regulatory decision making under the Japanese CSCL.

- ***Canada:*** It would be possible to use such a category/(sub)category for screening level risk assessment under Canada's Chemical Management Plan. The hazard assessment value (D-value) is not used in Canada. The margin of exposure (MOE) approach would be used and the uncertainty in the read across would be assessed against the magnitude of the MOE.

- ***Netherlands:*** D-value could be used as an indication of derived no-effect level (DNEL). This study is very valuable, as it gives examples of less toxic substances, which may be used as alternatives in certain uses. Due to legislative reasons it would be difficult to use the results of this study under EU's Registration, Evaluation, Authorisation of Chemicals (REACH). More information on other toxicological endpoints for all members showing similarities in toxicological profile could be helpful.

- ***EU/ECHA:*** Under REACH, it is responsibility of the registrant to provide data. If established, "high uncertainty" may be an obstacle to derive predictions. For data gap filling and generation of standard information requirements for repeated dose toxicity the justification seems insufficient, mainly because the structural differences are not commented on systematically as to how they can affect the read-across. ADME and the transcriptomics data can be used as supportive information but are not generated uniformly for all analogues. It is important to generate "bridging" information (e.g. transcriptomics data for all chemicals in the group) on both source and target, in order to be able to compare. The endpoints are varying (from different durations), so it would not be clear which information requirement is addressed.

Based on the experience reviewing this case study, the following areas were identified for potential guidance development:

- How to report transcriptomics data, and the description of the interpretation and related uncertainties

- Building a hypothesis for category formation that includes the use of genomics data

- Uncertainty analysis: It is not clear up to what level of uncertainty can be allowed without affecting the integrated conclusion

3.2. Case Study 2: Pesticide Cumulative Risk Assessment & Assessment of Lifestage Susceptibility [the United States]

The purpose of the case study is to highlight how AOP knowledge can be used to design a testing strategy to focus on potential for life susceptibility for an entire class of pesticides and how the associated data have been used to assess cumulative risk. The case study was developed based on a cumulative risk assessment for the organophosphate pesticides (OPs) by US Environmental Protection Agency (US EPA)[4] which was conducted based on the guidance document on Organophosphorous Pesticide Cumulative Risk Assessment (US EPA, 2006).

The OPs have been assessed for cumulative risk based on their shared ability to bind and to phosphorylate the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems. The inhibition of AChE has been used as the molecular initiating event for deriving benchmark doses (BMDs) and assessing lifestage susceptibility. The lifestage susceptibility has been evaluated using a specific study protocol called the comparative cholinesterase assay specifically designed to assess various early lifestages (fetal, pregnant females, post-natal) across duration.

Please refer to ENV/JM/MONO(2017)24, Series on Testing & Assessment No. 272 for the case study to put the following points into context.

The strongest aspects of the case study were identified as follows:

- A good overview of how relative potency factors of substances with a common mechanism can be derived and how lifestage susceptibilities can be taken into account
- Using a relatively simple measurement to assess risk for humans for a complex endpoint
- Justification of relevance of BMD over NOAEL/LOAEL
- Clearly defined endpoint and that the levels of AChE are measurable in various matrices

The main points discussed for revising the case study were as follows:

- In response to reviewer's comments detailed descriptions were added in the following points:
  - Description for comparative cholinesterase (CCA) study design and explanation of why the CCA design is preferred over the developmental neurotoxicity (DNT)
  - Definition of index chemical and equation for relative potency factors (RPF)
  - Generic structure of OP and importance of activation to the oxon metabolites
  - Citation of the AOP in the AOP Wiki and figure version of the AOP
  - Exposures from food, water and non-occupational exposures

[4] US EPA, Cumulative Assessment of Risk from Pesticides. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/cumulative-assessment-risk-pesticides

- The following points requested by reviewers to add detailed descriptions were not added as they were beyond of the scope of the case study

  - Analysis of relative sensitivity of AChE inhibition compared with other behavioral changes

  - Types of behaviours evaluated

  - Consideration of group boundary (e.g. Other chemicals which are AChE inhibitors with shared health outcomes instead of shared mechanism/AOP/ MOA)

- Specific comments were made on the benchmark dose analysis for setting the benchmark response (BMR) (i.e. such as the selection of the point of departure). However, no revisions were made to these comments for the reason that the difference in the interpretation of the approach was attributed to the difference in regulatory contexts between the author country and the reviewer country.

The main uncertainties identified for the case study were as follows:

- The enzyme levels may vary substantially over time (both during the day but also during the various phases of life). To account for variation across different parameters (including background levels of the AChE enzyme) would require a physiologically-based pharmacokinetic (PBPK) model for all the OPs—which are not available. However, this would not change the overall approach since the results of the CCAs would be preferred over the results of the DNT to develop such PBPK models.

The main comments on the use of the case study in other member countries' regulatory context are as follow:

- ***EU countries:*** the results of such a case study could be used in the regulatory context, provided that all regulatory requirements are satisfied.

Based on the experience reviewing this case study, the following areas were identified for potential guidance development:

- Defining boundaries for groups of substances, including how to take ADME differences into concern for substances with same modes of action

- How to describe the rationale for justification of the BMD and point of departure used

3.3. Case Study 3: 90-Day Rat Oral Repeated-Dose Toxicity for Selected n-Alkanols: Read-Across [ICAPO]

This case study has been designed to illustrate specific issues associated with read-across and to stimulate discussion on the topic. It is not intended to be related to any currently ongoing regulatory discussions on this group of compounds. A category consisting of congeneric series of selected n-Alkanols (C5-C13) was formed for the target endpoint of 90-day rat oral repeated-dose toxicity. In this case the chemical category represents analogues which are non-reactive and exhibit nonpolar narcosis, and metabolic products of the parent alcohols have no toxicological significance (i.e., these alkanols are direct-acting toxicants). It was concluded that the NOAEL value of 1000 mg/kg bw/d for 1-pentanol and 1-hexanol can be read across to fill the data gaps of the untested analogues in this category with acceptable uncertainty. The justification of read-across was discussed based on similarly in chemistry; toxicokinetics,

especially metabolism; toxicodynamics, especially lack of reactivity; receptor binding and systemic effects. The US EPA toxicity forecaster (ToxCast) program assays[5] and in silico profilers within the COSMOS Project of SEURAT-1[6] were used for reducing the uncertainty associated with the low/no toxicity prediction.

Please refer to ENV/JM/MONO(2017)25, Series on Testing & Assessment No. 273 for the case study to put the following points into context.

The strongest aspects of the case study were identified as follows:

- The similarity discussion, within the section on data matrices for assessing similarity, especially the discussions on similar toxicokinetics, metabolism and mechanistic plausibility
- The incorporation of new approach methodology data: The use of ToxCast data and the predictions from the COSMOS models
- The way to derive low/no toxicity prediction
- The way to analyse and report uncertainty

The main points discussed for revising the case study were as follows:

- It was not necessary to revise the case study based on review comments.
- There was a question as to why undecanol and dodecanol with a study (TG422) were not considered as source chemicals in addition to pentanol and hexanol with 90-day studies (TG408)[7]. The authors answered that they considered the TG 408 studies to be higher quality than the TG422 studies. Thus the read across was conducted from pentanol and hexanol with data for heptanol, undecanol and dodecanol providing *in vivo* weight of evidence.

