Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)36

Unclassified

English - Or. English

27 October 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

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

Series on Testing and Assessment,
No. 350

JT03483905

SERIES ON TESTING AND ASSESSMENT

NO. 350

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

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

Environment Directorate
ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT
Paris 2021

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 38 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in eleven different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides**; **Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents;** and **Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/chemicalsafety/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

This publication is available electronically, at no charge.

Also published in the Testing and Assessment link

For this and many other Environment, Health and Safety publications, consult the OECD's World Wide Web site (www.oecd.org/chemicalsafety/)

or contact:

OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

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 (Quantitative) Structure-Activity Relationships ((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 case study, listed below, submitted to the 2020 review cycle of the IATA Case Studies Project. The topics discussed in this document include the strongest aspects and uncertainties of the case study. The IATA Case Studies Project has also identified a variety of areas for developing further guidance on IATA.

1. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR THE SYSTEMIC TOXICITY OF PHENOXYETHANOL WHEN INCLUDED AT 1% IN A BODY LOTION, ENV/CBC/HA(2021)2,

This case study illustrates an example, and its publication as an OECD monographs does not translate into direct acceptance of the methodology for regulatory purposes across OECD countries. In addition, this case study should not be interpreted as an official regulatory decision made by the authoring member countries.

Table of contents

Tables

LIST OF ABBREVIATIONS

AOP: Adverse Outcome Pathway

BIAC: Business at OECD

CoCAP: Cooperative Chemicals Assessment Programme

HTS: High Throughput Screening

HTTK: High throughput toxicokinetic

IATA: Integrated Approaches for Testing and Assessment

ICAPO: International Council for Animal Protection in OECD Programmes

ITS: Integrated Testing Strategy

IVIVE: In Vitro-In Vivo Extrapolation

MDH: Malate Dehydrogenase

MoA: Mode of Action

MoE: Margin of Exposure

MoIE: Margin of Internal Exposure

MoS: Margin of Safety

NAM: New Approach Methodology

NGRA: Next Generation Risk Assessment

NM: Nanomaterial

NOTEL: No-Observed-Transcriptional Effect Level

OECD: Organisation for Economic Co-operation and Development

PAA: Phenoxyacetic Acid

PBK: Physiologically Based Kinetic

PoD: Point of Departure

(Q)IVIVE: (Quantitative) In Vitro-In Vivo Extrapolation

(Q)SAR: (Quantitative) Structure-Activity Relationship

SEURAT-1: Safety Evaluation Ultimately Replacing Animal Testing -1

SCCS: Scientific Committee on Consumer Safety

TTC: Threshold of Toxicological Concern

US EPA: United States Environment Protection Agency

UVCB: Unknown or Variable composition, Complex reaction products, or Biological material

WHO: World Health Organization

WPHA: Working Party on Hazard Assessment

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 OECD member countries.

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 the case study, uncertainty of the case study, areas for further developing guidance and possible 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 past five review cycle of the project (2015- 2019) included twenty-three case studies and five considerations documents, which have all been published (OECD, 2016a; 2016b; 2016c; 2016d; 2016e; 2017a; 2017b; 2017c; 2017d; 2017e; 2017f; 2018a; 2018b; 2018c; 2018d; 2018e; 2019a; 2019b; 2019c; 2020a; 2020b; 2020c; 2020d; 2020e; 2020f; 2020g; 2020h; 2020i).

In the sixth review cycle (2020), the one case study shown in Table 1 was reviewed. The final case study is published [ENV/CBC/HA(2021)2, Series on Testing and Assessment No.332]. The 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 member countries. In addition, the case studies should not be interpreted as official regulatory decisions made by the authoring member countries. This document describes the results of the case study and summarises the learnings and lessons stemming from the case study reviewed in the sixth review cycle.

Table 1. Case Study Reviewed in the Sixth Review Cycle (2020)

No.	Title	Lead	Purpose of Use	References

[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

1	Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion	Business at OECD (BIAC)	Exploration of whether comparing *in vitro* bioactivity with estimated consumer exposures is a workable solution for cosmetic ingredients to ensure a high level of consumer protection whilst also ensuring the risk assessment is animal-free.	OECD, 2021a

2. PROCESS FOR REVIEWING THE CASE STUDIES

The following eight countries/organisations participated in the review of this case study: Australia, Canada, Germany, Japan, the Netherlands, Sweden, International Council for Animal Protection in OECD Programmes (ICAPO), and the OECD secretariat.

The author of the sixth review cycle was requested to use the templates provided in 1Part IAnnex A and Annex 1 for the documentation of the case study. The template for the case study 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). 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 AOPs / Mode of Action (MoA), Defined Approaches (DAs), Workflows, Grouping/Read-Across. Both of the templates have been continuously updated based on the review experience of the case studies in the past review cycles.

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? 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?
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 approaches in this case study in your regulatory context? If no, explain whether this is due to scientific reasons or a specific regulatory constraint/requirement.
7. Does the template work well?
8. Are there tools in the case study that you would like the author to demonstrate?
9. Others?

In addition, the case study author was requested to 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 approaches in this case study in your regulatory context? If no, explain whether this is due to scientific reasons or a specific regulatory constraint.
5. Does the template work well?
6. Would you like to demonstrate in more detail the tools applied in your case study?
7. Other?

The reviewers' comments and the revised case study were discussed at the sixth meeting of the IATA Case Studies Project (17-18 November 2020), in order to finalise the case study and summarise the learnings and lessons.

3. SUMMARY OF REVIEW RESULTS

3.1. Case Study 2020-1: The Use of Integrated Approaches for Testing and Assessment for the Systemic Toxicity of Phenoxyethanol when Included at 1% in a Body Lotion

This case study is an exposure-based, next generation risk assessment (NGRA) for the preservative ingredient phenoxyethanol. The case study was guided by the Safety Evaluation Ultimately Replacing Animal Testing (SEURAT-1) assessment workflow (Berggren et al., 2017) and the International Cooperation on Cosmetics Regulation NGRA principles (Dent et al., 2018), with the aim of using only non-animal approaches to assure the systemic safety of this ingredient when present at an active level (1%) in a product with a high level of consumer use (body lotion).

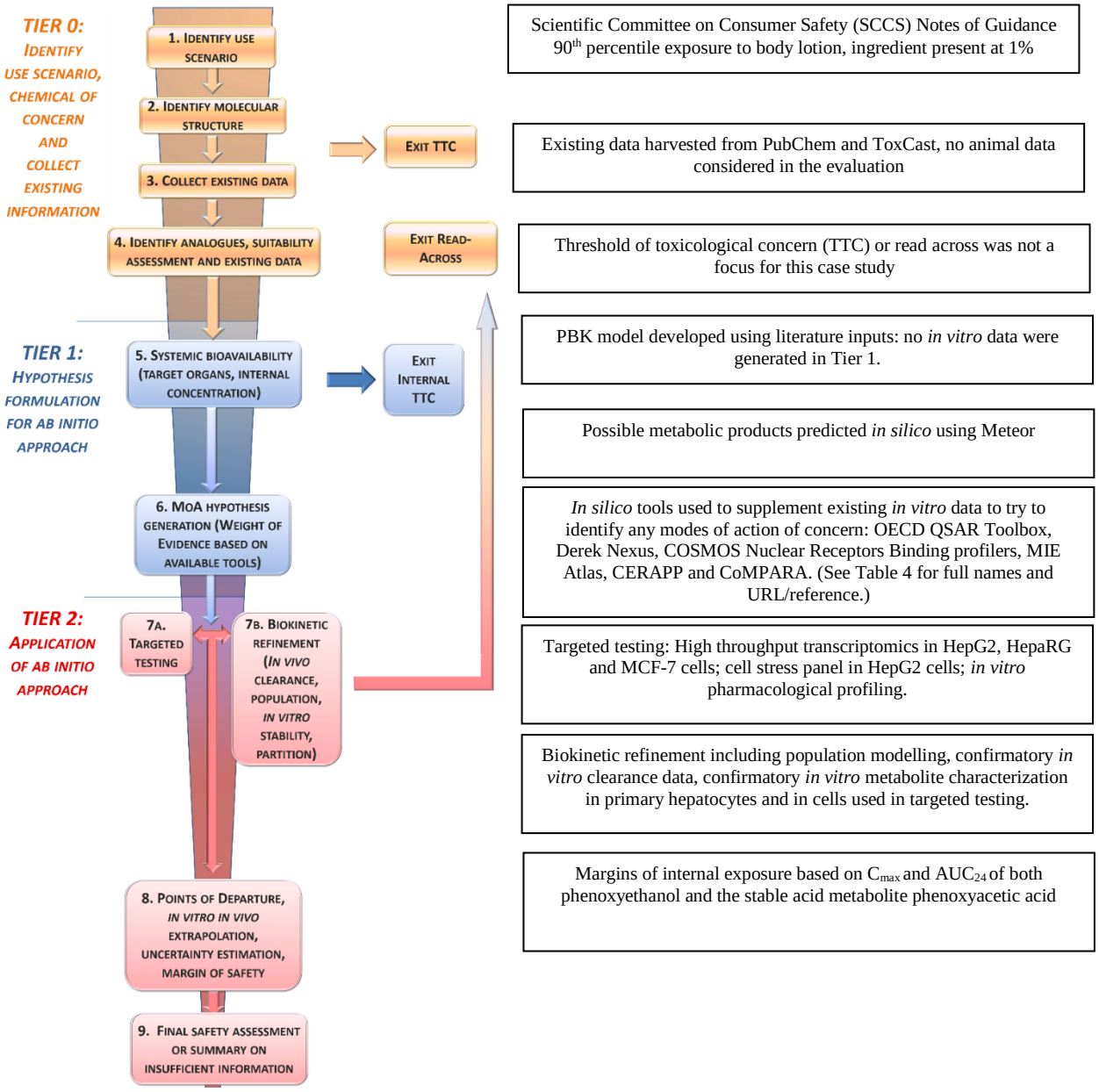


Figure 1. How the data used in this case study map to the Next Generation Risk Assessment workflow for systemic effects (Berggren et al., 2017), and the order in which the case study steps were performed (OECD, 2021a)

The overall strategy of the case study is one where *in vitro*/*in silico* approaches instead of animal-based approaches for hazard and exposure identification are used in the risk assessment. Instead of animal data, the approach involved the generation of new approach methodology (NAM) data on biokinetics and biodynamics.

In silico and *in vitro* approaches showed the major metabolite of phenoxyethanol to be phenoxyacetic acid (PAA), and physiologically based kinetic (PBK)[3] modelling was used to predict the 95th percentile population exposures of both phenoxyethanol and PAA in blood and tissues. These internal exposures were compared with points of departure (PoDs) derived from *in vitro* bioactivity assays. These included published non-animal data and new *in vitro* pharmacological profiling, cell stress, and transcriptomics data. The PoD exceeded the predicted internal exposure levels for both phenoxyethanol and PAA. This provided some assurance that *in vitro* bioactivity does not occur at consumer-relevant exposure levels, although the case study stresses that more experience is needed in the interpretation of these types of margins of exposure.

This case study illustrates one possible approach to assess the safety of both a parent chemical and its major stable metabolite in non-animal systemic toxicity risk assessment.

Please refer to ENV/CBC/HA(2021)2 Series on Testing & Assessment No. 332 for the case study to put the following points into context.

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

- The use of an exposure-led and hypothesis-driven safety assessment workflow that is performed without animal testing but combines various *in vitro* and *in silico* tools.
- The application of NGRA incorporating PBK modelling, the (Quantitative) *in vitro-in vivo* Extrapolation ((Q)IVIVE) approach and derivation of a margin of internal exposure (MoIE) based on no-observed-transcriptional effect level (NOTEL).
- Both *in silico* and *in vitro* assessments were performed to assess the metabolism of the parent compound; exposure estimates were carried out with conservative assumptions and included variability in the major metabolizing enzyme as a creative approach to addressing population variability to ensure that exposure estimates, and the resulting internal concentration predictions, were over-protective.
- The consideration of the breadth of biological coverage of non-animal tools and assays in evaluating the outcome of the workflow.
- Anticipation the MoA of the target chemicals based on its preservative action, such as inhibition malate dehydrogenase (MDH) in bacteria, and further analysed the capacity of the target chemical for human MDH inhibition using docking models and *in vitro* testing.

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

- Understanding of the level of biological coverage based on the *in vitro* tests and the cell lines selected and how this impacts on the uncertainty of the assessment.
- Fewer *in vitro* studies were available for the major metabolite, including lack of transcriptomics data in a kidney cell line (the main predicted target organ of the metabolite).
- Challenges in interpreting the adequacy of MoIE due to unfamiliar uncertainties and new concepts.
- The *in silico* tools applied in this study are usually not assessed for their toxicological relevance which contributes to the overall uncertainty.

[3] Physiologically based kinetic (PBK) is synonymous of physiology-based pharmacokinetic (PBPK), physiologically-based biokinetic (PBBK) and physiologically-based toxicokinetic (PBTK).

