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NO. 369

REPORT ON CONSIDERATIONS FROM CASE STUDIES 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 2022

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Foreword

OECD Member Countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Quantitative) Structure-Activity Relationships ((Q)SAR), chemical categories and *in vitro* testing as a part of Integrated Approaches for Testing and Assessment (IATA). The OECD also developed guidance on Adverse Outcome Pathways (AOPs) concept that supports the development of mechanism-based New Approach Methodologies (NAMs). 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 experiences across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience using IATA by developing case studies, which illustrate examples of chemical hazard predictions that may be considered for regulatory use. The case studies are reviewed on an annual cycle and discussed with experts who provide input on the technical and regulatory aspects. From all case studies reviewed in each cycle, general considerations are summarised in a document to help create a common understanding of using novel methodologies and to generate considerations/guidance.

This document reports the learnings and lessons obtained from the review experience of the eight case studies submitted in the seventh review cycle in 2021 of the IATA Case Studies Project. The topics discussed in this document include the strongest aspects and uncertainties of each case study. The IATA Case Studies Project has also identified a variety of areas for developing further guidance on IATA.

These case studies illustrate examples of using new methods, and their publication as OECD monographs does not indicate acceptance of these methodologies for regulatory purposes across OECD countries. In addition, these case studies should not be interpreted as an official regulatory decision made by the authoring Member Countries.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ADME	Absorption, Distribution, Metabolism and Excretion
AO	Adverse Outcome
AOP	Adverse Outcome Pathway
AUC24	24 hour area under the concentration-time curve
BFR	Brominated Flame Retardants
BIAC	Business at OECD
BMC	Benchmark concentration
BMD	Benchmark Dose
BPA	Bisphenol A
CE	Cosmetic Europe
CFD	Computational Fluid Dynamics
Cmax	Maximum concentration
CoCAP	Cooperative Chemicals Assessment Programme
DA	Defined Approach
DNT	Developmental Neurotoxicity
EC3	Effective concentration for a stimulation index [SI] of 3
EFSA	European Food Safety Authority
EKE	Expert Knowledge Elicitation
ER	Estrogen Receptor
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
HEC	Human Equivalent Concentration
HTS	High Throughput Screening
HTTK	High Throughput Toxicokinetic
HTTr	High-Throughput Transcriptomics
HOS	Human Observational Study
IATA	Integrated Approaches for Testing and Assessment
ICAPO	International Council for Animal Protection in OECD Programmes
ITS	Integrated Testing Strategy
IVB	*In Vitro* Battery
IVIVE	*In Vitro-In Vivo* Extrapolation
KE	Key Event
KER	Key Events Relationship
LDH	Lactate Dehydrogenase
LOC	Level of Concern
MIE	Molecular Initiating Event
MoA	Mode of Action
MoE	Margin of Exposure
MoIE	Margin of Internal Exposure
MucilAir™	3D *in vitro* human model of respiratory epithelium

nAChR	nicotinic Acetylcholine Receptor
NAM	New Approach Methodology
NGRA	Next Generation Risk Assessment
NIH	National Institutes of Health
NM	Nanomaterial
OECD	Organisation for Economic Co-operation and Development
PBK	Physiologically Based Kinetic. PBK is synonymous of physiology-based pharmacokinetic (PBPK), physiologically-biokinetic (PBBK) and physiologically-based toxicokinetic (PBTK).
POD	Point of Departure
OPFR	Organophoshorous Flame Retardant
(Q)SAR	(Quantitative) Structure-Activity Relationship
SARA	Skin Allergy Risk Assessment
SCCS	Scientific Committee on Consumer Safety
TCEP	Tris (2-chloroethyl) phosphate
TEER	Trans-epithelial Electrical Resistance
TTC	Threshold of Toxicological Concern
UN	United Nations
US EPA	United States Environment Protection Agency
UVCB	Unknown or Variable composition, Complex reaction products, or Biological material
WHO	World Health Organization
WoE	Weight of Evidence
WPHA	Working Party on Hazard Assessment

1. INTRODUCTION

OECD Member Countries are expanding the use of alternative methods for assessing chemicals. The OECD has developed guidance on using various alternative methods such as (Quantitative) Structure-Activity Relationships ((Q)SAR), chemical categories and *in vitro* testing as stand-alone approaches and as a part of Integrated Approaches for Testing and Assessment (IATA). The OECD also developed guidance on Adverse Outcome Pathways (AOPs) concept that supports the development of mechanism-based New Approach Methodologies (NAMs). There is a need to investigate the practical applicability of these approaches for different aspects of regulatory decision-making using case studies and assessment experience across OECD Member Countries.

The Cooperative Chemicals Assessment Programme (CoCAP)[1] was revised in 2014 to share experiences developing and applying IATA. The IATA Case Studies Project[2] was launched in 2015 as a forum for scientific exchange on application of novel approaches for evaluating chemical hazards. 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 outcome of these shared experiences helps to create common understanding of using NAMs and identify considerations/guidance stemming from these case studies.

Case studies are submitted and reviewed annually by experts from Member Countries and other stakeholders. The results of the reviews are discussed in a project meeting. The discussion includes the strongest aspects, uncertainty, areas for further developing guidance, and possible use of each case study in a regulatory context. Following each review cycle, approved case studies are published, along with a considerations document capturing the learnings and lessons stemming from all case studies in the annual cycle. The outcomes of the past six review cycles of the project (2015-2020) included 24 case studies and six considerations documents, which have all been published on the OECD website (https://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm). These case studies are illustrative examples, and their publication as OECD monographs in the OECD series on testing and assessment does not translate into acceptance of the methodologies for regulatory purposes across OECD Member Countries. In addition, these case studies should not be interpreted as official regulatory decisions made by the authoring Member Countries.

In the seventh review cycle (2021), eight case studies were reviewed (Annex Table 7). This document briefly summarises each case study, and the learnings and lessons stemming from all case studies reviewed in the seventh review cycle and all IATA Case Study review cycle.

[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

2. LEARNINGS AND LESSONS

2.1. Learnings from 7th Review Cycle

This section describes the learnings gained through the review of the eight case studies submitted in the 2021 seventh review cycle. Considerations identified from the review of these case studies should be taken into account for the development of future IATA. Three topics were selected at the 7th OECD IATA Case Study Project meeting based on the reviewer's comments. In this section, the four topics were identified for discussion. The first three of these may be generally relevant to IATAs and the fourth includes comments on seven of the eight IATAs for evaluating developmental neurotoxicity (DNT).

1. Approaches to quantify the uncertainty using the Bayesian network analysis approach.
2. Alternative approaches using NAM-based POD
3. Uncertainties in extrapolation between species.
4. IATAs for evaluating Developmental Neurotoxicity (DNT) using *in vitro* batteries

2.1.1. Approaches to quantify the uncertainty

Quantifying uncertainty using the Bayesian network analysis

The statistical models can be used to quantify uncertainties through derived confidence intervals and probability distributions. Other types of statistical methods (e.g. Bayesian methods) can be used for synthesising multiple sources of evidence.

The quantitative approaches can be used to characterise the uncertainty in KERs in an AOP and overall pathway approaches. Case Study 2021-8 utilised a DA (SARA model) based on a Bayesian multilevel modelling approach. The approach allows users to take into account the variability in the underlying observed data and the uncertainty in the strength of relationship that exists between the data sources. A Bayesian network approach allows quantification of uncertainty for each prediction based on the data and, thus, quantify confidence in the decisions. It also allows users to quantify the value of additional information before conducting more tests.

Case Study 2021-1 utilised a Bayesian network analysis approach to quantify the strength of the KER. A node that represents a random variable in a Bayesian network model was used to estimate the joint probability that all events (MIEs, KEs, AO) in the network will occur. High marginal probability (> 0.50) of an AOP string in the AOP network indicates that it is likely that the particular events (MIE, KE, adverse outcome (AO)) would be triggered by the target chemical at a minimum concentration/dose.

Quantitative expert judgement

In Case study 2021-1, Expert Knowledge Elicitation (EKE) was used to estimate the range of the lowest concentrations/doses of the target chemical judged to activate the MIEs, KEs and AO (EFSA, 2014). EKE methods have been developed for eliciting knowledge from experts in an as unbiased manner as possible. Only MIEs, KEs and AOs with an estimated probability of at least 66% were considered for the AOP

network postulation. The result of the EKE performed on the available data for the postulation of the AOP network was in line with the Bayesian network analysis result.

Machine learning techniques

In Case Study 2021-1, a Bayesian network approach was applied to quantify the uncertainties in the KERs and for the quantification of the overall WoE. 2021-8 also applied machine learning approaches to quantify uncertainty. The structure of the BN ITS in 2021-8 model represents abstracted AOP and is developed based on mechanistic knowledge with the aim to follow sequence of the mechanistic events in the existing AOP. Case Study 2021-1 and 2021-2 used a convolutional neuronal network (CNN) running on Keras implemented in Python 3 to quantify neuronal differentiation.

Table 1. Quantitative approaches to integrate evidence and/or to integrate different lines of evidence in order to reach a general conclusion

Approach	Model example	Case study example	Reference
Quantifying uncertainty in KERs and the overall pathway using the Bayesian network analysis	-Bayesian network analysis approach	Case Study 2021-1, 2021-8 (SARA model), 2019-5, 2019-6	Borenstein et al. (2009) ; Sutton and Abrams (2001) ; Higgins et al. (2015)
Quantitative expert judgement including multi-criteria decision analysis for integrating different types of studies		Case Study 2021-1	Linkov et al., 2011, 2015
Machine learning techniques	-Random Forest Models -Artificial neural network	Case Study 2021-1, 2021-2, 2021-8 (BN-ITS)	Li and Ngom, 2015

2.1.2. Alternative approaches using NAM-based POD

NAM-based PODs were calculated using *in vitro* data, *in silico* data and read across based on the properties of target chemicals. In some case studies, NAM-based PODs were compared to estimates for human exposure for prioritisation or risk assessment. This method enables the conduct of prioritisation or risk assessment of a chemical that has no *in vivo* data.

