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

Third Review Cycle (2017)

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REPORT ON CONSIDERATION FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA)- THIRD REVIEW CYCLE (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 four case studies, listed below, submitted to the 2017 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 INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) FOR ESTROGENICITY OF THE SUBSTITUTED PHENOLS, ENV/JM/MONO(2018)26, Series on Testing & Assessment No. 290.
2. PRIORITISATION OF CHEMICALS USING THE INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA)-BASED ECOLOGICAL RISK CLASSIFICATION, ENV/JM/MONO(2018)27, Series on Testing & Assessment No. 291.
3. CASE STUDY ON GROUPING AND READ-ACROSS FOR NANOMATERIALS GENOTOXICITY OF NANO-TiO_2, ENV/JM/MONO(2018)28, Series on Testing & Assessment No. 292.
4. A CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR SUB-CHRONIC REPEATED-DOSE TOXICITY OF SIMPLE ARYL ALCOHOL ALKYL CARBOXYLIC ESTERS: READ-ACROSS, ENV/JM/MONO(2018)29, Series on Testing & Assessment No. 293.

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 countries. In addition, these cases studies should not be interpreted as official regulatory decisions made by the authoring member countries.

This document has been prepared by a project team of the Working Party on Hazard Assessment and was endorsed at the 2nd meeting of the Working Party on Hazard Assessment in June 2018.

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 (Quantitative) Structure-Activity Relationships ((Q)SAR), chemical categories, Adverse Outcome Pathways (AOPs) and *in vitro* testing 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 each 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) and the second review cycle of the project (2016) included nine case studies and two considerations documents, which have all been published (OECD, 2016a; 2016b; 2016c; 2016d; 2016e; 2017a; 2017b; 2017c; 2017d; 2017e; 2017f).

In the third review cycle (2017), the four case studies shown in Table 1 were reviewed. The final case studies are published [ENV/JM/MONO(2018)26 -29, Series on Testing and Assessment No. 290-293]. 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 four case studies in the third review cycle and summarises the learnings and lessons stemming from the case studies reviewed in the three 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 Third Review Cycle (2017)

No.	Title	Lead	Purpose of Use	References
1	Estrogenicity of Substituted Phenols	Canada United States	Demonstration of a screening for estrogenic potential of chemical substances with *in silico* and *in vitro* data, and hazard characterisation of substituted phenols for estrogenicity.	OECD, 2018a
2	Prioritisation of chemicals using the Integrated Approaches for Testing and Assessment (IATA)-based Ecological Risk Classification	Canada	Identification of priorities for ecological risk assessment under the Canadian Environmental Protection Act, 1999 (CEPA)	OECD, 2018b
3	Case study on grouping and read-across for nanomaterials ? genotoxicity of nano-TiO_2	JRC[1]	Determination of genotoxic hazard potential for nano TiO_2 materials based on *in vitro* comet assay results, applying ECHA's workflow for grouping and read-across and Read-Across Assessment Framework for nanomaterials.	OECD, 2018c
4	A Case Study on the Use of Integrated Approaches for Testing and Assessment for Sub-Chronic Repeated-Dose Toxicity of Simple Aryl Alcohol Alkyl Carboxylic Esters: Read-Across	ICAPO[2]	Illustration of read-across based on similarities in toxicokinetics (metabolism and excretion) and toxicodynamics (lack of reactivity, receptor binding and systemic effects, as well as similar NOAELs for source substances).	OECD, 2018d

[1] JRC: European Union / Joint Research Centre
[2] ICAPO: International Council for Animal Protection in OECD Programmes

2. PROCESS FOR REVIEWING THE CASE STUDIES

The following 14 countries/organisations participated in the third review cycle: Australia, Canada, Denmark, Germany, Italy, 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).

The authors were requested to consider the templates provided in the Annex 1 and 2. The template for the case studies on read-across 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 updated based on review experience of the case studies in the first and second review cycles. The general template for IATA case studies based on building blocks was developed in order to fit the case studies using multiple IATA components, such as AOP/ Mode of Action (MOA), Defined Approaches, Workflows, Grouping/Read-Across.

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 third meeting of the IATA Case Studies Project (27-28 November 2017) in order to finalise the case studies and summarise the learnings and lessons.

3. SUMMARY OF REVIEW RESULTS

3.1. Case Study 1: Estrogenicity of Substituted Phenols [Canada/the United States]

This case study is intended to offer practical insights to inform the development of guidance for deriving and applying IATA in a regulatory context. The goal of this case study is to demonstrate that *in silico* and *in vitro* data can be used to screen for estrogenic potential of chemical substances, and that these data sources provide a good proxy for estimating the *in vivo* dose for a point of departure.

Three substituted phenols (partially hindered and non-hindered) were selected as target chemicals and the analogue chemicals were identified for each of the target chemicals. The available data, including QSAR predictions and the data from *in vitro* high throughput screening (HTS) and *in vivo* studies, were collected and used to determine the estrogenic potential of the target chemicals. The hypothesis was supported based on the case study; i.e. that the non-hindered substituted phenols are active for estrogenic potential, whereas the hindered phenols are inactive for estrogenic potential. Also, the case study exemplified the use of data from alternative methods and contributed to confidence in using this type of information by comparing the data from *in silico*, *in vitro* HTS and *in vivo* studies.

Please refer to ENV/JM/MONO(2018)26 Series on Testing & Assessment No. 290 for the case study to put the following points into context.

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

- Provides clear hypothesis of the IATA workflow.
- Systematic data gathering approach for chemical structure and *in vitro* and *in vivo* studies.
- This prioritisation scheme provides a real example of using an IATA workflow to consider New Approach Methodologies (NAMs), QSAR, chemical fingerprinting, read-across and mechanistic information. It was mentioned that this IATA is quite close to a Defined Approach due to its concrete data interpretation procedure.
- Points out possible use of HTS bioactivity data and High-Throughput Toxicokinetics (HTTK)-based *in vitro* to *in vivo* extrapolation for prioritisation.

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

- More explanation was requested on the section on Adverse Outcome Pathway (AOP) to clarify how estrogen receptor (ER) activation is mechanistically associated with *in vivo* effects, especially increases in uterine weight. However, there are no available AOPs relevant to estrogenic effects of the group of phenols. If relevant AOPs are developed, future applications of this IATA could be updated with this information.
- It was requested to specify how the assays linked with various MOAs. The authors added a table, which describes how the assays are used to measure steps in the estrogenic MOA.

- More explanation and illustration were requested to make it easier to understand how to select analogue chemicals. The authors expanded the description on this point and added an illustration.

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

- The uncertainty associated with analogue identification and evaluation was focused on in the case study. Two complementary approaches were used to identify source analogues for three target chemicals and provided different sets of analogues for each of the target chemicals with some overlaps. Application of the two approaches highlighted how to address the challenge of analogue identification and pointed out the importance of consideration for scaffolding the target chemicals during analogue selection. Further guidance could be developed for the use of the different methods and maybe also on the selection of appropriate models in general.
- There are uncertainties related to the domain of applicability when using QSAR model predictions for any given chemical space. The uncertainties need to be determined and were considered as relevant to the chemicals evaluated in this case study.
- Broadly, this case study highlighted the need to develop an approach to uncertainty analysis for NAMs data.

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

- ***Australia:*** The approach taken in this study regarding establishing an aggregate pool of phenol chemicals from various international inventories and the use of lower tier data to support hypothesis would be useful in our regulatory context. However, the application of two distinct yet complementary approaches to identify source analogues may not be practicable in the Australian regulatory context due to resources required. The result produced by the second approach to identify analogues (the global similarity method in the case studies) needs further investigation for application in a regulatory context.
- ***Canada****:* Canada is working to develop approaches to incorporate NAMs into regular risk assessment practices. Read-across is frequently used to address data gaps and assess groups of chemicals under Canada's Chemicals Management Plan (CMP)[3]; therefore, there is comfort in that area. The generation of this case study and others illustrating approaches toward the integration of NAM into risk assessment activities is building the foundation for using these results in Canada's regulatory context. Additional comfort will continue to be built around the use of *in silico* and *in vitro* data in Canada's regulatory risk assessments as we begin to introduce this IATA (or elements thereof as relevant) into the assessment of certain substituted phenols under the CMP.
- ***Japan:*** This approach would not be used in Japan but this is not a scientific reason rather a legislative reason. Screening level assessment of estrogenicity is not conducted in Japan's Chemical Substances Control Law (CSCL)[4].

[3] Canada, Chemicals Management Plan. https://www.canada.ca/en/health-canada/services/chemical-substances/chemicals-management-plan.html

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

- ***Netherlands:*** This approach may be used in screening. As there is no fixed definition for the regulatory endpoint endocrine disruption (or estrogenicity) under REACH, we are bound to use indirect reasoning to make the case for a substance. It is doubtful that the approach will be sufficient in itself e.g. to make the case of endocrine disruption for inclusion of a substance on the Registration, Evaluation, Authorisation of Chemicals (REACH)[5] Annex XV candidate listing due to the subjective selection, but it could very well form a part of the argumentation.
- ***Sweden:*** This approach can be used for screening purposes.

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

- Guidance on the use and reporting of results of HTS and HTTK assays
- Guidance on use, reporting and the identification and characterisation of uncertainty for new approach methodologies

3.2. Prioritisation of chemicals using the Integrated Approaches for Testing and Assessment (IATA)-based Ecological Risk Classification [Canada]

The purpose of the case study is to describe a novel prioritisation approach for organic chemicals based on an IATA, called the Ecological Risk Classification (ERC) approach, which has been used by Environment and Climate Change Canada (ECCC) to identify priorities for ecological risk assessment under the *Canadian Environmental Protection Act, 1999* (CEPA)[6].

The ERC is a risk-based prioritisation approach which employs a weighted consideration of multiple descriptors to establish both the hazard (potency) and the exposure profiles to determine a risk classification of a chemical substance. Hazard profiles were established based principally on descriptors regarding mode of toxic action, chemical reactivity, food web-derived internal toxicity thresholds, bioavailability, and chemical and biological activity. Descriptors considered in the exposure profiles include potential emission rate, overall persistence, and long-range transport potential. A risk matrix is used to assign a low, moderate or high level of potential concern for substances based on their specific hazard and exposure profiles. QSAR prediction and the data from *in vitro* and *in vivo* studies were used to determine hazard profiles. 640 organic substances were prioritised by using ERC and 5 percent of them were classified as having a high risk potential, 16% as moderate risk potential, and 79% as low risk potential[7].