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

- ***Australia:*** The approach taken in the case study is at a preliminary proof of concept stage and not currently suitable for our regulatory context. Since it is currently at an early stage, the overall approach lacks sufficient scientific validity for assessment purposes. However, as is, certain aspects of the case study could be utilised for screening or in assessment, such as use of *in silico* tools to identify mechanisms of action/metabolites and the weight of evidence consideration of available information. Constraints in using the approaches in the case study in a regulatory context are that most of the *in silico* tools and *in vitro* assays are not publicly available with very little information on them. Transparency and scientific validity of the tools have to be established before they can be used in a regulatory setting. In addition, as these are relatively new tools, the application, interpretation and reporting of the outcomes of the tools have to be standardised
- ***Canada (Health Canada):*** The various NAMs used herein could be considered to examine the potential for toxicity of a substance in screening and assessment activities. However, in practice, use would likely also involve integrated application with traditional risk assessment approaches as part of weight of evidence until a certain level of comfort is established within our regulatory context.
- ***Germany:*** Currently: No. Lack of sufficient validation of the underlying methodology and individual tools combined. Lack of information on predictively. As pointed out by the authors themselves, "many more case studies are required to test the validity of this assumption for substances with diverse modes of action".
- ***Japan:*** No.
- ***Netherlands:*** No. First it should be clear which organs/systems are included in the system toxicology and more comparable studies using other compounds are required to optimize *in vitro* versus *in silico* risk assessment.
- ***Sweden:*** No***.*** Could be more suitable for the assessment of cosmetic ingredients as animal testing is banned, but in the context of for e.g. REACH, this might not be possible, also due to the areas of uncertainty and their unknown impact.

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

- Assessment workflows using non-animal data
- Use of *in vitro* and *in silico* information for development of PBK models
- Coverage of biological space
- Docking exercise within the case study
- Examples of developing MoIEs based on bioactivity data and their interpretation

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 23 case studies from the past five review cycles. Table 2 shows a summary of the 24 case studies reviewed 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 (17 case studies), grouping of chemicals for cumulative risk assessment, not for read-across (Case Study 2016-2), safety assessment workflow (Case Study 2016-5, 2019-1 and 2020-1) and screening of chemicals (Case Study 2017-1, 2017-2 and 2018-2).

Case Study 2020-1 demonstrates the potential for determining the safety of a chemical by a safety assessment workflow that compares biological activity with estimated exposure using non-animal methods. Case Study 2016-5 and 2019-1 included the use of read-across approaches within the concept of a safety assessment workflow.

Although Case Study 2017-1 focused on a prioritisation scheme for potential endocrine active chemicals, it illustrated two systematic methodologies for identifying analogues with cheminformatics and data analysis tools. Case Study 2018-2 included the elements of the defined approach for identifying oestrogen receptor active chemicals, and Case Study 2019-1 focused on a read-across approach for exploring the endocrine activity of the target chemical.

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 these case studies were: repeated dose toxicity (12 case studies), neurotoxicity (3 case studies), reproductive toxicity (2 case studies), oestrogenicity (2 case studies), mutagenicity (1 case study), bioaccumulation (1 case study), genotoxicity (1 case study), ecotoxicity (1 case study) and developmental toxicity (1 case study).

Every case study addresses some challenging topics related to IATA, including use of MoA/AOP (17 case studies), capturing and communicating uncertainty (22 case studies), use of new approach methodologies (19 case studies) and low/no toxicity prediction (12 case studies).

Identified areas for further developing guidance from the 24 case studies are summarised in section 4.2.

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

Year-No. (Lead)	Assessment Approach	Endpoint	IATA Topics				Reference
			AOP[1]	UR[2]	NAM[3]	L/N[4]	
2020-1 (BIAC)	Safety assessment workflow	Repeated dose toxicity	X	X	X	X	OECD, 2021a
2019-1 (BIAC)	Safety assessment workflow Read-across	Reproductive toxicity	X	X	X	X	OECD, 2020a
2019-2 (BIAC)	Read-across	Repeated dose toxicity	X	X	X		OECD, 2020b
2019-3 (BIAC)	Read-across	Repeated dose toxicity	X	X			OECD, 2020c

2019-4 (BIAC)	Read-across	Repeated dose toxicity	X	X	X		OECD, 2020d
2019-5 (BIAC)	Read-across	Repeated dose toxicity	X	X	X	X	OECD, 2020e
2019-6 (BIAC)	Read-across	Developmental toxicity	X	X	X	X	OECD, 2020f
2019-7 (BIAC)	Read-across	Neurotoxicity	X	X	X		OECD, 2020g
2019-8 (BIAC)	Read-across	Neurotoxicity	X	X	X	X	OECD, 2020h
2018-1 (Japan)	Read-across	Reproductive toxicity	X	X			OECD, 2019b
2018-2 (US)	Prioritisation and screening	Oestrogenicity	X	X	X	X	OECD, 2019c
2017-1 (Canada/US)	Prioritisation and hazard characterisation	Oestrogenicity	X	X	X	X	OECD, 2018b
2017-2 (Canada)	Prioritisation of chemicals	Ecotoxicity	X	X	X	X	OECD, 2018c
2017-3 (JRC)	Read-across	Genotoxicity for nano-TiO_2		X	X		OECD, 2018d
2017-4 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2018e
2016-1 (Japan)	Read-across	Repeated dose toxicity		X	X		OECD, 2017b
2016-2 (US)	Grouping for cumulative risk 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 Guidance Development

In the past five review cycles, the following six areas for further developing guidance were identified as priority areas from the 23 case studies (OECD, 2016a, 2017a 2018a, 2019a and 2020i). Although these are areas in which potential guidance could be developed, the intent is not to address all of these aspects within future OECD guidance documents, but rather note a potential need that has been identified. In addition, activities have been undertaken that address some of these needs (e.g. Guidance Document on Characterisation, Validation and Reporting of PBK models for Regulatory Purposes (OECD, 2021b)).

1. Describing scope and context for read-across
2. Building hypotheses based on MoA/AOP

3. Definition of analogues/category boundaries
4. Justification of data gap filling
5. Uncertainty Analysis
6. Integrated Conclusion

The following areas were identified as for further guidance development based on the review experience of the case study in the sixth review cycle (Case Study 2020-1):

- Understanding the adequacy of the level of biological coverage when combinations of non-animal methods are used;
- Rationale for the derivation of *in vitro*-based MoIE;
- Assessment of metabolism *in vitro* with varying degrees of uncertainty.

In addition, the Overview of Concepts and Available Guidance Related to Integrated Approaches to Testing and Assessment (IATA) (OECD, 2020j) highlights existing gaps in IATA guidance as priority areas for consideration:

- Lack of harmonised definitions and interpretation of some frequently used terms, such as New Approach Methodology (NAM), Integrated Testing Strategy (ITS) and Sequential Testing Strategy (STS);
- Need for practical guidance on the use of NAMs, as well as guidance on how to integrate individual methods within IATA and how to use the results in an overall weight of evidence;
- Need for overall guidance on uncertainty characterisation and documentation at the assessment level, including how this derives from the uncertainties associated with the individual components;
- Need for an overarching roadmap of IATA related guidance to navigate users;
- Need for good modelling practices to support the mutual acceptance of QSAR predictions;
- Need for guidance on toxicodynamic models, such as quantitative AOP models;
- Need for high-level principles to inform the design and application of IATA;
- Need to further consider the relationship between IATA and Mutual acceptance of data (MAD), and in particular the extent to which IATA and their components need to be MAD compliant.

At the sixth meeting of the IATA Case Studies Project, the project members discussed the current path-forward for developing guidance based on the input from the Working Party on Hazard Assessment (WPHA) who had considered at their June 2020 meeting the areas highlighted by the IATA Case Studies Project, the Overview of Concepts and Available Guidance Related to Integrated Approaches to Testing and Assessment (IATA) and other on-going efforts. The areas identified as priorities by the WPHA were as follows:

- Exploring an update of the OECD guidance on grouping of chemicals including an uncertainty section, grouping based on metabolites, guidance on biological read-across etc. based on experience garnered in the IATA case studies project.
- Development of a document on IATA, including IATA principles, evidence integration and consideration of weight of evidence/uncertainty

The focus in 2021 will be on the update of the OECD guidance on grouping.

A summary of areas for further developing guidance in the different case studies incorporating the above issues is shown in **Error! Not a valid bookmark self-reference.** (the underlined and bold areas were identified in the 2020 review cycle):

Table 3. Summary List of the Areas for the Development of Further Guidance

Areas for the development of further guidance	Related case study
1. Describing scope and context for read-across	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2017-3, 2017-4, 2018-1, 2019-2, 2019-3, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8
a. Considerations for justifying focus of an IATA (e.g. choosing 'major' effect vs 'minor' effect); providing explanation why a certain effect is considered the most relevant (toxicological response observed at a lower dose), while others are minor (occurring at a higher dose)	2015-2, 2015-3, 2016-1, 2016-3, 2016-4, 2017-4, 2019-2, 2019-3
2. Building hypotheses based on MoA/AOP	2015-1, 2015-3, 2016-2, 2016-5, 2017-1, 2017-2, 2018-1, 2018-2, 2019-3, 2019-4, 2019-5, 2019-7, 2019-8, 2020-1
a. Hypothesis for category formation that includes the use of omics data	2016-1
3. Definition of analogue/category boundaries	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-2, 2016-3, 2016-4, 2017-1, 2017-3, 2017-4, 2018-1, 2019-1, 2019-2, 2019-3, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8
a. Defining boundaries based on- phys/chem properties, toxicokinetics, toxicodynamics, bioavailability and metabolism, or ,nanomaterials-specific parameters.	2015-1, 2015-3, 2015-4, 2016-1, 2016-2, 2016-3, 2016-4, 2017-1, 2017-3, 2017-4, 2018-1, 2019-3, 2019-4, 2019-7, 2019-8
4. Justification of data gap filling	All case studies
a. Reporting of (Q)SAR prediction results	2015-1, 2015-4, 2016-1, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-4, 2018-1, 2019-1, 2019-2, 2019-4, 2019-5, 2019-6
b. Use of NAM data, TTC approach and PBPK models (e.g. How to integrate NAM data – for example via linking to mechanistic relevance (interpretation))	2015-2, 2016-1, 2016-2, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4, 2018-2, 2019-1, 2019-2, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8, 2020-1
c. Guidance for describing NAM data in the context of IATA case studies	2015-2, 2016-1, 2016-2, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4, 2018-2, 2019-1, 2019-2, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8, 2020-1
d. Decision logic for low/no toxicity predictions	2015-4, 2016-3, 2016-4, 2017-1, 2017-2, 2017-4, 2018-2, 2019-1, 2019-5, 2019-6, 2019-8, 2020-1
e. Guidance on when *in vitro* data could be further generated to support read-across	2015-2, 2016-1, 2016-3, 2016-4, 2017-4, 2018-2, 2019-1, 2019-2, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8
f. Guidance for use and reporting of results of HTS and HTTK assays	2015-2, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-4, 2018-2, 2019-1, 2019-2, 2019-5, 2019-6, 2020-1
g. What is needed to address biological read-across	2019-1, 2019-2, 2019-5, 2019-6, 2019-7, 2019-8
h. **Understanding the adequacy of the level of biological coverage when combinations of non-animal methods are used**	2016-5, 2019-1, 2020-1
5. Uncertainty Analysis	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2017-1, 2017-2, 2017-3, 2017-4, 2018-1, 2018-2, 2019-1, 2019-2, 2019-3, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8, 2020-1
a. Exposure route, including route to route extrapolation	2015-4, 2016-5, 2017-1, 2017-4, 2019-1, 2019-2
b. Use of data from different test conditions for read-across for a target endpoint	2015-1, 2015-2, 2015-3, 2016-1, 2016-3, 2016-4, 2017-3, 2017-4

c. How uncertainties impact on overall conclusion	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4, 2018-1, 2018-2, 2019-1, 2019-2, 2019-3, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8, 2020-1
d. Guidance for evaluating the reliability/robustness of data including toxicokinetics/ toxicodynamic (TK/TD) data ● Similarity of metabolic pathways ● Whether differences in the structure of target chemicals would have any significant impact on the metabolic pathway ● When should information on metabolites be included?	2015-1, 2015-2, 2015-3, 2016-1, 2016-3, 2016-4, 2017-4, 2018-1, 2019-1, 2019-2, 2019-3, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8, 2020-1
e. Reporting of uncertainty of read-across (e.g. Ranking of uncertainty vs descriptive analysis/ quantitative vs qualitative analysis)	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2017-3, 2017-4, 2018-1, 2019-2, 2019-3, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8
f. How to define acceptable uncertainty	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2017-1, 2017-2, 2017-3, 2017-4, 2018-1, 2018-2
g. Uncertainty framework (Overall uncertainty in the assessment resulting from the combined uncertainties of the different IATA components and data types)	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4, 2018-1, 2018-2, 2019-1, 2019-2, 2019-3, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8 2020-1
h. Tips on using non-endorsed AOPs regarding documentation/uncertainty/terminology	2015-1, 2016-2, 2016-5, 2017-1, 2018-1, 2018-2, 2019-3, 2019-4, 2019-6, 2019-8
6. Integrated Conclusion	All case studies
a. Combining approaches/methodologies for predicting bioaccumulation	2015-4
b. Integrating (Q)SAR predictions, including when to use consensus modelling or not	2015-1, 2015-4, 2016-1, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-4, 2018-1, 2019-1, 2019-2, 2019-4, 2019-5, 2019-6
c. Guidance on deriving integrated conclusions from the different components of the IATA, including harmonised uncertainty assessment	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4, 2018-1, 2018-2, 2019-1, 2019-2, 2019-3, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8
7. Others	
a. UVCBs, multi-constituents coverage (composition coverage, methodology and other)	2015-2, 2017-2
b. Level of detail needed in case studies according to the defined purpose	All case studies
c. How to include data on/predictors for metabolism when building IATAs according to the defined purpose. **Assessment of metabolism *in vitro* with varying degrees of uncertainty**	2015-2, 2015-3, 2017-4, 2018-1, 2019-3, 2019-4, 2020-1
d. How to describe the rationale for justification of the benchmark dose (BMD) and PoD used	2016-2
e. Guidance on developing prioritisation scheme based on IATA	2017-1, 2017-2
f. Guidance on use or reporting new approach methods (chem-informatics tools, HTS, HTTK assays)	2015-2, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4, 2018-2, 2019-1, 2019-2, 2019-5, 2019-6, 2019-7, 2019-8 2020-1
g. Guidance on how to develop ITS and data interpretation procedures (DIP)	2018-2
h. Guidance on how to combine *in vitro* and computational information into an integrated report, including applicability domain	2018-2
i. Guidance for evaluating ToxCast data	2015-1, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-4, 2018-2, 2019-1, 2019-2, 2020-1
j. Guidance on reporting of docking/modelling approaches	2019-7, 2019-8, 2020-1
k. Coverage of key events (KEs) in AOP based testing strategy	2018-2, 2019-5, 2019-7, 2019-8
l. Establishing a list of chemicals (comprising data rich chemicals with various MoAs) to be used as standards for NAM validation	2015-2, 2016-1, 2016-2, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4,

	2018-2, 2019-1, 2019-2, 2019-4, 2019-5, 2019-6, 2019-7, 2019-8
m. **Rationale for the choice of an acceptable *in vitro*-based MoIE**	2019-1, 2019-2, 2020-1
7.1. Areas related to other working party	
a. Guidance on the interpretation of NM-related data *1	2017-3
b. Guidance for reporting from exposure simulation models (e.g. environmental concentrations) *2	2017-2

*1: The area is related to the Working Party on Manufactured Nanomaterials
*2: The area is related to the Working Party on Exposure Assessment

6.1. Considerations from the case studies in the sixth review cycle

This section describes the learnings gained through the review experience of the case study in the sixth review cycle in 2020.

