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

Fourth Review Cycle (2018)

Series on Testing and Assessment
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No. 307

REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) - FOURTH REVIEW CYCLE (2018)

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INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

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Paris 2019

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Forward

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

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

This document reports the learnings and lessons obtained from the review experience of the two case studies, listed below, submitted to the 2018 review cycle 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.

1. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR TESTICULAR OF ETHYLENE GRYCOL METHYL ETHER (EGME)-RELATED CHEMICALS, ENV/JM/MONO(2019)27, Series on Testing & Assessment No. 308.
2. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR ESTROGEN RECEPTOR ACTIVE CHEMICALS, ENV/JM/ MONO (2019)28, Series on Testing & Assessment No. 309.

These case studies are illustrative examples, and their publication as OECD monographs does not translate into direct acceptance of the methodologies for regulatory purposes across OECD countries. In addition, these cases studies should not be interpreted as official regulatory decisions made by the authoring member countries.

This document has been prepared by a project team of the Working Party on Hazard Assessment and was endorsed at the 3rd meeting of the Working Party on Hazard Assessment in June 2019.

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

Table of contents

Tables

1. Introduction

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

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

This project reviews case studies submitted from member countries every year. The review results are discussed in a project meeting. The discussion includes the topics of strongest aspects of case study, uncertainty of case study, areas for further developing guidance and possible use of each case study in a regulatory context. In every review cycle, the case studies approved will be published with a considerations document capturing the learnings and lessons stemming from case studies. The outcomes of the past three review cycle of the project (2015 - 2017) included thirteen case studies and three considerations documents, which have all been published (OECD, 2016a; 2016b; 2016c; 2016d; 2016e; 2017a; 2017b; 2017c; 2017d; 2017e; 2017f; 2018a; 2018b; 2018c; 2018d; 2018e).

In the fourth review cycle (2018), the two case studies shown in Table 1 were reviewed. The final case studies are published [ENV/JM/MONO(2019)27-28, Series on Testing and Assessment No. 308-309]. These case studies are illustrative examples, and their publication as OECD monographs does not translate into direct acceptance of the methodologies for regulatory purposes across OECD member countries. In addition, these cases studies should not be interpreted as official regulatory decisions made by the authoring member countries. This document describes the review results of each of the two case studies and summarises the learnings and lessons stemming from the case studies reviewed in the four review cycles.

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

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

Table 1. Case Studies Reviewed in the Fourth Review Cycle (2018)

No.	Title	Lead	Purpose of Use	References
1	Testicular Toxicity of Ethylene Glycol Methyl Ether (EGME)-Related Chemicals	Japan	Assessment of testicular toxicity of EGME-related chemicals for the hazard classification under the Chemical Substances Control Law (CSCL) by integrating metabolism information for the category assessment and read-across with use of toxicity data of EGME-related source chemicals.	OECD, 2019a
2	Case Study on the Use of an Integrated Approach to Testing and Assessment for Estrogen Receptor Active Chemicals	United States	Use a combination of 4 to 16 *in vitro* high throughput screening (HTS) assays and a computational model for estrogen receptor (ER) agonist activity, which could serve as an alternative to low and medium throughput *in vitro* and *in vivo* tests for ER activity, for screening and priority setting of environmental chemicals based on their ER agonist activity and for further evaluation of endocrine-related activity in higher tier *in vivo* tests.	OECD, 2019b

2. PROCESS FOR REVIEWING THE CASE STUDIES

The following 17 countries/organisations participated in the fourth review cycle: Australia, Canada, Denmark, Germany, Italy, Japan, the Netherlands, Norway, Sweden, the United Kingdom (UK), the United States (US), EU/Joint Research Centre (EU/JRC), EU/European Chemicals Agency (EU/ECHA), Business and Industry Advisory Committee to the OECD (BIAC), International Council for Animal Protection in OECD Programmes (ICAPO), Lhasa Limited and Liverpool John Moores University.