In past seven review cycles, IATA case studies that derived PODs using NAMs (NAM-based POD) are as follows:

- Case Study 2021-3 and 2021-6 screened and prioritised groups of data-poor substances using NAM-based PODs. Case Study 2021-3 prioritised a class of OPFRs, whose toxicity profile is unknown, using a battery of *in vitro* and complementary non-mammalian animal models (e.g. zebrafish) and ranked the toxicity of these compounds for further *in vivo* testing. PODs/ benchmark concentration measurements were compared across multiple assays. Then, HTTK models were used to extrapolate from chemical concentrations that were active in *in vitro* assays to estimated chemical concentrations in rodent models and human exposures. Case Study 2021-6 selected 25 BPA alternatives as target chemicals. Active test chemical concentrations were identified using *in vitro* transcriptomics and ToxCast ER model results. PODs were derived using HTTK models to extrapolate to *in vivo* chemical concentrations. The NAM-based PODs were compared to in vivo PODs when available and ranked to see ER potency from a hazard perspective.

- Case Study 2020-1, 2021-8 and 2016-5 calculated PODs using only *in vitro* and *in silico* data based on NGRA framework or the Safety Evaluation Ultimately Replacing Animal Testing (SEURAT-1) conceptual framework in order to conduct risk assessment of cosmetic products. In Case Study 2020-1, PODs were calculated from no-observed-transcriptional effect level (NOTEL) and maximum concentration (C_{max}) or the 24 hour area under the concentration-time curve (AUC24). PODs of Case Study 2021-8 were obtained from five DAs using the most conservative EC3 value (the estimated concentration of test chemical required to induce a threefold stimulation of draining lymph node cell proliferation compared with concurrent controls) (Table 10 of (OECD 2021h)), and compared to the consumer exposure for risk assessment of skin sensitisation.
- Case Study 2021-7 and 2016-2 calculated POD using *in vitro* assays. Case Study 2021-7 utilised an *in vitro* 3D human respiratory model (MucilAir™) that eliminates the need for a species extrapolation factor for exposure risk assessment. In Case Study 2016-2, for cumulative risk assessment, brain acetylcholinesterase AChE activity data were used as PODs since brain AChE has tighter confidence intervals and confers less uncertainty on cumulative risk estimates compared to relative potency estimates based on red blood cells.
- Case Study 2019-1, 2019-2, 2019-3 and 2019-5 calculated PODs of target chemicals by read across using *in vivo* data of analogue chemicals, and *in vitro* and *in silico* data. In Case Study 2019-1 and 2019-2, the PODs were compared with MoIE derived using PBPK for risk assessment.

2.1.3. Uncertainties in extrapolation between species

Some NAM approaches may actually help to reduce uncertainties in estimating effects in humans. For example, in Case Study 2021-7, CFD calculations aligned with human cell toxicity data (MucilAir™) were used to estimate inhalation exposure to Chlorothalonil. The MucilAir™ model uses human respiratory epithelial cells and CFD simulates where inhaled particles containing Chlorothalonil are expected to land in the human respiratory tract after inhalation Chlorothalonil containing particles, and thus, avoids the uncertainty associated with extrapolating from effects observed in lab animals to humans. This is a considerable strength of this approach given the cells and complexity of the rat nasal turbinate differs substantially from the human. This case study demonstrates a NAM approach that replaced an *in vivo* repeated dose respiratory toxicology study and may more accurately predict effects in humans.

On the other hand, there is more uncertainty associated with extrapolation between effects observed in the case studies in the seventh review cycle that used models zebrafish models. In Case Study 2021-2, 2021-4, and 2021-5 a zebrafish model was used in combination with *in vitro* assays, to evaluate possible DNT effects of chemical metabolites. Lack of metabolic competence of *in vitro* assays is a frequent limitation of NAM approach, and this may be overcome by using the zebrafish model, although characterisation of zebrafish metabolism is needed to determine how effectively the model can approximate human metabolism. More experimental work is needed to address molecular and cellular effects in zebrafish. The authors also mentioned that additional work is needed to capture DNT effects mediated by hormonal changes and metabolites, and species-to-species kinetics extrapolation models will reduce these uncertainties (Table 2).

Table 2. Uncertainties of Case Studies in extrapolation between species

Case study	Test system	Uncertainties in interspecific extrapolation	Additional works to reduce uncertainty in the future

2021-1, 2	Zebrafish model *(in vivo*)	No interpretation of metabolism between human and zebrafish	Applying species-to-species kinetics extrapolation models
2021-4, 5	Zebrafish embryo assay (*in vivo*)	No mechanistic interpretation between human cell data and the zebrafish data	More experimental work to address molecular and cellular effects in zebrafish.
2021-5	*In vitro metabolism studies*	In human hepatocytes, formation of some metabolites of imidacloprid were observed but because metabolism of parent imidacloprid was slow, the disappearance of imidacloprid was not accurately quantifies and an *in vitro* intrinsic clearance was not calculated.	Studies to characterize the metabolism of imidacloprid to determine the rate and extent of the metabolism of imidacloprid to the desnitro metabolite
2021-7	Human cell model (*in vitro*) CFD simulates	More accurately predict effects in humans than *in vivo* data	-

2.1.1. IATAs for evaluating developmental neurotoxicity (DNT) using in vitro batteries

As detailed in Annex Table 8, five of eight case studies in the 2021 seventh review cycle focused on developmental neurotoxicity. The various case studies used an *in vitro* battery of assays to evaluate the effects of chemicals on key neurodevelopmental processes. While some of the IATA approaches varied, several of the case studies applied the same approach for different chemicals. From the examination of these case studies, a few high-level points for consideration for *in vitro* batteries to evaluate developmental neurotoxicity, and general considerations regarding IATAs based on *in vitro* batteries, are summarised below (Table 3).

Table 3. Summary of considerations regarding *in vitro* batteries used to evaluate developmental neurotoxicity.

Considerations specific to DNT-IVB	Considerations relevant to *in vitro* batteries (IVBs)
Grouping of assays in the DNT-IVB according to key neurodevelopmental process (e.g. neurite outgrowth, migration) does not imply that confirmation of a finding is necessary across all these assays. The assays grouped by key neurodevelopmental process represent a different window of development and/or measure different brain cellular population. Advancing the testing of more chemical classes through the DNT-IVB might allow to clarify any redundancy among the assays within a group and the battery as a whole.	In lack of a comprehensive AOP network, building a battery with relevant assays to cover great extent of the biological space is recommended. This is advisable particularly for complex endpoints and in case an animal model does not exist/perform poorly or in vivo data is scarce.
Having hits across all or a specific number of assays within the DNT IVB is not necessary. Even a hit in one of the assays of the DNT IVB could mean that a certain chemical has the potential to disrupt the nervous development and that further investigation is needed. Experience showed that chemicals can impact different stages of developing brain.	Having hits across all or a specific number of within a battery may or may not be necessary. The underlying biology and scientific knowledge should dictate the necessity of how many hits are necessary across the assays of a battery.
DNT-IVB data was successfully used to fill data gaps in an AOP-informed IATA.	Filling data gaps in an AOP-informed IATA is likely more achievable by using data from a science based IVB rather than assembling data from random individual assays.
Data from the DNT-IVB helped in the interpretation of human data by providing a mechanistic linkage to adverse outcome(s)	A well designed IVB can improve mechanistic understanding and contextualization of existing human data
DNT-IVB has unknown metabolic capacity and this uncertainty should be taken into consideration when data derived from DNT-IVB is used beyond screening and prioritization purposes	The metabolic capacity of an IVB is accepted that is limited and in most cases unknown. Integration of alternative species such as zebrafish can be an option.

	Another option would be the development of an agreed strategy to deal with the issue e.g. testing metabolites

2.2. Lessons learned from all IATA Case Studies reviewed

This section describes the learnings gained through the review of all IATA Case Studies submitted to date. An overview of general considerations and a high-level summary of strengths and limitations of all case studies are includes by topic, with the objective of identifying additional guidance and information that may be needed to use IATA approaches to address specific regulatory scenarios and improve the fitness for purpose of NAMs used for regulatory decision making.

2.2.1. Providing a context for uncertainties (e.g. uncertainties are not described for most traditional, animal toxicity tests and may contribute to an erroneous impression on the (limited) utility of methods).

2.2.2. How to build confidence in NAMs and application in a specific regulatory context (e.g. well characterised text systems, with appropriate controls and expected results; running test according to standards; how to apply principles to batteries of assays)

2.2.3. How to interpret negative results from NAMs/NAM batteries

2.2.4. How to take lessons learned from DNT in vitro battery and make the information more generically applicable

2.2.5. How to transition from "predictive" to "protective"

2.3. Topics identified for further guidance development

Over the seven review cycles, the considerations documents have identified priorities for further guidance. There is not an intention to address all these topics in OECD Guidance Documents, but rather, to note that a potential need was identified. In addition, activities have been undertaken to address some of these topics (e.g. Guidance Document on Characterisation, Validation and Reporting of PBK models for Regulatory Purposes (OECD, 2021b)).

A summary of areas for developing further guidance and related case studies is shown in Table 4.