Case Study 2020-1 is an exposure-based NGRA, using non-animal data, for the preservative ingredient phenoxyethanol.

The approach to the safety assessment is to utilise a broad suite of human-relevant bioactivity data and ensure that human exposures remain below the level that results in *in vitro* bioactivity. This is facilitated by comparison of the bioactivity PoD to estimated exposure adjusted using PBK modelling to derive a MoIE.

The advantages identified in these approaches should be taken into account for the development of future IATA. As a result, the following five topics are discussed in this section:

1. Assessment workflows using non-animal data
2. Use of *in vitro* and *in silico* information for development of PBK models
3. Coverage of biological space
4. Use of docking models in the context of chemicals assessment.
5. Examples of developing MoIEs based on bioactivity data and their interpretation

6.1.1. Assessment workflows using non-animal data

Assessment workflows provide a framework for an evaluation that allow for clearer communication of the steps and parameters considered as well as a consistent approach for chemicals across which the workflow is applied. Case Study 2020-1 uses an assessment workflow, as have Case Study 2016-5 and 2019-1 which included the use of read-across approaches within the concept of a safety assessment workflow.

Case Study 2020-1 is an exposure-based case study based on the SEURAT-1 assessment workflow (Berggren et al., 2017) and the International Cooperation on Cosmetics Regulation NGRA principles (Dent et al., 2018) (Figure 1). The target is not to assess adverse effects or to develop an allowable daily intake (ADI), but to ensure the safety of phenoxyethanol by confirming that internal exposure from the cosmetic product is less than *in vitro* bioactivity. This approach did not use any animal data, but use a variety of *in vitro* and *in silico* data. Under certain regulations, cosmetic ingredients may not be tested on animals, and most existing chemicals do not have results based on animal tests. This exposure-based non-animal assessment can be used for prioritization and screening level risk assessments, or other assessment activity depending on the problem formulation and context for the assessment.

In order to increase confidence in the results of non-animal workflows in general, regulators will need to understand the limitations of the incorporated tools and be able to assess their scientific validity. This speaks to the need for transparency of the tools and to work towards the standardisation of their interpretation, reporting and use. For Case Study 2020-1, although the objective of this case study was to ensure breadth of biological coverage, it is not yet obvious whether the measures of bioactivity and cell lines are protective of all relevant MoAs that could lead to adverse health effects in consumers. Therefore assessment workflows are needed that use a battery of approaches that cover all of the relevant human biology, and further work is needed to identify a spectrum of methods that ensures full coverage.

6.1.2. Use of in vitro and in silico information for development of PBK models

When results from *in vitro* assays or bioactivity are used to develop PoDs, there is also a need to consider the exposure metric against which this PoD is compared. Traditionally, risk assessment approaches include the estimation of exposure to an organism via a particular route (oral, dermal, inhalation) or a particular media (water, air, soil, dust, food). These were then compared to applied doses from standard animal toxicology studies. PoDs derived from bioactivity at a cellular level in the context of *in vitro* assays could be compared as a first step to applied doses. However, to more precisely understand blood or tissue levels of exposure, PBK models are used as predictive tools to refine exposure estimates by calculating internal exposures or better understanding distribution to target tissues that can more readily compared to bioactivity.

As stated in the PBK model Annex to Case Study 2020-1, a MoIE has been estimated for phenoxyethanol using a PBK model to estimate blood concentrations following exposures to phenoxyethanol in humans that could be compared with concentrations that do not elicit cellular responses *in vitro*. The use of a PBK model reduces the uncertainty in the risk assessment by incorporating chemical-specific information on the uptake, distribution, metabolism and excretion of the chemical (Clewell et al. 2008). Typically, PBK models have been calibrated and evaluated using *in vivo* data, however the PBK models in Case Study 2020-1 were developed using only *in vitro* and *in silico* methods. This practice of using non-animal methods for the development of a PBK model supported the purpose of the case study to exemplify the development of an *ab initio* assessment using *in vitro* and *in silico* methods.

There are seven other case studies employing PBK models.

In Case Study 2019-1 and 2019-2, PBK models were also used to estimate internal exposure concentrations using available data including *in vivo* data.

In Case Study 2016-5, a six compartment PBK model, including a skin compartment, was built for the target chemical to simulate dermal exposure based on the human safrole model (Martati et al 2012, 2014) using non animal data. From the data gathered, molecular initiating events and target organs predicted by PBK modelling were identified as relevant bases for the assessment. The PBK models helped to define the target organs and internal concentrations applicable as well as to set the appropriate concentrations for the targeted testing.

In Case Study 2019-5 and 2019-6, PBK models for read-across compounds were developed in human and animals in order to predict the pharmacokinetics of source and target compounds in the read-across based on *in vitro* to *in vivo* extrapolation (IVIVE) PBK. In addition, PBK models determined the concentrations of compounds in target tissues and through reverse dosimetry translate *in vitro* toxicity assay data to an oral equivalent dose.

In Case Study 2019-7 and 2019-8, PBK modelling have been used to evaluate the relevance of the observed effects *in vitro* towards a likely *in vivo* exposure situation using available data including *in vivo* data.

During the review of Case Study 2020-1, an initial key uncertainty was the transparency of the PBK model that was used in the workflow. Many of these uncertainties were resolved with the provision of further information on the PBK model in the context of the review process. This speaks to the need for standardisation and reporting of PBK models. To support this, the OECD has recently developed a Guidance Document on the Characterisation, Validation and Reporting of PBK Models for Regulatory Purposes (OECD, 2021b). The document focuses on PBK models derived from *in vitro* and *in silico* methods and aims to provide a clear and consistent model assessment framework for facilitating the dialogue between the developers and proponents of PBK models and regulators who review and adopt the use of PBK models. Figure 2 outlines the workflow for PBK model development, validation, reporting and dissemination that the document addresses.

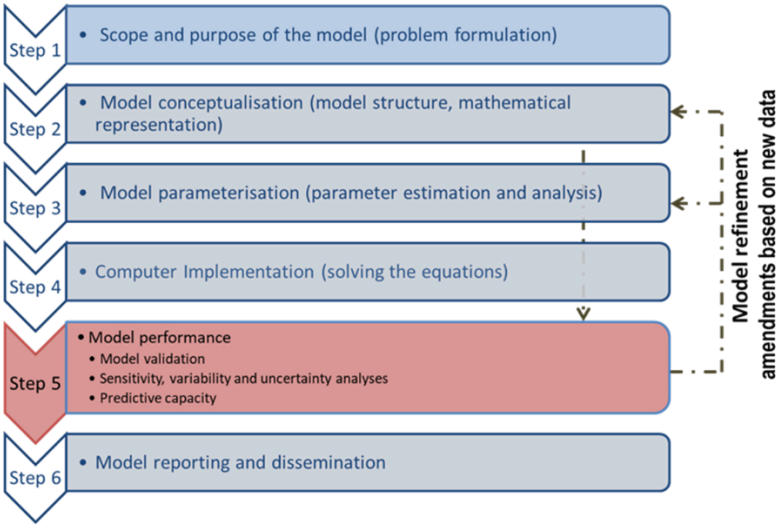


Figure 2 Workflow for PBK model development, validation, reporting and dissemination (OECD, 2021b, forthcoming)

6.1.3. Coverage of biological space

Based on the discussions of Case Study 2020-1, it is clear that an outstanding issue for the acceptance of the use of PoDs based on bioactivity is an understanding of the level of coverage of biological space by the assays that are utilised. If key pathways of toxicity are not part of the battery, this leads to uncertainty in the protectiveness of the approach, even if the aim is not to predict apical outcomes.

Case study 2020-1 was intended to apply a protective approach for human health by examining relevant bioactivity data (Table 4) including the use of *in vitro* and *in chemico* data via SafetyScreen44™ (cell-free profiling), cell stress and transcriptomics data. Although transcriptomics aim to provide an across genome

approach, there are still questions as to the sufficiency of use of a limited number of cell lines. Coming to an understanding of the combination of tests that would reduce uncertainty for regulators by improving the biological coverage would help to advance these applications.

Table 4. Models used for bioactivity assessment in Case study 2020-1

Tier	Methods
0	The literature search was conducted in • PubChem database (https://pubchem.ncbi.nlm.nih.gov) • US EPA's Computational Toxicology Dashboard (https://comptox.epa.gov/dashboard) • SCCS opinion on the safety of phenoxyethanol in cosmetics
1	*In silico* tools that were used to identify potential MoA of phenoxyethanol were • OECD QSAR Toolbox v. 4.1 (https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm), • Derek Nexus v 5.0.2 Lhasa Ltd., • COSMOS nuclear Receptors Binding profilers • MIE (Molecular initiating event) Atlas (Allen et al., 2018), • Models of the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) (Mansouri et al., 2016) • Collaborative Modeling Project of Androgen Receptor Activity (CoMPARA) (Grisoni, Consonni and Ballabio, 2019).
2	Several types of bioactivity data were generated • SafetyScreen44™ pharmacological profiling • Cell stress panel • Transcriptomics (TempO-Seq) (dose response)

6.1.4. Docking exercise within the case study

The use of three-dimensional ligand-receptor docking computational methods have not been traditionally used for chemical risk assessment. However, as the knowledge of structures of additional receptors and ligands continues to increase, and docking modelling programs become more accessible, these methods can contribute to a weight of evidence assessment for a chemical substance. In fact, molecular docking information has been used in five case studies thus far, also to provide information on different aspects of the assessment.

In reviewing Case Study 2020-1, member countries reflected that a strong aspect of the case study was to anticipate the MoA of the chemical based on its preservative action (inhibition of MDH in bacteria). A docking model was used in order to visualise if phenoxyethanol could potentially inhibit human isoforms of MDH and the docking indicated that it was unlikely to be a competitive inhibitor of the docking site. The results from this analysis were then combined with the results from *in vitro* studies in the IATA.

Case Study 2019-1 and 2019-2 also used docking approaches, but rather to evaluate the molecular initiating event and the probability of a chemicals to bind oestrogen receptors.

Case Study 2019-7 examined a target chemical and its read-across analogue in terms of the similarity of their receptor binding, visualised using molecular docking. It was envisioned that this could then also enable the future creation of a structure-based pharmacophore model a screening tool for similar binding propensities.

Finally, Case Study 2019-8 used molecular docking in two ways. It was used to further understand the chemical-target interactions for the molecular initiating event, to help identify the probability of such interactions with the binding site for other chemicals. It was also used for understanding the mechanism of different binding modes.

These varied examples demonstrate that the use of docking models to inform various aspects of chemical assessment has potential to be further exploited.

6.1.5. Examples of developing MoIEs based on bioactivity data and their interpretation

A MoIE approach was used in Case Study 2020-1, Case Study 2019-1 and Case Study 2019-2. A MoIE is calculated using a measure of internal exposure, such as blood concentration or target-tissue dose, rather than comparing the externally applied dose or ingested dose (Bessems et al., 2017). In Case Study 2017-1, the oral doses that would result in a steady-state blood concentration equivalent to the half-maximal activity concentration (AC50) from the ToxCast assays were estimated. An administered dose equivalent (ADE) was determined through the application of reverse dosimetry.

As discussed above (Section 4.3.2), PBK information is needed to support the conversion of applied exposure metrics to internal exposure or vice versa. This can be in the form of a PBK model or sufficient kinetic information to inform a (Q)IVIVE.

Table 5 provides examples of MoIEs derived in some of the case studies and, where calculated, with the margin of safety (MoS) or the margin of exposure (MoE) that may have been derived using traditional animal approaches. Other analysis shows that NAM-derived PoDs are often more conservative than animal-derived PoDs (Paul Friedman et al., 2020).

In terms of interpreting the magnitude of the MoIE, in Case Studies 2019-1 and 2019-2, a MoIE of 25 was considered equivalent to the default external dose MoE of 100. In cosmetic safety evaluation according to SCCS (2018), the risk assessment is basically calculated by a MoE (or MoS). The MoE is based on the PoD/Exposure, and a safe risk ratio (RR) is generally considered to be a MoE ≥ 100 when the PoD is derived in an animal study. A MoIE differs from a traditional MoE because it is calculated as the ratio of a measure of internal exposure (Bessems et al. 2017). In particular, calculation of internal exposures with a PBK model can be used to replace the default uncertainty factor of 4 for interspecies differences in TK differences (IPCS 2005, WHO 2010).