Profiles of three substances, phenol, 2-(1-methylpropyl)-4,6-dinitro- (CAS RN 88-85-7); phosphoric acid, triphenyl ester (CAS RN 115-86-6); and benzenamine, N-phenyl- (CAS RN 122-39-4), identified by ERC as high, moderate, and low priorities, respectively, were provided as examples to illustrate hazard and exposure prioritisation profiles and risk classification.

Please refer to ENV/JM/MONO(2018)27, Series on Testing & Assessment No. 291 for the case study to put the following points into context.

[5] EU, Registration, Evaluation, Authorisation of Chemicals (REACH). https://echa.europa.eu/regulations/reach/understanding-reach

[6] Canada, Canadian Environmental Protection Act, 1999. http://laws-lois.justice.gc.ca/eng/acts/C-15.31/

[7] ECCC, Science approach document: ecological risk classification of organic substances. http://www.ec.gc.ca/ese-ees/A96E2E98-2A04-40C8-9EDC-08A6DFF235F7/CMP3%20ERC_EN.pdf

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

- Clear and transparent presentation of the steps, concepts and classification rules in the ERC.
- Good example of the use of IATA in prioritisation, particularly for hazard assessment.
- Use of many different types of information effectively to derive reliable prioritisation result.
- Fit-for-purpose approach, allowing to process a high number of chemicals and to apply tailored regulatory approaches suited to the level of effort/resources required.
- Retrospective analysis comparing the ERC with the original prioritisation approach, showing that the IATA approach is better able to identify substances of potential concern regarding hazard/exposure.

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

- Clarification of ERC concept was requested for the following points:
  - How to calculate the Hazard Assessment Factor (HAF)[8] used
  - Differences between regional and local scale exposure
  - Tonnage thresholds used in ERC
  - Local field adjustment procedure
  - Application of DNA and Protein binding descriptors

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

- The domain of applicability of each model was ranked as a medium uncertainty because each of the profiled chemicals was not manually checked to determine if they were in the domain of the model. However, ERC was based on the weight-of-evidence approach, and the impact of uncertainties was reduced by not relying on a single hazard or exposure metric. In some cases, model results were not available for all organic substances due to limitations of the model domain and results were considered only as supporting information.
- Extrapolating from mechanistic data to an adverse outcome in an organism was ranked as medium uncertainty. The results from the models used for profiling chemical reactivity and for bioactivity suggested a potential for interaction with biological tissues. However, apical endpoints may or may not be realised depending on the fate and toxicokinetics of the substance. As a result, protein and DNA binding results were applied as an adjustment to the preliminary hazard classification and bioactivity results were considered only as supporting information.

[8] Arnot JA, Mackay D. 2008. Policies for chemical hazard and risk priority setting: Can persistence, bioaccumulation, toxicity and quantity information be combined? Environ. Sci. Technol. 42(13): 4648-4654. https://pubs.acs.org/doi/abs/10.1021/es800106g

- Although the fluctuations to the quantities in commerce and prediction of long-range transport in air were ranked as low uncertainties, the preliminary exposure classes are proxies for exposure. However, preliminary exposure classes were based on available data, such as fate, emissions rates to the environment and Canadian use pattern data. Further refinement of exposure potential would be examined in risk assessment, not prioritisation.

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

- ***Netherlands*:** The results from this case study could not be used for prioritisation of substances under REACH, as there is a different prioritisation system in EU[9]; however, the principle (taking into account emissions, persistency, fat and hazard) is similar. Nevertheless, it was very useful to learn about different prioritisation criteria, and system based on IATA and alternative methodologies.
- ***Sweden*:** This approach could possibly be used in a screening context.
- ***ECHA:*** Possibly, but exposure would probably be assessed in more detail.

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

- Guidance on developing prioritisation scheme based on IATA would be helpful to improve and harmonise the prioritisation methods
- Guidance for reporting from exposure simulation models (e.g. environmental concentrations)

3.3. Case Study 3: Case study on grouping and read-across for nanomaterials – genotoxicity of nano-TiO_2 [JRC]

The case study is applying the workflow for grouping and read-across proposed in the ECHA REACH guidance update for nanomaterials (NMs)[10] and exploring the extent to which ECHA's Read-Across Assessment Framework (RAAF)[11] is applicable to NMs for identifying sources of uncertainty associated with the read-across. The purpose of the case study is to determine the genotoxic hazard potential of two target TiO_2 NMs, based on *in vitro* comet assay results from other TiO_2 NMs.

The dataset of the analogues includes six NMs with different properties: different primary and crystallite sizes (5-100nm), different crystalline types (anatase and rutile) and surface characteristics (coated or uncoated). The grouping hypothesis derived from the physicochemical characteristics and *in vitro* comet assay results is that uncoated TiO_2 NMs may damage DNA, but this potential would be masked by non-reactive coating and large

[9] The Dutch National Institute for Public Health and the Environment (RIVM), Prioritisation in processes of the European chemical substances regulations REACH and CLP. http://www.rivm.nl/bibliotheek/rapporten/601352001.pdf

[10] ECHA, (2017), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals. https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf/71ad76f0-ab4c-fb04-acba-074cf045eaaa.

[11] ECHA, (2017), Read-Across Assessment Framework (RAAF). https://echa.europa.eu/documents/10162/13628/raaf_en.pdf.

amounts of impurities on the surface. A set of cheminformatics tools were used to identify the physicochemical properties that differentiate the analogues, determine their similarity and that may drive genotoxicity. The outcome of the *in vitro* comet assay was predicted for two target TiO_2 NMs, negative for the coated one, and positive for the uncoated one. These results were verified by experimental literature data.

Please refer to ENV/JM/MONO(2018)28, Series on Testing & Assessment No. 292 for the case study to put the following points into context.

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

- Practical application of the ECHA REACH guidance workflow for NMs.
- Concrete examples of how to build and justify the hypotheses for read-across for NMs (read-across not based on the chemical structure).
- Cheminformatics data analysis
- Evaluation of uncertainties and application of ECHA's RAAF to NMs

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

- Adaptation of the structure of the document for a better flow of the description of the read-across and streamlining of the tables
- Clearer description of what physical properties were related to grouping and read-across for these NMs for the endpoint examined (genotoxicity based on *in vitro* comet assay).
- The list of comet assays considered in the case study.

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

- There is uncertainty associated with the nanoform identification and physicochemical characterisation, which is subject to high variability in measurements (different experimental conditions, result ranges). The analysis of the variability of the physiochemical property measurements has shown that particle size distribution and zeta potential is dependent on the test method such as the dispersion medium used or different sonication methods.
- For the *in vitro* comet assay, an OECD test guideline is not available. Generally, the assessment of quality, reliability and relevance to human health endpoints of measured toxicity data as well as their interpretation is difficult, partly due to the uncertainty in applying existing testing protocols to NMs.
- The set of analogues considered in this case study was limited because of the scarcity of the data available or insufficient quality. Only the nanoforms that were completely identified by means of fundamental parameters like solubility, hydrophobicity, zeta potential, dispersability were considered. This led to a dataset with only six TiO_2 NMs. Thus, the hypothesis is based on a small dataset and considers a single *in vitro* endpoint (comet assay).
- There is some uncertainty related to the mechanism for genotoxicity for TiO_2. The majority of studies examined supported the hypothesis that the genotoxic effect of TiO_2 is masked by the presence of coating. However, the way in which the coating can prevent DNA damage is not entirely clear.

- The complexity and diversity of nanoforms is an issue to be considered in nanomaterial hazard assessment, compared to "conventional" chemicals.

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

- ***Australia:*** Yes, results of such a case study could be used for highlighting a hazard concern or weight of evidence conclusion, but may not be used for a regulatory decision such as recommending a hazard classification (as this case study is only a single assay to be used in genotoxicity evaluation).
- ***Canada:*** This IATA would be more useful in a regulatory setting if the scope was widened to increase the number of genotoxicity assays and the purpose was to read-across to make a call on overall genotoxic potential of NMs. A case study such as this one could be used as supporting information for justifying read-across in a risk assessment, but would not be the only factor considered. There was also a limited amount of well-conducted/appropriate toxicological tests and lack of physical-chemical characterisation of NM variants. The case study exemplified how ECHA's RAAF could be adapted for NMs as was suggested at the end of the case study.
- ***Netherlands:*** There is no standardised protocol available for the *in vitro* Comet assay, which hampers comparisons of studies and thus its use in read-across approaches. Therefore, the results presented can only be used as part of a weight-of-evidence.
- ***Sweden:*** Yes, it is possible. However, the legislation, data requirements and guidance documents need to be updated in order to ensure a regulatory use.
- ***ECHA:*** REACH foresees the possibility for adaptation using read-across. Possibly yes, the result of similar case/read-across study may be accepted, depending on the quality of the data available for the phys-chem characterisation of the nanoforms and for the toxicological endpoint considered. (NB: the *in vitro* comet assay addressed in this case study is not part of the data required under REACH).