The main uncertainties identified for the case study were as follows:

- Over all, the uncertainty of the case study is thought to be low. A few remaining uncertainties were as follows:
  - Uncertainty due to the difficulty to prove a negative, which is a critical issue for low/no toxicity predictions
  - The availability of complete toxicokinetics data across the category appears limited.

[5] US EPA, Toxicity Forecasting: https://www.epa.gov/chemical-research/toxicity-forecasting

[6] EU, SEURAT-1: http://www.seurat-1.eu/, COSMOS : http://www.cosmostox.eu/

[7] TG 408 is a 90-day repeated dose toxicity study, which is able to provide a satisfactory estimation of a no-effect level. TG 422 is a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in which males should be dosed for a minimum of four weeks and females should be dosed throughout the study (approximately 54 days).

The main comments on the use of the case study in other member countries' regulatory context are as follow:

- ***Australia:*** As the category members, justifications for data gap filling, and uncertainties are thoroughly discussed and transparent the results of such a case study would be useful in our regulatory context.

- ***Canada:*** The uncertainty associated with this read-across is considered low and the category as a whole is well studied. The read-across as described could be used to support a screening level human health risk assessment in Canada under the Chemicals Management Plan.

Based on the experience reviewing this case study, the following areas were identified for potential guidance development:

- How best to support a low/no toxicity read across prediction: A low/no toxicity prediction will be extremely important in regulatory decisions. Once other such cases are developed it will be easier to establish a "best" strategy.

- How to use of new approach methodology data such as high-throughput screening data and omics data:

  - How to use the data as supportive data for justifying a category based on an AOP.

  - How to describe and incorporate the data that do not point to a specific pathway (i.e. how to discuss activity when it only relates to cell viability assays).

  This was thought to be an issue that is independent of the case study and could be better addressed after several case studies have been developed using different types of new approach methodology data.

- How to report new approach methodology and its data

- How to capture and describe uncertainty related to category justification elements

3.4. Case Study 4: 90-Day Rat Oral Repeated-Dose Toxicity for Selected 2-Alkyl-1-alkanols: Read-Across [ICAPO]

This case study has been designed to illustrate specific issues associated with read-across and to stimulate discussion on the topic. It is not intended to be related to any currently ongoing regulatory discussions on this group of compounds. A category consisting of congeneric series of selected 2-Alkyl-1-alkanols (C5-C13) was formed for the target endpoint of 90-day rat oral repeated-dose toxicity. In this case the chemical category represents analogues which are non-reactive and exhibit nonpolar narcosis, and metabolic products of the parent alcohols have no toxicological significance (i.e., these alkanols are direct-acting toxicants). It was concluded that a no systemic toxic conclusion, with a NOAEL of 125 mg/kg bw/d, can be read across with high confidence to untested 2-ethyl- and 2-propyl-1-alkanols in the category and that a no systemic toxic conclusion, with a NOAEL of 125 mg/kg bw/d, can be read across as the "worst possible scenario" to untested 2-methyl-1-alkanols in the category. The justification of read-across was discussed based on similarly in chemistry; toxicokinetics, especially metabolism; toxicodynamics, especially lack of reactivity; receptor binding and systemic effects. ToxCast assays[5] and in silico profilers within the COSMOS Project of SEURAT-1[6] were used for reducing the uncertainty associated with the prediction.

Please refer to ENV/JM/MONO(2017)26, Series on Testing & Assessment No. 274 for the case study to put the following points into context.

The strongest aspects of the case study were identified as follows:

- The similarity discussion, within the section on data matrices for assessing similarity, especially the discussions on similar toxicokinetics, metabolism and mechanistic plausibility
- The incorporation of new approach methodology data: The use of ToxCast data and the predictions from the COSMOS models
- The way to analyse and report uncertainty

The main points discussed for revising the case study were as follows:

- It was not necessary to revise the case study based on review comments.
- There was a request to add more information on the 18 month mice carcinogenicity study. The authors answered that the longer term mice studies were added for completeness. The authors did not consider their results to be related to endpoint (90-day rat oral repeated-dose toxicity) being evaluated.

The main uncertainties identified for the case study were as follows:

- Overall, the uncertainty of the case study is thought to be low. A few remaining uncertainties were as follows:
  - The uncertainty on the inclusion of 2-methyl substituted derivatives within the category: While the inclusion of these analogues in the group is supported, the association is a generous one considering the lack of in vivo experimental data. This uncertainty could be reduced by the availability of additional data on the metabolism of these derivatives. Such data could change the outcome by either reducing uncertainty for the derivatives or excluding them from the category.
  - The uncertainty on the limitation of toxicokinetics data. For example, the claim that 2-alkyl-alkanols have longer half-life in the body than n-alkanols, leading to higher internal concentration (and therefore lower NOAELs) is plausible but in this case study only qualitative, and it should/could be supported by experimental data.
  - There is only data "in the middle" of the category, but no data on the extremes. This kind of extrapolation adds additional uncertainty.

The main comments on the use of the case study in other member countries' regulatory context are as follow:

- ***Australia:*** Similar case studies could be used as part of a weight of evidence approach to risk assessment of industrial chemicals.
- ***Netherlands:*** It is not thought that this case study could be used for REACH for legislative reasons. In addition, it is difficult to prove what will happen at higher doses: The REACH legislation allows the category approach, but in this case more supporting experimental data will

be necessary to support the claims (similar toxic mechanism of action, similar uptake, similar metabolism/excretion). The arguments are given why it has to be similar, but the supporting data is too little (lacking data on the category boundaries).

Based on the experience reviewing this case study, the following areas were identified for potential guidance development:

- How to use new approach methodology data such as high-throughput screening data and omics data (See Section 3.3).
- How to report new approach methodologies and their data
- Guidance and templates for a differentiated/quantitative assessment of uncertainties would be helpful (based on the tables in the case studies)

3.5. Case Study 5: Chemical Safety Assessment Workflow Based on Exposure Considerations and Non-Animal Methods [JRC/BIAC]

This case study presents a general workflow, which was developed based on the SEURAT-1[6] conceptual framework for safety assessment (White and Knight, 2013; Daston G et al. 2015), in an attempt to structure knowledge and data in a logical sequence for an integrated chemical safety assessment relying specifically on alternative methods and based on exposure considerations. The workflow presented consists of 3 tiers:

TIER 0: Identification of the use scenario, chemical of interest and collection of existing information: the exposure scenario and chemical identity are defined and existing data is collected. Based on the collected information and data, the applicability of the Threshold of Toxicological Concern (TTC) approach and a read-across assessment are evaluated as exit points for Tier O, which can also be reconsidered at a later stage when more relevant information becomes available.

TIER 1: Hypothesis formulation for *ab initio* approach: Systemically available concentrations are predicted in different body compartments and relevant target organs are identified for further assessment according to these concentrations, and contribute to formulating the MOA hypothesis together with results from the in silico and (existing) in vitro profile.