The authors were requested to consider the templates provided in Annex 1 and Annex 2. The template for the case studies on read-across was developed based on the reporting format in the OECD Guidance on Grouping of Chemicals (OECD, 2014a) and a case study document (OECD, 2014b) and was updated based on review experience of the case studies in the past three review cycles. The general template for IATA case studies, based on building blocks, was developed in order to fit the case studies using multiple IATA components, such as AOPs / Mode of Action (MOA), Defined Approaches (DAs), Workflows, Grouping/Read-Across and was updated based on review experience of the case studies in the third review cycle.

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

1. Is the purpose of the case study clear?
2. Are the justifications presented in the different sections sound? (e.g. hypothesis; analogue selection; justification for data gap filling; integrated conclusion; uncertainty discussion; other). If not, suggest how to improve it.
3. Are there specific topic areas in the case study that could benefit from the development of further guidance for application or interpretation? (e.g. building the hypothesis; identifying important IATA elements for the endpoint; selecting analogues; deriving integrated conclusion; uncertainty communication. etc.)
4. What are the strongest aspects of the case study?
5. What are the dominant and most relevant areas of uncertainty and how do you think they could be reduced? Could their reduction lead to a different conclusion of the case study?
6. Would you use the results of such a case study in your regulatory context? If no, why not (legislative/policy/scientific reasons)?
7. Does the template work well?
8. Other?

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

1. Which areas of the case study was the most difficult to justify and why?
2. What information would have helped you in developing the case study?

3. Would the availability of guidance or tools in a particular area have helped you in developing the case study?
4. Would you use the results of such a case study in your regulatory context? If no, why not (legislative/policy/scientific reasons)?
5. Does the template work well?
6. Other?

The reviewer's comments and the revised case studies were discussed at the fourth meeting of the IATA Case Studies Project (27 November 2018) in order to finalise the case studies and summarise the learnings and lessons.

3. SUMMARY OF REVIEW RESULTS

3.1. Case Study 1: Case Study on the Use of Integrated Approaches for Testing and Assessment for Testicular Toxicity of Ethylene Glycol Methyl Ether (EGME)-Related Chemicals

This case study was developed to address how read-across can be applied to fill data gaps in the reproductive toxicity endpoint for screening assessment under the Japanese Chemical Substances Control Law (CSCL)[3]. A category approach was applied to assessing the testicular toxicity of ethylene glycol methyl ether (EGME)-related chemicals. Based on toxicity information for EGME and related chemicals and accompanied by possible adverse outcome pathway information on the testicular toxicity of EGME, the category members were defined as chemicals that are metabolised to methoxy- or ethoxyacetic acid, which are responsible for testicular toxicity.

Fifteen chemicals were obtained from the Japanese chemical inventory as a shortlist for the category and were categorised into three groups. Integration of the different types of available data from *in vivo*, *in vitro* and *in silico* in the literature shows that chemicals for which information is available on the metabolic formation of methoxy- or ethoxyacetic acid possess testicular toxicity. Using read-across based on three category members with experimental data, D-values for testicular toxicity of other 12 category members were derived. The results suggest that testicular toxicity is a concern for the untested chemicals which are predicted to produce one of the toxic metabolites.

Please refer to ENV/JM/MONO(2019)27 Series on Testing & Assessment No. 308 for the case study to put the following points into context.

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

- The IATA approach is well developed, with clear explanation. The uncertainty of category justification and prediction by read-across is thoroughly described. Therefore read-across is convincing and the uncertainties are low.
- The rationale for IATA and read-across is supported by metabolism studies and knowledge of the role of active metabolites is a strong point.
- *In vivo* data are available for 7 chemicals which are used for a read-across for 10 substances; thus the basis of data is sound. However, this is also supported by consideration of *in silico* data, *in vitro* data and the coherence between *in silico, in vitro* and *in vivo* results.
- The use of quantitative read-across to derive NOAELs.
- Use of the Hazard Evaluation Support System Integrated Platform (HESS)[4] database and a rat cellular metabolism simulator, with a custom filter to search for

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

[4] Hazard Evaluation Support System Integrated Platform, National Institute of Technology and Evaluation (NITE). https://www.nite.go.jp/en/chem/qsar/hess-e.html

relevant compounds/metabolites, in order to build the category of similar chemicals.