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

- Guidance on defining boundaries of NMs
- Identification of the specific parameters relevant to grouping and read-across for nanomaterials particularly for grouping hypotheses based on similar physicochemical properties and consideration of coatings
- Guidance on the interpretation of NM-related data
- Guidance on use or reporting of cheminformatics tools including guidance on how much detail of the underlying models should be described
- General guidance on NMs
  - Standardised guidelines for characterisation and testing of NMs, including dispersion protocols for NMs
  - NM-specific and relevant protocols, in order to increase data availability/quality

3.4. A Case Study on the Use of Integrated Approaches for Testing and Assessment for Sub-Chronic Repeated-Dose Toxicity of Simple Aryl Alcohol Alkyl Carboxylic Esters: Read-Across [ICAPO]

The purpose of this case study is to fill data gaps with sufficient confidence in the predictions that may be used by member countries in risk assessment or other regulatory processes. The aim of this investigation is to examine the sub-chronic repeated-dose toxicity of three subcategories of similar aryl alkanoates by the use of read-across. The sub-category is defined as the C2 to C12 benzyl alkanoates, C2 to C12 2-phenethyl alkanoates and C2 to C12 3-phenpropyl alkanoates. In this category approach, the common features of the category members are common hydrolysis products, the C2 to C12 range of the simple saturated carboxylic acids and either benzyl alcohol, 2-phenethyl alcohol or 3-phenpropyl alcohol. It is hypothesised that read-across can be performed, within the category, ester to ester, as well as the common alcohol metabolite. It was illustrated where *in silico*, *in vitro* and *in vivo* metabolism of all the analogues in the chemical category is extremely similar and plays a key role in toxicity as well as how *in vivo* data for the target chemicals and key metabolites may be used to reduce uncertainties. It was concluded that a NOAEL derived from the in vivo data of Benzyl acetate, Benzyl alcohol, 2-Phenethyl alcohol and carboxylic acids can be read across to the other chemical substances within the subcategory or category based on toxicokinetic (TK) and toxicodynamic (TD) similarity.

Please refer to ENV/JM/MONO(2018)29, Series on Testing & Assessment No. 293 for the case study to put the following points into context.

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

- Data gap filing by read-across for a large number of target chemicals based on a small number of source chemicals by effectively using the information on common/similar metabolites shared by the category members. Also, the study uses read-across principles that extend well beyond structure and include elements such as bioavailability and reactivity resulting in a broader weight-of-evidence approach.
- Use of different experimental data, such as TK/TD data, for weight-of-evidence in order to strengthen the grouping hypothesis
- Clear description of the hypothesis with sufficient details and detailed description of the available experimental data.

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

- In response to reviewer's comment, the following points were added:
  - A figure of the common cytosolic metabolic pathway to visualise the pathway and explain that the major metabolites of benzyl acetate are benzoic acid and hippuric acid, and not the aryl alcohol.
  - A summary of *in silico* metabolic simulator results to compare the hypothesis with results from *in silico* toxicokinetics modelling.

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

- Overall, the identified uncertainties were classified as low. The following two uncertainties were classified as medium:

- Number of analogues used for read-across: Although the category members consisted of 42 chemicals, read-across was performed based on the *in vivo* toxicity data for two chemicals in the category and metabolites (two alcohols and carboxylic acid). However, the TK/TD similarities within the category members were confirmed by using the data from *in vivo*, *in vitro* and *in silico* for all chemicals in category. Therefore, the authors concluded that the number of analogues used for read-across was adequate.

- Overall uncertainty of the read- across predictions for 3-phenpropyl esters: There is no *in vivo* data available for 3-phenpropy esters, and the hazards of 3-phenpropyl esters were predicted based on the benzyl alkane esters and 2-phenethyl alkane esters.

- It was pointed out that there was significant uncertainty associated with the quality of the toxicity data reported for the source chemicals because the relevant studies were conducted 30-50 years ago, prior to the establishment of criteria for robust study designs. The reliable data, such as the studies conducted following by OECD Test guidelines in the Good Laboratory Practice (GLP), would be needed if the case study was applied for regulatory purposes in some countries.

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

- ***Australia:*** The approach taken in this study regarding selection of category members, use of sub-chronic repeated dose toxicity as health endpoint, use of toxicokinetic and repeated-dose toxicity data from the common metabolite to fill the data gap, provision of strong justification of data gap filling and low uncertainty of the approach would be useful in our regulatory context.
- ***Canada:*** The results of this case study would be useful in some of Canada's regulatory programmes. In other regulatory contexts, legislation requires studies to be conducted as GLP and to demonstrate a robust study design. For this reason, an evaluation of study reliability and relevance would be favourable. Establishing a category for read across demands even greater rigour given that the category will potentially serve as the model for numerous substances.
- ***Netherlands:*** Use of the benzyl alkane esters sub-category under REACH could be possible. For two other sub-categories, 2-Phenetyl alkane esters and 3-phenyl alkane esters, probably some additional evidence would be required.
- ***Sweden:*** Yes, it is possible. However, the legislation, data requirements and guidance documents need to be updated in order to ensure a regulatory use.
- ***ECHA:*** The proposed hypothesis and evidence presented is not suitable for data gap filling under REACH. The case study does not provide adequate elaboration on remaining uncertainties regarding the impact of structural differences (e.g. side chain of ester) and prediction of kinetics (esterase activity) in the absence of experimental evidence with the analogues. There is missing any relevant experimental data as reference for the phenpropyl esters. The value for screening and prioritisation is limited due to the limited number of so called analogues registered, and for obvious scientific variables that were not addressed, or addressed partially. Maybe refining the scope and definitions could bring advantages for screening purposes.

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

- Guidance on addressing and quantifying uncertainties
- Guidance on when *in vitro* data could be further generated to support read-across
- Guidance for evaluating the reliability of data including TK/TD data

4. LEARNINGS AND LESSONS

4.1. Summary of the Case Studies Reviewed in All the Review Cycles

This chapter summarises learnings and lessons stemming from the case studies of the project including the nine case studies from the past two review cycles. Table 2 shows a summary of the 13 case studies reviewed in the all review cycles to date.

The assessment approaches illustrated by the case studies are classified into four types: data-gap filling by read-across based on grouping of chemicals (9 case studies), grouping of chemicals for cumulative risk assessment, not for read-across (Case Study 2016-2), safety assessment workflow (Case Study 2016-5), and prioritisation of chemicals (2017-1, 2017-2). Although case study 2017-1 focused on a prioritisation scheme for potential endocrine active chemicals, it illustrated two systematic methodologies for identifying the analogues with cheminformatics and data analysis tools. Case study 2017-3 illustrated the approach on read-across for nano-TiO_2 considering nano-specific properties, such as crystal type, surface coating and size.

The target endpoints of the case studies were: repeated dose toxicity (7 case studies), neurotoxicity (one case study), mutagenicity (one case study), bioaccumulation (one case study), genotoxicity (one case study), estrogenicity (one case study) and ecotoxicity (one case study).

Every case study addresses some challenging topics related to IATA including use of MOA/AOP (6 case studies), capturing and communicating uncertainty (11 case studies), use of new approach methodologies (10 case studies) and low/no toxicity prediction (5 case studies).

Identified areas for further developing guidance from the 13 case studies are summarised in section 4.2.

Table 2. Summary of the Case Studies Reviewed in the Three Review Cycles

Year-No. (Lead)	Assessment approach	Endpoint	IATA topics				References
			AOP*1	UR*2	NAM*3	L/N*4	

2017-1 (Canada/US)	Prioritisation and hazard characterisation	Estrogenicity	X	X	X	X	OECD, 2018a
2017-2 (Canada)	Prioritisation of chemicals	Ecotoxicity	X	X	X		OECD, 2018b
2017-3 (JRC)	Read-across	Genotoxicity for nano-TiO_2		X	X		OECD, 2018c
2017-4 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2018d
2016-1 (Japan)	Read-across	Repeated dose toxicity		X	X		OECD, 2017b
2016-2 (US)	Grouping for cumulativerisk assessment	Neurotoxicity	X		X		OECD, 2017c
2016-3 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2017d
2016-4 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2017e
2016-5 (JRC/BIAC)	Safety assessment workflow	Repeated dose toxicity	X		X		OECD, 2017f
2015-1 (Canada/US)	Read-across	Mutagenicity	X	X			OECD, 2016b
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 and second review cycle, the following 9 areas for further developing guidance were identified as high priority areas from the 9 case studies (OECD, 2016a and 2017a):

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. Incorporation of new approach methodologies
6. Decision logic for low/no toxicity predictions
7. Uncertainty analysis and reporting
8. Integrated conclusion
9. Reporting templates for IATA based on a building block approach

The new areas for further developing guidance were identified based on the case studies in the third review cycle as follows:

1. **Uncertainty framework:** This area was identified from several case studies and was highlighted by JRC's presentation at the third meeting: Overview of current

IATA guidance. The overall uncertainty associated with IATAs is generated as the result of combining uncertainties of the individual IATA components (e.g. *in vitro* and *in vivo* test guidelines, new approach methodologies, QSAR), the various data types (e.g. measured or predicted data) and the associated quality and reliability of the data. This needs to be considered for use of IATA for regulatory purpose. An uncertainty framework would be helpful for consideration of the overall uncertainty in the IATAs.

2. **Use of data including new approach methodologies:** All case studies integrated various types of data generated by NAMs. Also, the importance of the reliability of the existing data (including *in vivo data*) used for forming a chemical category was highlighted during the discussion of the case study developed by ICAPO. To use the data, including NAMs, appropriately, the following areas were identified as needs for further guidance development:
   - Guidance on when *in vitro data* could be further generated to support read-across
   - Guidance for evaluating the reliability and robustness of data including TK/TD data
   - Guidance on the use and reporting of new approach methodologies (cheminformatics tools, HTS, HTTK assays)
3. **Guidance related to prioritisation schemes including exposure simulation models:** The two case studies 2017-1 and 2017-2 were addressing prioritisation using IATA. Case study 2017-1 illustrated the prioritisation scheme for potential endocrine disrupting chemicals based on *in silico, in vitro* and *in vivo* data for estrogenicity. Further, the *in vitro* HTS bioactivity data were also used to estimate an oral equivalent dose (OED) which when compared with estimates of exposure can be used as an approach for lower tier risk-based screening and assessment. The case study 2017-2 focused on prioritisation for chemicals from the view of ecotoxicity using the data from *in silico* and *in vitro*. Also, the case studies described the exposure classification with exposure simulation models. Therefore, the following two areas were identified based on the two case studies:
   - Guidance on developing prioritisation schemes based on IATA
   - Guidance for reporting from exposure simulation models (e.g. environmental concentrations)
4. **Guidance related to nanomaterials:** The case study 2017-3 was addressing read-across for NMs. The read-across for NMs relies on NMs-specific characterisation. The case study suggested that continued development of guidelines for characterisation and testing for NMs including NMs-specific protocols are needed. This will be useful to increase data availability and quality related to NMs. Also, it was suggested that guidance on the interpretation of NMs-related data is necessary.