Table 5. Comparison of the outcome of the 'traditional' risk assessment and the NGRA

Case study	Exposure	PoD	MoS or MoE	MoIE
2020-1 (NGRT)	6.2 µM	171 µM	-	28
2020-1 (traditional risk assessment)	1.23 mg/kg/day	357 mg/kg/day (animal data)	MoS 290	-
2019-1	2.0E-2µM	2.1 µM	-	284
2019-2	51 µM	311 µM (animal data)	MoE 7	25.4

The challenge is with the interpretation of the margins for NGRA. There is rich experience in interpreting margins in the context of traditional risk assessment and how large this margin should be based on uncertainties in the evaluation or aspects such as intra and inter species differences. However, experience needs to be gained in the interpretation of the adequacy of margins in NGRA and how the relevant uncertainties can be accounted for in the size of this margin.

7. USEFUL TOOLS FOR IATA

This chapter highlights useful tools for IATA, which were presented and demonstrated at webinars. The webinar for demonstration of IATA tools was agreed at the fourth meeting of the IATA Case Studies Project in 2018 in order to share additional information on IATA tools within the project team and to promote the use of these tools for developing IATAs. So far, three webinars were held and the following six tools were introduced and demonstrated:

1. Hazard Evaluation Support System Integrated Platform (HESS) [Japan]
2. Computational model for Estrogen Receptor (ER) pathway [the United States]
3. Consexpo [the Netherlands]
4. European Union System for the Evaluation of Substances (EUSES) [the Netherlands]
5. AOP-informed Cumulative Risk Assessment (CRA) tool [Norway]
6. RISK Identification And Ranking (RAIDAR)[Canada]

Table 6 provides more details on the IATA tools demonstrated at the webinars.

Table 6. IATA tools demonstrated at the webinar

Tool	Description
Hazard Evaluation Support System Integrated Platform (HESS)*1 (Japan)	HESS allows chemicals to be categorised on the basis of structural, physicochemical and mechanistic similarities and helps predict the repeated dose toxicity of untested chemicals by means of the categor approach.
Computation model for Estrogen Receptor (ER) pathway*2 (the United States)	The model used 16 *in vitro* assays which are a subset of a larger collection of assays used in the US EPA ToxCast program to identify and quantify the ER agonist activity of a chemical. The application of the model is for screening of environmental chemicals based on their ER agonist activity and for determining whether further evaluation of endocrine-related activity in higher tier *in vivo* tests (e.g., female pubertal assay, two generation reproductive toxicity study) is needed.
Consexpo*3 (the Netherlands)	A computer program that enables the estimation and assessment of exposure to substances from consumer products such as paint, cleaning agents and personal care products;
European Union System for the Evaluation of Substances (EUSES)*4 (the Netherlands)	Model for the evaluation of the risks of substances to man and the environment. The main outputs of EUSES are local and regional risk characterisation ratios (RCRs) for several environmental compartments: air, surface water, sediment, soil, biota.
AOP-informed Cumulative Risk Assessment (CRA) tool*5 (Norway)	CRA tool facilitates rapid and consistent hazard and risk assessment of single chemicals and mixtures. The tools utilise concepts outlined by AOPs to compile, assemble, integrate and visualise data from different levels of biological organisation and support users for identification of risk drivers, relevant toxic endpoints, susceptible species and species sensitivity distributions for a given aquatic exposure scenario.
RISK Identification And Ranking (RAIDAR) *6 (Canada)	RAIDAR is an evaluative, regional-scale, mass balance model for screening level exposure and risk assessment. The model simulates chemical fate and transport in the environment, bioaccumulation in a range of species, food web bioaccumulation, far-field exposures to humans and representative ecological species, and effects (risk).

*1: https://www.nite.go.jp/en/chem/qsar/hess-e.html
*2:https://www.epa.gov/endocrine-disruption/use-high-throughput-assays-and-computational-tools-endocrine-disruptor
*3: https://www.rivm.nl/en/consexpo
*4: https://ec.europa.eu/jrc/en/scientific-tool/european-union-system-evaluation-substances
*5: https://www.niva.no/en/projectweb/radb
*6: https://arnotresearch.com/RAIDAR/

8. CONCLUSION

One case study was reviewed in the sixth review cycle of the project in 2020. Case Study 2020-1 is an exposure-based NGRA case study for the preservative ingredient phenoxyethanol, with the aim of using only non-animal approaches to assure the systemic safety of this ingredient when present at an active level (1%) in a product with a high level of consumer use (body lotion). This case study illustrates one possible approach to safety assess both a parent chemical and its major stable metabolite in non-animal systemic toxicity risk assessment.

The case study exemplified the use of a number of different NAMs, including *in silico*, *in chemico* and *in vitro* data. As discussed, the main uncertainties that remain in the use of this type of method include the coverage of biological space by the assays as well as the interpretation of the MoIE. These areas require further standardisation and understanding in regards to application in a regulatory context. Further case studies addressing these aspects would also help to advance the discussion.

Based on the review experience of the case study, new areas for further developing guidance were identified. This included:

- Understanding the adequacy of the level of biological coverage when combinations of non-animal methods are used;
- Rationale for the derivation of an acceptable *in vitro*-based MoIE;
- Assessment of metabolism *in vitro* with varying degrees of uncertainty.

The document further highlighted considerations regarding five topics including;

- Assessment workflow using non-animal data;
- Use of *in vitro* and *in silico* information for development of PBK models;
- Coverage of biological space;
- Docking exercise within the case study;
- Examples of developing MoIEs based on bioactivity data and their interpretation.

In summary, although there was the one case study in the sixth review cycle, the considerations obtained from the case study provided new knowledge on the non-animal assessment concept of the IATA. This knowledge shows the possibility of the IATA assessment for many chemicals that do not have any results based on animal tests in the regulatory context especially early stage assessment such as prioritization and screening level of risk assessments.

9. REFERENCES

Allen, T. E. H. et al. (2018) 'Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events', Toxicological Sciences, 165(1), pp. 213–223. doi: 10.1093/toxsci/kfy144.

Berggren, E. et al. (2017) 'Ab initio chemical safety assessment: A workflow based on exposure considerations and non-animal methods', Computational Toxicology. Elsevier, 4, pp. 31–44. doi: 10.1016/J.COMTOX.2017.10.001.

Bessems, J. G. M. et al. (2017) 'The margin of internal exposure (MOIE) concept for dermal risk assessment based on oral toxicity data - A case study with caffeine.' Toxicology, 392, pp. 119–129. doi: 10.1016/j.tox.2017.03.012.

Blackburn, K. and S.B. Stuard (2014) A Framework to Facilitate Consistent Characterization of Read Across Uncertainty. Regulatory Toxicology and Pharmacology. Vol. 68, No. 3, pp 353-62.

Clewell RA and Clewell HJ 3rd. 2008 Development and specification of physiologically based pharmacokinetic models for use in risk assessment. Regul Toxicol Pharmacol. 50(1):129-43. DOI: 10.1016/j.yrtph.2007.10.012

Dent, M. P. et al. (2018) 'Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients', Computational Toxicology, 7, pp. 20–26. doi: 10.1016/J.COMTOX.2018.06.001.

ECHA (2017a), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals, ECHA-17-G-17-EN, ECHA, Helsinki.

ECHA (2017b), Read-Across Assessment Framework (RAAF), ECHA-17-R-01-EN, ECHA, Helsinki.

EFSA (2018a), Guidance on Uncertainty Analysis in Scientific Assessments, EFSA Journal 2018;16(1):5123, 39 pp. https://doi.org/10.2903/j.efsa.2018.5123

EFSA (2018b) Principles and methods behind EFSA's Guidance on Uncertainty Analysis in Scientific Assessment. EFSA Journal 2018;16(1):5122, 282 pp. https://doi.org/10.2903/j.efsa.2018.5122

EU-ToxRisk (2018), Recommendations of the EU-ToxRisk Regulatory Advisory Board (RAB) on how to document case studies for regulatory evaluation

Grisoni, F., Consonni, V. and Ballabio, D. (2019) 'Machine Learning Consensus To Predict the Binding to the Androgen Receptor within the CoMPARA Project', Journal of Chemical Information and Modeling. American Chemical Society, 59(5), pp. 1839–1848. doi: 10.1021/acs.jcim.8b00794.

IPCS (2005), International Programme on Chemical, Safety Workshop on Incorporating Uncertainty Variability into Risk Assessment (2000: Berlin, Germany); Chemical-Specific Adjustment Factors for Interspecies Differences and Human Variability: Guidance Document for Use of Data in Dose/Concentration-Response Assessment, Geneva, World Health Organization. https://apps.who.int/iris/handle/10665/43294.

Mansouri, K. et al. (2016) 'CERAPP: Collaborative Estrogen Receptor Activity Prediction Project.', Environmental health perspectives, 124(7), pp. 1023–33. doi: 10.1289/ehp.1510267.

Martati E, Boersma MG, Spenkelink A, Khadka DB, van Bladeren PJ, Rietjens IM, Punt A (2012) Physiologically based biokinetic (PBBK) modeling of safrole bioactivation and detoxification in humans as compared with rats. Toxicol Sci 128(2): 301-16

Martati E, Boonpawa R, van den Berg JH, Paini A, Spenkelink A, Punt A, Vervoort J, van Bladeren PJ, Rietjens IM (2014) Malabaricone C-containing mace extract inhibits safrole bioactivation and DNA adduct formation both in vitro and in vivo. Food Chem Toxicol 66: 373-84

OECD (2014a). Guidance on Grouping of Chemicals, Second Edition, Series on Testing & Assessment No. 194. ENV/JM/MONO(2014)4, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2014b). Weight of Evidence Assessment for the Skin Sensitisation Potential of 4-Isopropylaniline (Cumidine, CAS 99-88-7), Series on Testing & Assessment No. 199. ENV/JM/MONO(2014)5, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2014c). c ENV/JM/MONO(2014)35, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2016a). Report on Considerations from Case Studies on Integrated Approaches for Testing and Assessment (IATA), First Review Cycle (2015), Case Studies on Grouping Methods as a Part of IATA, Series on Testing & Assessment No. 250. ENV/JM/MONO(2016)48, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2016b). Case Study on the Use of Integrated Approaches for Testing and Assessment for *In vitro* Mutagenicity of 3,3' Dimethoxybenzidine (DMOB) Based Direct Dyes, Series on Testing & Assessment No. 251. ENV/JM/MONO(2016)49, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2016c). Case Study on the Use of Integrated Approaches for Testing and Assessment for Repeat Dose Toxicity of Substituted Diphenylamines (SDPA), Series on Testing & Assessment No. 252. ENV/JM/MONO(2016)50, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2016d). Case Study on the Use of an Integrated Approach to Testing and Assessment for Hepatotoxicity of Allyl Esters, Series on Testing & Assessment No. 253. ENV/JM/MONO(2016)51, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2016e). Case Study on the Use of an Integrated Approach to Testing and Assessment of the Bioaccumulation Potential of Degradation Products of 4,4'-Bis(Chloromethyl)-1,1'- Biphenyl, Series on Testing & Assessment No. 254. ENV/JM/MONO(2016)52, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2016g). Users' Handbook supplement to the Guidance Document for developing and accessing Adverse Outcome Pathways, Series on Testing & Assessment No. 233, OECD Series on Adverse Outcome Pathways No. 1, ENV/JM/MONO(2016)12, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2016h). Guidance Document on the Reporting of Defined Approaches to Be Used within Integrated Approaches to Testing and Assessment No. 255, ENV/JM/MONO(2016)28, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2016i). Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to Be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation, Series on Testing and Assessment No. 256, ENV/JM/MONO(2016)29, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2017a). Report on Considerations from Case Studies on Integrated Approaches for Testing and Assessment (IATA), Second Review Cycle (2016), Series on Testing & Assessment No. 270. ENV/JM/MONO(2017)22, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2017b). Case Study on the Use of an Integrated Approach to Testing and Assessment for Repeated-Dose Toxicity of Phenolic Benzotriazoles, Series on Testing & Assessment No. 271. ENV/JM/MONO(2017)23,

OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2017c). Case Study on the Use of Integrated Approaches for Testing and Assessment for Pesticide Cumulative Risk Assessment & Assessment of Lifestage Susceptibility, Series on Testing & Assessment No. 272. ENV/JM/MONO(2017)24, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2017d). Case Study on the Use of Integrated Approaches for Testing and Assessment of 90-Day Rat Oral Repeated-Dose Toxicity for Selected n-Alkanols: Read-Across, Series on Testing & Assessment No.273. ENV/JM/MONO(2017)25, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2017e). Case Study on the Use of Integrated Approaches for Testing and Assessment of 90-Day Rat Oral Repeated-Dose Toxicity for Selected 2-Alkyl-1-alkanols: Read-Across, Series on Testing & Assessment No. 274. ENV/JM/MONO(2017)26, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2017f). Chemical Safety Assessment Workflow Based on Exposure Considerations and Non-Animal Methods, Series on Testing & Assessment No. 275. ENV/JM/MONO(2017)27, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2018a). Report on Considerations from Case Studies on Integrated Approaches for Testing and Assessment (IATA), Third Review Cycle (2017), Series on Testing & Assessment No. 289. ENV/JM/MONO(2018)25, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2018b). Case Study on the Use of Integrated Approaches for Testing and Assessment (IATA) for Estrogenicity of Substituted Phenols, Series on Testing & Assessment No. 290. ENV/JM/MONO(2018)26, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2018c). Prioritisation of chemicals using the Integrated Approaches for Testing and Assessment (IATA)-based Ecological Risk Classification, Series on Testing & Assessment No. 291. ENV/JM/MONO(2018)27, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2018d). Case Study on Grouping and Read-across for Nanomaterials Genotoxicity of Nano-TiO_2, Series on Testing & Assessment No. 292. ENV/JM/MONO(2018)28, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2018e). 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, Series on Testing & Assessment No. 293. ENV/JM/MONO(2018)29, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2019a). Report on Considerations from Case Studies on Integrated Approaches for Testing and Assessment (IATA), Fourth Review Cycle (2018), Series on Testing & Assessment No. 307. ENV/JM/MONO(2019)26, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2019b). Case Study on the Use of an Integrated Approach to Testing and Assessment for Testicular Toxicity of Ethylene Glycol Methyl Ether (EGME)-Related Chemicals, Series on Testing & Assessment No. 308. ENV/JM/MONO(2019)27, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2019c). Case Study on the Use of Integrated Approaches for Testing and Assessment for Estrogen Receptor Active Chemicals, Series on Testing & Assessment No. 309. ENV/JM/MONO(2019)28, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2019d), Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment, Series on Testing and Assessment No. 311, ENV/JM/MONO(2019)31, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020a). Case Study on use of an Integrated Approach to Testing and Assessment (IATA) and New Approach Methods to Inform a Theoretical Read-Across for Dermal Exposure to Propylparaben from Cosmetics, Series on Testing & Assessment No. 320. ENV/JM/MONO(2020)16, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020b). Case Study on the use of Integrated Approaches for Testing and Assessment for Systemic Toxicity Arising from Cosmetic Exposure to Caffeine, Series on Testing & Assessment No. 321. ENV/JM/MONO(2020)17, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020c). Case Study on the Use of Integrated Approaches for Testing and Assessment for 90-Day Rat Oral Repeated-Dose Toxicity of Chlorobenzene-Related Chemicals, Series on Testing & Assessment No. 322. ENV/JM/MONO(2020)18, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020d). Case Study on the Use of Integrated Approaches for Testing and Assessment to Inform Read-Across of p-Alkylphenols: Repeated-Dose Toxicity, Series on Testing & Assessment No. 323. ENV/JM/MONO(2020)19, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020e). Case Study on the Use of Integrated Approaches to Testing and Assessment for Prediction of a 90 day Repeated Dose Toxicity Study (OECD 408) for 2-Ethylbutyric acid Using a Read-Across Approach to Other Branched Carboxylic acids, Series on Testing & Assessment No. 324. ENV/JM/MONO(2020)20, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020f). Case Study on the Use of Integrated Approaches to Testing and Assessment for Read-Across Based Filling of Developmental Toxicity Data Gap for Methyl Hexanoic acid, Series on Testing & Assessment No. 325. ENV/JM/MONO(2020)21, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020g). Case Study on the Use of Integrated Approaches to Testing and Assessment for Identification and Characterisation of Parkinsonian Hazard Liability of Deguelin by an AOP-based Testing and Read Across Approach., Series on Testing & Assessment No. 326. ENV/JM/MONO(2020)22, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020h). Case Study on the Use of Integrated Approaches to Testing and Assessment for Mitochondrial Complex-III-Mediated Neurotoxicity of Azoxystrobin - Read-Across to Other Strobilurins, Series on Testing & Assessment No. 327. ENV/JM/MONO(2020)23, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

OECD (2020i). Report on Considerations from Case Studies on Integrated Approaches for Testing and Assessment (IATA) - Fifth Review Cycle on Testing & Assessment No. 328. ENV/JM/MONO(2020)24, OECD, Paris. Available at http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2020)24&doclanguage=en

OECD (2020j). Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA), Series on Testing and Assessment No. 329, OECD, Paris. Available at http://www.oecd.org/chemicalsafety/risk-assessment/concepts-and-available-guidance-related-to-integrated-approaches-to-testing-and-assessment.pdf

OECD (2021a). Case Study on the Use of the use of Integrated Approaches for Testing and Assessment for the Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion, Series on Testing & Assessment No. 332. ENV/CBC/HA(2021)2, OECD, Paris.

OECD (2021b). Guidance Document on Characterisation, Validation and Reporting of PBK models for Regulatory Purposes, Series on Testing & Assessment No. 331, OECD, Paris.

Paul Friedman, K. et al. (2020) 'Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization', Toxicological Sciences. Oxford University Press (OUP), 173(1), pp. 202–225. doi: 10.1093/toxsci/kfz201.

Schultz TW, Amcoff P, Berggren E, et al. (2015). A strategy for structuring and reporting a read-across prediction of toxicity. Regul Toxicol Pharmacol. Vol. 72, No. 3, pp. 586‐601. doi:10.1016/j.yrtph.2015.05.016

SCCS (2018) 'Scientific Committee on Consumer Safety (SCCS) Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation 10th revision, 24-25 October 2018, SCCS/1602/18'.

Terry W. Schultz, Andrea-Nicole Richarz, Mark T.D. Cronin (2019) Assessing uncertainty in read-across: Questions to evaluate toxicity predictions based on knowledge gained from case studies. Computational Toxicology, Vol. 9, pp. 1-11 https://doi.org/10.1016/j.comtox.2018.10.003

U.S. EPA. (2006) Approaches For the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data In Risk Assessment (Final Report). U.S. Environmental Protection Agency, Washington, D.C., EPA/600/R-05/043F

U.S. EPA (2011), Recommended use of body weight3/4 as the default method in derivation of the oral reference dose. Risk Assessment Forum. United States Environmental Protection Agency, Washington, D.C., EPA/100/R11/0001.

WHO (2010) Harmonization Project Document No. 9: Characterization and application of physiologically based pharmacokinetic models in risk assessment. Geneva: World Health Organization.

WHO (2018), Guidance document on evaluating and expressing uncertainty in hazard haracterization, 2nd edition. https://apps.who.int/iris/handle/10665/259858

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 Analogs for SAR-based toxicological assessments. Regulatory Toxicology and Pharmacology. Vol. 56, No 1, pp 67-81.

Annex A. Template for IATA Case Studies on Chemical Grouping (Read-across)

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

NOTE: 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 subsections 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 changed as necessary. The template will be revised based on experience with use. A case study based on the template is expected to be assessed as stand-alone, thus needs to contain all necessary information and appropriate links for a detailed assessment.

Abstract / Synopsis / Executive summary

This section should provide a brief overview of the case study, including the objectives, concepts, methodologies, outcomes and conclusion in about 300 words. Please refer to Executive Summary in Case Study 2018-1 (OECD, 2019b) and 2018-2 (OECD, 2019c), and Summary in 2017-3 (OECD, 2018d) as examples.

Table of Contents

Abbreviations and acronyms

1. Introduction

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

2. Purpose

a. Purpose of use

Specify the purpose of use of the IATA (e.g. regulatory context: hazard identification, hazard characterisation, risk assessment, screening etc.). If the IATA is used for low toxicity prediction, please define what is meant by 'low toxicity' for the purposes of the particular case study. If in a regulatory context, provide a short but sufficient description of any (e.g. legal) requirements for the IATA approach to be accepted.

b. Target chemical(s)/category definition [See 3.2.3.1 "Chemical identity and composition" 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 (e.g. in chemical structures); composition including impurities; and if known, any limitations in the information.

c. Endpoint(s)

- Identify the endpoint(s) for which the analogue/category approach is applied. Endpoint-specific considerations/approaches may be needed if more than one endpoint is addressed by the read-across.

d. Exposure information (if needed)

- Provide the considered exposure for the grouping/read-across, such as route(s) of administration covered by the experimental model (e.g. oral), the population of interest (.e.g. human, ecological), and as relevant, any route to route or *in vivo/in vitro* extrapolations that were applied to inform the grouping/read-across

Tip

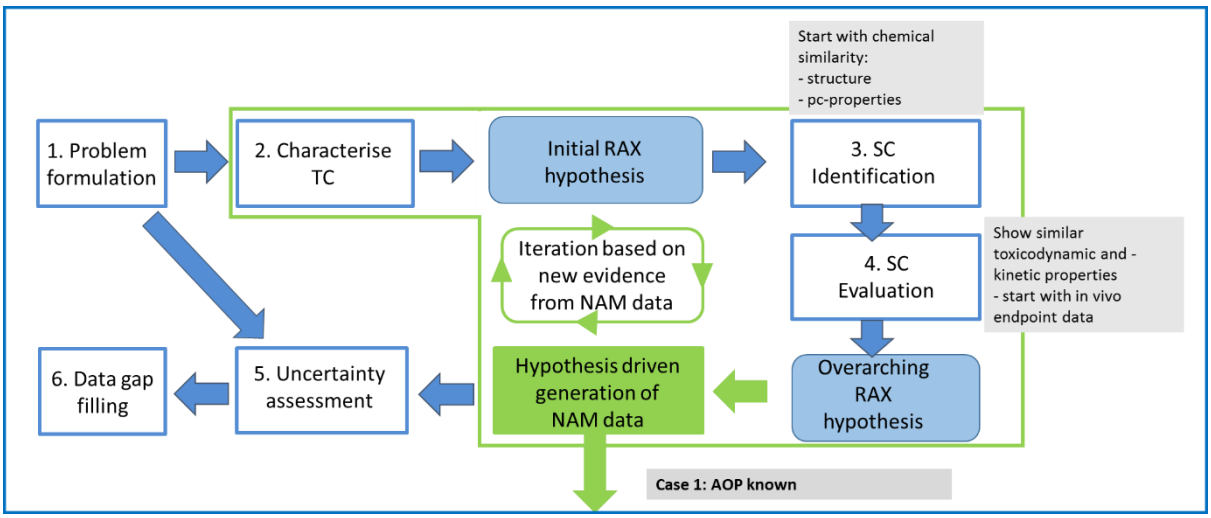
- The description of the purpose of use is important for considering the acceptable uncertainty of the case study, which could be linked to the uncertainty assessment. For example, if the conclusion derived by case study is renewable in a framework such as tiered-approach, this needs to be clearly stated (see case studies OECD, 2016b and 2016c).
- As the goal of the OECD IATA Case Studies project is to discuss case studies which would lead to regulatory application a description of the regulatory relevance is important to contextualise the case and discuss the further development of guidance and how use IATA for regulatory purpose.
- It is recommended to specify the analogues and justification for data gap filling, used for each addressed endpoint, in order to identify for what endpoints is the analogue/category being applied.

Tip for nanomaterials

- The parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints are for example surface chemistry, size, shape and surface area, along with physical/chemical properties. (See *"1.2 Target chemicals"* of the case study 2017-3 (OECD, 2018d))
- For the complete list of parameters and more information on grouping of nanomaterials please, see "ECHA (2017a), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals", Appendix I, Table 2: Key physicochemical parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints.

3. Hypothesis for the analogue approach/category [See 2.4 "The mechanistic basis of using analogues or chemical categories" and 3.2.1 "Hypothesis and evidence based approaches" of the grouping guidance (OECD, 2014a)] If many steps are included in the IATA, include a figure for the workflow of the IATA applied in the case study to make IATA approach clear.

Please refer to Figure 1 in Case Study 2019-4 (OECD, 2020d) and Figure 2 under section 4.1 "Testing and assessment strategy" in Case Study 2019-5 (OECD, 2020e).



Example of Workflow Figure, which was used in Case Study 2019-5

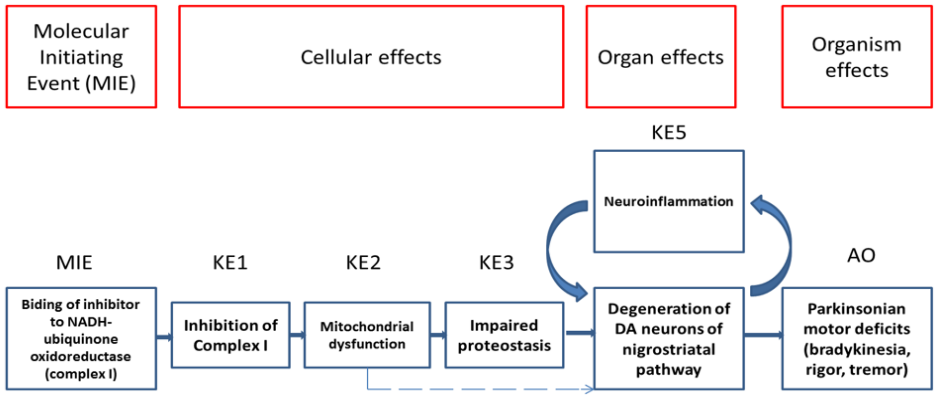
- For an analogue approach, describe the characteristics that a substance must have to be suitable as a source substance, including a description of the composition of the source substance (e.g. level of purity). Provide the hypothesis for why read-across can be performed between the source and target chemicals [See 4.2.2 "Step 1: Identification of potential analogues" of the grouping guidance (OECD, 2014a)].
- 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 "Step 1: Develop category hypothesis and definition and identify category members" of the grouping guidance (OECD, 2014a)].
- These hypotheses can be argued by a number of elements as follows [See 3.2.3 "Elements for a read-across justification" of the grouping guidance (OECD, 2014a)]. Chemical identity and composition, including level of purity
  - Chemical identity and composition, including level of purity [See 3.2.3.1 "*Chemical identity and composition* of the grouping guidance (OECD, 2014a)]
  - Physical-chemical properties and other molecular description [See 3.2.3.2 "*Physical-chemical properties* of the grouping guidance (OECD, 2014a)]
  - Kinetics: Absorption, distribution, metabolism and excretion [See 3.2.3.3 "*Absorption, distribution, metabolism and excretion* of the grouping guidance (OECD, 2014a)]
  - Mode/Mechanism of action or adverse outcome pathways (MoA/AOP) [See 3.2.3.4 "*Mode/ mechanisms of action or adverse outcome pathways (MoA/AOP)* of the grouping guidance (OECD, 2014a)]
  - Chemical / biological interaction [See 3.2.3.5 "*Chemical / biological interaction* of the grouping guidance (OECD, 2014a)]
  - Toxicological and epidemiological information, along with information from new approach methodologies (NAMs) [See 3.2.3.6 "*Responses found in in vitro methods* of the grouping guidance (OECD, 2014a)]
  - 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 relevant for the assessment should be addressed. In addition, it is recommended to describe how the (combination of) elements support the hypothesis (see for more detail OECD, 2014a).