Table 4. 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, 2021-1, 2021-2, 2021-3, 2021-4, 2021-5, 2021-6, 2021-7, 2021-8
a. Hypothesis for category formation that includes the use of omics data	2016-1, 2021-3, 2021-4, 2021-5, 2021-6
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. 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, 2021-2, 2021-7, 2021-8
b. What is needed to address biological read-across	2019-1, 2019-2, 2019-5, 2019-6, 2019-7, 2019-8
c. Understanding the adequacy of the level of biological coverage when combinations of non-animal methods are used	2016-5, 2019-1, 2020-1, 2021-4, 2021-5, 2021-6, 2021-8
4.1 NAMs	
a. 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
b. 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, 2021-4, 2021-5, 2021-6
c. Guidance on how to combine *in vitro* and computational information into an integrated report, including applicability domain	2018-2, 2021-2, 2021-8
d. 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, 2021-1, 2021-2, 2021-3, 2021-4, 2021-5, 2021-6, 2021-7, 2021-8
c. Guidance on how to develop ITS and data interpretation procedures (DIP)	2018-2
f. Coverage of key events (KEs) in AOP based testing strategy	2018-2, 2019-5, 2019-7, 2019-8, 2021-1, 2021-2, 2021-3, 2021-4, 2021-5, 2021-8
g. Rationale for the choice of an acceptable *in vitro*-based MoIE	2019-1, 2019-2, 2020-1
h. 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, 2021-1, 2021-2, 2021-3, 2021-4, 2021-5, 2021-6, 2021-7, 2021-8
i. The application, interpretation and limitations of the Bayesian Network analysis in the quantitative assessment of the WoE	2019-5, 2019-6, 2021-1, 2021-2. 2021-8
j. The extrapolation from the derived from *in vitro* POD via such as IVIVE and HTTK modelling.	2016-5, 2020-1, 2021-3, 2021-6, 2021-7
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, 2021-1, 2021-2, 2021-3, 2021-4, 2021-5, 2021-6, 2021-7, 2021-8
a. Exposure route, including route to route extrapolation	2015-4, 2016-5, 2017-1, 2017-4, 2019-1, 2019-2, 2020-1. 2021-8
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 2021-1. 2021-2, 2021-3, 2021-4, 2021-5 ,2021-6, 2021-7 2021-8

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, 2021-1, 2021-3, 2021-4, 2021-5, 2021-6, 2021-7,
e. 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
f. 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, 2021-1, 2021-2, 2021-3, 2021-4, 2021-5 ,2021-6, 2021-7, 2021-8
g. 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, 2021-1, 2021-2, 2021-4, 2021-5
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, 2021-6
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, 2021-1, 2021-2, 2021-3, 2021-4, 2021-5, 2021-6, 2021-7, 2021-8
7. Reporting	
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, 2021-6
b. 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, 2021-1, 2021-2, 2021-3, 2021-4, 2021-5, 2021-6, 2021-7, 2021-8
c. 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, 2021-3, 2021-4, 2021-5, 2021-6
d. 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
e. How to describe the rationale for justification of the benchmark dose (BMD) and PoD used	2016-2, 2021-1, 2021-2, 2021-3, 2021-6, 2021-7, 2021-8
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, 2021-3, 2021-4, 2021-5, 2021-6
g. Guidance on reporting of docking/modelling approaches	2019-7, 2019-8, 2020-1, 2021-4, 2021-5
h. 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, 2021-3, 2021-6
8. Application of Regulatory use	

a. The application of machine learning approaches in a regulatory setting.	2021-1, 2021,2, 2021-3, 2021-6
b. The justification of the use of a specific DA and how regulators select the best DA.	2021-8
9. 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. Guidance on developing prioritisation scheme based on IATA	2017-1, 2017-2, 2021-3, 2021-6
9.1. Areas related to other working parties	
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*

3. CONCLUSION

Eight case studies were reviewed in the seventh review cycle of the project in 2021.

- Case Studies 2021-1, 2021-2, 2021-3, 2021-4 and 2021-5 were developed to be included in the OECD Guidance Document under development by the DNT expert group of the OECD. These case studies demonstrate the applicability of an IVB for DNT testing based on AOP networks using different target chemicals.
- Case Study 2021-6 prioritised BPA Alternatives that have little data using NAMs-based PODs.
- Case Study 2021-7 utilised an *in vitro* 3D human respiratory model (MucilAir™) and CFD for respiratory operator exposure risk assessment.
- Case Study 2021-8 applied five DAs for risk assessment of skin sensitisation, one of which is included in the DASS guideline 497 (OECD 2021d).

Based on the review experience of the eight case studies, new areas for further developing guidance were identified including:

- The application, interpretation and limitations of the Bayesian Network analysis in the quantitative assessment of the WoE.
- The application of Machine learning approach in a regulatory setting.
- The extrapolation from the derived *in vitro* POD via IVIVE and HTTK modelling.
- The justification of the use of a specific DA and how regulators select the most appropriate DA.

Four consideration topics from the seventh review cycle are discussed in this document.

- Approaches to quantify the uncertainty.
- Alternative approaches using NAM-based POD
- Approaches to reduce uncertainties in extrapolation between species.
- IATAs for evaluating Developmental Neurotoxicity (DNT) using *in vitro* batteries.

In summary, the considerations obtained from the eight case studies in seventh review cycle provided a new knowledge on how to prioritise and assess risk of chemicals using NAMs, and how to integrate new *in vitro* test batteries with existing data based on AOPs. These lessons and learnings provide key considerations for the use of the NAMs in the context of the IATAs as well as improvements of read-across approaches.

The case studies reviewed in all review cycles are summarized in Annex 3.

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Annex 1. Questions for authors and reviewers of Case Studies included in the 7th cycle

Eleven countries/stakeholders (Australia, Canada, France, Germany, Italy, Japan, the Netherlands, the Unites States, International Council for Animal Protection in OECD Programmes (ICAPO), Business and Industry Advisory Committee (BIAC) and the OECD secretariat) participated in the seventh review cycle. Authors used templates for the documentation of the case studies (Annex 5 and Annex 6). The template for the case studies on read-across (Annex 5) is based on the reporting format in the OECD Guidance on Grouping of Chemicals (OECD, 2014a) and an example using read-across in a weight of evidence approach (OECD, 2014b). The general template for IATA case studies (Annex 6) was developed to fit case studies with multiple components, such as adverse outcome pathways (AOPs), Mode of Action (MoA), Defined Approaches (DAs), Workflows, and Grouping/Read-Across. The templates are continuously updated based on the case studies reviews.

Questions were developed to guide the review of case studies and to get feedback from case study authors. The questions for authors and reviewers are also updated, based on experiences gained in the review cycle. The questions in the seventh review cycle are indicated below (Annex Table 5 and Annex Table 6).

Annex Table 5. Guided reviewer questions for seventh (2021) IATA Case Study review cycle

Guided Reviewer Questions	
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?

Annex Table 6. Guided author questions for seventh (2021) IATA Case Study review cycle

Guided Author 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 studies were discussed at the seventh meeting of the IATA Case Studies Project (16-18 November 2021), in order to finalise the case studies and summarise the learnings and lessons.

Annex 2. Summary of results of the review of Case Studies included in the 7th cycle

All case studies in the seventh review cycle are summarized in Annex Table 7.

Annex Table 7. Case Studies Reviewed in the Seventh Review Cycle (2021)

No.	Title	Lead	Purpose of Use	References
2021-1	Case study for the integration of *in vitro* data in the developmental neurotoxicity hazard identification and characterisation using Deltamethrin as a prototype chemical	EFSA	Demonstrate the applicability of an *in vitro* battery (IVB) with existing *in vivo*, *in vitro* and human observational study data using Deltamethrin	OECD, 2022a
2021-2	Case study for the integration of *in vitro* data in the developmental neurotoxicity hazard identification and characterisation using Flufenacet	EFSA	Demonstrate the applicability of an IVB with existing *in vivo*, *in vitro* and human observational study data using Flufenacet	OECD, 2022b
2021-3	Case study on the use of integrated approaches to testing and assessment for DNT to prioritize a class of compounds using Organophosporus flame retardants	US	Demonstrate the applicability of an *in vitro* battery (IVB) with *in vitro* and complementary non-mammalian animal models (e.g. zebrafish) for prioritisation of a class of organophoshorous flame retardants (OPFRs).	OECD, 2022c
2021-4	Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity hazard characterisation of Acetamiprid	EU ToxRisk	Demonstrate the applicability of an *in vitro* battery (IVB) with existing *in vivo* and *in vitro* data using the pesticide Acetamiprid	OECD, 2022d
2021-5	Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity hazard characterisation of Imidacloprid and the metabolite Desnitro-imidacloprid	EU ToxRisk	Demonstrate the applicability of an *in vitro* battery (IVB) with existing *in vivo* and *in vitro* data using the pesticide Imidacloprid and the metabolite.	OECD, 2022e
2021-6	Case Study on the use of Integrated Approaches to Testing and Assessment for potential Systemic Toxicity and Estrogen Receptor Activation of a Group of Bisphenols and Select Alternatives	Health Canada	Examine 25 potential Bisphenol A (BPA) alternatives using NAMs.	OECD, 2022f
2021-7	Case Study Using a New Approach Methodology (NAM) for Refining Inhalation Risk Assessment from Point of Contact Toxicity of the Pesticide, Chlorothalonil.	ICAPO / Syngenta	Demonstrate how an *in vitro* 3D human respiratory model, *in silico* computational fluid dynamics, and *in vivo* human operator exposure measurements can be utilised to replace an *in vivo* repeated dose respiratory toxicology study.	OECD, 2022g
2021-8	Case Study on the Use of Integrated Approaches for Testing and Assessment for skin sensitisation: Demonstrating the Next Generation Risk Assessment Framework using Geraniol	Cosmetic Europe	Demonstrate the applicability of the Next Generation Risk Assessment (NGRA) using of Geraniol at 0.1% via a face cream.	OECD, 2022h

Case Studies 2021-1, 2021-2, 2021-3, 2021-4 and 2021-5 are also referenced in the OECD Guidance Document under development by the DNT IVB expert group, the draft document includes guidance on the evaluation of data from the DNT-IVB.

3.1. Case Study 2021-1: Case study for the integration of *in vitro* data in the developmental neurotoxicity hazard identification and characterisation using Deltamethrin as a prototype chemical from EFSA

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

- Comprehensive overview of available *in vitro* models to assess (developmental) neurotoxicity
- Application of a risk of bias assessment tool for weighing evidence from available studies
- Documentation of the AOP and linkages to other developed AOPs
- Statistical approach to quantify the strength of the KER using a Bayesian network analysis approach

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

- Lack of biological plausibility in KERs.
- Lack of confidence in empirical evidence that was based on human observational study data for the KERs of the higher biological organisation in the AOP, due to inadequate assessment of confounding selection bias and suboptimal measurements of exposure with a nonspecific biomarker.
- The subjectivity of the expert judgements. A different group of experts would end up with different conclusions, in terms of uncertainty assessment and numerical estimates for the various probability measures.