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 NAM data

3. Definition of analogues/category boundaries chemical similarity

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

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 for read-across

5. Decision logic for low/no toxicity predictions

6. Guidance on when *in vitro* data could be further generated to support read-across

7. Guidance on use and reporting of results of HTS and HTTK assays

5. Uncertainty Analysis

1. Exposure route, including route to route extrapolation

2. Use of data from different test conditions for read-across for a target endpoint

3. Impact on conclusion

4. Guidance for evaluating the reliability/robustness of data including TK/TD data

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

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

7. Uncertainty framework (Overall uncertainty in the assessment resulting from the combined uncertainties of the different IATA components and data types)

6. Integrated Conclusion

1. Combining approaches/methodologies for predicting bioaccumulation

2. Integrating QSAR predictions, including when to use consensus modelling

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

4. How to define acceptable uncertainty

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

7. Nanomaterials

   - Standardised guidelines for characterisation and testing of NMs, also e.g. dispersion protocols for NMs
   - NM-specific and relevant protocols
   - increase data availability/quality

8. Guidance on the interpretation of NM-related data
9. Guidance for reporting from exposure simulation models (e.g. environmental concentrations)
10. Guidance on developing prioritisation scheme based on IATA
11. Guidance on use or reporting new approach methods (cheminformatics tools, HTS, HTTK assays)

4.3. Considerations from the case studies in the third review cycle

This section describes the new considerations gained through the review experience of the four case studies in the third review cycle. The case studies in the third review cycle addressed the main three areas: the read-across for repeated dose toxicity, prioritisation of chemicals, and considering NMs properties for read-across. This section consists of three subsections.

4.3.1. Considerations on read-across

The following three issues were identified in the third review cycle as matters to be considered in the read-across approach:

1. Different approaches for identifying analogues for grouping and read-across (cheminformatics, TK/TD, nano-specific parameters)
2. The opportunity for using the QSAR Toolbox and other tools for identifying analogues.
3. Considerations for route to route extrapolation for same substance in the context of read-across

1. Different approaches for identifying analogues for grouping and read-across (cheminformatics, TK/TD, nano-specific parameters,)

Identifying appropriate analogues is important for grouping and read-across. Setting of criteria is needed to define the category members. Generally, category members are identified by structural similarity such as common boundaries or functional group. Additional information could be used to assess the similarity between category members to support the strength of the grouping hypothesis. As shown in Table 3, ten case studies have demonstrated how different information types are used for identifying analogues or for supporting the strength of the grouping hypothesis. These include cheminformatics, TK/TD and NMs-specific parameters. It was suggested that cheminformatics information can be used to develop a hypothesis to select analogues and also for approaches to assess the similarity within the identified chemical categories.

Table 3. Different Approaches for Identifying Analogues for Grouping and Read-across

Year-No. (Lead)	Endpoint	Supporting Information for identifying analogues	How to use information for grouping and read-across
2015-1 (Canada)	Mutagenicity	Physicochemical properties (*in silico*) Metabolism (*in vivo*, *in vitro*, *in silico*) Mechanistic consideration Biological activity (empirical and predicted)	With respect to relevant physicochemical properties, the category members were generally similar. The metabolic data from *in vivo*, *in vitro*, *in silico* demonstrated that the category members can undergo reductive metabolism at the azo bond to form aromatic amines including 3,3' dimethoxybenzidine (DMOB). The mechanistic consideration suggested that DMOB Azo Direct Dyes contain substructures that alert for potential DNA binding through the formation of a reactive metabolite, which is believed to be the major mechanism responsible for mutagenicity of category members. Data from *in silico* (Ames mutagenicity) demonstrated the similar effect between category members and were used as supporting data for read-across.
2015-2 (Canada)	Repeated dose toxicity	Physicochemical properties (*in silico*) Toxicokinetic parameters (*in silico*) Metabolism (*in silico*) Toxicity data (*in vitro, in vivo*)	There was a general trend for physicochemical parameters (logKow, water solubility, melting and boiling points, and vapour pressures) depending on the length of the side chain and number of substitutions between the same subcategory members.The differences in branching were not expected to have a significant impact on the physicochemical parameters expect melting point. There was variation with respect to the predicted kinetic parameters (Oral bioavailability, C_{max}, T_{max}, AUC) based on the SDPA with variable number of substitutions. The similar metabolic profiles in mammalian organisms were observed within subgroup with the metabolic simulator. Of the SDPAs tested, similarities in effects were observed with the target organ being mainly the liver. Each of effects related to blood, thyroid or spleen were seen for some SDPAs.
2015-3 (Japan)	Repeated dose toxicity	Metabolism (*in vitro*, *in vivo*, *in silico*) Toxicity data (*in vivo*)	The data from *in vitro*, *in vivo*, *in silico* suggested that allyl alcohol is generated by the metabolic hydrolysis of category members. Available *in vivo* data for some of category members showed similar hepatotoxic effects.
2015-4 (Japan)	Bioaccumulation	Physicochemical properties Bioaccumulation	The values of logPow for target chemicals were considered for justifying the bioaccumulation potential. Predicted bioaccumulation values between source chemicals and target chemicals were used as supportive information.
2016-1 (Japan)	Repeated dose toxicity	Metabolism (*in silico*) Transcriptomic profiles Toxicity effect	Predicted metabolism data were used as supporting data for subcategorising. Transcriptomic profiles and toxicity data were used for subcategorising.

2016-3,4 (ICAPO)	Repeated dose toxicity	Physicochemical property (*in silico*) Toxicokinetic data (*in vivo*) Metabolism (*in silico*) Toxicophore (*in silico*) Mechanistic plausibility The data from new approach methods	Physicochemical properties, with the exception of density and pKa, trend in values related to C-atom number within a scaffold. The values for density and pKa are the same within the category members. While available data were limited, the data demonstrated that toxicokinetic understanding of category members is complete. The data from *in silico* metabolism demonstrated that it is highly likely that all of the category members undergo metabolism via the same pathway. None of the category members are associated with any toxicophore based on *in silico* modelling. The category members were associated with the simple narcosis mechanism of toxicity that is equivalent to depressant anaesthetics. ToxCast data and receptor binding simulations results indicated no activity of category members associated with a specific mode of action.
2017-1 (Canada/U.S.)	Estrogenicity	Cheminformatics information such as decompositions, unique fragments, similarity matrix, chemical structural fingerprint *in vitro* ER activity Physicochemical property.	Cheminformatics information was used for identifying and selecting analogues. The data from i*n vitro* ER activity were analysed for data quality. Physicochemical properties, which are expected to be relevant for ER binding, were used for filtering analogues.
2017-3 (JRC)	Genotoxicity	Nano-specific parameters	Nano-specific parameters were considered to develop the read-across grouping hypothesis, such as surface characteristics, crystalline types, size.
2017-4 (ICAPO)	Repeated dose toxicity	Physicochemical property Toxicokinetic data (*in vivo*, *in vitro*, *in silico*) Toxicodynamic data (*in vivo*, *in silico*, new approach methods)	Physicochemical properties, with the exception of density, trend in values related to C-atom number within a scaffold. The values for density are the same within the category members. The toxicokinetic data from *in silico*, *in vitro*, and *in vivo* demonstrated that category members undergo metabolism via the same pathway. The *in vivo* toxicodynamic data demonstrated similar effects levels and clinical signs. The *in silico* toxicodynamic data demonstrated no activity within category members which are associated with a specific toxicity. ToxCast data results indicated no activity of category members associated with a specific mode of action.

2. The opportunity for using the QSAR Toolbox and other tools for identifying analogues

The QSAR Toolbox[12] is a decision support tool for hazard assessment and includes numerous databases, profilers (mechanistic alert), metabolic simulators and QSAR models. This information can be used as supportive information for grouping chemicals and read-across. Also, there are other potential useful tools for supporting identification of analogues. As described in Table 4, case studies demonstrated the opportunities for using the QSAR toolbox and other tools for identifying analogues.

The QSAR Toolbox can be used to search analogues with the endpoint data based on its query function. Also, the profilers in the QSAR Toolbox can be useful for assesses the

[12] OECD QSAR Toolbox. http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm

similarity between the category members in regards to toxicological category, toxicokinetics and toxicodynamics. The other QSAR software, metabolic simulator and expert systems demonstrated their utility for identifying analogues to assess the similarity in the view of physicochemical property, toxicokinetics and toxicodynamics. In addition, integration of the data predicted by multiple tools is helpful for justification of the grouping hypothesis. Cheminformatics tools can be used for searching analogues and filtering analogues as well as assessing the strength of the grouping hypothesis.