TIER 2: Application of *ab initio* approach: Following up on the indications on target organs/tissues obtained in Tier 1, if a well-known AOP is concerned, the respective key events are investigated to confirm the hypothesis. Furthermore, quantitative (dose-response) estimates of biological effects are derived under mimicked realistic conditions. A point of departure for safety assessment is predicted based on the relevant AOP incorporating kinetics and biomarker data from repeated dose assays.

Piperonyl butoxide (PBO) was selected to illustrate the case study in a hypothetical exposure scenario as a new ingredient introduced in a daily applied body lotion. A six compartment physiologically based kinetic (PBK) model was built for PBO including a skin compartment to simulate dermal exposure. From the data gathered, pointing at respective molecular initiating events, and target organs predicted by PBK modelling, the AOPs for liver steatosis and liver fibrosis were identified as relevant basis for the assessment. Based on the AOPs and their key events, relevant *in vitro* assays were identified for targeted testing to confirm the MOA hypothesis. The Virtual Cell Based Assay (VCBA) was used for estimating the realistic corresponding dose/concentration in the *in vitro* assays. .

Please refer to ENV/JM/MONO(2017)27, Series on Testing & Assessment No. 275 for the case study to put the following points into context.

The strongest aspects of the case study were identified as follows:

- Starting the development of a tool to guide the risk assessment through various methods in order to facilitate decision making
- Consideration of exposure early in the application of the decision logic, IATA approach not limited to hazard characterisation and estimation of systemic bioavailability
- Integration of multiple methods for safety assessment (TTC, read-across, QSAR, *in vitro*, omics etc.)
- Use of non-animal test methods
- Contributes to more focused strategies to advance alternative assessment approaches
- Steps for refinement considered at each tier
- Illustrates important issue of IATA approach: how to gather additional data, especially new types of data
- Identification of many challenges and considerations for estimation of the internal dose and comparing this against activity seen *in vitro* (e.g. consideration of targeted *in vitro* test systems, IVIVE, metabolism, clearance, etc).

The main points discussed for revising the case study were as follows:

- There was a question as to why the study focused only on specific effects although the selected endpoint is repeated dose toxicity for human health safety assessment. The authors clarified that the safety assessment workflow is set out to be general (applicable to different endpoints, chemicals, exposure); however the specific example chosen as an illustration focuses on liver toxicity (because of the context of the SEURAT-1 initiative), and was not intended to be a complete risk assessment.
- The following points with the workflow were revised:
  - Read-across is not only considered as exit in Tier 0 but can be reconsidered later in the workflow with more information collected, explicitly included in the workflow scheme.
  - Clarification that the possible metabolites identified are also subject to the hazard screening to add to the MOA hypothesis generation
  - Exit point for low internal exposure is considered (included in the workflow scheme)
  - Some descriptions were expanded and clarified

The main uncertainties identified for the case study were as follows:

- Highly complex, utilising multiple data streams: the application to real case assessments will require expert judgement and detailed justifications.

- More experience and guidance needed to understand the contribution of uncertainties as well as a harmonised way to report them for different types of data and methods.

- Generic (i.e. not substance-specific) PBK and *in vitro* to *in vivo* extrapolation (IVIVE) models needed.

The main comments on the use of the case study in other member countries' regulatory context are as follow:

- ***Canada:*** Health Canada is exploring the use of possible new approach methodologies in the assessment of existing substances under the Chemicals Management Plan. As experience builds, it is conceivable that similar workflows could be used that integrate multiple tools (TTC, read-across, *in vitro* assays, IVIVE, etc.) to support screening level risk assessment in Canada. We commend the authors for starting the development of a tool to guide the risk assessment through various methods in order to facilitate decision making.

- ***Netherlands:*** Since this case study explores the development of a general workflow in structuring knowledge in a rational order while focusing on alternative methods, it is not readily applicable in Netherland's context. The authors of the case study also state "that the intention of the case study is not to be an assessment of a specific chemical in view of regulatory acceptance…"

- ***Sweden:*** Not presently (legislative/policy/scientific). Perhaps in the future if this type of study is further developed and agreed upon by ECHA.

- ***BIAC:*** For an applicant (industry), such approach will be quite time demanding and couldn't be applied as a routine. However, it could be a very useful tool to clarify some effects observed, for example in screening tests, avoiding launching into further higher tier testing.

Based on the experience reviewing this case study, the following areas were identified for potential guidance development:

- Guidance in deriving integrated conclusions, integrating multiple data streams

- Identification and characterisation of overall uncertainty in a safety assessment, resulting from combined uncertainties of the different IATA components and data types

- Use of AOPs in a regulatory context: AOPs agreed at OECD level and/or accepted for use in a regulatory context would increase confidence in conclusions only based on *in vitro* and *in silico* data.

  - Reliability assessment of data coming from new types of tests (in-vitro) and models (PBK models) that is fit for regulatory use.

- Challenges in estimation of the internal dose, IVIVE, metabolism, clearance.

4. LEARNINGS AND LESSONS

4.1 Summary of the Case Studies Reviewed in the First and Second Review Cycles

This chapter summarises learnings and lessons stemming from the case studies of the project including the five case studies of the last review cycle. Table 2 shows a summary of the 9 case studies reviewed in the first and second review cycles.

The assessment approaches illustrated by the case studies are classified into three types: data-gap filling by read-across based on grouping of chemicals (7 case studies), grouping of chemicals for cumulative risk assessment, not for read-across (Case Study 2016-2), and safety assessment workflow (Case Study 2016-5). The workflow contains read-across assessment, but the focus of the case study is the workflow concept.

The target endpoints of the case studies were: repeated dose toxicity (6 case studies), neurotoxicity (one case study), mutagenicity (one case study) and bioaccumulation (one case study). Five of nine case studies illustrate read-across for repeated dose toxicity.

Every case study addresses some challenging topics related to IATA including use of MOA/AOP (4 case studies), capturing and communicating uncertainty (5 case studies), use of new approach methodologies (5 case studies) and low/no toxicity prediction (3 case studies).

Identified areas for further developing guidance from the 9 case studies are summarised in section 4.2. In Section 4.3, considerations obtained from five case studies that illustrate read-across for repeated dose toxicity are described.