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

- It was requested to include information on the testicular toxicity of ethoxyacetic acid. The author provided the available toxicity information on ethoxyacetic acid in hypothesis section even though it is limited in comparison to methoxyacetic acid.
- Two substances containing an aromatic moiety were removed from this study because they are structurally diverse from other members and not enough information is available to demonstrate that other possible metabolites or the parent structures do not have different mechanism of action and impacts on the toxicity levels.
- In response to the requests from reviewers, the author provided a summary data of available metabolism studies including metabolism maps.
- The data matrix table for EGME and related chemicals from HESS were revised by adding related chemicals such as Ethylene glycol propyl ether (EGPE), methoxy- and ethoxyacetic acid and including information on the experimental study quality (test guideline and GLP compliance).
- The uncertainty regarding the MOA/AOP was added to the table describing uncertainty for the category justification and prediction by read-across.
- Additional information such as EU Harmonised Classification data for 10 category members and information on possible MoA/AOP of EGME-induced testicular toxicity was provided.

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

- The most relevant area of uncertainty is that caused by more complex substances which contain an EGME or methoxy-acetic acid as only one of the moieties. There are uncertainties regarding if the remaining metabolites from disparate moieties may also contribute to the overall toxicity. However, the author provided additional information to show that varying components other than the common core structure (e.g. dicarboxylate metabolites and alpha–beta unsaturated double bond moiety) are not likely to cause testicular toxicity based on available toxicity data of several substances.
- Uncertainty regarding the MoA/AOP was additionally mentioned. The investigation in this case study was largely based on the metabolism leading to a toxic moiety. Knowledge of the exact mechanisms is not crucial for grouping and does not affect the conclusions. Metabolic formation of methoxy- or ethoxyacetic acid is sufficient.

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

- ***Australia:*** The results of this study could be very useful in our regulatory context once the AOP has been endorsed by the OECD.
- ***Canada:*** A very useful approach for category building based on metabolism. Prefer a slightly more restrictive category with fewer but more similar members.

- ***Germany:*** The results could be used for screening purposes and could also be used for mixture toxicity of groups of chemicals with a common metabolite.
- ***Netherlands:*** Possibility to use for read-across for Classification and Labelling under REACH
- ***Sweden:*** Yes. Similar data/assumptions has recently been used in the EU within the CLP regulation (Classification, Labelling and Packaging) for classification of Tetraglyme as a reproductive toxicant.
- ***Japan (author):*** This case study was intended to address how read-across assessment can be applied to screening assessment under the CSCL. If similar grouping will be performed for developmental toxicity, it is possible to use the combined results for regulatory purpose in the near future.
- In addition, the participants at the project meeting expressed that this category approach in the case study will be useful for the assessment chemicals in their regulatory context, such as assessment for mixtures.

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

- Guidance for evaluating the reliability/robustness of data including TK/TD data
    - Similarity of metabolic pathways
    - The degree to which differences in the structure of target chemicals, beyond the moiety of interest, would have a significant impact on the similarity of a metabolic pathway

3.2. Case Study 2: Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Estrogen Receptor Active Chemicals

This case study was developed to evaluate potential estrogenic activity of environmental chemicals. The IATA presented in this document is consistent with a defined approach (DA) and describes an integrated testing strategy (ITS) for the identification of endocrine disruption by a substance primarily for the purposes of screening and prioritisation without the use of animal testing. The data interpretation procedure (DIP) is designed to provide information on whether the substance tested may act as an agonist of estrogen receptor activation. The combination of test methods used covers multiple key events (KEs) of the pathway leading to Estrogen Receptor (ER) agonist activity. The prediction model combines results from at least four *in vitro* assays designed to address different KEs of the pathway with different technologies to determine the final classification. Computational model classifications were derived for 1812 substances, and model performance was compared to high quality *in vitro* and *in vivo* data for specific reference chemicals. Depending on the combination of tests used, this prediction model generally achieved accuracies of 84 to 93%, as compared to reference chemical data from US EPA's Endocrine Disruptor Screening Program (EDSP) [5]Tier 1 guideline *in vitro* tests and uterotrophic assay

[5] US EPA's Endocrine Disruptor Screening Program (EDSP) https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-overview

(OECD TG440[6]). These results compellingly verify the applicability of this testing strategy as an IATA for identification of estrogenic chemicals.