- Especially, hypothesis of mechanism(s) (AOP/MOA) for how the target chemical induces target endpoint toxicity need to be described in this section. Hypothesis of structural boundaries and limitations for the approach should also be clearly described, including possible impact of structural dissimilarities. The graphical representation of the AOP would be helpful for the reader and key references (See "Graphical Representation of the AOP" at section 1- AOP Description (OECD, 2016g)). If an AOP together with testing of various MIE/KE/AO is used in the case study, a figure demonstrating the alignment of the AOP with the various tests should be included. Please refer to Figure 1 in Case Study 2018-2 (OECD, 2019c), Figure 3 in Case Study 2019-4, Figure 7 in Case Study 2019-5, Figure 2 (A and B) in Case study 2019-7 and Figure 5.1 (A and B) in Case Study 2019-8.

A)



B)

Key event	MIE	KE1	KE2	KE3	KE4	AO
Assay	Receptor Docking studies	Seahorse assay (complex inhibition and whole cell)	Mitochondrial membrane potential assay	Protease activity assay	Viability assays (Resazurin, PI, ATP)	
	Similarity studies			CHOP-GFP expression	Neuronal health (outgrowth and degeneration)	
Key event					KE5	
Assay					No assay available	

Example of AOP figure together with MIE/KE/AO, which was used in Case Study 2019-7

The tools in the AOP-KB[4]should be referred to as appropriate (e.g. AOP wiki[5], Effectopedia [6]etc.). Identifying the relevant AOP from AOP wiki is required. Please provide the AOP number, status on AOP-wiki and the link. For AOPs that are not documented, consider the "Users' Handbook supplement to the Guidance Document for developing and accessing Adverse Outcome Pathways" (OECD, 2016g) - although an entire AOP description is not the purpose here. If needed, the entire AOP can be described in Annex.

- Describe how a data gap is intended to be filled for the purpose of read-across (the prediction model used - worst case scenario, regression etc.). Here it could also be justified as to why read-across is sufficient, and why further testing is not needed.

Tip

- Hypothesis needs to be described as a testable format.
- For the hypothesis that metabolite induces target effect, the effects induced by other metabolites other than the toxicant need to be considered (see "("*2.2 Elements for a read-across hypothesis* of the case study 2015-3" (OECD, 2016d)).

[4] AOP-KB. https://aopkb.oecd.org/

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

[6] Effectopedia. https://www.effectopedia.org/

Tip for nanomaterials

- Provide an explanation which parameters are critical for the analogue approach/category hypothesis.
- Hypothesis could be argued using for example the following physicochemical and chemical properties (list is not exhaustive) (see for example"2.2 Characterisation of the analogue nanoforms" of 2017-3 (OECD 2018d)):
  - Chemical composition
  - Surface chemistry (including coating chemicals and the coating ratio)
  - Impurity
  - Size (including primary particle diameter)
  - Shape (including surface chemistry)
  - Surface area
  - Solubility
  - Hydrophobicity
  - Zeta potential
  - Dispersibility
  - Dustiness
  - Physical hazard
  - Biological (re)activity
  - Photoreactivity
- For the complete list of parameters and more information on grouping of nanomaterials please, see "ECHA (2017a), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals", Appendix I, Table 2: Key physicochemical parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints.

4. Source chemicals/Category members [See 2.3 "Selecting analogues/Creating chemical categories and setting boundaries", 4.2.2 "Step 1: Identification of potential analogues" and 5.2.2 "Step 1: Develop category hypothesis and definition and identify category members" 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 B, that were used to identify the source chemicals/category members.
- Provide the rationale for selection of analogue(s)/category members with respect to the defined purpose and endpoint.
- Provided consideration of a selection bias in the choice of source chemicals when using the analogue or category approach (e.g. data quality and 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.
- Recommend to use positive and negative reference chemicals if possible, especially in the case of testing that it is done to support the IATA.

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 "Examples of categories and structural relationships" 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 when selecting analogues. A clear description of boundaries is also important.

5. Justification of data gap filling

a. Data gathering [See 4.2.3 "Step 2: Data gathering for the analogues" and 5.2.3 "Step 2: Gather data for each category member" of the grouping guidance (OECD, 2014a)]

- Provide a summary of the methods used for gathering the data for target and source chemicals/category members (e.g. selection criteria of the data, data source). More detailed information on the methods can be included in the Annex.

b. Data and methods [See 4.2.4 "Step 3: Evaluation of available data for adequacy", 4.2.5 "Step 4: Construct a matrix of data availability" (analogue approach); 5.2.4 "Step 3: Evaluate available data for adequacy." 5.2.5 "Step 4: Construct a matrix of data availability" (category approach) of the grouping guidance (OECD, 2014a)]. Provide a matrix of data (see data matrix template) with the following:

- 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 a summary of the essential data. Recommended to include the detailed data 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[7]. One of the example is Case Study 2018-1 (OECD, 2019b). More detailed or supporting information can be included in an Annex.
- 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 the OECD guidance document No. 211 (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) [8]and U.S. EPA Toxicity ForeCaster (ToxCast™) Data [9]. More detailed information on the methods can be included in an Annex.
- If (Q)SAR data are included, provide the name, version, owner of the models used for deriving (Q)SAR estimation data. If not described elsewhere, (Q)SAR models 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). A QMRF inventory is maintained by JRC that can be utilised as a resource of QMRFs and its reference number can be referred to JRC QSAR Model databases[10]. QPRF(s) and QMRF should be included in an Annex.

[7] OECD Integrated Approaches to Testing and Assessment (IATA). http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm#casestudies

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

[9] U.S. EPA, Toxicity ForeCaster (ToxCast™) Data https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data

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

- 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 255 and 256 (OECD, 2016h; 2016i). In this section, please describe the individual information sources used and data interpretation procedure applied (See "6. Description of the individual information sources used (see Annex II)" and "7. Data interpretation procedure applied" of the OECD guidance (OECD, 2016h)). Detailed information on the defined approaches can be included in Annex. Please refer to the section "4. Data/Information Gathering" of the case study 2018-2 (OECD, 2019c).
- Provide justification/purpose for each assay/information used. Only necessary information should be provided, avoid giving information not directly useful for your Case Study (do not provide data just because you have it).
- Provide all available suitable information regarding the defined purpose, including the data from the different IATA components (*in silico*, *in vitro* and *in vivo*, if applicable). If possible, the cells of the data matrix should also indicate the available key study results.

c. Justification [See 2.5 "Robustness of a chemical category and of an analogue approach", 2.6 "The interdependence between categories and (Q)SARs.", 4.2.6 "Step 5: Assess the adequacy of the analogue approach and fill the data gap" and 5.2.6 "Step 5: Perform a preliminary evaluation of the category and fill data gaps" of the grouping guidance (OECD, 2014a)]

- Based on the data matrix, summarise how these data support the hypothesis described in section 3.
- Identify similarities/trends in the experimental data of the endpoint(s) for the chemicals in the data matrix and verify their concordance with the hypothesis described in section 3.
- 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

Please include a summary text box at the end of each section with the key highlights or conclusions of the section, which would impact on the conclusion, if authors believe this would help the readers. Summary text box applied in section "*CHEMICAL SAFETY ASSESSMENT WORKFLOW PROPOSED*" in Case Study 2016-5 can be referred (OECD, 2017f).

ernative
ata and

Tip

- Reliability of each (Q)SAR prediction result needs to be described in terms of the applicability domain of (Q)SARs. For example, it can be discussed by the coverage of the fragments in the training sets (See the case study 2015-4 (OECD, 2016e)).
- It is recommended that every approach be described separately, e.g. if read-across, (Q)SAR and *in vitro* tests are used, every one of these approaches would need to be described separately before combining in IATA.
- Please explain how satisfying comprehensiveness/coverage of the data gathering is achieved.
- For transparency, the data reporting is an important aspect. For example, if estimation relies on qualitative/semi-quantitative estimation, it is important to explain how these support quantitative estimations where needed for that purpose. Further, to demonstrate coherence of findings and similarity/trend/strength of effects sufficient reporting of the experimental data is needed (e.g. type, degree and dose levels). If data reveal inconsistencies or similar studies show different concerns this would also benefit from explanation.
- Please, try to ensure maximal use of existing experimental information before considering (Q)SAR predictions.
- Alert-based system work best for predicting an alert and not lack of it, unless there are structure-specific definitions for lack of activity

Tip for nanomaterials (See "5. JUSTIFICATION OF DATA GAP FILLING" of the case study 2017-3 (OECD, 2018d))

- Describe methods used for measuring the endpoints
- It is recommended to describe which methodologies for measurements of the relevant parameters are applied, and to describe what are differences between the methodologies are, if applicable.
- Identify which parameters are relevant to which endpoints, if possible.
- For the complete list of parameters and more information on grouping of nanomaterials, please see ECHA (2017a) "Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals", Appendix I, Table 2: Key physicochemical parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints.

6. Strategy for and integrated conclusion of data gap filling

a. Uncertainty

- Discuss the uncertainty of each factor for the read- across. For the given purpose, it seems that the consideration of uncertainty may start from the choice of hypothesis (like in Appendix 1). Another consideration includes severity of effect, if it is present. (e.g. Does the number of targets matter? Could all targets meet all sources? How read-across could be addressed (e.g. subgrouping)?)
- Aspects can include uncertainty and confidence associated with all type of the data used in the IATA, including the underlying data used for read across from the source chemicals (e.g. applicability domain, type and quality) as well as assumptions used to develop the similarity rationale of the analogues/category members and uncertainty.

- The following table provides an example of reporting uncertainty (Please modify as appropriate and also it is recommended to describe what is not addressed.): Examples of modified templates, which were used for past case studies, are shown in Appendix 1, 2,and 3. Appendix 4 lists a series of questions to guide through the assessment of uncertainties. Also, refer to the case studies published in the past[11].
- **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 (" Consideration of uncertainties associated with the application of the defined approach" of OECD, 2016h; " Consideration of uncertainties associated with the application of the defined approach" of CASE STUDY I-XII of OECD, 2016i) might be helpful for considering uncertainties related to non-guideline test methods
- If AOP is used, please discuss uncertainty on AOP (e.g. endorsed AOP: the AOP approved and published by OECD vs putative AOP; the AOP not approved by OECD and established based on the known knowledge.)
- For the application of WoE approach, the ECHA WoE template[12] provides a structured template for presenting the WoE approach/ uncertainty (EU-ToxRisk, 2018)
- The EFSA guidance documents (EFSA, 2018a; 2018b) could be considered for uncertainty assessment as a good starting point. In addition, for quantitative hazard assessments, the WHO Guidance on Evaluating and Expressing Uncertainty in Hazard Assessment (WHO, 2018) can provide further support (EU-ToxRisk, 2018)
- In application of WoE, please refer to the OECD WoE guidance document (OECD, 2019d), which provides universal Guiding Principles that should be considered when developing or augmenting systematic approaches to WoE for chemical evaluation and Key Elements to formulating a systematic approach to WoE

Factor	Uncertainty (low, medium, high)	Impact of uncertainty on hypothesis	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			

[11] OECD Integrated Approaches to Testing and Assessment (IATA). http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm#casestudies

[12] ECHA – Template for Weight of Evidence / Uncertainty in Hazard Assessment https://echa.europa.eu/documents/10162//17169198/template_for_weight_of_evidence_en.docx

Tip

- When using ranks to indicate uncertainties (e.g. low, medium, high), definitions should be provided.

Tip for nanomaterials

- In addition to the above mentioned aspects, the following should be considered in the characterisation of uncertainties related to the analogue/category approach for nanomaterials (See "7. UNCERTAINTY ASSESSMENT" of the case study 2017-3 (OECD, 2018d)):
  - Complexity of nanostructures: similarity, category boundaries and members
  - Identity characterisation of the nanomaterials
  - Variability of the measurements, test system relevance for nanomaterials and possible nanospecific artefacts in assays

For more information on grouping of nanomaterials please, see "ECHA (2017a), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals"

b. Integrated conclusion

- Provide the strategy used to fill the data gap and integrated conclusion of data gap filling, including description how the data gap is actually filled (e.g. average, most sensitive, similarity weighted, qualitative). In case of category approach, indicate proposed conclusion/value for each data gap. If prediction models were used, please describe the satisfaction with parameters related to the prediction.
- Give discussion of remaining uncertainties and how they might be addressed.
- Finally, provide a short conclusion wrapping up the outcome of the evaluation with linking to the given purpose.

References

Annex

- Author can include supplemental or background data in an Annex in order to increase readability of case study if the data supports a particular aspect of the case study. The below table is an example of a summary table for *in vivo* data (Reference of Case Study 2019-4).

References	
Species/strain	
Sex	
Route of admin.	
Exposure period	
Doses	
GLP	
Test substance	
NOAEL	
Result	
Other findings	

- Author can provide a summary of methods and tools used in the case study, that a regulator may be less familiar with, such as an *in vitro* method, *in silico* ((Q)SAR) model or high throughput assay; or provide links to references of these methods for further information in order to increase readability of case study. The description should be sufficient for an expert, which a regulator may consult to get approval and better understanding of the methodology.

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 (OECD, 2016b; 2016c).

- 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 Analogs for SAR-based toxicological assessments. Regulatory Toxicology and Pharmacology. Vol. 56, Issue 1, pp 67-81. https://doi.org/10.1016/j.yrtph.2009.09.006
- Blackburn, K. and S.B. Stuard (2014) A Framework to Facilitate Consistent Characterization of Read Across Uncertainty. Regulatory Toxicology and Pharmacology. Vol. 68, Issue 3, pp 353-62. https://doi.org/10.1016/j.yrtph.2014.01.004

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 (OECD 2017d; 2017e) as well as in case study 4 in 2017 (OECD 2018e).