Table 2. Summary of the Case Studies Reviewed in the First and Second Review Cycles

Year-No. (Lead)	Assessment approach	Endpoint	IATA topics				References
			AOP[*1]	UR[*2]	NAM[*3]	L/N[*4]	
2016-1 (Japan)	Read-across	Repeated dose toxicity		X	X		OECD, 2017a
2016-2 (US)	Grouping for cumulative risk assessment	Neurotoxicity	X		X		OECD, 2017b
2016-3 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2017c
2016-4 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2017d
2016-5 (JRC/BIAC)	Safety assessment workflow	Repeated dose toxicity	X		X		OECD, 2017e
2015-1 (Canada/US)	Read-across	Mutagenicity	X	X			OECD, 2016b

Year-No. (Lead)	Assessment approach	Endpoint	IATA topics				References
			AOP[*1]	UR[*2]	NAM[*3]	L/N[*4]	
2015-2 (Canada)	Read-across	Repeated dose toxicity		X	X		OECD, 2016c
2015-3 (Japan)	Read-across	Repeated dose toxicity	X	X			OECD, 2016d
2015-4 (Japan)	Read-across	Bioaccumulation		X		X	OECD, 2016e

*1: AOP: Use of mode of action/adverse outcome pathways
*2: UR: Uncertainty reporting
*3: NAM: Use of new approach methodologies
*4: L/N: Low/no toxicity prediction

4.2. Update of the Identified Areas for Further Developing Guidance

In the first review cycle the following 6 areas for further developing guidance were identified from the read-across case studies (OECD, 2016a):

1. Describing scope and context for read-across
2. Building hypotheses based on MOA/AOP
3. Definition of analogues/category boundaries
4. Justification of data gap filling
5. Uncertainty analysis and reporting
6. Integrated conclusion

The main additional aspects in the areas for further developing guidance identified in the second review cycle are as follow:

1. ***Building hypotheses based on MOA/AOP:*** This area was identified from read-across case studies of the first review cycle. Different aspects regarding this area were obtained from two case studies of the second review cycle, which do not use read-across:

   – How to apply a category approach for risk assessment, i.e. how to use cumulative risk assessment of substances acting through the same AOP (Case Study 2016-2)

   – How AOP information can be incorporated in IATA to address issues other than similarity in grouping (e.g. targeted *in vitro* testing based on AOPs in Case Study 2016-5).

2. ***Decision logic for low/no toxicity predictions:*** Read-across approaches have been developed for positive predictions (e.g. grouping based on molecular initiating event causing target toxicity). On the other hand, a promising harmonised approach for read-across for low/no toxicity has not

been developed due to its intrinsic difficultly. However, a low/no toxicity prediction will be important in future regulatory decisions. Case Studies 2016-3 and 2016-4 have successfully demonstrated such predictions. Decision logic for low/no toxicity predictions would be one of the areas for further developing guidance.

3. ***Incorporation of new approach methodologies:*** All the five case studies of the second review cycle showed different examples of the use of new approach methodology data in IATA. Based on the review experience the following points were identified for further developing guidance in this area.

   - How to describe new approach methodology: The OECD guidance document for describing non-guideline in vitro test methods (OECD, 2016c) can be used as background information. The template for reporting individual information sources in the OECD guidance document on the reporting of defined approaches to be used within IATA (OECD, 2016f) could be used for reporting of new approach methodologies or kinetics models. However, some modifications of the template are needed for applying it to the types of IATA Case Studies reviewed in the project.

   - How to report results and data from new approach methodologies in the context of the case studies

   - How to use new approach methodology data (See Subsection 4.3.2).

   - How to address uncertainty issues of new approach methodology

4. ***Uncertainty analysis and reporting:*** This area was identified in the first review cycle as one of the highest priority areas for further developing guidance. The importance of capturing and communicating uncertainty was also demonstrated in the case studies of the second review cycle. It might be possible to start developing guidance on uncertainty of read-across based on the experience gained from the reviewed case studies. Uncertainty guidance for read-across should be part of the OECD guidance on grouping of chemicals. An annex could be developed to the guidance for incorporating the uncertainty issue. The existing uncertainty templates (Wu et al., 2010; Blackburn & Stewart, 2014; Schultz et al. 2015) used in Case Studies 2016-3, 2016-4, 2015-1 and 2015-2 would be a good starting point for development.

5. ***Reporting templates for IATA based on a building block approach:*** In the second review cycle two case studies other than read-across were developed (Case Studies 2016-2 and 2016-5). The template for read-across (Annex) was not suitable to these case studies although some parts of the template were applicable (e.g. purpose, data gathering). The appropriate structure depends on the type of case study and some new sections that are not in the template for read-across are needed (e.g. topics related to exposure assessment). Therefore, it was proposed to integrate existing reporting templates to develop a more comprehensive and flexible template based on a building block approach. For example, the template for reporting individual information sources in the guidance on reporting of defined approaches (OECD 2016f; 2016g) could be used (with some modification) for reporting of new approach methodologies or kinetics models.

The summary of updated areas for further developing guidance incorporating the above issues is as follows:

1. Describing scope and context for read-across

1) Rationale for the selected endpoint

2) Considerations for justifying focus of an IATA (e.g. choosing major' effect vs 'minor' effect) or explaining why you are choosing one

2. Building hypotheses based on MOA/AOP

1) Hypothesis for category formation that includes the use of omics data

3. Definition of analogues/category boundaries chemical similarity

1) Defining boundaries - phys/chem properties, toxicokinetics, toxicodynamics, bioavailability and metabolism

4. Justification of data gap filling

1) Reporting of QSAR prediction results

2) How much to report on reliability

3) Use of new approach methodology data, TTC approach and PBPK models (e.g. How to integrate new approach methodology data – linking to mechanistic relevance (interpretation))

4) Guidance for describing new approach methodology data in the context of IATA case studies

5) Decision logic for low/no toxicity predictions

5. Uncertainty Analysis

1) Exposure route

2) Use of data from different test conditions for read-across for a target endpoint (e.g. durations of dosing, species and administration route in repeated dose toxicity test data)

3) Impact on conclusion

4) Reporting of uncertainty of read-across (e.g. Ranking of uncertainty vs descriptive analysis/ quantitative vs qualitative analysis)

5) Consider approaches in: AOP handbook (OECD, 2016h) and scientific papers (Wu et al., 2010; Blackburn & Stewart, 2014; Schultz et al., 2015)

6. Integrated Conclusion

1) Combining approaches/methodologies for predicting bioaccumulation

2) Integrating QSAR predictions, including when to use consensus modelling or not

3) How to define acceptable uncertainty

4) Guidance on deriving integrated conclusions from the different components of the IATA, including harmonised uncertainty assessment

7. Others

1) Relevance of change in pH to prediction of degradation products (e.g. in the environment)

2) UVCBs, multi-constituents coverage (composition coverage, methodology and other)

3) Level of detail needed in case studies according to the defined purpose

4) How to include data on/predictors for metabolism when building IATAs according to the defined purpose

5) How to describe the rationale for justification of the BMD and point of departure used

6) Reporting template for IATA based on a building blocks approach

4.3. Considerations on Read-across for Repeated Dose Toxicity

Table 3 shows the list of five case studies on read-across for repeated dose toxicity reviewed in the first and second review cycles. This section consists of two subsections.

Subsection 4.3.1 describes the lessons learned from ICAPO's Case Studies, which were developed for the purpose of illustrating specific issues associated with assessing similarities and uncertainties in read-across and to stimulate discussion on the topics.

Subsection 4.3.2 picks up five specific issues on read-across for repeated dose toxicity, which should be addressed, and describes how each of the five case studies addressed them.