Please refer to ENV/JM/MONO(2019)28 Series on Testing & Assessment No. 309 for the case study to put the following points into context.

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

- Use of a defined approach including ITS and DIP based on AOP/mechanistic understanding.
- Combination of multiple *in vitro* high throughput screening assays covering molecular initiating events (MIE) and multiple key events (KEs) of the ER pathway.
    - As few as 4 *in vitro* assays can accurately predict the estrogenic potential of a chemical, as long as they probe diverse points in the ER pathway and, human proteins and cell types.
    - There is flexibility in constructing the integrated battery of *in vitro* assays
    - A computational model contributes to WoE and increases confidence in the ER pathway model developed.
    - Extension of the applicability domain of single assays by integrating multiple assays that encompass multiple steps of the toxicity pathway.
- Detailed explanation of the objectives, methodology (e.g. *in vitro* assays and computational modelling) and analysis of uncertainties and limitations, with helpful figures illustrating the approach and detailed *in vitro* assays in Annex. In particular, the authors identify specific OECD TGs that this IATA can replace, and they have developed an approach that is user-friendly (in that expert knowledge is not required for data interpretation).
- The potential to screen large number of chemicals for ER agonist without animal testing.
- Comparison to the *in vitro* and *in vivo* reference set chemicals is strongly supporting the model as are the more than 1800 chemicals run through the HTS assays.
- The peer-reviewed publications of model and performance as well as the availability of data.

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

- The relationship of MIE/KEs/Key Event Relationships (KERs) with the *in vitro* assays was improved in the report.
- A flowchart was added in order to demonstrate how this IATA can be applied for rapid screening for ER agonist activity.

[6] OECD Test No. 440. Uterotrophic Bioassay in Rodents: A Short-Term Screening Test for Oestrogenic Properties. https://doi.org/10.1787/9789264067417-en .

- It was clarified that a subset of as few as 4 assays can be used as an alternative approach to the current guideline (OECD TG493[7], TG455[8] and TG440[6]) and that the subset of assays can be any assays that address different key events of the ER pathway and that incorporate different technologies. In addition, an Annex was added to describe the 9 subset models with 7 or fewer assays that achieve ≥94% balanced accuracy for all chemicals and the *in vitro* and *in vivo* reference chemical sets.
- Benefits of using a subset of assays in this case study was articulated, specifically the flexibility it gives users by allowing the use of any assays that fit the described criteria (as long as there is at least one an assay representing each KE).
- In response to the reviewers comments, the following changes were addressed:
    - More details of the *in vitro* assays
    - More detailed figure descriptions
    - Adding qualitative uncertainty analysis

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

- Technical aspects of the assays:
    - Domain of applicability of the assays (e.g. chemical compatibility with vehicle).
    - Lack of metabolic competence in the assays (e.g., no detection of bioactivated ER agonists). The author mentioned that further research is ongoing to address the issue on the lack of metabolic competence of the assays.
- Simplified assays for inferring integrated physiological responses. However, the data from multiple assays and technologies were used, which allows for detection of false positives and a more confident assessment of the test chemical's "true" *in vitro* ER bioactivity.
- There is uncertainty regarding the reliability of the *in vivo* data, whether or not the studies were conducted as GLP as per OECD recommendations for safety assessment of chemicals.

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

- ***Australia:*** Yes, the approach described in this study regarding screening and prioritising of chemicals for potential ER activity would be useful in our regulatory context. However, the application of the approach as such may be limited by the

[7] OECD Test No. 493: Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) *In vitro* Assays to Detect Chemicals with ER Binding Affinity. http://dx.doi.org/10.1787/9789264242623-en

[8] OECD Test No. 455: Performance-Based Test Guideline for Stably Transfected Transactivation *In vitro* Assays to Detect Estrogen Receptor Agonists and Antagonists. http://dx.doi.org/10.1787/9789264243040-en

resources required and the lack of the ability to recommend a hazard classification for these chemicals.