- 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 (2015), A Strategy for Structuring and Reporting a Read-across Prediction of Toxicity. Vol. 72, Issue 3, pp 586-601. https://doi.org/10.1016/j.yrtph.2015.05.016

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	Table Cell (Alt+E)		
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

Appendix 3. Examples of Reporting uncertainty Following the ECHA Read-Across Assessment Framework (RAAF)[13] (3)

Examples of assessment elements (AEs) for an analogue approach, for all RAAF read-across scenarios and detailed description of the AEs see (ECHA, 2017b).
Assessment Elements for Scenario 1 (analogue approach for read-across based on hypothesis for (bio)transformation to common compound(s))

AE A.1 Common AE: Identity and Characterisation of the source substance
AE A.2 Common AE: Link of structural similarities and differences with the proposed prediction
AE A.3 Common AE: Reliability and adequacy of the source study
AE 1.1 Scenario-specific AE: Formation of common (identical) compound(s)
AE 1.2 Scenario-specific AE: The biological targets for the common compound(s)
AE 1.3 Scenario-specific AE: Exposure of the biological target(s) to the common compound(s)
AE 1.4 Scenario-specific AE: The impact of parent compounds
AE 1.5 Scenario-specific AE: Formation and impact of non-common compounds
AE A.4 Common AE: Bias that influences the prediction

Assessment Elements for Scenario 2 (analogue approach for read-across based on hypothesis that different compounds have the same type of effects)

AE A.1 Common AE: Identity and Characterisation of the source substance
AE A.2 Common AE: Link of structural similarities and differences with the proposed prediction
AE A.3 Common AE: Reliability and adequacy of the source study
AE 2.1 Scenario-specific AE: Compounds the test organism is exposed to
AE 2.2 Scenario-specific AE: Common underlying mechanism, qualitative aspects
AE 2.3 Scenario-specific AE: Common underlying mechanism, quantitative aspects
AE 2.4 Scenario-specific AE: Exposure to other compounds than to those linked to the prediction
AE 2.5 Scenario-specific AE: Occurrence of other effects than covered by the hypothesis and justification
AE A.4 Common AE: Bias that influences the prediction

[13]ECHA, Grouping of substances and read-across

https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

Appendix 4. Examples for reporting uncertainty (4)

30 questions relating to 12 types of uncertainty were identified to be addressed in assessing uncertainties of a read-across in the following study:

- Terry W. Schultz, Andrea-Nicole Richarz, Mark T.D. Cronin (2019) Assessing uncertainty in read-across: Questions to evaluate toxicity predictions based on knowledge gained from case studies. Computational Toxicology, Vol. 9, pp. 1-11 https://doi.org/10.1016/j.comtox.2018.10.003

Uncertainty in Read-Across	Uncertainty in Read-Across
The context of, and relevance to, the regulatory use of the read-across prediction as defined by appropriate problem formulation	• Is the regulatory purpose of the read-across prediction clearly defined? • Is the acceptable level or degree of uncertainty for the stated purpose defined? • Is the stated acceptable level or degree of uncertainty appropriate for the stated regulatory purpose?
Type of category/group including the definition of the applicability domain	• Is the read-across approach (e.g., analogue or category) clearly reported? • Are the target and source chemicals clearly identified? • Is the applicability domain of the analogue or category defined? • Do target and source chemicals fit within the defined applicability domain?
The premise or hypothesis of the read-across.	• Is the hypothesis on which the read-across is based clearly stated and presented in sufficient detail to be assessed?
Mechanistic plausibility including completeness of the understanding of the MoA or AOP	• How clearly does the hypothesis state the chemical and biological mechanisms underpinning the toxic effect being read across? • Is there sufficient experimental information provided to support the proposed chemical and toxicological mechanisms? • How extensively does the experimental information provided support the mechanistic plausibility and / or the AOP or MoA on which the read-across is based?
Similarity in chemistry	• Are the chemical structures (i.e., 2D structure, isomers, SMILES and molecular formula) reported for the derivatives used in the read-across? • Are the dissimilarities in chemical structure reported and are they toxicologically relevant? • Are the relevant molecular and physico-chemical properties (e.g., for molecular size, hydrophobicity, solubility, volatility, degradation etc.) reported for the derivatives used in the read-across? • Are the dissimilarities in molecular and physico-chemical properties reported and are they toxicologically (or pharmacokinetically) relevant?
Toxicodynamic similarity	• Is there sufficient and consistent toxicodynamic information provided to establish similarity in the hazard of the derivatives used in the read-across?
Toxicokinetic similarity	• Is there sufficient ADME information provided to establish toxicokinetic similarity for the derivatives used in the read-across? • Are any dissimilarities in ADME properties (and, as appropriate, metabolism / degradation) toxicologically relevant?
The quality of the apical endpoint data used to fill the data gap	• Is the performance (e.g., reliability, accuracy, precision, repeatability and reproducibility) of the data read across reported clearly? • Has the quality of the data to be read across been assessed and are they sufficient to meet the purpose of the exercise i.e., complete and of sufficient quality?
The consistency in the effects and severity of the apical *in vivo* hazard and their concordance with regards to the intermediate and apical effects and potency data	• Is the qualitative expression of the data reported and is it consistent among the source chemicals? • Is the potency of the hazard reported and is it consistent among the source chemicals? • What are the temporal relationships between relevant endpoints? • What are the dose–response relationships between relevant endpoints?
Strength or robustness of the supporting datasets	• How extensively are the relevant or key events either empirically measured and/or modelled by appropriate *in silico*, *in chemico* and *in vitro* data? • Is the performance (e.g., reliability, accuracy, precision, repeatability and reproducibility) of the supporting methods adequately reported?
The Weight-of-Evidence (WoE) supporting the prediction	• Is there consistency in the supportive information (e.g., structural alerts) between analogues or within the category? • How many and how large are the dissimilarities in the supporting information (i.e., data gaps)?
Documentation and written evidence provided	• Is the read-across prediction adequately documented? • Does the evidence support the hypothesis that the uncertainty is acceptable for the stated purpose (as per Question 1)?

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								
	Defind approach of IATA								
	…								

* 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
	Defind approach of IATA								
	…								

* More relevant metabolite such as toxicant

**General outline of relative comparative kinetics

Annex 1. General Template for IATA case Studies - Building Blocks

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

NOTE: 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 subsections 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 changed as necessary. The template will be revised based on experience with use.

Abstract / Synopsis / Executive summary

This section should provide a brief overview of the case study, including the objectives, concepts, methodologies, outcomes and conclusion in about 300 words. Please refer to Executive Summary in Case Study 2018-1 (OECD, 2019b) and 2018-2 (OECD, 2019c), and Summary in 2017-3 (OECD, 2018d) as examples.

Table of Contents

Abbreviations and acronyms

1. Introduction

This should include a summary of the background/problem formulation, purpose, endpoints covered and description of the target chemical(s)/category, assessment approach

2. 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 specify the purpose as d) other. If the IATA is used for low toxicity prediction, please define what is meant by 'low toxicity' for the purposes of the particular case study.

If in a regulatory context, provide a short but sufficient 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 [See 3.2.3.1 "*Chemical identity and composition* of the grouping guidance (OECD, 2014a)]) and chemical structure(s) of the target substance(s). In some case studies, target chemicals may be entire chemical classes or the IATA illustrated may be generic. Or if there are no specific target chemicals, example chemicals can be used to illustrate the IATA (SEE "1. PURPOSE" or "3. RESULTS OF ERC PRIORITISATION" of the case study 2017-2 (OECD, 2018c) and "1.2. Target Chemical(s)" at the section "A. Purpose" of the case study 2018-2(OECD, 2019c)).

c. Endpoint(s)

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

d. Exposure information (if needed)

Provide the considered exposure, such as route of exposure (dermal, oral and inhalation), type of exposure (consumer, occupational and environment), for example, if the case study addresses prioritisation or chemical assessment work flows. The inclusion of this section and its level of detail/quantification will depend on the case study.

If relevant, please describe extrapolation from *in vitro* into *in vivo*.

Tip

- The description of the purpose of use is important for considering the acceptable uncertainty of the case study, which could be linked to the uncertainty assessment. 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 OECD, 2016b and 2016c).
- As the goal of the OECD IATA Case Studies project is to discuss case studies which would lead to regulatory application a description of the regulatory relevance is important to contextualise the case and discuss the further development of guidance and how to use the IATA for regulatory purpose.

3. 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.
- If many steps are included in the IATA, include a figure for the workflow of the IATA applied in the case study in order to provide an overview on how the IATA work through. Please refer to Figure 1 in Case Study 2019-4 (OECD, 2020d) and Figure 2 under section 4.1 “Testing and assessment strategy” in Case Study 2019-5. (OECD, 2020e). The below figure used in Case Study 2019-5 is an example.

Example of Workflow Figure, which was used in Case Study 2019-5

4. Approaches used (Potential Blocks for Inclusion)

Describe which approaches are applied for assessing the chemicals under the provided hypothesis:

- **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 (See “Graphical Representation of the AOP” at section “1- AOP Description” of “User’s Handbook supplement to the Guidance Document for developing and accessing Adverse Outcome Pathways”

(OECD, 2016h)). The tools in the AOP-KB[14]should be referred to as appropriate (e.g. AOP wiki[15], Effectopedia[16] etc.).

Identifying the relevant AOP from AOP wiki is required. Please provide the AOP number, status on AOP-wiki and the link. For AOPs that are not documented, consider the "Section 1-AOP Description" of "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. If needed, the entire AOP can be described in Annex.

If an AOP together with testing of various MIE/KE/AO is used in the case study, a figure demonstrating the alignment of the AOP with the various tests should be included. Please refer to Figure 1 in Case Study 2018-2 (OECD, 2019c), Figure 3 in Case Study 2019-4 (OECD, 2020d), Figure 7 in Case Study 2019-5 (OECD, 2020e), Figure 2 (A and B) in Case study 2019-7 (OECD, 2020g) and Figure 5.1 (A and B) in Case Study 2019-8 (OECD, 2020h). The below figure is an example of the figure demonstrating the alignment of the AOP with the various tests, which was used in Case Study 2019-7. The figure indicated where the assay is available and not available.

Example of AOP figure together with MIE/KE/AO, which was used in Case Study 2019-7

- **Defined Approach**: If a defined approach is included, please refer to the ANNEX I: TEMPLATE FOR REPORTING DEFINED APPROACHES TO TESTING AND ASSESSMENT BASED ON MULTIPLE INFORMATION SOURCES" of "Guidance Document on the Reporting of Defined Approaches to be used within Integrated Approaches to Testing and Assessment" (OECD, 2016h). Please copy into this section the "*5. Rationale underlying the construction of the defined approach*" from the above mentioned template (OECD, 2016h), completed with proper explanations. The elements described in the section "3. Approaches Used" of the case study 2018-2 (OECD, 2019c) can be helpful for development of an IATA using Defined Approach.
- **Workflow**: If an IATA workflow is included, provide a schematic and explanation of the elements of the workflow including input, decision and exit points. If prioritisation is the goal of IATA workflow, provide an explanation of how to classify the hazard and exposure profiling and potential risk classification. Please refer to the section "CHEMICAL SAFETY ASSESSMENT WORKFLOW" of the case study 2016-5 (OECD, 2017f), "3.3 IATA Workflow" of the case study 2017-1 (OECD, 2018b) and the section "2. PRIORITISATION OF CHEMICALS USING AN IATA-BASED ERC APPROACH" of the case study 2017-2 (OECD, 2018c) and the section "2. PRIORITISATION OF CHEMICALS USING AN IATA-BASED ERC APPROACH" of the case study 2017-2 (OECD, 2018c).
- **Read-across**: If a read-across is included, use elements of the template for IATA case studies on Read-Across or the grouping guidance (OECD, 2014a). Please refer to "*4. Identification of analogues, suitability assessment and existing data*" of the case study 2016-5 (OECD, 2017f) and "4.1. Analogue chemicals" of the case study 2017-1 (OECD, 2018b)

[14] AOP-KB. https://aopkb.oecd.org/

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

[16] Effectopedia. https://www.effectopedia.org/

5. Data/Information gathering

In this section, please describe the test methods or data sources used for gathering data for target chemicals

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. More detailed information on the methods can be included in an Annex. A template for the description is available in an 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)[17] and U.S. EPA Toxicity ForeCaster (ToxCast™) Data[18].
- If (Q)SAR data are included, provide the name, version, owner of the models used for deriving (Q)SAR estimation data. If not already described elsewhere (Q)SAR models should be reported using the QSAR Model Reporting Format (QMRF)[19], and individual predictions, if applicable, should be reported using the QSAR Prediction Reporting Format (QPRF)[20]. A QMRF inventory is maintained by JRC that can be utilised as a resource of QMRFs and its reference number can be referred to JRC QSAR Model databases[21]. QPRF(s) and QMRF should be included in Annex.
- If the exposure elements are included, provide the methods used for the data generation (e.g. data source, exposure models/tools.) Please refer to "2. Identification of the use scenario of the case study 2016-5 (OECD, 2017f)" and "*Exposure profiling*" of the case study 2017-2 (OECD, 2018c)
- If a defined approach is included, please refer to the template of "Guidance Document on the Reporting of Defined Approaches to be used within Integrated Approaches to Testing and Assessment" (OECD, 2016h). In this section, please describe the individual information sources used and data interpretation procedure applied (See *"6. Description of the individual information sources used (see Annex II)"* and *"7. Data interpretation procedure applied"* of the OECD guidance (OECD, 2016h). Detailed information on the defined approaches can be included in the Annex. Please refer to the section "4. Data/Information Gathering" of the case study 2018-2 (OECD, 2019c).
- If high throughput or omics data are used then indicate how the data has been applied in the specific case study i.e. to support *in vivo*/*vitro* data or any other reason etc.
- Provide justification/purpose for each assay/information used. Only necessary information should be provided, avoid giving information not directly useful for your Case Study (do not provide data just because you have it).