Table 3. List of Case Studies on Read-across for Repeated Dose Toxicity

Year-No.	Lead	Target group of chemical	Reference
2016-1	Japan	Phenolic benzotriazoles	OECD, 2017a
2016-3	ICAPO	n-Alkanols	OECD, 2017c
2016-4	ICAPO	2-Alkyl-1-alkanols	OECD, 2017d
2015-2	Canada	Substituted diphenylamines	OECD, 2016c
2015-3	Japan	Allyl esters	OECD, 2016d

4.3.1. Lessons Learned from ICAPO's Case Studies

ICAPO's case studies (2016-3 and 2016-4) were developed based on the authors' work related to the SEURAT-1 project[6]. Their work over the past several years with cosmetic–related substances has revealed

that, while read-across is conceptually simple, in practice it is difficult, especially for complex health endpoints such as repeated-dose toxicity. The authors summarised the following quick learnings on read-across from their experience of SEURAT-1.

1. Acceptance of a read-across is more likely when done on an endpoint-by-endpoint basis since overarching hypothesis used for read-across depends on the endpoint. Acceptance is basically driven by three aspects as follows:

    1) Quality and quantity of the read across data;

    2) Confidence (e.g., adequacy and reliability) associated with the underlying similarity hypothesis;

    3) Good supporting information and weight-of-evidence, including data from in vitro methods.

2. The confidence of a read-across prediction can be increased by decreasing uncertainty from the following viewpoints:

    1) ***Transparency:*** This is fundamental to accepting read-across predictions. One needs to justify the prediction by explaining, in a scientific manner, how the prediction was derived and why it is justified for the intended purpose. The level of details has to fit the purpose

    2) ***Mechanistic probability:*** This is important to accepting read-across predictions. It is needed to show toxicological relevance. It provides a means of linking in vitro effects to the in vivo endpoint of interest. AOP can suggest drivers of apical endpoints.

    3) ***Weight-of-evidence (WOE):*** Increased WOE reduces uncertainties both in relation to the similarity justifications and the completeness of the read-across argument. A many-to-one prediction is better than a one-to-one prediction. In vitro and alternative approach data may improve WOE.

    4) ***Hypothesis testing:*** Incorporation of in vitro methods into computational approaches allows for the addition of relatively rapid and inexpensive hypothesis-driven testing and evaluation. It has the advantage of doing targeted rather than universal tests and has a particular application in reducing toxicodynamic uncertainty and acceptance of a "low/no toxic" prediction where a higher level of certainty is likely to be required.

From the Case studies 2016-3 and 2016-4 five other lessons were derived:

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

2. While there can be an over-arching rationale for grouping organic substances based on molecular structure and chemical properties, these similarities alone are often not sufficient to justify a RA prediction. This is especially the case for sub-chronic and chronic health effects. Further information is often required typically including considerations of toxicokinetic- and toxicodynamic-related issues (e.g., metabolism, mechanistic plausibility).

3. Sources of uncertainty must be addressed. It is not enough to just identify them. Uncertainty includes a variety of elements which are typically divided into two main issues:

- The uncertainty associated with the similarity justification (e.g. use of data from different test conditions).

- The uncertainty associated with the completeness of the read-across argument (e.g. mechanistic plausibility).

4. While it is not always possible to definitively state a mode-of-action, less uncertainty is directly linked to strong mechanism plausibility. Confidence in mechanistic plausibility can be increased by toxicologically-relevant in vitro or alternative methods data to support the toxicodynamics. The case studies demonstrate this point with the case of nonpolar narcotics toxicity of which mode-of-action is not fully described.

5. The limitations to quantifying read-across include:

   - The availability of suitable in vivo data to be read across.

   - The lack of toxicologically-relevant in vitro or alternative methods data to support the toxicodynamics.

   However, a major limitation to using read-across for repeated-dose endpoints is the lack of toxicokinetics data and understanding.

4.3.2. Examples of How to Address Specific Issues

This subsection describes how each of five case studies addressed specific issues on read-across for repeated dose toxicity in order to capture concrete examples to be potentially used for further guidance. The following five specific issues were picked up for the exercise based on the review experience of the case studies:

1. How to define the target effect

2. How to use the data with different test conditions

3. How to explain the impact of structural differences on toxicity

4. How to use new approach methodology data

5. How to reduce uncertainties

1. How to define the target effect

Since repeated dose toxicity tests contain a lot of different types of effects observed in the whole organism, the target effect (not only its toxicity level), needs to be defined for read-across. In order to define the target effect, it is needed to compare observed effects in the test data of different compounds to find similarity in effect. Table 4 summarizes how each of the five case studies defined the target effect from the observed effects. In the case studies, the target effects were defined by considering the importance of toxicity effects from the following viewpoints:

- Frequency of the observation of the effect

- Types of effect (e.g. histopathological findings or organ weight)

- Systemic effect or local effects
- Direct effect or secondary effect
- Dose level of the effect
- Grade of the effect

Table 4. How to Define the Target Effect from the Observed Effects

Year-No. (Lead)	Defined Target effect	Observed effects used for defining the target effect	Observed effects not to be considered as target effect (reason)
2016-1 (Japan)	Hepatotoxicity	Histopathological changes such as hypertrophy, degeneration and necrosis of hepatocyte and bile duct hyperplasia accompanied by the organ weight increase.	Kidney effects (Observed in limited studies of category members typically at a higher dose than that of liver effects)
2016-3,4 (ICAPO)	Nonpolar narcotics acting in a manner similar to depressant anaesthetics	Mild changes consistent with low-grade effects including decreased body weight, accompanied by clinical chemical and haematological changes but generally without concurrent histopathological effects.	-
2015-2 (Canada)	Liver and spleen effects	Vacuolation, hepatocyte enlargement and/or minimal hypertrophy generally accompanied by associated clinical chemistry parameters related to liver function Histopathology findings in the spleen	Haematopoietic effects related to blood clotting and thyroid effects (Secondary to liver toxicity)
2015-3 (Japan)	Hepatotoxicity	Hepatocyte degeneration/necrosis and bile duct hyperplasia	Forestomach hyperplasia (non-systemic toxicity)

2. How to use the data with different test conditions

In general, it is difficult to directly compare repeated dose toxicity data derived using different test conditions such as durations of dosing, species and administration route due to problems such as differences in metabolites by administration periods/dose levels. Therefore, according to the IATA concept the appropriate purpose for using the data with different test conditions need to be considered and the acceptable variation in test conditions used for each purpose.

Table 5 shows the distribution of test conditions and toxicity levels of each category/subcategory in each case study. In all case studies, these data were used for identifying the similarities in effects within categories/subcategories for defining the target effect (Table 4). On the other hand, variations in toxicity

levels in a category were often observed. This point was considered for selecting source chemical data for data-gap filling taking into account the reliability of test data. For example, only TG 408 data were used as source chemical data for read-across in Case Studies 2016-3 and 2016-4, which require lower uncertainty level due to their purpose of low/no toxicity prediction, for the reason that these data exhibit qualitative and quantitative consistency between and within rodent species.