- ***Canada (Health Canada):*** Yes, it is possible that this model could be used in a weight of evidence approach to chemical risk assessment but this case study would likely not be used to satisfy current data requirements under the Health Canada New Substances Notification Regulations (NSNR).
- ***Canada (Environment and Climate Change Canada):*** Yes. Information on the ER agonist activity (based on QSAR model results), assuming conservation of pathways across species, has been used in the ecological prioritisation of organic chemicals in the third phase of the Chemicals Management Plan (ECCC 2016). In addition, bioactivity demonstrated in ToxCast assays was considered as supporting information to identify chemicals characterised by a reactive mechanism(s) of action for the CMP3 eco-prioritisation purpose. Similar efforts are being undertaken for post 2020 ecological prioritisation. Therefore, results of this case study could potentially be used to generate additional line(s) of evidence in both prioritisation of chemicals and in risk assessment.
- ***Germany:*** Yes, for prioritisation and probing if a chemical could be estrogenic from a mechanistic perspective.
- ***Netherlands:*** Yes, for triggering further testing. The results, however, cannot be used as the only evidence for identification of endocrine disruptors.
- ***EU/JRC:*** Yes, the ToxCast ER Bioactivity Model has been included for the E-Modality (as equivalent to OECD TG440[6]) in the strategy to investigate EATS-related endocrine activity described in the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (ECHA, EFSA 2018).
- ***United States (author):*** Yes. As noted in the case study, this work has been accepted by the US EPA as an alternative to accepted guidelines studies as alternatives to some EDSP Tier 1 testing (binding TG 493[7], ERTA TG 455[8], uterotrophic TG 440[6]).

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

- Guidance on how to develop integrated testing strategies (ITS) and data interpretation procedures (DIP).
- Guidance on how to combine *in vitro* and computational information into the integrated report with the same level of transparency, including applicability domain of QSARs and *in vitro* assays.
- Guidance on how to report individual data sources for a variety of NAMs – omics, QSAR, *in silico*, etc.

4. LEARNINGS AND LESSONS

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

This chapter summarises learnings and lessons stemming from the case studies of the project including the thirteen case studies from the past three review cycles. Table 2 shows a summary of the 15 case studies reviewed up to date.

The assessment approaches illustrated by the case studies are classified into five types: data-gap filling by read-across based on grouping of chemicals (10 case studies), grouping of chemicals for cumulative risk assessment, not for read-across (Case Study 2016-2), safety assessment workflow (Case Study 2016-5), prioritisation of chemicals (Case Studies 2017-1, 2017-2) and defined approach (Case Study 2018-2). Although case study 2017-1 focused on a prioritisation scheme for potential endocrine active chemicals, it illustrated two systematic methodologies for identifying analogues with cheminformatics and data analysis tools. In addition, case study 2016-5 includes the read-across approach in the concept of a safety assessment workflow. For more information, case study 2017-3 illustrated the approach on read-across for nano-TiO_2 considering nano-specific properties, such as crystal type, surface coating and size.

The target endpoints of the case studies were: repeated dose toxicity (7 case studies), estrogenicity (2 case studies), neurotoxicity (1 case study), mutagenicity (1 case study), bioaccumulation (1 case study), genotoxicity (1 case study), ecotoxicity (1 case study) and reproductive toxicity (1 case study).

Every case study addresses some challenging topics related to IATA, including use of MOA/AOP (8 case studies), capturing and communicating uncertainty (13 case studies), use of new approach methodologies (11 case studies) and low/no toxicity prediction (5 case studies).

Identified areas for further developing guidance from the 15 case studies are summarised in section 4.2.