Table 4. Tools for identifying analogues

Year-No. (Lead)	Tools used for identifying analogues	How to use tools for identifying analogues
2015-1 (Canada)	OECD QSAR Toolbox*1 (v3.3) ACD Percepta*2 (2015- v2726) DEREK Nexus*3 (v 3.01) Toxtree*4 (v2.5) Case Ultra*5 (v1.4) Leadscope Model Applier *6 (v1.6) VEGA*7 (v2.1.12) OASIS TIMES*8 (v2.27.5)	The QSAR Toolbox was used to predict microbial metabolism of category members by using CATABOL microbial metabolic simulator. The other tools were used for the prediction of physicochemical properties, microbial metabolism, and Ames mutagenicity of chemical members. Expert systems (Derek Nexus, Toxtree) were used to profile category members of alerts related to mutagenicity. The prediction and profiling data were used for assessing the similarity between the selected chemical members.
2015-2 (Canada)	OECD QSAR Toolbox*1 (v 3.3) EPI Suite*9 (v4.11) DEREK Nexus*3 (v4.1) ACD Percepta*2 (2012- v2076) OASIS TIMES*8 (v2.27.5)	The QSAR Toolbox was used to search for additional SDPAs with required endpoint data, which met the criteria (e.g. diphenylamine (DPA) substructure, no other functional groups and saturated alkyl chain and/or phenyl substitutions).Also, HESS*10 profiler in the QSAR Toolbox was used for molecular profiling related to the category hypothesis. The other tools were used for the prediction of physicochemical properties, metabolic pathway and toxicokinetic parameters of chemical members. Expert systems (Derek Nexus) were used to screen category members for alerts related to systemic effects. The prediction and profiling data were used for assessing the similarity between the selected chemical members.
2015-3 (Japan)	HESS version*10 (v3.0)	HESS was used to identify structural alerts of category members and what kinds of MOA were assumed between category members. The data from HESS showed that all of analogues belong to same toxicological category (allyl esters), which supported identification of analogues.
2015-4 (Japan)	OECD QSAR Toolbox*1 (v 3.2.) EPI Suite*9 (v4.11) ChemBioDraw Ultra*11 (v14) CATALOGIC*12 (v.5.11.13)	The QSAR Toolbox was used to extract other source chemicals with target endpoint data from the selected database by using the query function (e.g. biphenyl structure, log Pow = 4.52 and known BCF value for fish). Other tools were used to estimate physicochemical properties relevant to the endpoint and biodegradation products of parent chemical. The predicted physical chemical properties were used for justification.
2016-1 (Japan)	OECD QSAR Toolbox*1 (v 3.3.) EPI Suite*9 (v4.11) HESS*10 (v3.2) DEREK Nexus*3	The QSAR Toolbox was used to calculate structural similarity and to predict formation of metabolites from category members based on the rat liver S9 metabolism simulator. Also, HESS profiler in the QSAR Toolbox was applied to find the possible toxicological category for systemic effects. The data from other tools were used as supportive information for identifying analogues on the basis of physicochemical properties (logKow) and toxicological categorisation for systemic effects.
2016-3,4 (ICAPO)	EPI Suite*9 (v4.1) ACD/ Percepta*2 OECD QSAR Toolbox*1 (v 3.3) MetaPrint2D-React software *13 SMARTCyp *14 (v 2.4.2) Meteor Nexus*15 COSMOS*16 profilers	The QSAR Toolbox was used to predict metabolites based on liver metabolism simulators (Rat liver S9 and Skin metabolism) for comparison of potential metabolic products within category members. Also, the profilers (DNA binding by OECD and protein binding by OECD) were applied to compare toxicophores within category members. The predicted data from the tools were used for assessing the similarity between category members in the view of physicochemical properties, metabolism and toxicopheres.
2017-1 (Canada/U.S.)	Cheminformatics tool and data analysis tool such asIndigo*17, Pandas*18.	To select chemicals similar to the target chemicals both structurally and chemically, by using the method for decomposing structures in order to assess similarity.
	Cheminformatics tool such as PubChem*19 fingerprints, the Jaccard distance/Tanimoto index*20, MOE software*21	To identify analogues based on a global filtering protocol including the three steps: select analogues, evaluate sources of uncertainty, filter analogues to improve validity (Pradeep et al., 2017)

2017-3 (JRC)	Cheminformatics method	Cheminformatics methods, such as hierarchical clustering*22, principal component analysis*23 and random forest as variable selection*24, were used for assessing the strength of the grouping hypothesis.
2017-4 (ICAPO)	OECD QSAR Toolbox*1 (v 3.4) OASIS TIMES*8 MetaPrint2D-React*13 SMARTCyp*14 (v2.4.2) Meteor software *15 COSMOS *16 DEREK Nexus *3 EPI Suite*9 v4.1	The QSAR Toolbox was used to confirm similarity in regards to *in silico* toxicokinetics based on the liver metabolism simulators (Rat liver S9 and Skin metabolism) and *in silico* toxicodynamics based on the mechanistic and endpoint profilers The other tools were used for the prediction of physicochemical properties, toxicokinetic properties and toxicodynamic properties. The prediction data were used for assessing the similarity between the selected chemical members.

*1: OECD QSAR Toolbox. http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm
*2: Advanced Chemistry Development (ACD) Percepta.; http://www.acdlabs.com/products/percepta/
*3: Deductive Estimation of Risk from Existing Knowledge (DEREK) Nexus software. Lhasa Limited. https://www.lhasalimited.org/products/derek-nexus.htm
*4: Toxtree. IDEAconsult Ltd. http://toxtree.sourceforge.net/
*5: CASE Ultra. Multicase Inc.. http://www.multicase.com/case-ultra
*6: Leadscope Model Applier, Leadscope, Inc.. http://www.leadscope.com/product_info.php?products_id=98
*7: Virtual models for property Evaluation of chemicals within a Global Architecture (VEGA). Istituto di Ricerche Farmacologiche Mario Negri Milano. https://www.vegahub.eu/
*8: TIssue MEtabolism Simulator (TIMES). Bourgas Prof.Assen Zlatarov University, Laboratory of Mathematical Chemistry. http://oasis-lmc.org/products/software/times.aspx
*9: Estimation Programs Interface (EPI) Suite, US Environmental Protection Agency. https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
*10: Hazard Evaluation Support System Integrated Platform, National Institute of Technology and Evaluation (NITE). https://www.nite.go.jp/en/chem/qsar/hess-e.html
*11: ChemBioDraw Ultra 14, PerkinElmer, Inc.. http://www.perkinelmer.com/product/chemdraw-professional-chemdrawpro
*12: CATALOGIC, Bourgas Prof.Assen Zlatarov University, Laboratory of Mathematical Chemistry. http://oasis-lmc.org/products/software/catalogic.aspx
*13: MetaPrint2D-react. http://www-metaprint2d.ch.cam.ac.uk/metaprint2d-react
*14: SMARTCyp, University of Copenhagen . https://smartcyp.sund.ku.dk/
*15: Meteor Nexus, Lhasa Limited. https://www.lhasalimited.org/products/meteor-nexus.htm
*16: COSMetics to Optimise Safety (COSMOS). http://cosmosspace.cosmostox.eu/app/home
*17: Indigo. http://lifescience.opensource.epam.com/indigo
*18: Pandas. http://pandas.pydata.org/
*19: PubChem. https://pubchemdocs.ncbi.nlm.nih.gov/about
*20: Tanimoto index. An Elementary Mathematical theory of Classification and Prediction. Internal IBM Technical Report.
*21: Molecular Operating Environment (MOE) software. https://www.chemcomp.com/MOE-Molecular_Operating_Environment.htm
*22: Suzuki, R., Shimodaira, H., 2006. Pvclust: an R package for assessing the uncertainty in hierarchical clustering, Bioinformatics, 22, pp. 1540–1542.
*23: Husson, F. et al., 2011, Exploratory multivariate analysis by example using R, CRC Press.
*24: Liaw, A., Wiener, M., 2002, Classification and Regression by random Forest. R News, 2(3), pp. 18–22.

3. Considerations for route to route extrapolation for a substance in the context of read-across

Route to route extrapolation for a substance should done with care when conducting read-across since there are possible differences in absorption, distribution, metabolism and excretion (ADME) characteristics between the administration routes. When the target exposure route is different from the administered route in the *in vivo* toxicity test, information can be required to support route to route extrapolation for a substance, such as *in vivo* toxicity data on an analogue chemical and metabolic data for the substance employing both the target exposure route and administered route.

Case study 2017-4 illustrated how to consider data which can support route to route extrapolation. In the case study, *in vivo* TK data with different administration routes such as gavage, feed and dermal, as well as *in vitro* and *in silico* TK data were considered to assess the similarity of the metabolites within categories. These TK data suggested that the same substance is metabolised through the same pathway regardless of the exposure route. Meanwhile, the gavage studies for benzyl acetate (target chemical) and benzyl alcohol (hydrolysis products) and a dermal study for 2-phenethyl alcohol (hydrolysis products) were used for identification of NOAELs. In the results of these studies, the NOAEL for benzyl acetate and benzyl alcohol were observed at the level of 100 and 250 mg/kg bw/d respectively and NOAEL for 2-phenethyl alcohol was observed at the level of 0.50 ml/kg bw/d (i.e., 500 mg/kg bw/d). The metabolic similarity and the NOAEL data for the category members would support the route to route extrapolation within the category members.

4.3.2. Considerations for Prioritisation Based on IATA including Exposure modelling

This subsection describes considerations for prioritisation of chemicals based on IATA as well as the consideration of exposure modelling. Two case studies from third review cycle (2017-1, 2017-2) illustrated approaches to prioritisation based on IATA including the consideration of exposure classification; also referred to as risk-based prioritisation and/or assessment. Also, case study 2016-5 addressed definition of the exposure scenario. The following four considerations are described in this subsection:

1. Retrospective analysis of prioritisation approaches using persistence, bioaccumulation and inherent toxicity (PBiT) hazard criteria versus the Ecological Risk Classification (ERC) for organic substances based on hazard and exposure criteria;
2. How to use IATA for prioritisation;
3. Use of exposure simulation models and referencing existing guidance;
4. Considerations for extrapolation from *in vitro* to *in vivo.*

1. Retrospective analysis of prioritisation approaches using PBiT hazard criteria versus the ERC based on hazard and exposure criteria

In 2006, categorisation of the Canadian Domestic Substance List (DSL)[13] was completed to identify the chemical substances for further assessment activity in the Chemicals Management Plan (CMP). Ecological categorisation was based on the three criteria for persistence (P), bioaccumulation (B) and inherent toxicity to non-human organisms (iT)

[13] Canada, Domestic substances list: https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/substances-list/domestic.html

(PBiT ecological Categorisation approach). The substance hazard profiles were categorised by using empirical data, QSAR models, analogue approach or read-across data. For chemicals prioritised using the PBiT ecological Categorisation approach (i.e., PiT, BiT, or PBiT) and assessed in the first two phases of CMP, fewer than 20% of resulted in a toxic conclusion under the *Canadian Environmental Protection Act, 1999* (CEPA).

The Ecological Risk Classification (ERC) approach based on an IATA and weight of evidence was developed to further prioritise and screen the remaining 640 organic substances with greater efficiency for risk assessment and the result was published in 2016 (ECCC, 2016).