Table 5. Distribution of Test Conditions and Toxicity Levels

Year-No. (Lead)	Sub category	Test condition	# of studies/ # of chemicals		Toxicity levels
2016-1 (Japan)	1	28d, rat, gavage TG422, rat, gavage 90d, rat, feed 90d, dog, feed	1/1 4/3 3/3 2/2	10/4	NOAEL: <0.5 mg/kg/d NO(A)EL: 0.1, 2.5, 2.5, 4 mg/kg/d NOAEL: 5, <100, 100 ppm NOAEL: <15, 30 mg/kg/d
	2	90d, rat, feed	1/1	1/1	NOAEL: 50 ppm
	3	TG422, rat, gavage 90d, rat, feed 90d, dog, feed 104w, rat, feed	1/1 1/1 1/1 1/1	4/1	NOEL: <30 mg/kg/d NO(A)EL: 100 mg/kg/d NOEL: 32 mg/kg/d NOEL: 47 mg/kg/d
	4	30d, rat, feed	1/1	1/1	NOAEL: 5658 mg/kg/d
2016-3 (ICAPO)		TG422, rat, gavage TG422, rat, feed **90d rat, gavage***	2/2 1/1 2/2	5/5	NOAEL: 1000 mg/kg/d NOAEL: 2000 mg/kg/d **NOAEL: 1000, 1100 mg/kg/d***
2016-4 (ICAPO)		11d, rat gavage 28d, rat gavage **90d, rat, gavage*** **90d, mice, gavage*** sub-chronic, rat, gavage 18m, mice, gavage 2y, rat, gavage	1/1 1/1 2/2 1/1 1/1 1/1 1/1	8/2	NOAEL: 100 mg/kg/d NOAEL: 100 mg/kg/d **NOAEL: 125, 100 mg/kg/d*** **NOAEL: 125 mg/kg/d*** NOAEL: 25 mg/kg/d NOAEL: 200 mg/kg/d NOAEL: 200 mg/kg/d
2015-2 (Canada)	1	28d, rat, gavage	1/1	1/1	NOAEL: 15 mg/kg/d
	2	TG422, rat, gavage 90d, rat, gavage	2/2 1/1	3/3	NOAEL: 5, 25 mg/kg/d NOAEL: <100 mg/kg/d
	3	TG422, rat, gavage	1/1	1/1	NOAEL: 25 mg/kg/d
	4	28d, rat, gavage TG422, rat, gavage	1/1 1/1	2/2	NOAEL: 50 mg/kg/d NOAEL: 40 mg/kg/d
2015-3 (Japan)	1	14w, rat, gavage 18w, rat, gavage 18w, rat, feed	1/1 1/1 1/1	3/3	NOEL: <6 mg/kg/d NOEL: 15 mg/kg/d NOEL: -
	2	13w, rat, gavage	1/1	1/1	NOEL: 31 mg/kg/d

*Only 90 day data (TG408 study and similar studies) are used as source chemical data for read-across.

3. How to explain the impact of structural differences on toxicity

As mentioned in the considerations document of the last review cycle this is one of the important issues shared by read-across approaches for all endpoints (OECD, 2016a). In order to make a reliable category, the impact of the differences in chemical structure on the target effect should be explained. Table 6 shows how each of the five case studies have addressed this.

Similarities in effects are typically explained by the commonality in structure (e.g. common mode of action induced by common functional group) and similarities/differences in toxicity levels are typically explained by the similarities/differences in kinetics (e.g. differences in physico-chemical properties by the differences in alkyl chain length) as can be seen in Case Studies 2016-3, 2016-4 and 2015-3.

On the other hand, more complex cases were found in Case Studies of 2016-1 and 2015-2. For example, similarities and differences in observed effects were found at the same time in Case Study of 2016-1. In the two case studies, subcategorization was applied based on detailed similarity analysis for clarifying the relationship between the differences in chemical structures and in toxicity effects/levels.

Table 6. How to Explain the Impact of Structural Differences on Toxicity

Year-No. (Lead)	Commonality in structure / Variation in structure	Main factor impacting toxicity effect	Observed trend in toxicity
2016-1 (Japan)	Phenolic Benzotriazole	Distribution	Similarity in primary target organ: liver
	Substituent	Mode of action of hepatotoxicity suggested by transcriptomic profiles Kinetics (e.g. reactivity of functional group by the effect of steric hindrance)	Differences in liver toxicity levels
2016-3 (ICAPO)	n-Alcohol	Mode of action: nonpolar narcotics	Similarity in effect: mild liver effects
	Chain length	Kinetics (affects most physico-chemical properties (e.g. Low Kow values increase with increasing chain length))	Similarity in toxicity levels (The narrow range of chain length for the applicability domain limits the impact on kinetics)*
2016-4 (ICAPO)	2-Alkyl-1-alkanol	Mode of action: nonpolar narcotics	Similarity in effect: mild liver effects
	Chain length	Kinetics (affects most physico-chemical properties (e.g., Low Kow values increase with increasing chain length)	Similarity toxicity levels (The narrow range of chain length for the applicability domain limits the impact on kinetics)*

Year-No. (Lead)	Commonality in structure / Variation in structure	Main factor impacting toxicity effect	Observed trend in toxicity
2015-2 (Canada)	Diphenyl amine	Mode of action induced by common functional group	Similarity in primary target organ: liver
	Substituent without functional group	Kinetics	Differences in liver toxicity levels Differences in target organ: spleen
2015-3 (Japan)	Allyl ester	Mode of action by common toxicant of metabolite: allyl alcohol	Similarity in liver effect: hepatocyte and bile duct
	Chain length , degree of branch	Hydrolysis rate to produce the toxicant of allyl alcohol	Differences in liver toxicity levels

* Difference in the trend of the toxicity levels between n-alcohol and 2-alkyl-1-alkanol is due to the difference in kinetics between the two groups.

4. How to use new approach methodology data

As mentioned in Subsection 4.3.1, mechanistic probability is important for accepting read-across predictions. New approach methodologies are expected to be used for enhancing the evidence for the argument in toxicological relevance. As shown in Table 7, four case studies demonstrate promising ways to use of new approach methodology data such as investigating possible mode of action and reducing uncertainty for low/no toxicity prediction by increasing weight of evidence.

Table 7. How to Use New Approach Methodology Data

Year-No. (Lead)	Used data	Main result	Purpose of use
2016-1 (Japan)	Transcriptome data (in vivo, mouse liver) generated by the authors 5 chemicals	Activation of some nuclear receptors and induction of oxidative stress are associated with observed effects in the liver. Some members have different profiles.	Subcategorization by possible mode of action
2016-3 (ICAPO)	Tox Cast data 8 chemicals, 700 assays, 3315 studies	Only < 2.7% (88/3315 studies) of the ToxCast assays showing any activity and none of the active assay being associated with specific bioactivity.	Reduce the uncertainties associated with low/no toxicity prediction by increasing WOE in the confidence in the mechanistic relevance and completeness of the read-across
	In silico profilers of the COSMOS Project of SEURAT-1 9 chemicals	The-alkanols have no predicted potential of nuclear receptor binding	

Year-No. (Lead)	Used data	Main result	Purpose of use
2016-4 (ICAPO)	Tox Cast data 2 chemicals, 250 and 602 assays, respectively	Only 10 of the 852 studies showing any activity and none of the active assays being associated with specific bioactivity	Reduce the uncertainties associated with low/no toxicity prediction by increasing WOE in the confidence in the mechanistic relevance and completeness of the read-across
	In silico profilers of the COSMOS project of SEURAT-1 12 chemicals	No potential receptor binding was predicted.	
2015-2 (Canada)	ToxCast/Tox21™ high throughput screening program data 1 chemical,169 assays	Six active assays: Perturbation in biological activity of liver cells in vitro Not active in any of the cytotoxicity assays	Discussion of possible mechanism of target effect

5. How to reduce uncertainties

Uncertainty analysis was reported in each case study. The case studies demonstrated that uncertainties were able to be reduced in different ways. Table 8 shows examples of how uncertainty was reduced in each case study.