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

Year-No. (Lead)	Assessment Approach	Endpoint	IATA Topics				Reference
			AOP[1]	UR[2]	NAM[3]	L/N[4]	
2018-1 (Japan)	Read-across	Reproductive toxicity	X	X			OECD, 2019a
2018-2 (US)	Prioritisation and screening	Estrogenicity	X	X	X		OECD, 2019b
2017-1 (Canada/US)	Prioritisation and hazard characterisation	Estrogenicity	X	X	X	X	OECD, 2018b
2017-2 (Canada)	Prioritisation of chemicals	Ecotoxicity	X	X	X	X	OECD, 2018c
2017-3 (JRC)	Read-across	Genotoxicity for nano-TiO_2		X	X		OECD, 2018d
2017-4 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2018e
2016-1 (Japan)	Read-across	Repeated dose toxicity		X	X		OECD, 2017b
2016-2 (US)	Grouping for cumulative risk assessment	Neurotoxicity	X		X		OECD, 2017c
2016-3 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2017d
2016-4 (ICAPO)	Read-across	Repeated dose toxicity		X	X	X	OECD, 2017e
2016-5 (JRC/BIAC)	Safety assessment workflow	Repeated dose toxicity	X		X		OECD, 2017f
2015-1 (Canada/US)	Read-across	Mutagenicity	X	X			OECD, 2016b
2015-2 (Canada)	Read-across	Repeated dose toxicity		X	X		OECD, 2016c
2015-3 (Japan)	Read-across	Repeated dose toxicity	X	X			OECD, 2016d
2015-4 (Japan)	Read-across	Bioaccumulation		X		X	OECD, 2016e

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

4.2. Update of the Identified Areas for Further Developing Guidance

In the past three review cycles, the following 9 areas for further developing guidance were identified as priority areas from the 13 case studies (OECD, 2016a, 2017a and 2018a). Note that although these are areas in which potential guidance could be developed, the intent is not to address all of these aspects within future OECD guidance documents, but rather note a potential need that has been identified.

1. Describing scope and context for read-across
2. Building hypotheses based on MOA/AOP
3. Definition of analogues/category boundaries
4. Justification of data gap filling
5. Incorporation of new approach methodologies
6. Decision logic for low/no toxicity predictions

7. Uncertainty analysis and reporting
8. Integrated conclusion
9. Reporting templates for IATA based on a building block approach

The following areas for further developing guidance were identified based on the review experience of the two case studies in the fourth review cycle:

1. **Guidance for evaluating the reliability/robustness of data including TK/TD data:** In Case Study 2018-1, the metabolic products play a key role in the hazard characterisation and evaluating the similarity of the metabolic pathway between the target chemicals is important for the hazard assessment in this case study. Therefore, the project members agreed that there is a need to evaluate the similarity of metabolic pathways and to evaluate the impact of the differences in the structure of target chemicals on the metabolic pathway.
2. **Guidance related to defined approaches:** Case Study 2018-2 is the first case study which includes a potential defined approach within the IATA. Therefore, the following new areas were identified for the development of further guidance, which can provide benefit for application or interpretation of the IATA.

    - Guidance on how to develop integrated testing strategies (ITS) and data interpretation procedures (DIP)
    - Guidance on how to combine *in vitro* and computational information into an integrated report with the same level of transparency, including applicability domain of QSARs and *in vitro* assays.
    - Guidance on how to combine NAMs and report individual information sources– omics, QSAR, *in silico*, etc

    Although this case study illustrates how to develop an integrated testing strategy (ITS) and data interpretation procedures (DIP), a reference document for the development of the ITS and DIP would be beneficial for consistency across defined approaches. The case study also reported information in combination using individual *in vitro* methods and a computational model. In general, it was expressed that there is a need to consider the applicability domains of both assays and computational models and transparently present both types of methods within an IATA. Finally, the project members discussed the importance of transparency of the individual methods (*in vitro*) in order to be able to understand the relevance and limitations of each method. Identifying the essential component to be described would be helpful to understand the individual *in vitro* method and information sources and its applicability within an IATA. However, this could already build upon the Guidance Document 255 on the Reporting of Defined Approaches and Individual Information Sources to be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation (OECD, 2016g).