The authors compared the results from prioritisation approaches using the PBiT ecological Categorisation approach and the ERC approach to investigate whether classification between the two approaches would be different and whether greater efficiency in risk assessment may be gained by prioritisation using an IATA. A subset of 433 discrete organic substances was used for the comparison. The results of comparison were as following:

- Of 433 substances, 169 substances were classified as high or moderate priorities by the PBiT ecological Categorisation approach and 70 substances were classified as high or moderate priorities by the ERC approach. The ERC approach resulted in fewer substances being classified as high or moderate priorities (approximately 41% less, 70/169)
- Of 433 substances, 264 substances were classified as low priorities by the PBiT ecological Categorisation approach and 363 substances were classified as low priorities by the ERC approach. The ERC approach resulted in more substances classified as low priority (approximately 37% more, 363/264).
- A subset of the substances categorised as high/moderate priorities by the ERC approach (approximately 66%, or 47 substances) did not meet the original PBiT ecological categorisation criteria, or a categorisation conclusion could not be made at the time due to empirical and model limitations.

The following findings were gained by analysing the result of comparison:

- The ERC approach more effectively captures substances with high hazard potential.
- Priorities categorised by the ERC approach better aligned with priorities categorised as human health only in 2006
- ERC approach was able to classify “eco uncertain” substances that could not be prioritised by PBiT ecological Categorisation approach due to QSAR limitations
- Factoring in exposure descriptors contributed to 80% of 640 organics becoming low priorities analysed using ERC approach, and allowed for gaining efficiency with risk assessment activities

It was found out that the PBiT ecological Categorisation approach can have limitations due to following reasons:

- The criteria cannot deal with challenging chemistries (e.g., PFOS, pharma, other ionogenics)
- They are somewhat redundant (B is part of iT)
- The criteria may not capture very potent relatively persistent chemicals (“continuously present”)

– PBT criteria are not integrated and thus outcomes are subject to the weakness of a "bright line" decision process, such as uncertainty of pass/fail thresholds, and in vs out for analogous chemicals

In conclusion of the retrospective analysis of prioritisation approaches using PBiT hazard criteria versus the ERC based on hazard and exposure criteria, PBT criteria are still useful, but care should be taken to examine the domain of applicability of these criteria. Although prioritisation based only on PBiT criteria can have certain limitations, ERC approach showed that PBT properties can be integrated into chemical prioritisation schemes to better understand the potential for adverse effects.

2. How to use IATA for prioritisation

In the two case studies, the data from *in silico*, *in vitro* and *in vivo*, were used effectively to prioritise the chemicals for a given purpose as shown in Table 4. The data generated from each of the *in silico* models or *in vitro* data includes some uncertainties such as domain of applicability and extrapolating from mechanistic data to an adverse outcome in an organism. However, using this information in combination reduces the uncertainties associated with each of the individual data points from *in silico*, *in vitro* and *in vivo* methods and makes it possible to apply a weight-of-evidence approach. Also, the combination of different types of data is useful to provide support for a hypothesis and to build confidence in the use of data from alternative methods.

Table 5. The Data Used for Hazard Classification in Prioritisation

Year-No. (Lead)	Target Endpoint	Hazard Classification	Gathered Data
2017-1 (Canada/US)	Estrogenicity	1. ER complex formation	1. ToxCast*1 cell-free protein binding assays, CERAPP*2, OASIS TIMES*3, ACD Percepta*4, OECD QSAR Toolbox*5
		2. DNA binding and trans-activation of gene expression	2. ToxCast*1 ERE reporter gene assays,OASIS TIMES*3, ACD Percepta*4
		3. Protein expression	3. Western blot; proteomics (not assessed herein)
		4. Altered levels of circulating hormones	4. ELISA (enzyme-linked immunosorbant assay (not assessed herein)
		5. Organ-level changes	5. Uterotrophic assay (TG 440*6); Derek Nexus Expert System*7
		6. Organismal-level changes	6. Two-generation reproductive toxicity assay (TG 416*8); Derek Nexus Expert System*7
2017-2 (Canada)	Ecotoxicity	1. Mode of Action	1. OASIS MoA profiler*5, MoATox database by US EPA*9, Critical Body Residue (CBR) toxicity ratio*10, Chemical activity
		2. Hazard Assessment Factor (HAF)	2. Integrated P, B, iT metric for aquatic and terrestrial food webs; RAIDAR model*11
		3. Chemical reactivity (ER binding, DNA and protein binding)	3. Estrogen Receptor (ER) binding*5, DNA binding*5, Protein binding*5, Androgen receptor (AR) binding*3
		4. Bioavailability	4. log Kow or log D
		5. Chemical activity	5.Calculation as fish LC50 divided by water solubility (Empirical data or QSARs to estimate LC50 values and water solubility values)
		6. Chemical reactivity (AR binding and EDKB)	6. AR binding*3,*in vitro* assay Endocrine Disruption Knowledge Base (EDKB)*12
		7. Bioactivity	7. ToxCast*1, Tox21

*1: ToxCast. https://actor.epa.gov/dashboard/

*2: Collective Estrogen Receptor Activity Prediction Project (CERAPP). https://www.epa.gov/chemical-research/cerapp-collaborative-estrogen-receptor-activity-prediction-project-0
*3: OASIS TIMES. http://oasis-lmc.org/products/software/times.aspx
*4: Advanced Chemistry Development (ACD) Percepta. http://www.acdlabs.com/products/percepta/
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*7: Derek Nexus Expert System. https://www.lhasalimited.org/products/derek-nexus.htm
*8: OECD (2001), Two-Generation Reproduction Toxicity. OECD Guideline for the testing of chemicals 416. http://www.oecd-ilibrary.org/environment/test-no-416-two-generation-reproduction-toxicity_9789264070868-en
*9: Baron MG, Lilavois CR, Martin TM. 2015. MOATox: A comprehensive mode of action and acute toxicity database for predictive model development. Aquat. Toxicol. 161: 102-107.
*10: CBR toxicity ratio = CBRnarcosis ÷ CBRfishLC50 = 3.0 mmol/kg ÷ [(Fish LC50 × BCF) ÷ MW]
*11 RAIDAR (Risk Assessment, Identification and Ranking). http://www.arnotresearch.com/index_download1.html#!/page_Downloads
*12: Ding D, Xu L, Fang H, Hong H, Perkins R, Harris S, Bearden ED, Shi L, Tong W. 2010. The EDKB: an established knowledge base for endocrine disrupting chemicals. BMC Bioinformatics. 11 Suppl 6:S5.

3. Use of exposure simulation models and referencing existing guidance

Exposure assessment approaches can be different in countries due to different regulatory contexts. However, physical chemical properties (e.g. water solubility, Henry's law constant), environmental fate (persistency) and the information on the use of chemical (e.g. tonnage, use patterns) should be considered for exposure assessment. The exposure classification criteria and the exposure models used in the case study 2017-2 are summarised in the Table 5.

In the case study, several exposure models were used for exposure classification. As shown in Table 5, two of three exposure classification criteria were classified based on the results of more than one model. The use of these multiple models allowed the application of a weighted approach to exposure profiling. The case study referred to one OECD guidance document (OECD 2004) regarding overall persistence. However additional guidance may be helpful for describing how to estimate the quantity of releases to the environment.

Table 6. Exposure Classification Criteria and Models Used in the Case Study 2017-2

Exposure Classification Criteria	Use in ERC	Models used for the Criteria	Description
Quantities in commerce	No model	-	-
Overall persistence (Pov)	Overall Persistence (Pov)	RAIDAR v2.5*1	An evaluative, regional-scale, mass balance model for screening level exposure and risk assessment. Model includes approaches suitable for ionogenic substances.
	Generating biodegradation half-lives for input into RAIDAR model	BIOWIN v4.10*2	Biodegradation Probability Program for Microsoft Windows in EPISUITE v4.11
Long-range transport in air	Determining half-life in air	AOPWIN v1.92*2	atmospheric, gas-phase reaction predictor in EPI Suite v4.11
	Air-water partition coefficient	HENRYWIN v3.2*2	Henry's law constant predictor in EPISUITE v4.11
Determining the ratio of the critical to actual emission rates	Wastewater removal rate predictions (%) to determine the emission rate	SimpleTreat v3.0*3	Software program developed by The National Institute for Public Health and the Environment (RIVM) for sewage treatment plant removal predictions

*1: RAIDAR (Risk Assessment, Identification and Ranking). http://www.arnotresearch.com/index_download1.html#!/page_Downloads
*2: EPI Suite. http://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
*3: SimpleTreat. https://www.rivm.nl/en/Topics/S/Soil_and_water/SimpleTreat

4. Considerations for extrapolation from in vitro to in vivo

While the results from *in vitro* testing are often used to measure potential interaction with biological tissue, the results from *in vivo* testing are often used to derive the dose information, such as NOAEL, LOAEL. The dose information from *in vitro* testing cannot be directly used as a surrogate for dose information *in vivo*. Therefore, a model is necessary to extrapolate from *in vitro* to *in vivo* with consideration of toxicokinetics.

The case studies 2016-5 and 2017-1 addressed extrapolation from *in vitro* to *in vivo* by using the various methods shown in Table 6. In both of the case studies, *in vitro* assay concentrations were used to estimate the concentration in target organs, such as blood and liver, in a population of healthy individuals based on Monte Carlo simulation.

Table 7. Examples of Extrapolation from *in vitro* to *in vivo*

Year-No. (Lead)	The Models	Description	Purpose of use
2016-5 (JRC/BIAC)	Virtual Cell Based Assay (VCBA)	The VCBA has been developed within the COSMOS project to simulate a chemical's fate in an in vitro assay and comprises five interconnected models: fate and transport, chemical partition inside the cell, cell growth, toxicity and effects, as well as the experimental set up.	Applied for obtaining the dissolved concentration that could enter the cell to extrapolate in vitro dosimetry to intracellular concentration.
	Physiologically-Based Kinetic (PBK)	PBK mathematically describe interconnected compartments representing the human body, considering absorption, distribution, metabolism and excretion (ADME) properties of a chemical within the organism.	Predict concentrations of target chemical in six different compartments: lungs, skin, liver, richly perfused, slowly perfused and kidney.
2017-1 (Canada/U.S.)	The method described in Wetmore, et al. 2012*[1]	The method can estimate the daily human oral dose, called the oral equivalent dose (OED) or applied dose equivalent (ADE), based on extrapolating from the in vitro assay concentration. Oral equivalent doses would result in a steady-state blood concentration equivalent to the AC50 (concentration at 50% of maximum activity) from the ToxCast assay	To calculate oral equivalent doses from the in vitro bioactivity (HTTK data).