As mentioned in Subsection 4.3.1, one of the viewpoints for reducing uncertainty is transparency for explaining how the prediction was derived. From this viewpoint, uncertainties can be reduced without generating new data. For example, the uncertainties pointed out by the reviewers were able to be reduced in many cases by providing more detailed discussions and showing more details of repeated dose toxicity data.

Another viewpoint is to increase of WOE for reducing uncertainties both in relation to the similarity justifications and the completeness of the read-across argument. The case studies demonstrated that new approach methodology data and QSAR data can be used for increasing the WOE, however, the relevance of these data to the mechanistic probability of the toxicity need to be explained as shown in each case study. In this respect, developing guidance for reporting new methodology data would be important.

Table 8. Examples of reduced uncertainty

Year-No. (Lead)	Reduced uncertainty	How to reduce the uncertainty
2016-1 (Japan)	Factor for the differences in the toxicity levels of the hepatotoxicity induced by phenolic benzotriazoles	Generation of transcriptome data to investigate the mode of action of the hepatotoxicity for subcategorization (See Table 7).

Year-No. (Lead)	Reduced uncertainty	How to reduce the uncertainty
2016-3,4 (ICAPO)	Uncertainties associated with low/no toxicity prediction	The concordance of in vivo, in vitro, USEPA toxicity forecaster (ToxCast) results and other new-methods data (See Table 7).
2015-2 (Canada)	(Before revision) Observed or potential differences in: • Chemical structure (alkyl vs. phenyl substitution) • Range in physicochemical properties across all SDPAs • Bioavailability • Systemic Effects	Provided more in-depth analysis to establish subgroups based on the differences. Supported the bioavailability and metabolism comparison with predictive tools. Significantly expanded the study summaries to give the case study more details on the observed effects.
2015-3 (Japan)	(Before revision) The hypothesis that metabolites other than the toxicant (carboxylic acids) do not induce other toxic effects.	Enhanced the discussion and present result of toxicity studies of two branched carboxylic acids was presented

5. CONCLUSION

Five case studies were reviewed in the second review cycle of the project. Three of them are case studies on read-across, one is a case study on cumulative risk assessment based on grouping of chemicals, and the other is a case study on an exposure-based chemical safety assessment workflow that does not rely on animal testing. The review exercise of the case studies by the project team clarified that all the five case studies illustrate pragmatic use of alternative methods within IATA by different approaches and contain a lot of valuable knowledge that should be shared by member countries for promoting the use of IATA.

Based on the review results of the five case studies, the identified areas for further developing guidance, which was developed in the first review cycle, were updated. Especially, two new areas are identified as high priority areas. One is the use of new approach methodologies. All the five case studies illustrate good examples of use of new approach methodologies for enhancing mechanistic plausibility of IATA. The other is the area of low/no toxicity. No harmonised approaches for read-across for low/no toxicity have been developed due to its intrinsic difficultly. Two case studies of this review cycle have successfully demonstrated such predictions. In addition to the new areas uncertainty analysis/reporting was still identified as high priority area also in the review cycle.

The considerations of the review cycle especially focuses on read-across for repeated dose toxicity since five case studies of this area have been obtained from the first and second review cycles. First, lessons learned from two case studies developed by ICAPO were summarised. The lessons include the principles for increasing acceptance of a read-across prediction for complex endpoints and the opportunities for reducing uncertainty to increase confidence of a read-across prediction. Secondly, it is described how each of the five case studies addressed specific issues such as use of new approach methodology and reported uncertainty in order to capture concrete examples to be potentially used for further guidance.

In summary, the considerations for developing further guidance on IATA have been advanced from that of the first review cycle by adding the review experience of five new case studies of the second review cycle. A certain level of experience has been accumulated in the area of read-across especially in the topic of uncertainties.

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ANNEX: TEMPLATE USED FOR THE 2016 CASE STUDIES

Title: Case Study on the use of Integrated Approaches for Testing and Assessment for "Target Endpoint(s)" of "Target Chemical(s)/Category"

(N.B. The following template should not be viewed as a strict structure, but rather identifies the information that should be included in this type of case study. The template will be revised based on experience with use and depending on the specific case study additional information may be required or particular sections may not apply).

Foreword

(This should include a very short summary of the purpose, endpoints covered and description of the target chemical(s)/category)

Table of Contents

1. Purpose

1.1. Purpose of use

- Specify the purpose of use of the IATA (eg. regulatory context, hazard identification, hazard characterization, risk assessment, screening etc.). If in a regulatory context, provide a short description of any (eg. legal) requirements for the IATA approach to be accepted.

1.2. Target chemical(s)/category definition [See 3.2.3.1 of the grouping guidance]

- For analogue approach, provide the chemical descriptor common identifiers (including CAS number, name and composition including impurities) and chemical structure(s) of the target substance(s).
- For category approach, provide a summary of the common features of the category members; describe the boundaries; allowed variations (eg. in chemical structure); composition including impurities; and if known, any restrictions.

1.3. Endpoint(s)

- Identify the endpoint(s) for which the analogue/category approach is applied.

2. Hypothesis for the analogue approach/category [See 2.4 and 3.2.1 of the grouping guidance]

- For an analogue approach, describe the characteristics a substance must have to be suitable as a source substance. Provide the hypothesis for why read-across can be performed between the source and target chemical [See 4.2.2 of the grouping guidance].
- For a category approach, provide the hypothesis for why the category was formed including the relational features of the category. Provide the hypothesis for why read-across can be performed within the category [See 5.2.2 of the grouping guidance].

- These hypotheses can be argued by the number of elements as follows [See 3.2.3 of the grouping guidance].
    - Chemical identity and composition
    - Physical-chemical properties and other molecular description
    - Kinetics: Absorption, distribution, metabolism and excretion
    - Mode/Mechanism of action or adverse outcome pathways (MOA/AOP)
    - Chemical / biological interaction
    - Responses found in alternative assays
    - Information obtained from other endpoints/species/routes
    - Information on fate in the environment (hydrolysis, biodegradation)
    - The route and duration of expected exposure

  Ideally, all elements should be addressed, if relevant for the assessment.

- Especially, hypothesis of mechanism(s) (AOP/MOA) for that the target chemical induces target endpoint toxicity need to be described in this section. Hypothesis of structural bounders for the mechanism should also be described.
- Describe how a data gap is intended to be filled if this is the purpose.

3. Source chemicals/Category members [See 2.3, 4.2.2 and 5.2.2 of the grouping guidance]

3.1. Identification and selection of source chemicals/category members

- Provide the selection criteria, based on the hypothesis described in section 2, that were used to identify the source chemicals/category members.
- Provide rational for selection of analogue(s)/category members with respect to the defined purpose and endpoint.
- Consider selection bias selecting source chemicals in relation to employment of the analogue and of the category approach (e.g. data completeness, support for hypothesis etc.).
- Describe the methods used to identify the source chemicals/category members (e.g. inventories and tools used should be provided). Listing search criteria to establish initial pool of candidate analogues is helpful.