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

- ***Australia:*** While the overall approach described in this study would be helpful for DNT hazard characterisation, the use of complex human cell-based DNT *in vitro* tests is relatively new and the AOP requires further development to reduce significant uncertainties in the KERs to support scientific validation for regulatory assessment purposes. The postulated mechanism is biologically plausible however certain limitations such as the *in vivo* study conducted according to the OECD TG did not show a positive effect. Also, the HOS have several limitations and these have been described in the case study. The limitations of the studies limit their usefulness in a regulatory context.
- ***Canada (Health Canada):*** Yes, we could use aspects of the case study in our regulatory context. *In vitro* data may be considered within the assessment WoE in our regulatory program. While AOP development is not a routine aspect of the assessment process, the approaches and strategies described in the IATA (e.g. for the literature review, screening for reliability/relevance, and the critical appraisal of evidence retrieved in the scientific literature) may be useful to consider in our regulatory context.
- ***France:*** Some of the elements of the approach are easily usable, others, notably in the analysis of uncertainties, are quite advanced and require specific means and skills that will be difficult to implement.

- ***Germany:*** Yes, although the specific requirements for statistics and database modelling are time and expertise limiting.
- ***Italy:*** Overall, the case study shows the applicability for hazard identification and characterisation.
- ***Netherlands: :*** No for the following reasons:

  • In case younger subjects are more vulnerable for exposure to chemicals, which concentration will prevails in regulations defining exposure limits? By definition the lowest? Or should special categories for developmental (neuro) toxicants be created for different age groups and pregnant women? This issue should be mentioned and included for discussion.

  • From the cellular response to organ response to organism response, and finally to the human setting, many extrapolations are required. Can (suggestions for) margins of safety special for developmental neurotoxic compounds be included in the AOP?

  • Microelectrode Array (MEA) recordings using human cells may result in less assessment factors/margins of safety to calculate a safe level and probably makes it more suitable to meet regulatory requirements. Should the use of human cells for MEA recordings be prioritised?

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

- The application, interpretation and limitations of the Bayesian Network analysis in the quantitative assessment of the WoE.

Overviews of the case study are as follows.

This case study aims to demonstrate the applicability of integrating a new *in vitro* test battery with existing data such as *in vivo, in vitro* and human observational study (HOS) data to develop a postulated AOP for DNT hazard characterisation using Deltamethrin, which can cause neurotoxicity effects after acute and repeated oral administration. Understanding and integrating mechanistic information determined by relevant *in vitro* assays helps to reduce the uncertainty in the DNT hazard identification/characterisation of Deltamethrin.

In conclusion, the uncertainty of the approach adopted in this IATA case study, such as evidence-based postulation of an AOP network informing IATA through a probabilistic quantification of the WoE, was considered acceptable, however many reviewers responded this approach alone was not suitable for regulatory decision making. .

Please refer to ENV/CBC/HA(2022)3 Series on Testing & Assessment No. XXX.

3.2. Case Study 2021-2: Case study for the integration of *in vitro* data in the developmental neurotoxicity hazard identification and characterisation using Flufenacet from EFSA

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

- Demonstration of the IATA with limited data available.
- The detailed description of the systematic literature review and the uncertainty analysis performed on the methods used in the case study.

- Supports the alternative model zebrafish in the DNT hazard identification and characterisation process.

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

- The observed neurotoxic effects in dog may be caused by metabolites rather than the parent compound. Moreover, it has been shown that mixtures of parent compounds and metabolites may result in additive activation, potentiation or inhibition of neurotransmitter receptors (Hendriks et al., 2010).
- A limitation of the case study is that very few relevant studies were identified in the literature search.

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

- ***Australia:*** Systematic literature reviews are an established methodology, and while they are not routinely used in our regulatory system, could play a role in our assessment of chemicals. In contrast the DNT-IVB requires further validation and would not be used as the basis for regulatory decision making. In its current state, it could play a role as supporting information as part of a WoE approach.
- ***Canada:*** Yes, we could use aspects of the case study in our regulatory context. *In vitro* data may be considered within the assessment WoE in our regulatory program. While AOP development is not a routine aspect of the assessment process, the approaches and strategies described in the IATA (e.g. for the literature review, screening for reliability/relevance, and the critical appraisal of evidence retrieved in the scientific literature) may be useful to consider in our regulatory context.
- ***France:*** Yes, in its principle, this method could be useful, but in practice there is little interest in demonstrating the absence of a given effect outside of a specific method validation exercise as is the case here.
- ***Germany:*** Yes.
- ***Italy:*** The same as Case Study 2021-1
- ***Netherlands:*** No. See our comments on this question for Case Study 2021-1

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

- How to evaluate a target chemical with limited data available.

Overviews of the case study are as follows.

This case study was developed using Flufenacet, which is not expected to have a DNT concern, following the same approach as Case Study 2021-1. The aims of this case study are also the same as Case study 2021-1 to demonstrate the applicability of integrating results of a new *in vitro* test battery with existing data to develop a postulated AOP for DNT hazard characterisation using Flufenacet.

This case study used zebrafish as an alternative model to assess hormonal and metabolite-mediated DNT effects. Negative results in Flufenacet-treated zebrafish reduced the uncertainty that DNT endpoints could be affected through a secondary mechanism caused by disturbance of hormone homeostasis or a neurotoxic metabolite. Further work to understand hormone and metabolite-mediated DNT effects and differences between zebrafish and human metabolism is needed. This case study helps to show the toxicological applicability domain of the DNT-IVB and supports the use of alternative model, zebrafish in the DNT hazard identification and characterisation process.

Please refer to ENV/CBC/HA(2022)4 Series on Testing & Assessment No. XXX.

3.3. Case Study 2021-3: Case study on the use of integrated approaches to testing and assessment for DNT to prioritize a class of compounds using Organophosporus flame retardants from the United States

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

- The comparison of the OPFRs to two brominated flame retardants and halogenated OPFRs that were data rich.
- The use of HTTK models for comparisons across the various exposure scenarios.
- A thorough uncertainty analysis of the approaches used, and suggestions for further refinement.
- The number of assays being used in the case study and the consideration of all relevant information and use of comprehensive NAM coverage to assess the potential for DNT.

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

- As identified by the authors, 1) lack of data across assays, 2) assay confidence, 3) number of hits within the battery, 4) lack of directionality, 5) metabolism, 6) underlying principles with *in vitro in vivo* correlation (IVIVC) and 7) weighting of assays, contributed to uncertainty.
- High-throughput toxicokinetic (HTTK) model, which was used to convert the data.

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

- ***Australia:*** The approach described in this study would be helpful for the prioritisation of chemicals with potential DNT characteristics for further study. However, it may be premature to use the IATA case study for purposes beyond prioritisation at present due to uncertainty around the accuracy of the predictions.
- ***Canada:*** Yes, we could use aspects of this case study in our regulatory context. For example, we use class-based assessments in some contexts. We also use data from multiple sources, including *in vitro* assays, to prioritise chemical substances. It could be used in our weight of evidence approach.
- ***Germany:*** Currently: Yes, however, the study only provides the rather general conclusion that the OPFRs as a class are active. Further differentiation and discussion of the available *in vivo* data might be helpful.
- ***Italy:*** Yes but only for prioritisation or WoE.
- ***Netherlands:*** No not yet, more information on exposure is needed and follow-up to further investigate prioritised OPFRs.
- ***US:*** Some approaches could be used to provide information and improve recommendations to sponsors, but not to provide official regulatory guidance. Computational methods, *in vitro* models with human cells that show concordance with *in vivo* models and alternative animal models are NAMs under consideration for potential application to support decision-making in regulatory toxicology.

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

- The extrapolation from the derived *in vitro* BMC via HTTK modelling.
- A prioritisation of compounds taking into account their relative toxicity.
- The toxicity data in relation to structural features, e.g. the influence of more substituents on the phenyl ring or the influence of phenyl versus alkyl substituents, to see how the toxicity varies within the class of compounds.
- Minimum required criteria for use of NAMs for different regulatory purpose (prioritisation in this case) to ensure an accurate data analysis and interpretation, harmonisation of protocols, etc.

Overviews of the case study are as follows.

This case study was developed to show how a battery of *in vitro* and complementary non-mammalian animal models (e.g. zebrafish) can be used to prioritise OPFRs for further testing. Available *in vivo* neurobehavioral data and human exposure data were collected, and pharmacokinetic models were used to prioritise and rank the toxicity of these OPFRs for further *in vivo* testing.

Results suggested that the *in vitro* BMC of some of the flame retardants such as Tiphenyl phosphate (TPHP), 2,2′ 4,4′ -Brominated diphenyl ether (BDE-47), Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), and 3,3′ ,5,5′ -Tetrabromobisphenol A (TBBPA) is within the range of plasma concentrations estimated from human exposure. For other compounds such as Trimethyl phenyl phosphate (TMPP), Isodecyl diphenyl phosphate (IDDP), 2-Ethylhexyl diphenyl phosphate (EHDP), Isopropylated phenyl phosphates (IPP), Tert-butylated phenyl diphenyl phosphate (BPDP), and Tris(2-chloroethyl) phosphate (TCEP), the biological activity *in vitro* occurred at concentrations higher than those estimated from human exposure data (Blum et al., 2019). However, there are limited exposure data for most OPFRs.

Please refer to ENV/CBC/HA(2022)5 Series on Testing & Assessment No. XXX for the case study to put the following points into context.

3.4. Case Study 2021-4: Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity hazard characterisation of Acetamiprid from EU ToxRisk

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

- The use of *in vitro* tests to investigate the hypothesised mechanism of nicotine receptor-induced developmental neurotoxicity. Such an approach is especially useful in contributing to a WoE approach for developmental neurotoxicity.
- Outlining the NAM methods used to generate data to fill data gaps in the putative, developed AOP, as well as describing the results and analysis of uncertainties.
- From a risk assessment perspective, the use of PBPK models to contextualise the *in vitro* data to a relevant human dose.
- The hazard of the compound using both human cells and zebrafish.