*1: Wetmore, B.A., Wambaugh, J.F., Ferguson, S.S., Sochaski, M.A., Rotroff, D.M., Freeman, K., Clewell, H.J.,3rd, Dix, D.J., Andersen, M.E., Houck, K.A., Allen, B., Judson, R.S., Singh, R., Kavlock, R.J., Richard, A.M., Thomas, R.S., 2012. Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicol. Sci. 125, 157-174.

4.3.3. Grouping and Read-Across for Namonmaterials

Case study (2017-3) was developed by applying the workflow for grouping and read-across proposed in the ECHA REACH guidance (ECHA, 2017a) which was updated for nanomaterials. This case study also illustrated the extent to which ECHA's Read-Across Assessment Framework (RAAF) (ECHA, 2017b) is applicable to nanoforms for identifying sources of uncertainty associated with the read-across. Read-across was conducted to determine the genotoxic hazard potential of two nano-TiO_2 target substances based on *in vitro* comet assay results from other TiO_2 nanoforms. The authors identified the following nanospecific issues related to the read-across for nanomaterials in this case study:

- Similarity based on chemical structure to be replaced with appropriate and relevant other parameter(s), i.e. consideration of physical form and key physicochemical properties
- Definition of the "similar or different" compounds should consider factors such as coating, size, and length
- It is important to define the substance that the organism is exposed to and leading to an adverse effect. In the case of nanomaterials it is not only a distinction between parent compounds and (bio)transformation products such as metabolites, but also needs to consider nanomaterial specific issues such as impurities/coating and released metals.
- There may be specific kinetics of exposure which have to be taken into consideration.

- High variability of the measurements and possible nano-specific artefacts in assays are issues specific to NMs to be considered in the assessment.

The following consideration was gained from this case study:

1. Applicability of the ECHA workflow
    1) It was shown that the workflow in the ECHA guidance for grouping and read-across of NMs (ECHA 2017a) worked well, with slight adaptations for the case study. It generally allowed presenting all the information collected to build and test the grouping hypothesis. Since the hypothesis was strongly reliant on the analysis of the gathered data (physicochemical properties), it was an iterative process between data gathering and hypothesis development/refinement.
2. Relevance of computational methods in grouping for read-across
    1) The case study showed the utility of supervised and unsupervised cheminformatics techniques. It was shown how unsupervised techniques like hierarchical clustering and principal component analysis (PCA) can support the grouping hypothesis by identifying the differences between nanoforms and by supporting the weight of evidence in the read-across hypothesis. Supervised techniques were shown to be useful to assess and/or develop the grouping hypothesis as they can help in identifying the properties that are more relevant to classify the substances in the corresponding groups.
    2) The supervised and unsupervised techniques should be considered as feeding information into an overall weight of evidence, rather than being conclusive.
3. Uncertainty in grouping NMs for read-across
    1) The case study shows that the RAAF is generally applicable to NMs and helpful to systematically identify uncertainties associated with the read-across for nanomaterials.
    Uncertainties were related to the identification of the (non-)nanoforms, experimental variability associated with the physicochemical and toxicological information and due to the lack of measurement protocols for NMs, and, finally, to the lack of knowledge on the mechanisms of genotoxic action of the NMs.
    2) A key aspect that would need to be extended for the application of the RAAF to NMs is the concept of similarity, as in the RAAF (following REACH) it is based on structural similarity. For NMs other principles for similarity, such as physicochemical properties, should be included.

The case study highlighted the need for more reliable testing methods as well as more concrete guidance related to data treatment, dealing with data variability, use of cheminformatics methods, the selection of analogues taking into account the NM specificities, grouping not based on chemical structures.

5. CONCLUSION

Four case studies were reviewed in the third review cycle of the project. The case studies in the third review cycle addressed new areas, such as prioritisation based IATA, read-across for nanomaterials and the new endpoints such as estrogenicity and ecotoxicity. The lessons and learnings gained from the review experience of the case studies by the project team demonstrate new possibilities for the use of IATA in various objectives and also promote the application of IATA in the regulatory contexts of member countries.

Based on the review experience of the four case studies, new areas for further developing guidance were identified. Mainly, the identified areas are relevant to prioritisation schemes based on IATA and guidance related to nanomaterials. In two case studies from the third review cycle, prioritisation schemes were established by using the data from non-animal testing methods such as *in vitro* and *in silico*. These case studies illustrate good examples of prioritisation schemes based on IATA. Moreover, the areas related to exposure estimation and classification were highlighted as a part of the prioritisation schemes. It is necessary to develop guidance on how to report the exposure simulation models and how to extrapolate from *in vitro* to *in vivo* dosing information. One case study demonstrated read-across for NMs. In the case study, the read-across was performed considering nano-specific characteristics. The case study provides additional input to areas for which further guidance is needed for read-across for NMs, for example in an update of the OECD guidance document on grouping and read-across (OECD, 2014a). Use of data including new approach methodologies were also identified for further development of guidance in order to use the data including NAMs appropriately. The area includes the needs for the guidance for evaluating the reliability/robustness of data in order to establish the uncertainty of the quality of the data used to build the chemical category. The uncertainty framework is highlighted to capture overall uncertainty associated with IATAs, which are generated as the result of combining uncertainties of the individual IATA components and data types. The overall uncertainty needs to be considered for use of IATA for regulatory purpose.

Broad areas of learnings and considerations were obtained from the third review cycle. This included considerations related to read-across, such as use of different approaches for identifying analogues, route to route extrapolation in read-across and considerations for read-across of nanomaterials. Secondly, lessons learned from two case studies addressing prioritisation schemes provided insight to the tools that can be used during prioritisation based on IATA and the incorporation of exposure considerations.

In summary, new lessons and learnings were obtained from the review experience of four new case studies in the third review cycle. These lessons and learnings can continue to build the foundation for the application of NAMs and the learnings should be shared within member countries to promote the use of IATA.

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Pradeep, P., Mansouri, K., Patlewicz, G., Judson, R. (2017), A systematic evaluation of analogs and automated read-across prediction of estrogenicity: A case study using hindered phenols. Computational Toxicology, Vol. 4, pp. 22-30.

ANNEX 1: The template for the case studies on read-across used in 2017

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. Depending on the specific case study additional information/(sub)section(s) may be required or particular (sub)sections may not apply. The order of the (sub)sections of the template can be changed and two or more (sub)sections of the template can be merged, as necessary. The titles of a (sub)section can be change as necessary. The template will be revised based on experience with use).

Introduction

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

Table of Contents

A. Purpose

a. Purpose of use

- Specify the purpose of use of the IATA (eg. regulatory context, hazard identification, hazard characterisation, 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.

b. Target chemical(s)/category definition [See 3.2.3.1 of the grouping guidance (OECD, 2014a)]

- 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.

c. Endpoint(s)

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

Tip

- The description of the purpose of use is important for considering the acceptable uncertainty of the case study. For example, if the conclusion derived by case study is renewable in a framework such as tiered-approach, this needs to be clearly stated [See case studies 1 and 2, 2015(OECD, 2015b, 201c)].

B. Hypothesis for the analogue approach/category [See 2.4 and 3.2.1 of the grouping guidance (OECD, 2014a)]

- 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 (OECD, 2014a)].
- These hypotheses can be argued by the number of elements as follows [See 3.2.3 of the grouping guidance (OECD, 2014a)].
    - 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.

Tip

- Hypothesis needs to be described as a testable format.
- For the hypothesis that metabolite induces target effect, the effects induced by other metabolites than the toxicant need to be considered [See case study 3, 2015 (OECD, 2016d)].

C. Source chemicals/Category members [See 2.3, 4.2.2 and 5.2.2 of the grouping guidance (OECD, 2014a)]

a. 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.

b. 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 (OECD, 2014a); example of the chemical identifiers for UVCBs)

Tip

- Not only structural similarity but also impacts of structural differences to the target effect need to be considered for selecting analogues.

D. Justification of data gap filling

a. Data gathering [See 4.2.3 and 5.2.3 of the grouping guidance (OECD, 2014a)]

- Provide the methods used for gathering the data for target and source chemicals/category members (eg. selection criteria of the data, data source).

b. Data and methods [See 4.2.4, 4.2.5, 5.2.4 and 5.2.5 of the grouping guidance (OECD, 2014a)]

- Provide a matrix of data (see data matrix template).

- If mass unit such as mg/kg-bw is used in the data, it should also be expressed in molar units such as mmol/kg-bw.
- Provide detailed data, as necessary (in case that the detailed data are used for the justification of the hypothesis). The appropriate degree of detail of the data should be considered in the context of the purpose of case study. Examples of reports of detailed data can be found in past IATA case studies.
- If data from non-guideline test methods are included, provide descriptions of the methods or links to sources that summarise the methods. The appropriate degree of detail of the description should be considered in the context of the purpose of the case study. A template for the description is available in a OECD guidance document (OECD, 2014c) Examples of description using the template can be found in JRC EURL ECVAM Database service on Alternative Methods to animal experimentation (DB-ALM)[14]
- If QSAR data are included, provide the name, version, owner of the models and reference number of QMRF inventory maintained by the JRC[15] used for deriving QSAR estimation data. QSAR models if not already described elsewhere should be reported using the QSAR Model Reporting Format (QMRF), and individual predictions, if applicable, should be reported using the QSAR Prediction Reporting Format (QPRF).
- If data derived from defined approaches of IATA are included, provide the descriptions of the defined approaches. A template for the description and case study examples are available in OECD guidance documents (OECD, 2016f; 2016g).
- Provide analysis of the available information for suitability regarding the defined purpose. If possible, the cells should also indicate the available key study results.

c. Justification [See 2.5, 2.6, 4.2.6 and 5.2.6 of the grouping guidance (OECD, 2014a)]

- 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 (OECD, 2014a): 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.