    The summary of updated areas for further developing guidance incorporating the above issues is shown in Table 3 (the underlined and bold areas were added in the 2018 review cycle):

Table 3. Summary list of the areas for the development of further guidance

Areas for the development of further guidance	Related case study
1. Describing scope and context for read-across	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2017-3, 2017-4, 2018-1
a. Rationale for the selected endpoint	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2017-3, 2017-4, 2018-1
b. 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
2. Building hypotheses based on MOA/AOP	2015-1, 2015-3, 2016-2, 2016-5, 2017-1, 2017-2, 2018-1, 2018-2
a. Hypothesis for category formation that includes the use of omics data	2016-1
3. Definition of analogue/category boundaries	2015-2, 2015-3, 2015-4, 2016-1, 2016-2, 2016-3, 2016-4, 2017-1, 2017-3, 2017-4, 2018-1
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
4. Justification of data gap filling	All case studies
a. Reporting of QSAR prediction results	2015-1, 2015-4, 2016-1, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-4, 2018-1
b. How much to report on reliability	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2017-3, 2017-4, 2018-1, 2018-2
c. Use of New Approach Methodology (NAM) data, TTC approach and PBPK models (e.g. How to integrate NAM data – for example via linking to mechanistic relevance (interpretation))	2015-2, 2016-1, 2016-2, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4, 2018-2
d. Guidance for describing New Approach Methodology 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
e. Decision logic for low/no toxicity predictions	2015-4, 2016-3, 2016-4, 2017-1, 2017-4
f. Guidance on when *in vitro* data could be further generated to support read-across	2015-2, 2016-1, 2016-3, 2016-4, 2017-4, 2018-1
g. 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
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
a. Exposure route, including route to route extrapolation	2015-4, 2016-5, 2017-1, 2017-4
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
d. Guidance for evaluating the reliability/robustness of data including TK/TD data ● **Similarity of metabolic pathways** ● **Whether differences in the structure of target chemicals would have any significant impact on the metabolic pathway**	2015-1, 2015-2, 2015-3, 2016-1, 2016-3, 2016-4, 2017-4, 2018-1
e. Reporting of uncertainty of read-across (e.g. Ranking of uncertainty vs descriptive analysis/ quantitative vs qualitative analysis)	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2017-3, 2017-4, 2018-1
f. Consider approaches in: AOP handbook (OECD, 2016h) and scientific papers (Wu et al., 2010; Blackburn & Stewart, 2014; Schultz et al., 2015)	2015-1, 2015-2, 2016-3, 2016-4
g. Uncertainty framework (Overall uncertainty in the assessment resulting from the combined uncertainties of the different IATA components and data types)	2015-1, 2015-2, 2015-3, 2015-4, 2016-1, 2016-3, 2016-4, 2016-5, 2017-1, 2017-2, 2017-3, 2017-4, 2018-1, 2018-2
6. Integrated Conclusion	All case studies
a. Combining approaches/methodologies for predicting bioaccumulation	2015-4
b. Integrating QSAR 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

	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
	d. 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
7.	Others	
	a. Relevance of change in pH to prediction of degradation products (e.g. in the environment)	2015-4
	b. UVCBs, multi-constituents coverage (composition coverage, methodology and other)	2015-2, 2017-2
	c. Level of detail needed in case studies according to the defined purpose	All case studies
	d. How to include data on/predictors for metabolism when building IATAs according to the defined purpose	2015-2, 2015-3, 2017-4, 2018-1
	e. How to describe the rationale for justification of the BMD and point of departure used	2016-2
	f. Reporting template for IATA based on a building blocks approach	2016-2, 2016-5, 2017-1, 2017-2, 2018-2
	g. Guidance on developing prioritisation scheme based on IATA	2017-1, 2017-2
	h. 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
	i. Guidance on how to develop integrated testing strategies (ITS) and data interpretation procedures (DIP)	2018-2
	j. Guidance on how to combine *in vitro* and computational information into an integrated report, including applicability domain	2018-2
8.	Areas related to other working party	
	a. Nanomaterials (NMs) *1 • Standardised guidelines for characterisation and testing of NMs, • NM-specific and relevant protocols • Increase data availability/quality	2017-3
	b. Guidance on the interpretation of NM-related data *1	2017-3
	c. 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*

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

This section describes the learnings gained through the review experience of the two case studies in the fourth review cycle in 2018. The case studies in the fourth review cycle addressed the two main areas: read-across for reproductive toxicity and a defined approach to identify potential endocrine disruptors. In addition, at the meeting, an analysis of the read-across case studies within the IATA Case Studies Project was discussed, which aimed at reviewing the issues of uncertainty in read-across with a complex health endpoint, including the sources and types of uncertainty. The following four topics are discussed in this section:

1. Use of metabolism information in read-across
2. How to use information from an AOP under development (status on AOP-wiki[9])
3. Use of combinations of high-throughput screening assays and computational models in Defined Approach/IATA, including documentation.