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

- Many of the parameters used within the PBPK model, due to the paucity of the data available to construct the models for Acetamiprid and its desmethyl metabolite.

- The lack of a clear linkage between the effects measured on intracellular free calcium, the neural responses, and the neurodevelopmental outcomes of those responses.
- The lack of AO report in humans and the overall uncertainty of the putative AOP.
- The heterogeneity of the available mammalian and human *in vivo* data

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

- ***Australia:*** The DNT-IVB and the AOP described would require significant further development and validation in order to form the basis of regulatory decision making. There is potential for elements of the DNT-IVB to be used as supporting information as part of a WoE approach within our regulatory context.
- ***Canada:*** In general, test data submitted to meet our regulatory requirements must be conducted according to (or sufficiently similar to) approved test guidelines (e.g., from the OECD, US EPA, etc). Additional data may be used as supplementary data in context of use. The approach of informing hazard assessment using NAMs tailored for different components of an AOP (where there is no data), and combining it with PBPK modelling, may be considered for screening purposes or in support of a WoE in the context of risk assessment in program areas where there are no data submission requirements. We strongly support work to continually develop approved new test guidelines to incorporate advances in science to meet regulatory requirements.
- ***Germany:*** The investigation of activation of the nAChR is clear and could be used as an *in vitro* mechanistic method.
- ***Italy***: Overall, the approaches developed by this case study can be used in WoE in scientific assessments. The level of remained uncertainties makes difficult use of the results in a regulatory context.
- ***Japan:*** The concept of the DNT-IVB would be useful. However, these assays are not routinely used. DNT-IVB should be fully validated for further regulatory consideration. In addition, context of use would be discussed toward regulatory context.
- ***Netherlands:*** No. Predominantly for scientific reasons. The cell models are not characterised to a level that is reassuring, and there is an apparent disconnect between the KEs.
- ***US:*** We could use several approaches to provide information and improve recommendations to sponsors, but not to provide official regulatory guidance. Systematic reviews, well established *in vitro* tests, *in silico* models and alternative animal models are new tools under consideration for potential application to support decision-making in regulatory toxicology.

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

- The evaluation methods of the PBTK and the docking predictions.
- The translation and interpretation of the kinetic data.
- The rationale for selecting the most appropriate *in vitro* NAMs and for developing the AOP.

Overviews of the case study are as follows.

The purpose of this case study was as follows;

1) Develop an example of the use and application of the DNT-IVB for a single substance, Acetamiprid.

2) Address the uncertainties identified by EFSA in some of the published *in vitro* and *in vivo* data to demonstrate that NAM data in an IATA context (integrating existing information) may be sufficient to characterise its DNT hazard.

3) Include consideration of exposure and a risk component through the use of PBK modelling for Acetamiprid, in addition to a hazard characterisation.

The approaches of this case study were as follows;

1) Assembling of evidence using a systematic literature review approach, extracting, appraising and assessing existing human observational, *in vivo* and *in vitro* evidence related to DNT endpoints.

2) Screening for evidence.

3) Integration of the evidence by an AOP-based approach.

4) Generation of additional mechanistic evidence and contextualisation (PBPK, binding to the nAChR (docking experiments)).

5) Data gap and uncertainty analysis.

In conclusion, Acetamiprid induces Ca^{2+} signaling in neuronal systems mediated by nAChRs at concentrations that are predicted by PBPK modeling to occur at 0.1-0.2 mg/kg, which is 4-10 times higher than the current acute reference doses (ArfD) set in the EU. This could potentially support a plausible mechanistic link to more firmly established human-relevant adverse outcomes.

Please refer to ENV/CBC/HA(2022)6 Series on Testing & Assessment No. XXX for the case study.

3.5. Case Study 2021-5: Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity hazard characterisation of Imidacloprid and the metabolite Desnitro-imidacloprid from EU ToxRisk

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

- The use of *in vitro* tests to investigate the hypothesised mechanism of nicotine-receptor induced developmental neurotoxicity. Significant detail is provided for how the *in vitro* methods were conducted and also the results obtained using imidacloprid and its metabolite. Such an approach is especially useful in contributing to a WoE approach for developmental neurotoxicity.
- The fact that it is AOP-anchored, thereby forcing ensuing research to be hypothesis-driven. Hypothesis-driven research is less likely to be biased than the alternative.

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

- The lack of *in vivo* data derived from robustly designed and performed experiments (and therefore including both negative and positive controls, multiple dose levels from which dose-responses could be ascertained, sufficiently low doses and sufficiently lengthy durations, etc.).
- The lack of information regarding KEs (i.e. between the molecular initiating events and the adverse outcomes) and the many layers and cross-talk that may exist between them;

- The lack of human cell model systems more closely approaching the *in vivo* human conditions than can cancer cells (such as human SH-SY5Y neuroblastoma cells) or fish embryos (zebrafish embryos);
- The lack of data regarding the various transient alterations such as DNA methylation, histone de-acetylation and/or others that may play roles difficult to uncover due to their transient nature or their subjectivity to influences such as ethnicity through for example short nucleotide polymorphisms.

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

- ***Australia:*** The DNT-IVB and the AOP described would require significant further development and validation in order to form the basis of regulatory decision making. Significant uncertainties regarding the later KEs, including a specific adverse outcome limit the use currently in a regulatory context. There is potential for elements of the DNT-IVB to be used as supporting information as part of a WoE approach within our regulatory context.
- ***Canada:*** While we would very much appreciate the opportunity to use the approaches employed in this case study in my regulatory context, we would not be able to do so. The case study, as presented does not provide confidence in the methodology and needs to be rewritten before consideration.
- ***Germany:*** -
- ***Italy:*** Overall, the approaches developed by this case study can be used in WoE in scientific assessments. The level of remained uncertainties makes difficult use of the results in a regulatory context.
- ***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 optimise *in vitro* versus *in silico* risk assessment.
- ***US:*** Some approaches of the study may be used to provide information and improve recommendations to sponsors, but not to provide official regulatory guidance. Application of NAMs, including *in vitro* and alternative *in vivo* models, and *in silico* models are new tools under consideration for potential application to support decision-making in regulatory toxicology, to improve our ability to predict risk, safety, and efficacy and to replace, reduce, and/or refine animal testing.

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

- Weighing of individual assay results and combination in the IATA
- Translation and interpretation of the kinetic data
- The step from AOP to quantitative AOP. The current AOP contains a number of KEs which are loosely connected, the step towards quantification of the relationship between the different KEs would be a great improvement.

Overviews of the case study are as follows.

This case study was to assess developmental neurotoxicity hazard characterisation using Imidacloprid and its metabolite, Desnitro-imidacloprid. The purpose and the approach for conducting this case study is the same as Case Study 2021-4. Case Study 2021-5 assessed the target chemical, and its metabolite, and used reverse modelling to calculate a brain concentration after an intake.

In conclusion, Imidacloprid/Desnitro-imidacloprid induce Ca2+ signalling in neuronal systems mediated by nAChRs. Applying reverse HTTK modelling revealed, that a brain concentration of 2 µM Imidacloprid, which was considered a POD from our *in vitro* studies, would be reached after an intake of 0.2 mg/kg body weight in an average human. This could potentially support a plausible mechanistic link to more firmly established human-relevant adverse outcomes.

Please refer to ENV/CBC/HA(2022)7 Series on Testing & Assessment No. XXX.

3.6. Case Study 2021-6: Case Study on the use of Integrated Approaches to Testing and Assessment for potential Systemic Toxicity and Estrogen Receptor Activation of a Group of Bisphenols and Select Alternatives from Canada (Health Canada)

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

- Evaluating NAM-based PODs from various perspectives and setting the most appropriate ones
- The use and exploration of several approaches to derive minimal bioactivity concentrations;
- The multi-factorial approach for determining POD NAM_ER and POD NAM_Sys. Utilising multiple lines of evidence allows for selection of the most sensitive endpoint for the chemical.

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

- Uncertainties surrounding the NAMs chosen for this case study to derive the minimal bioactivity concentration. For example, incomplete biological coverage from the approaches used, and the lack of metabolic competencies in *in vitro* systems;
- Uncertainty for the IVIVE model used, for which certain components are not well-defined like exposure and *in vitro*-derived clearance parameters;
- The suitability of the workflow used to identify analogues for read-across of kinetic parameters.
- For eight of the 25 compounds in this case study, testing was available for all 16 assays in the ToxCast ER Bioactivity model. However, no additional substance had results in all four assays representing the minimal four assay set thereby introducing uncertainty in the assignment of ER active compounds.
- QSAR models trained independently leading to variance in results for ER activity.

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

- ***Australia:*** The overall aim described in this study is to use NAMs for chemical screening and prioritisation which is very useful for us. Currently, we use the QSAR *in silico* models and read across approaches for chemical grouping and as supporting information/WoE for data poor chemicals. The challenge with using approaches in this case study is that it requires a certain level of computational expertise (for example, proficiency in the HTTK R package), and therefore, some level of training will be necessary. In addition, some of the models used in the case study for example Ingenuity Pathway Analysis (IPA) and adsorption, distribution, metabolism, excretion, and toxicity (ADMET) Predictor are

commercial models and may not be available for use by everyone. It may be preferable to use programs/models that are freely available through this may have limitations.

- ***France:*** This approach could be used, as it provides ranking and information about toxicity of substitutes, before carrying out risk assessment.
- ***Germany:*** Yes, we would as well use information from the ToxCast data (nevertheless, it is more in focus on endocrine disruptor related e.g., ER or AR activities) and would welcome accessibility to the HTTr data generated by the Canada Health.

  However, use of *in vitro* data on general toxicity from ToxCast DB or HTTr data is not yet fully in consideration particularly in the assessment procedure of pesticides or biocides regulation.