[14] JRC, EURL ECVAM Database service on Alternative Methods to animal experimentation (DB-ALM). https://ecvam-dbalm.jrc.ec.europa.eu/

[15] JRC, QSAR Model Database. https://qsardb.jrc.ec.europa.eu/qmrf/

Tip

- Reliability of each QSAR prediction result needs to be described in terms of the applicability domain of QSARs. For example, it can be discussed by the coverage of the fragments in the training sets [See Case Study 4 in 2015 (OECD, 2016e)].

5. E. Strategy for and integrated conclusion of data gap filling

a. 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): Examples of the modified templates, which were used for past case studies, are shown in Appendix 1and 2.

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

Tip

- When use ranks to indicate the uncertainties, these definitions need to be clarified.

b. 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

Appendix 1: Example of Reporting Template of Uncertainty (1)

The template was prepared based on the following frameworks and was used for the case studies 1&2 in 2015 of the project.

- Wu, S., K. Blackburn, J. Amburgey, J. Jaworska and T. Federle (2010) A Framework for Using Structural, Reactivity, Metabolic and Physicochemical Similarity to Evaluate the Suitability of Analogues for SAR-based toxicological assessments. Regulatory Toxicology and Pharmacology. Vol. 56, Issue 1, pp 67-81.
- Blackburn, K. and S.B. Stuard (2014) A Framework to Facilitate Consistent Characterisation of Read Across Uncertainty. Regulatory Toxicology and Pharmacology. Vol. 68, Issue 3, pp 353-62.

An overview of the template is shown below. Please refer to the original papers and the case studies above for details.

Part 1: Analogue suitability rating for read-across [a]

Evaluation Criteria [b]	Question [c]	Uncertainty [d]
Structure and reactivity	Do the target & analogue have similar structural features& chemical reactivity?	
Metabolism	Do the target & analogue have similar metabolic pathways?	
Physicochemical Properties	Do the target & analogue have similar phys-chem properties?	
…….		
Overall "suitability rating" [e]		

[a] This table is based on the decision tree of the framework by Wu *et al.* (2010)
[b] Criteria used for evaluating the suitability of analogues.
[c] Question and answer used for evaluating the criteria.
[d] Description of the uncertainties in the answer to the question.
[e] Rank (Suitable, Suitable with interpretation, Not suitable, Suitable with preconditions) derived from the decision tree.

Part 2: Uncertainty associated with the prediction of hazard using read across [e]

Analogue Data Set Characteristics [f]	Comment [g]
Number of analogues contributing data	
Robustness of analogue data set	
Concordance of effect(s)	
…….	
Overall uncertainty of read across prediction [h]	

[e] This table is based on the framework by Blackburn and Stuard (2014).

[f] Analogue data set characteristics used for evaluating overall uncertainty of read across prediction.
[g] Description of the evaluation results of the analogue data set characteristics obtained by answering the questionnaire of the framework.
[h] Rank of overall uncertainty of read across prediction derived from the evaluation results of analogue data set characteristics (Low, Low to Moderate, Moderate, High) with the description of the reason.

Appendix 2: Example of Reporting Template of Uncertainty (2)

The template was developed in the following framework and was used for the case studies 3&4 in 2016 of the project.

- Schultz, T.W., P. Amcoff, E. Berggren, F. Gautier, M. Klaric, D.J. Knight, C. Mahony, M. Schwarz, A. White and M.T.D. Cronin (2014), A Strategy for Structuring and Reporting a Read-across Prediction of Toxicity. Vol. 72, Issue 3, pp 586-601.

An overview of the template is shown below. Please refer to the original paper and the case studies above for details.

Part 1: Parameters and associated uncertainty used to justify category membership

Justification Parameter [a]	Data Uncertainty [b]	Strength of Evidence [c]	Comment [d]
Structural Similarity			
Phys/Chem Properties			
Metabolic Similarity			
Mechanistic Similarity			
Trends in Effects			
…….			
Overall uncertainty in similarity of category members			

[a] Similarity parameter used for justifying the category.
[b] Rank of uncertainty (low, medium, high) associated with underlying data used for analysis
[c] Rank of consistency (low, medium, high) within the data
[d] Description of the reason for the assignment of the ranks of the uncertainty and strength of evidence
[e] Rank of overall uncertainty (low, medium, high) and description of the reason

Part 2: Uncertainty associated with the prediction of hazard and dose-response using read-across

Factor [e]	Uncertainty [f]	Comment [g]
Number of analogues contributing data		
Robustness of analogue data set		
Concordance of effects		
Concordance of potency		
Severity of critical effect		
…….		

Overall uncertainty of read-across (low, medium, high)

[e] Uncertainty factor associated with the prediction of hazard and dose-response using read-across.
[f] Rank of uncertainty (low, medium, high)
[g] Description of the reason for the assignment of the ranks of the uncertainty
[h] Rank of overall uncertainty (low, medium, high) and description of the reason

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

ANNEX 2: General Template for IATA case Studies - Building Blocks used in 2017

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

(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. Depending on the specific case study additional information/(sub)section(s) may be required or particular (sub)sections may not apply. The order of the (sub)sections of the template can be changed and two or more (sub)sections of the template can be merged, as necessary. The titles of a (sub)section can be change as necessary. The template will be revised based on experience with use).

Introduction

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

Table of Contents

A. Purpose

a. Purpose of use

Indicate the regulatory relevance (i.e. intended application) of the IATA. This may be: a) screening for priority setting in view of further evaluation; b) hazard identification/characterisation; c) risk assessment; d) other (please specify). If more than one purpose is possible, please state this under d).

If in a regulatory context, provide a short description of any (e.g. legal) requirements for the IATA approach to be accepted.

b. Target chemical(s)

Provide the chemical descriptor common identifiers (including CAS number, name and composition including impurities) and chemical structure(s) of the target substance(s).

c. Endpoint(s)

Identify the endpoint(s) for which the IATA is applied.

B. Hypothesis for performing IATA

- Provide the hypothesis for performing IATA for the identified purpose
- Describe how the IATA will be performed for the specific purpose.

C. Approaches used (Potential Blocks for Inclusion)

- **AOP/MOA**: Description of potential mechanism(s) for the target chemicals to induce target endpoint toxicity. In particular, the graphical representation of the AOP would be helpful for the reader and key references. The tools in the AOP-KB should be referred to as appropriate (e.g. AOP wiki[16], Effectopedia etc.). For AOPs that are not documented, consider the "Users' Handbook supplement to the Guidance Document for developing and accessing Adverse Outcome Pathways" (OECD, 2016h) - although an entire AOP description is not the purpose here.
- **Defined Approach**: If a defined approach is included use the template of "Guidance Document on the Reporting of Defined Approaches to be used within Integrated Approaches to Testing and Assessment" (OECD, 2016f).
- **Workflow:** If an IATA workflow is included, provide a schematic and explanation of the elements of the workflow including input, decision and exit points.
- **Read-across**: If a read-across is included use elements of the template for IATA case studies on Read-Across.

D. Data/Information gathering

a. Data/Information

- Provide the methods used for gathering the data for target chemical(s) (e.g. selection criteria of the data, data source).
- Provide the data gathered using appropriate reporting format. The levels details for reporting the data should be considered depending on the purpose of the IATA.
- If data from non-guideline test methods are included, provide descriptions of the methods or links to sources that summarise the methods. The appropriate degree of detail of the description should be considered in the context of the purpose of the case study. A template for the description is available in an OECD guidance document (OECD, 2014a) Examples of description using the template can be found in JRC EURL ECVAM Database service on Alternative Methods to animal experimentation (DB-ALM)[17]
- If QSAR data are included, provide the name, version, owner of the models and reference number of QMRF inventory maintained by the JRC[18] used for deriving QSAR estimation data. QSAR models if not already described elsewhere should be reported using the QSAR Model Reporting Format (QMRF), and individual predictions, if applicable, should be reported using the QSAR Prediction Reporting Format (QPRF).

[16] AOP Wiki. https://aopwiki.org/

[17] JRC, EURL ECVAM Database service on Alternative Methods to animal experimentation (DB-ALM). https://ecvam-dbalm.jrc.ec.europa.eu/

[18] JRC, QSAR Model Database. https://qsardb.jrc.ec.europa.eu/qmrf/

b. Analogue chemicals.

- If the data of analogue chemicals were used for the IATA, provide the selection criteria that were used to identify the analogue chemicals. This can be based on the hypothesis described in section B.
- Provide rational for selection of analogue(s) with respect to the defined purpose and endpoint.
- Consider selection bias selecting analogue chemicals in relation to employment of the IATA (e.g. data completeness, support for hypothesis etc.).
- Describe the methods used to identify the analogue chemicals (e.g. inventories and tools used should be provided). Listing search criteria to establish initial pool of candidate analogues is helpful.
- Provide the common chemical identifiers (including CAS number, name and composition including impurities) and chemical structure(s) of the analogue chemicals.

E. Application of IATA

a. Summary of data

- Provide a summary of data in a suitable format for the purpose of IATA.
- Reliability of data should be discussed.
- The applicability domain of each estimation method including QSAR and alternative methods should be discussed
- Provide analysis of the available information for suitability regarding the defined purpose. If possible, the available key study results should be indicated.

b. Application of IATA

- Describe how to apply IATA based on the hypothesis and the data gathered.
- Describe the result of IATA.
- Refine the hypothesis used, if necessary.

c. Uncertainty

- Discuss the uncertainty of each element of the IATA.
- Aspects can include uncertainty and confidence associated with the data and assumptions.
- The magnitude and impact of the sources of uncertainty should be considered and to the extent possible, how the individual sources of uncertainty affect the overall uncertainty in the final outcome of the IATA. OECD guidance documents on defined approaches of IATA (OECD, 2016f; 2016g) might be helpful for considering uncertainties related to non-guideline test methods as well as the approaches outlined in the template for IATA case studies on Read-Across

d. Strategy and integrated conclusion

- Describe the strategy used to develop the integrated conclusion.
- Discuss how/if to further address the uncertainties.
- Finally, provide a short conclusion wrapping up the outcome of the evaluation.

References

Annex