3.2. List of source chemicals/ category members

- Provide the common chemical identifiers (including CAS number, name and composition including impurities) and chemical structure(s) of the source chemicals/category members. (See 3.2.3.1.3 of the grouping guidance; example of the chemical identifiers for UVCBs)

4. Justification of data gap filling

4.1. Data gathering [See 4.2.3 and 5.2.3 of the grouping guidance]

- Provide the methods used for gathering the data for target and source chemicals/category members (eg. selection criteria of the data, data source).
- Provide the name, version, owner of the models and reference number of QMRF inventory maintained by the JRC (http://qsardb.jrc.it/qmrf/) used for deriving QSAR estimation data.

4.2. Data matrix [See 4.2.4, 4.2.5, 5.2.4 and 5.2.5 of the grouping guidance]

- Provide a matrix of data (see data matrix template).
- Provide detailed data in an annex, as necessary (in case that the detailed data are used for the justification of the hypothesis).
- Provide analysis of the available information for suitability regarding the defined purpose. If possible, the cells should also indicate the available key study results.

4.3. Justification [See 2.5, 2.6, 4.2.6 and 5.2.6 of the grouping guidance]

- Based on the data matrix, summarise how these data support the hypothesis described in section 2.
- Identify similarities/trends in the experimental data of the endpoint(s) for the chemicals in the data matrix and verify their concordance with hypothesis described in section 2.
- Identify which elements drive the toxicity/endpoint.
- For category approach, describe the set of inclusion and/or exclusion rules that identify the boundaries within which reliable estimations can be made for category members. A broader consideration including mechanistic information, profiling computational methods, screening with non-standard in vitro tests should be given. Clearly indicate the boundaries of the category and for which substances the category does not hold [See 5.2.4 of the grouping guidance: example of outlier].

- The applicability domain of each estimation method including QSAR and alternative methods should be discussed based on the consistency between the estimation data and the experimental data of the source chemical(s)/category members.

5. Strategy for and integrated conclusion of data gap filling

5.1. Uncertainty

- Discuss the uncertainty of each factor for the read-across.
- Aspects can include uncertainty and confidence associated with the data and assumptions used to develop the similarity rationale of the analogues/category members and uncertainty and confidence associated with the underlying data used for read across from the source chemicals.
- The following is an example of reporting uncertainty (Please modify it appropriately):

Factor	Uncertainty (low, medium, high)	Comment
Hypothesis used for the read across		
Structural similarity		
Similarity of physico-chemical properties		
Similarity of toxicokinetics data		

Factor	Uncertainty (low, medium, high)	Comment
Similarity of other supportive data (e.g. data related to key event)		
Number of analogues used for the read across		
Quality of the target endpoint data used for the read across		
Similarity of the target endpoint data (among source chemicals)		
Concordance and weight of evidence of all data used for justifying the hypothesis		
Overall uncertainty of the read across		

5.2. Integrated conclusion

- Provide the strategy used to fill the data gap and integrated conclusion of data gap filling. In case of category approach, indicate proposed conclusion/value for each data gap.
- Give discussion how to further address the uncertainties.
- Finally, provide a short conclusion wrapping up the outcome of the evaluation.

References

Annex

Data matrix for analogue approach

Data matix, IATA for "indication of title of case study"

Chemical ID									
		Source1	Target	Source2	Source3	Source4	Source5	Outlier1	Outlier2
CAS									
Name									
Structure									
Summary of data gap filling									
		Source1	Target	Source2	Source3	Source4	Source5	Outlier1	Outlier2
Target endpoint1	Experimental result	value, unit, test method (eg. test guide line)		value, unit, test method (eg. test guide line)		value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)
	Integrated conclusion (eg. read-across)		derived result						
Target endpoint2	Experimental result	value, unit, test method (eg. test guide line)		value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)		value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)
	Integrated conclusion (eg. read-across)		derived result						
Molecular profiling related to the analogue approach hypothesis									
Parent chemical	Profiler 1 (name, version)								
	Expert system 1 (name, version)								
Metabolite*	Profiler 1 (name, version)								
	Expert system 1 (name, version)								
Physical-chemical data									
Melting point									
Boiling point									
Density									
logPow (measured value)									
logPow (calculated value)									
…									
Kinetics**									
Absorption									
Distribution									
Metabolism									
Excretion									
Supporting data related to the target endpoint(s)									
		Source1	Target	Source2	Source3	Source4	Source5	Outlier1	Outlier2
In vivo	Toxicogenomics								
	…								
In vitro	Alternative method A								
	…								
In chemico	…								
In silico	QSAR1 (Target endpoint1)								
	QSAR2 (Target endpoint1)								
	QSAR3 (Target endpoint2)								
	QSAR4 (In vitro endpoint)								
	…								
Other data	Battery approach								
	…								

* More relevant metabolite such as toxicant

**General outline of relative comparative kinetics

Data matrix for category approach

Data matix, IATA for "indication of title of case study"

Chemical ID									
		Member 1	Member 2	Member 3	Member 4	Member 5	Member 6	Member 7	Member 8
CAS									
Name									
Structure									
Summary of data gap filling									
		Member 1	Member 2	Member 3	Member 4	Member 5	Member 6	Member 7	Member 8
Target endpoint1	Experimental result	value, unit, test method (eg. test guide line)		value, unit, test method (eg. test guide line)		value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)
	Integrated conclusion (eg. read-across)		derived result		derived result				
Target endpoint2	Experimental result	value, unit, test method (eg. test guide line)		value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)		value, unit, test method (eg. test guide line)	value, unit, test method (eg. test guide line)
	Integrated conclusion (eg. read-across)		derived result				derived result		
Molecular profiling related to the category hypothesis									
Parent chemical	Profiler 1 (name, version)								
	Expert system 1 (name, version)								
Metabolite*	Profiler 1 (name, version)								
	Expert system 1 (name, version)								
Physical-chemical data									
Melting point									
Boiling point									
Density									
logPow (measured value)									
logPow (calculated value)									
…									
Kinetics									
Absorption									
Distribution									
Metabolism									
Excretion									
Supporting data related to the target endpoint(s)									
		Member 1	Member 2	Member 3	Member 4	Member 5	Member 6	Member 7	Member 8
In vivo	Toxicogenomics	result	result	result	result	result	result	result	result
	…								
In vitro	Alternative method A		result	result	result				
	…								
In chemico	…								
In silico	QSAR1 (Target endpoint1)	result	result	result	result	result	result	result	result
	QSAR2 (Target endpoint1)	result	result	result	result	result	result	result	result
	QSAR3 (Target endpoint2)	result	result	result	result	result	result	result	result
	QSAR4 (In vitro endpoint)	result	result	result	result	result	result	result	result
	…								
Other data	Battery approach	result	result	result	result	result	result	result	result
	…								

* More relevant metabolite such as toxicant

**General outline of relative comparative kinetics