  Further, the data acquisition procedure is quite interesting but could need to be standardized and get more reproducible in regulatory context. As seen in the appendix open source software (Python, R) was used for data processing/acquisition. Sharing or describing the scripts could increase reproducibility and acceptance.
- ***Italy***: Yes, in the framework of a WoE assessment of environmental contaminants.
- ***Japan:*** Currently No, Not sure whether the new approaches were validated by this case study due to uncertainty in every aspect.
- ***US:*** There's potential merit. Limitation to utilising this approach in regulatory context: The current list of studies of existing *in vivo* hazard data (LOECs, etc.) in Table 4 in (OECD, 2022f) is limited to seven chemicals; this does not constitute a strong enough 'n' for comparison with the NAM POD values for the 25 BPA alternatives to evaluate whether the NAM PODs are more conservative/protective than *in vivo* hazard values.

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

- NAM-based POD for general systemic toxicity. Various lines of case studies will provide the guidance of appropriate margins based on uncertainty.
- The extrapolation from the derived *in vitro* POD via IVIVE and HTTK modelling.

Overviews of the case study are as follows.

The purpose of this case study is to examine the potential risk of this 25 BPA alternatives using NAMs, and to demonstrate that the NAM-based PODs obtained using *in vitro* transcriptomics are protective for this substance group.

NAM-based PODs for general systemic toxicity and ER activation were calculated using data from ToxCast, High-throughput Transcriptomics (HTTr), HTTK and IVIVE. The NAM-based PODs were compared to *in vivo* PODs from repeated dose, reproductive, and developmental toxicity animal studies extracted from the United States Environment Protection Agency (US EPA) ToxVal database. The NAM-based PODs were found to be protective estimates of both general *in vivo* systemic effects and ER-specific effects using *in vitro* endpoints.

In addition, a WoE approach was conducted using both *in silico* predictions and *in vitro* data from the ER pathway to create qualitative hazard for those bisphenols and alternatives that interact with the ER.

The results suggested that 25 BPA alternatives have endocrine activity. All bisphenols showed the potential for ER signal disruption but non-bisphenol alternatives were not observed to interact with the ER.

Please refer to ENV/CBC/HA(2022)X Series on Testing & Assessment No. XXX..

3.7. Case Study 2021-7: Case Study Using a New Approach Methodology (NAM) for Refining Inhalation Risk Assessment from Point of Contact Toxicity of the Pesticide, Chlorothalonil from ICAPO

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

- Innovative approach combining *in vitro* and *in silico* data (animal-free risk assessment).
- The test system (MucilAir™) tissues are derived from the human nasal tissue which is also a strength of the case study, as well as computational fluid dynamics-based aerosol dosimetry modelling.
- The CFD airflow model with the 3D respiratory test system data used to determine the HEC, without any need of interspecies extrapolation.
- The application of AOPs in selection of appropriate assays and benchmark dose (BMD) determination.

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

- Toxicological effect on pulmonary alveoli (for small particle size): The tissue used in the MucilAir™ test is derived from nasal tissue which is considered to be similar in cellular composition to the conducting respiratory tract. In contrast, the epithelium of the pulmonary alveoli is very different in terms of composition and structure. Therefore, the potential effects on pulmonary alveoli may not be assessed by MucilAir™ model. In addition, the MucilAir™ tissues only represented 5 individual healthy donors and these may not be considered representative of the human population.
- Toxicodynamic relevance / sensitivity of the *in vitro* model; uncertainties of the CFD based dosimetry model.
- Mainly cytotoxicity is measured using Trans-epithelial Electrical Resistance (TEER), Lactate Dehydrogenase (LDH) and resazurin assays. Subtle changes in cellular metabolism and liberation of reactive oxygen species may not be observed with these assays. Moreover, the variability of the TEER values is rather high. Thus morphology and other biomarker endpoints need to be considered.
- The use of liquids instead of aerosols; in the case study, the compound is dissolved in a liquid and subsequently added to the tissues, while human exposure is via aerosols. However, air-liquid interface (ALI) aerosol exposure systems exist and are being used for *in vitro* hazard characterisation of air contaminants.

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

- ***Australia:*** A similar approach could be used for industrial chemicals with an appropriate *in vitro* 3D human respiratory model and *in silico* CFD in place of an inhalational toxicity study if the MOA for the irritation is similar. It is important in using these approaches for selection of an appropriate model that is applicable to the MOA for the local irritation effect. Given that the MucilAir™ assay has been reviewed and accepted by a regulatory agency gives more confidence in the use of the assay and the approach described in the case study. The limitations of the *in vitro* studies in assessing all possible systemic effects from repeated inhalation exposure would limit the use of this study to those chemicals with only local effects. As pesticides are regulated by another scheme, we are unable to comment on the acceptability under that scheme.

- ***Canada:*** Yes, the logic behind this approach seems sound. Since the vestibule is still part of the inhalation process and since the highest retained aerosol doses were found for this area, it may be worth using a dermal model to see if the HED from the vestibule info is lower than the inhalation models used in the case study (to ensure that the HED calculated is conservative). With such high retained doses within the vestibule, dermally absorbed amounts and the potential of cumulative exposure through daily work need to be considered from a risk assessment perspective. Additionally, the Level of Concern (LOC) approach is not used within the Existing Substances Risk Assessment Bureau at Health Canada for risk assessments. According to this document from the US EPA (https://www.epa.gov/pesticide-science-and-assessing/pesticide-risks/technical-overview-ecological-risk-assessment-risk), the LOC seems to go up to 1.0, more clarification is needed on how the uncertainty factor (UF) led to the LOC derivation of 3. Basing the MOE to be greater than the LOC as a threshold for no concern, is not familiar and requires more clarification of a reference document. The approach is relatively scientifically sound and defensible, and could be used in the regulatory context of the New Substances Assessment and Control Bureau of Health Canada (New Substances Notification Regulations). The results are also expected to be reproducible. There are limitations in the *in vitro* tests in that they don't address systemic responses, but for a known tissue irritant, it would appear to be a suitable method. Additionally, we are less familiar with the CFD model and the empirical validation of it. This model also does not examine deep lung penetration or damage, and therefore, consideration may be given to multiple path particle deposition (MPPD) model or other models for a more biologically comprehensive prediction when the particle size warrants such an investigation.
- ***France***: While the approach appears interesting and promising, some steps still lack clarity and justification. A comparison between this method and the *in vivo* results should be more fully documented for a more complete demonstration of the relevance of the method. It would be necessary to reduce the uncertainties identified as "medium" level at the end of the document. At this stage, the approach is not sufficient regarding to Registration, Evaluation, Authorisation and Restriction of CHemicals (REACH) requirements.
- ***Germany:*** No. The EU pesticides regulation framework requires validated methods and GLP. Up to now, reliability was not sufficiently demonstrated (e.g. by providing information on robustness, use of adequate uncertainty factors). Moreover, experimental quality standards must be met and certified. However, only TEER, LDH and resazurin assays are not sufficient for a weight-of-evidence approach, the 3D cell model and CFD model would have to combine with additional bioassays and biomarker analysis.
- ***Italy:*** Yes.
- ***Netherlands:*** No, in the introduction it is mentioned that *in vitro* and *in silico* models can be used to determine respiratory operator exposure risk for a US EPA submission. How is this aligned with criteria and regulation in Europe and other parts of the world? In addition, this case study is only applicable when already sufficient information on the mode of action of a compound exist. However, in general there is a lack of information on the mode of action and hazard of chemicals. It should be mentioned that information obtained from the AOP should be integrated with other sources of information for regulatory decision-making.

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

- Use of the *in silico* CFD and the *in vitro* 3D human cell model,
- An MOE level that is not a cause for concern

Overviews of the case study are as follows.

The purpose of the case study is to demonstrate how NAMs such as an *in vitro* 3D human respiratory model, particle size distribution (PSD), *in silico* computational fluid dynamics (CFD), and *in vivo* human operator exposure measurements can be utilised to replace an *in vivo* repeated dose respiratory toxicology study (OPPTS 870.3465, 40 CFR Part 798, OECD TG 412, 413).

A 3D *in vitro* human model of the respiratory epithelium (MucilAir™) was exposed to Chlorothalonil at different concentrations and analysed for toxicity. CFD calculations were used to identify where inhaled particles would land in the respiratory tract of the human and thus result in potential toxicity identified in the MucilAir™ tests. Rats are very sensitive to Chlorothalonil and the mode of action of Chlorothalonil toxicity leads to squamous metaplasia of the larynx in rats. Human derived MucilAir™ was chosen to more accurately reflect human exposure during operator use. This case study has a direct *in vitro* - *in vivo* correlation because human cell toxicity data is measure from exposure in the conducting airway utilising CFD and breathing information measured directly from operators performing the spraying. This means that there is no interspecies extrapolation required. Human equivalent concentrations (HECs) were calculated using these data which were then used to perform a formal regulatory risk assessment.

This case study helps determine respiratory operator exposure risk for a US EPA pesticide submission. The US EPA Scientific Advisory Panel (SAP) report (US EPA, 2019) was broadly supportive of this NAM and the testing strategy with no concerns about the reliability of the test system (MucilAir™). Following this report, additional testing was performed and both tests are described alongside the formal EPA risk assessment.

Please refer to ENV/CBC/HA(2022)10 Series on Testing & Assessment No. XXX.

3.8. Case Study 2021-8: Case Study on the Use of Integrated Approaches for Testing and Assessment for skin sensitisation: Demonstrating the Next Generation Risk Assessment Framework using Geraniol from Cosmetic Europe

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

- Clear and concise yet detailed descriptions of 5 DAs including calculation of a Margin of Exposure for each approach and integration of several DAs within an IATA.
- The practical application of the skin sensitisation AOP on which the IATA is based.
- The use of several *in vitro*, *in chemico* and mathematical models to integrate available data decreases the uncertainties and improves predictions on consumer risks of exposure to selected chemicals in cosmetics.

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

- The low number of substances in the datasets of the defined approaches used. For example, the SARA approach only has 81 chemicals in the dataset. Most DAs provide some degree of uncertainty assessment concerning input data and *in vivo* results used as data basis.
- The validation of the *in silico* models.
- Exposure assessment. The case study used the SCCS exposure estimate for face creams but SCCS notes that assessments made by other bodies (IFRA/RIFM) are generally higher.