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

[18] U.S. EPA, Toxicity ForeCaster (ToxCast™) Data https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data

[19] QMRF is available: https://community.oecd.org/docs/DOC-144256

[20] QPRF is available: https://community.oecd.org/docs/DOC-144257

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

Please include a summary text box at the end of each subsection with the key highlights or conclusions of the subsection, which would impact on the conclusion, if authors believe this would help the readers. Summary text box applied in section "*CHEMICAL SAFETY ASSESSMENT WORKFLOW PROPOSED*" in Case Study 2016-5 can be referred (OECD, 2017f)

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 3.
- 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.
- Recommend to use positive and negative reference chemicals if possible, especially in the case of testing that is done to support the IATA.

6. 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 (Q)SAR 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. We recommend to use a table to describe the uncertainty of each element. The following table provides an example of reporting uncertainty (Please modify as appropriate and also it is recommended to describe what is not addressed.) Also, you can refer the past case studies which the general template was applied. (Case Study 2017-2 (OECD, 2018c); Case Study 2018-2 (OECD, 2019c))
- Aspects can include uncertainty and confidence associated with the data and assumptions used to develop hypothesis.
- 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 *("Consideration of uncertainties associated with the application of the defined approach"* OECD, 2016h; "*Consideration of uncertainties associated with the application of the defined approach"* of CASE STUDY I-XII of OECD, 2016i) might be helpful for considering uncertainties related to non-guideline test methods. The uncertainty approaches outlined in the template for IATA case studies on Read-Across would be helpful for performing the uncertainty analysis.

- If AOP is used, please discuss uncertainty on AOP (e.g. endorsed AOP: the AOP approved and published by OECD vs putative AOP; the AOP not approved by OECD and established based on the known knowledge.).
- For the application of WoE approach, the ECHA WoE template [22]provides a structured template for presenting the WoE approach/ uncertainty (EU-ToxRisk, 2018).
- The EFSA guidance documents (EFSA, 2018a; 2018b) could be considered for uncertainty assessment as a good start point. In addition, for quantitative hazard assessments, the WHO Guidance on Evaluating and Expressing Uncertainty in Hazard Assessment (WHO, 2018) can provide further support (EU-ToxRisk 2018).
- In application of WoE, please refer to the OECD WoE guidance document (OECD, 2019d), which provides universal Guiding Principles that should be considered when developing or augmenting systematic approaches to WoE for chemical evaluation and Key Elements to formulating a systematic approach to WoE

Factor	Uncertainty (low, medium, high)	Impact of uncertainty on hypothesis	Comment
Hypothesis			
Used Approach (e.g. AOP/MOA, Defined Approach, workflow, read-across etc.)			
Methods/assays used in the IATA			
Data/information gathered in the IATA			
Quality of the data/information used in the IATA			
Concordance and weight of evidence of all data used for justifying the hypothesis			

Tip
- When using ranks to indicate uncertainties (e.g. low, medium, high), definitions should be provided.

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.

7. References

[22] ECHA – Template for Weight of Evidence / Uncertainty in Hazard Assessment https://echa.europa.eu/documents/10162//17169198/template_for_weight_of_evidence_en.docx

(*See OECD style guide third edition, p.56 "Bibliographical referencing: Sources and citations"*)

Annex

• Author can include supplemental or background data in an Annex in order to increase readability of case study if the data supports a particular aspect of the case study. The below table is an example of a summary table for *in vivo* data (Reference to Annex I and II in Case Study 2018-1 (OECD, 2019b); Annex IV in Case Study 2019-4 (OECD, 2020d)).

References	
Species/strain	
Sex	
Route of admin.	
Exposure period	
Doses	
GLP	
Test substance	
NOAEL	
Result	
Other findings	

• Author can provide a summary of methods and tools used in the case study, that a regulator may be less familiar with, such as an *in vitro* method, *in silico* ((Q)SAR) model or high throughput assay; or provide links to references of these methods for further information in order to increase readability of case study. The description should be sufficient for an expert, which a regulator may consult to get approval and better understanding of the methodology.

Appendix 5. List of Case Studies from Previous Cycles used as Example in the Template

Case Study No.	Case Study Title	Referred Information	Relevant template section	Why this example works well
2017-3	Case study on grouping and read-across for nanomaterials genotoxicity of nano-TiO_2	SUMMARY, Page 8	Abstract / Synopsis / Executive summary	This summary is concise and includes the elements described in this template.
2018-1	Case Study on the use of Integrated Approaches for Testing and Assessment for Testicular Toxicity of Ethylene Glycol Methyl Ether (EGME)-Related Chemicals	Executive Summary, Page 7	Abstract / Synopsis / Executive summary	This summary is concise and includes the elements described in this template.
2018-2	Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Estrogen Receptor Active Chemicals	Executive Summary, Page 7	Abstract / Synopsis / Executive summary	This summary is concise and includes the elements described in this template.
2015-1	*In Vitro* Mutagenicity of 3,3' Dimethoxybenzidine (DMOB) Based Direct Dyes	1.1. Purpose of use, Page 10	2. Purpose; Purpose of use	The section provides a clear and concise overview of the purpose of use including the regulatory purpose. This helps the readers understand how much extent of the uncertainty is acceptable in the case study, which could be linked to the uncertainty assessment.
2015-2	Repeat Dose Toxicity of Substituted Diphenylamines (SDPA)	1.1. Purpose of use, Page 9	2. Purpose; Purpose of use	The section provides a clear and concise overview of the purpose of use including the regulatory purpose. This helps the readers understand how much extent of the uncertainty is

				acceptable in the case study, which could be linked to the uncertainty assessment.
2017-3	Case study on grouping and read-across for nanomaterials genotoxicity of nano-TiO_2	1.2. Target chemicals, Page 12	2. Purpose; Tip for nanomaterials	This section explains how to define the parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints.
2019-4	Case Study on the Use of Integrated Approaches for Testing and Assessment for Repeated-Dose Toxicity of p-Alkylphenols	Read-across workflow in this case study, Fig.1	3. Hypothesis for the analogue approach/category; Figure for a Workflow	The figure provide a clear and concise workflow in this case study, which helps to guide the reader through.
2019-5	Prediction of a 90 day repeated dose toxicity study (OECD 408) for 2-Ethylbutyric acid using a read-across approach to other branched carboxylic acids	Overview of the six traditional assessment steps within the read-across assessment, Fig.2	3. Hypothesis for the analogue approach/category; Figure for a Workflow	The figure provide a clear and concise workflow in this case study, which helps to guide the reader through.
2018-2	Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Estrogen Receptor Active Chemicals	Representation of the ER pathway and computational model, Fig.1, Page 16	3. Hypothesis for the analogue approach/category; AOP	The figure provides a clear and concise overview of the putative AOP along with testing for MIE/KE/AO applied in the case study, which helps to guide the reader through.
2019-4	Case Study on the Use of Integrated Approaches for Testing and Assessment for Repeated-Dose Toxicity of p-Alkylphenols	Overview of hepatotoxic mechanism of p-alkylphenols, Fig.3	3. Hypothesis for the analogue approach/category; AOP	The figure provides a clear and concise overview of the putative AOP along with testing for MIE/KE/AO applied in the case study, which helps to guide the reader through.

2019-5	Prediction of a 90 day repeated dose toxicity study (OECD 408) for 2-Ethylbutyric acid using a read-across approach to other branched carboxylic acids	Overview on test systems used for hazard characterization, Fig.7	3. Hypothesis for the analogue approach/category; AOP	The figure provides a clear and concise overview of the putative AOP along with testing for MIE/KE/AO applied in the case study, which helps to guide the reader through.
2019-7	Identification and characterization of parkinsonian hazard liability of deguelin by an AOP-based testing and read across approach	AOP on inhibition of the mitochondrial complex I of nigrostriatal neurons leading to parkinsonian motor deficits, Fig.2	3. Hypothesis for the analogue approach/category; AOP	The figure provides a clear and concise overview of the endorsed AOP along with testing for MIE/KE/AO applied in the case study, which helps to guide the reader through.
2019-8	Waiving of repeat-dose neurotoxicity study (TG 424) for azoxystrobin based on Read-Across to other strobilurins	AOP on the inhibition of mitochondrial complex III leading to neurotoxic effects, Fig.5.1	3. Hypothesis for the analogue approach/category; AOP	The figure provides a clear and concise overview of the putative AOP along with testing for MIE/KE/AO applied in the case study, which helps to guide the reader through.
2015-3	Hepatotoxicity of Allyl Ester Category	2.2. Elements for a read-across hypothesis, Page 10	3. Hypothesis for the analogue approach/category; Tip	The section provides an example how to describe the consideration for the effects induced by other metabolites in the IATA, whose hypothesis is that metabolite induces target effect.
2017-3	Case study on grouping and read-across for nanomaterials genotoxicity of nano-TiO_2	2.2 Characterisation of the analogue nanoforms, Page 14-16	3. Hypothesis for the analogue approach/category; Tip for nanomaterials	The section provides an explanation which parameters are critical for the hypothesis and argues the hypothesis with the physicochemical

				and chemical properties.
2018-1	Case Study on the use of Integrated Approaches for Testing and Assessment for Testicular Toxicity of Ethylene Glycol Methyl Ether (EGME)-Related Chemicals	4.2. Justification; A data matrix, Table 3, Page 19	5. Justification of data gap filling	This data matrix provides an overview of the essential data for the read-across in the IATA.
2018-2	Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Estrogen Receptor Active Chemicals	4. Data/Information Gathering, Page 17-24	5. Justification of data gap filling; Defined Approach	This section describes an integrated battery of *in vitro* assays and a computational model with figures and tables, which provides an overview of data/information gathering procedure.
2016-5	Chemical Safety Assessment Workflow Based on Exposure Considerations and Non-animal Methods	CHEMICAL SAFETY ASSESSMENT WORKFLOW PROPOSED, Page 11-24	5. Justification of data gap filling; Summary text box	The summary textboxes provides a conclusion under each section, which makes readers understand what conclusion is observed.
2015-4	Bioaccumulation Potential of Biodegradation Products of 4,4'-Bis (chloromethyl)-1,1'-biphenyl	Data items and QSAR software versions, Table 4, Page 15	5. Justification of data gap filling; Tip	The table provide a summary of the estimated values by QSAR models, including the reliability of prediction of each models.
2017-3	Case study on grouping and read-across for nanomaterials genotoxicity of nano-TiO_2	5. JUSTIFICATION OF DATA GAP FILLING, Page 27-34	5. Justification of data gap filling; Tip for nanomaterials	The section provide an overview of justification of data gap filling for nanomaterials, by describing the methodologies for similarity assessment within category members and Identifying which parameters are relevant to the target endpoint.

2017-3	Case study on grouping and read-across for nanomaterials genotoxicity of nano-TiO_2	7.1. Evaluation of the read-across according to the Read-Across Assessment Framework, Table 8, Page 36-42	6. Strategy for and integrated conclusion of data gap filling; Uncertainty; Tip for nanomaterials	This uncertainty table provides a clear overview of the uncertainty elements including the nano-specific uncertainty.
2018-1	Case Study on the use of Integrated Approaches for Testing and Assessment for Testicular Toxicity of Ethylene Glycol Methyl Ether (EGME)-Related Chemicals	Annex I and Annex II. Page 35-59,	Annex: A summary table for *in vivo* data	The summary table provides a robust summary for *in vivo* assay.
2019-4	Case Study on the Use of Integrated Approaches for Testing and Assessment for Repeated-Dose Toxicity of p-Alkylphenols	Annex IV	Annex: A summary table for *in vivo* data	The summary table provides a robust summary for *in vivo* assay.
2015-1	*In Vitro* Mutagenicity of 3,3' Dimethoxybenzidine (DMOB) Based Direct Dyes	5.2 Uncertainty, Table 5-1, 5-2, Page 24-26	Appendix 1: Example of Reporting Template of Uncertainty (1)	This uncertainty tables provides a clear overview of the uncertainty analysis to capturing and reporting the uncertainty elements.
2015-2	Repeat Dose Toxicity of Substituted Diphenylamines (SDPA)	5.2 Uncertainty, Table 5-6, 5-7, 5-8, 5-9, 5-10, Page 31-36	Appendix 1: Example of Reporting Template of Uncertainty (1)	This uncertainty tables provides a clear overview of the uncertainty analysis to capturing and reporting the uncertainty elements.
2016-3	90-Day Rat Oral Repeated-Dose Toxicity for Selected n-Alkanols: Read-Across	4. STATEMENT OF UNCERTAINTY, Table 4. and Table. 5, Page 19-22	Appendix 1: Example of Reporting Template of Uncertainty (2)	This uncertainty tables provides a clear overview of the uncertainty analysis to capturing and reporting the uncertainty elements.
2016-4	90-Day Rat Oral Repeated-Dose Toxicity for Selected	4. STATEMENT OF UNCERTAINTY,	Appendix 1: Example of Reporting	This uncertainty tables provides a clear overview of

	2-Alkyl-1-alkanols: Read-Across	Table 4 and Table. 5, Page 23-26	Template of Uncertainty (2)	the uncertainty analysis to capturing and reporting the uncertainty elements.
2017-4	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	5.1. Uncertainty, Table 2, Page 34	Appendix 1: Example of Reporting Template of Uncertainty (2)	This uncertainty tables provides a clear overview of the uncertainty analysis to capturing and reporting the uncertainty elements.