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

- ***Australia:*** Yes the approaches could be used in the regulatory context. The tools need to be freely available and all the tests (as in this case) validated by the OECD or other validating schemes. It is recognised that this case study is only focusing on one use but this is not the case in a regulatory context. Cosmetics are used in several product types and at different concentrations resulting in different exposure patterns. Therefore this approach would need to be applied for the different uses separately to identify safe concentrations in a regulatory context. This would be a cumbersome approach to use in a regulatory context. It is also not easy or straight forward to use the same approach with data poor new chemicals. In addition in a regulatory context the cosmetic that submitted for authorisation of use will also be used in children when marketed and this needs to be considered as well by regulators.
- ***Canada:*** The approaches used in this case study (both the defined approaches and the NGRA framework methodology) could be used as part of a WoE assessment. The overall framework developed in this case study as well as elements specific to skin sensitisation can be applied in a screening and assessment context as well as to meet the legislated requirements.
- ***Germany:*** Yes, one of the Defined Approaches is already part of TG 497. Others are part of OECD GD 256 and could be used on a case-by-case basis for well specified active substances with good quality exposure data.
- ***Italy:*** Yes, in a WoE framework
- ***Japan:*** In general, risk assessment is currently not required for skin sensitization in regulatory context in Japan. From the aspect of practical use, this meta-model consisting of 5 approaches is too much to perform for small and medium-sized enterprises (SMEs).
- ***Netherlands:*** The DAs that are included in the new OECD test guideline DASS are usable for hazard assessment, but they cannot currently be used for risk assessment in a NGRA. This is due to the facts that these methods are not designed for risk assessment and the step used to calculate a POD warrants further scientific scrutiny. In addition, it should be kept in mind that regulations also require insight in other (repeated dose) endpoints for a risk assessment.

  Another note for NGRAs in general that I want to emphasize is that whether you can apply exposure based waiving is a policy decision that has no place in a general hazard/risk assessment framework.
- ***US:*** No specific comment.

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

- Guidance on exposure assessment, e.g. regarding mixed exposures, exposures from several sources, and exposures that may not be reliably quantifiable (in case of cosmetics and personal care products, e.g. creams for special face/body areas where use may not be restricted to the prescribed skin area, or generally improper use may occur).
- Machine learning approaches in the regulatory context.
- The justification of the use of a specific DA and how regulators select the best DA.

Overviews of the case study are as follows.

The purpose of this case study is to demonstrate the applicability of the tiered Next Generation Risk Assessment (NGRA) framework (containing NAMs and Defined Approaches (DAs) in order to replace animal models to assess the potential risk of consumer exposure for Geraniol at 0.1% via a face cream.

Selected DAs based on the NAMs data are as follows;

1: Sensitiser potency categorization based on test methods addressing KE 1+3 and *in silico* prediction (ITSv1 DA)

2: Sensitiser potency prediction based on KE 1+2+3: The artificial neural network model for predicting Local Lymph Node Assay (LLNA) EC3

3: Sequential testing strategy for hazard identification (Tier 1) and potency (United Nation (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) cat. 1A / 1B) categorization (Tier 2) of skin sensitisation

4: Sensitiser potency prediction based on KE 1+2+3: Bayesian Network ITS/DS (BN-ITS) for hazard and potency identification of skin sensitisers

5: DIP for Skin Allergy Risk Assessment (SARA)

Outputs obtained from the DAs were converted to PODs and the margins of exposure (MOE) were calculated (Tier 2). The POD was derived from different DAs, but each has sufficient conservatism. The final outcome differed between DAs. The result of prediction is that Geraniol is a weak, moderate or UN-GHS Category 1B sensitiser. Moreover, risk assessments based on predictions from four applied DAs concluded that use of 0.1% Geraniol in a face cream would be safe.

The risk assessment based on another DA, the BN-ITS predictions resulted in a borderline conclusion (MOE of 91).

Please refer to ENV/CBC/HA(2022)8 Series on Testing & Assessment No. XXX for the case study to put the following points into context.

Annex 3. Summary of the case studies reviewed in all review cycles

This Annex summarises learnings and lessons from the 32 case studies of the IATA Case Studies project including the 24 case studies from the past six review cycles. The assessment approaches used in the case studies can be classified into four general categories: i) data-gap filling by read-across based on grouping of chemicals (17 case studies), ii) grouping of chemicals for cumulative risk assessment (chemicals that are not appropriate for read-across) (one case study, 2016-2), iii) safety assessment workflow (four case studies), and iv) hazard screening of chemicals (nine case studies).

The target endpoints[3] included:

- repeated dose toxicity (13 case studies),
- neurotoxicity (eight case studies),
- reproductive toxicity (two case studies),
- estrogenicity (three case studies),
- mutagenicity (one case study),
- bioaccumulation (one case study),
- genotoxicity (one case study),
- ecotoxicity (one case study) and
- developmental toxicity (six case studies).

A variety of assessment approaches have been used in the IATAs reviewed to date (Annex Table 8). Approaches include:

- read-across (18 case studies),
- screening (e.g. prioritisation, hazard characterisation, both (11)),
- safety assessment workflow (4), and
- grouping for cumulative assessment (1).

In addition, case studies reviewed also demonstrated examples of using;

- MoA/AOP approaches (25 case studies),
- describing uncertainty (30),
- application of new approach methodologies (27), and
- deriving low/no toxicity prediction (15)

[3]Some case studies include more than one endpoint.

Annex Table 8. Summary of the case studies reviewed in the past seven review cycles

Year-No. (Lead)	Assessment Approach	Endpoint	IATA Topics				Reference
			AOP[1]	UR[2]	NAM[3]	L/N[4]	
2021-1 (EFSA)	Screening (Hazard characterisation)	Developmental neurotoxicity	X	X	X		OECD, 2022a
2021-2 (EFSA)	Screening (Hazard characterisation)	Developmental neurotoxicity	X	X	X	X	OECD, 2022b
2021-3 (BIAC)	Screening (Prioritisation)	Developmental neurotoxicity	X	X	X		OECD, 2022c
2021-4 (EU ToxRisk)	Screening (Hazard characterisation)	Developmental neurotoxicity	X	X	X		OECD, 2022d
2021-5 (EU ToxRisk)	Screening (Hazard characterisation)	Developmental neurotoxicity	X	X	X		OECD, 2022e
2021-6 (Canada)	Screening (Prioritisation)	Repeated dose toxicity Estrogenicity	X	X	X		OECD, 2022f
2021-7 (ICAPO)	Screening (Identification of safety)	Repeated dose respiratory toxicology	X	X	X	X	OECD, 2022g
2021-8 (CE)	Safety assessment workflow	Skin sensitisation	X	X	X	X	OECD, 2022h
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)	Screening (Prioritisation and screening)	Estrogenicity	X	X	X	X	OECD, 2019c
2017-1 (Canada/US)	Screening (Prioritisation and hazard characterisation)	Estrogenicity	X	X	X	X	OECD, 2018b
2017-2 (Canada)	Screening	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

In the seventh cycle:

- Seven of eight case studies identify chemical hazard (hazard characterisation and prioritisation). All eight case studies included AOP concepts and three concluded that the target chemicals have low/no toxicity.
- One case study (2021-8) described a safety assessment workflow. The potential for determining the safety of skin sensitisation of a chemical was demonstrating by comparing biological activity with estimated exposure using five DAs.

Annex 4. Useful tools for developing IATAs

This chapter highlights useful tools for IATA, which were presented and demonstrated at webinars. The webinar for demonstration of IATA tools was launched in 2019 in order to share additional information on IATA tools within the project team and to promote the use of these tools for developing IATAs. In this review cycle (2021), four tools were introduced and demonstrated, and summarised in Annex Table 9. Annex Table 10 provides all IATA tools demonstrated at the webinars since 2019.

Annex Table 9. IATA tools demonstrated in 2021

	Tool	Demonstrator	Endpoint	Description	Presentation slides
1	Collaborative Modelling Project of Androgen Receptor Activity (CoMPARA)	NIH	Androgen Receptor Activity	The CERAPP combined multiple models developed in collaboration with groups in the United States and Europe to predict ER activity of a common set of over 30,000 chemical structures. Quantitative structure–activity relationship models and docking approaches were employed, mostly using a common training set of over 1,000 chemical structures provided by the US EPA, to build a total of 40 categorical and 8 continuous models for binding, agonist, and antagonist ER activity.	https://community.oecd.org/docs/DOC-97372 **(Access is limited to project members)**
2	Collaborative Estrogen Receptor Activity Prediction Project (CERAPP)	NIH	Estrogen Receptor Activity	The CoMPARA list of screened chemicals built on CERAPP's list of over 30,000 chemicals to include additional chemicals of interest, as well as simulated ToxCast™ metabolites, totalling 55,450 chemical structures in order to predict AR activity. Computational toxicology scientists from 25 international groups contributed 91 predictive models for binding, agonist, and antagonist activity predictions. Models were underpinned by a common training set of over 1,000 chemicals compiled from a combined data set of 11 ToxCast™/Tox21 HTS *in vitro* assays.	
3	Collaborative Acute Toxicity Modelling Suite (CATMoS)	NIH	Acute Toxicity	CATMoS is the acute toxicity model and compiled an acute oral toxicity data inventory for 11,992 chemicals, split into training and evaluation sets, and made available to 35 participating international research groups that submitted a total of 139 predictive models.	
4	Cell stress panel	BIAC	Toxicological mode of action	The cell stress panel is a combination of endpoints that delivers a toxicity profile for novel therapeutics providing evidence of the toxicological mode of action. It consists of 36 biomarkers that represent cellular stress signalling pathways, organelle health and cellular cytotoxicity.	

Annex Table 10. IATA tools demonstrated at the webinar

Tool [Presenter]	Use free	Description	toxicity mechanism	metabolism	toxicokinetics
Apical endpoints					
Collaborative Acute Toxicity Modelling Suite (CATMoS) [US]*9	Free	acute toxicity model and compiled an acute oral toxicity data inventory	✓		
Hazard Evaluation Support System Integrated Platform (HESS)*1 [Japan]	Free	categorising on the basis of structural, physicochem and mechanistic similarities and helping predict the repeated dose toxicity of untested chemicals by me the category approach.	✓	✓	
Endocrine disruptors					
Computation model for Estrogen Receptor (ER) pathway*2 [US]	-	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.			
Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) [US] *7	Free	combined multiple models developed in collaboration with groups in the United States and Europe to predict ER activity			
Collaborative Modeling Project of Androgen Receptor Activity (CoMPARA)[US] *8	Free	Models predict AR activity and were underpinned by a common training set of over 1,000 chemicals compiled from a combined data set of 11 ToxCast™/Tox21 HTS *in vitro* assays.			
Risk assessment					
NIVA Risk Assessment Database tool*5 [Norway]	-	Hazard and risk assessment tools that utilize concepts outlined by the Aggregate Exposure Pathway (AEP) and AOP.	✓		
Cell stress panel [BIAC] *10	-	An *in vitro* Next Generation Risk Assessment (NGRA) Tool and Intended to cover off non-specific modes of toxicity action that lead to cell stress or mitochondrial toxicity	✓		
European Union System for the Evaluation of Substances (EUSES)*4 [the Netherlands]	Free	Quantitative environmental exposure and risk assessment tool for industrial chemicals and biocides.			
RISK Identification And Ranking (RAIDAR) *6 [Canada]	Free	Combines mass balance fate and bioaccumulation models		✓	✓
Exposure assessment					
Consexpo*3 [the Netherlands]	Free	Exposure estimation and assessment from consumer product			

*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://echa.europa.eu/support/dossier-submission-tools/euses

*5: https://www.niva.no/en/projectweb/radb

*6: https://arnotresearch.com/RAIDAR/

*7: https://ehp.niehs.nih.gov/doi/10.1289/ehp.1510267

*8: https://ehp.niehs.nih.gov/doi/10.1289/EHP5580

*9:https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/acute-systemic-tox/models/index.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tox-models

*10: https://www.cyprotex.com/product_sheets/Cell_Stress_Panel_Product_Sheet.pdf

Annex 5. 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.

The overview document (OECD, 2020i) helps understanding of IATA, by explaining key concepts and providing basic definitions, and to support easier access to existing resources.

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.

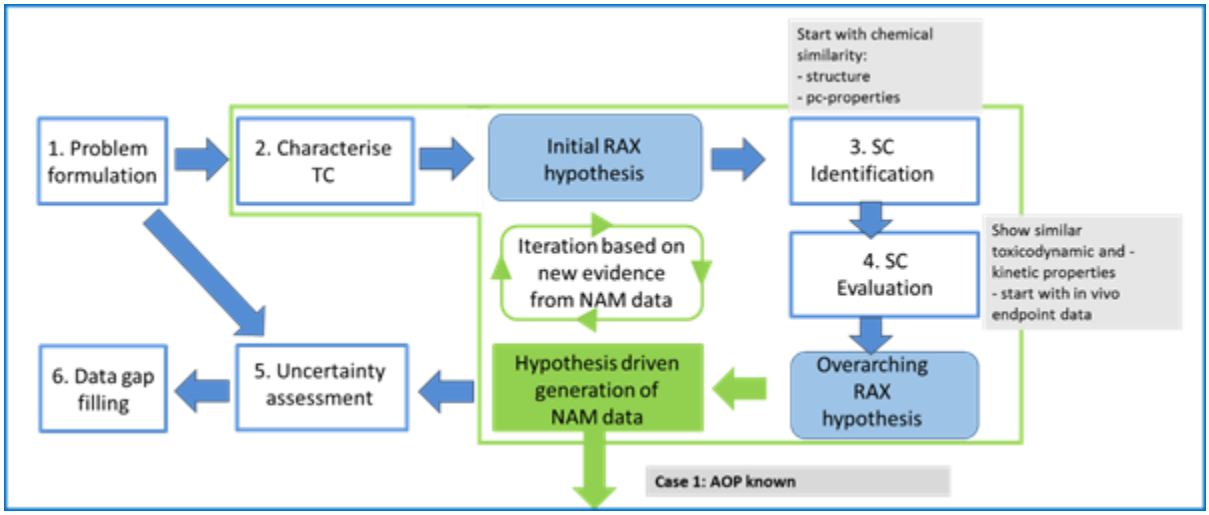
Tip for nanomaterials

- 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)]

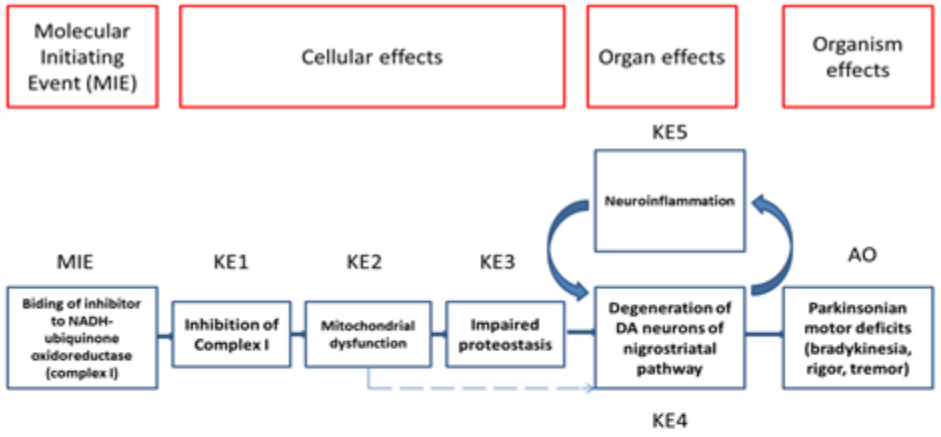
- Chemical / biological interaction [See 3.2.3.5 "*Chemical / biological interaction* of the grouping guidance (OECD, 2014a)]
- Toxicological and epidemiological information, along with information from new approach methodologies (NAMs) [See 3.2.3.6 "*Responses found in in vitro methods* of the grouping 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.

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

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	

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.

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

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

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

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)).

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

[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

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.
- 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 category does not hold i.e. substances outside scope of predictions e.g. by endpoint [See 5.2.4 "Step 3: Evaluate available data for adequacy" of the grouping guidance (OECD, 2014a): example of outlier].

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

[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/

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).

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)			

[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

Concordance and weight of evidence of all data used for justifying the hypothesis			
Overall uncertainty of the read-across			

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.

7. 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 Characterisation 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 6. 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.

The overview document (OECD, 2020i) helps understanding of IATA, by explaining key concepts and providing basic definitions, and to support easier access to existing resources.

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*.

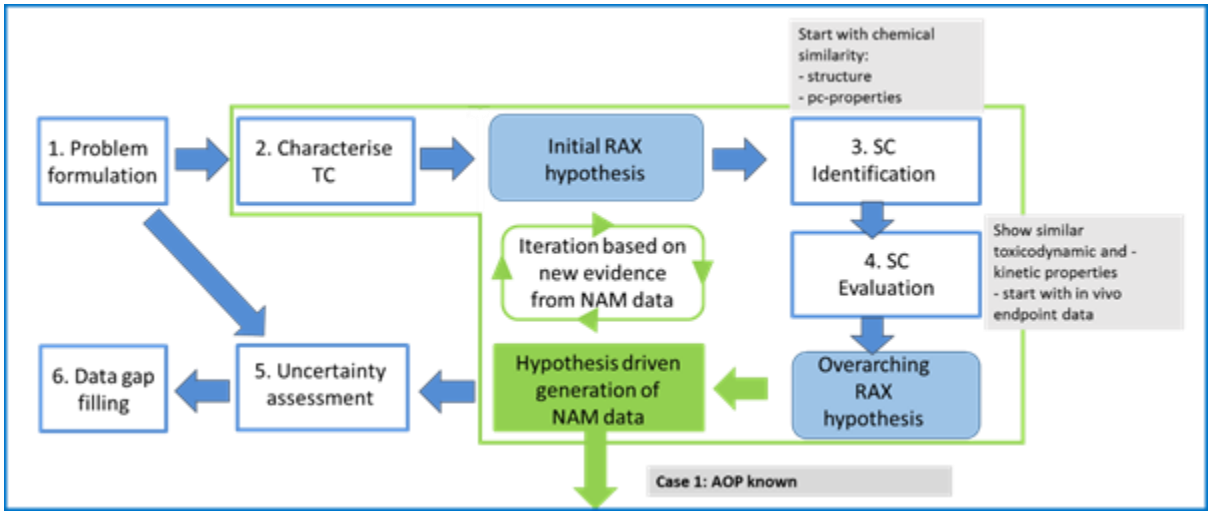
Tip

- 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.

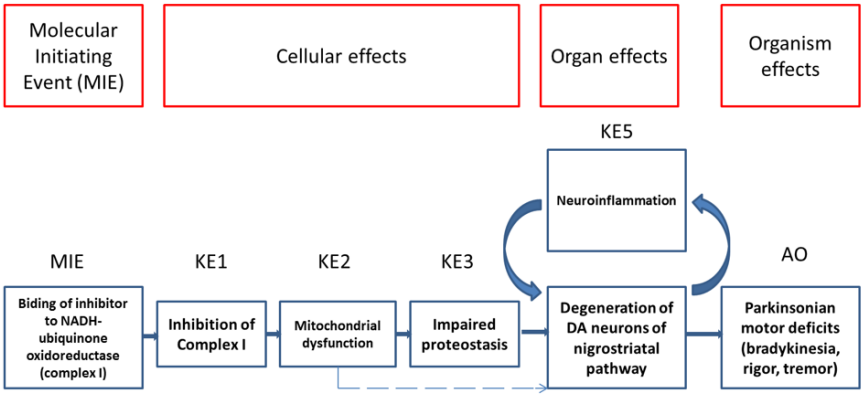
[14] AOP-KB. https://aopkb.oecd.org/

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

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

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

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	

- **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)

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 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 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			
Overall uncertainty of the IATA			

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

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

[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

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 characterisation, 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	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

	Identifying Estrogen Receptor Active Chemicals			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 2-Alkyl-1-alkanols: Read-Across	4. STATEMENT OF UNCERTAINTY, Table 4 and Table. 5, Page 23-26	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.

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.