[9] AOP-wiki https://aopwiki.org/

4. The analysis of the uncertainties in all of the read-across case studies in the IATA Case Studies Project.

4.3.1. Use of metabolism information in read-across

As mentioned at the section 3.1, Case Study 2018-1 addresses read-across based on common metabolites, which play a key role in the hazard characterisation. As a result of the discussion, the following issues were identified to use metabolism information in read-across:

- How to assess the similarity in a metabolic pathway?
- Impact of structural variation of analogues when a smaller core structure is the moiety of toxicological interest
- How to consider other toxicological endpoints of the metabolites other than the target endpoints?

To discuss the above issues, Table 4 shows the 4 case studies identified as case studies which use metabolic information for defining the target effect.

Table 4. Case studies using metabolic information for definition of the target effect

Case Study	Target Chemical	Key Metabolites	Target endpoints	Reference
2015-1	Azo direct dyes containing the 3, 3' dimethoxybenzidine (DMOB)	DMOB	Mutagenicity	OECD, 2016b
2015-3	Allyl Esters	Allyl alcohol	Hepatotoxicity	OECD, 2016d
2017-4	Simple aryl alkyl alcohols carboxylic esters	Phenyl alkyl alcohol Saturated carboxylic acids	Repeated dose toxicity	OECD, 2018e
2018-1	Ethylene Glycol Methyl Ether (EGME)-Related Chemicals	EGME or ethyl ether (EGEE) Methoxy- or ethoxyacetic acid	Testicular toxicity	OECD, 2019a

How to assess the similarity in a metabolic pathway

The types of data used to consider the similarity in a metabolic pathway across substances for the four case studies are summarised in Table 5. Generally, the available data from *in vivo* and *in vitro* studies were used as evidence to support the metabolic pathway of some of the category members in the case studies. *In silico* methods, such as prediction by metabolic simulators, were used as supportive information to illustrate that the category members without empirical data have the possibility to be metabolised through the same metabolic pathway.

Table 5. Assessment of similarity in metabolic pathway

Case Study	Metabolic pathway to produce key metabolites	Toxicokinetics data used for assessment	Uncertainty
2015-1 (Canada)	Azo reduction	*In vivo* (2 category members) *In vitro* (1 category member) *In silico* (all category members without empirical data)	There is uncertainty with respect to metabolism for the category members without empirical data due to the model domain issues.
2015-3 (Japan)	Hydrolysis	*In vivo* (3 category member) *in vitro* (6 category members) *in silico* (all category members without empirical data)	The influence of the degree of branching and structural variation on enzymatic hydrolysis is not clear.
2017-4 (ICAPO)	Hydrolysis	*In vivo* (1 category member) *In vitro* (1 category member) *In silico* (all category members and hydrolysed product (alcohol)) *in vivo* and *in vitro* data were used for the hydrolysed products	Hydrolysis is a universal metabolic step.
2018-1 (Japan)	Group 1, 3: Hydrolysis Group 2 :Oxidation	*In vivo* (2 category members) *In vitro* (3 category members) *In silico* (all category members without no empirical data) Supplemental data from *in vivo* and *in vitro* data of 5 analogue chemicals for the category members of group 1 without empirical data. Five analogue chemicals were excluded from category members based available 1 metabolic data of their analogue chemical.	For group 1 and 3, uncertainty seems to be low based on available metabolic data of varied structures. For group 2, it is likely that members 12 and 13 produce EGME or EGEE as a minor product although exact quantitative estimation of the production is difficult.

In Case Study 2018-1, available metabolic data from *in vivo* and *in vitro* studies for chemicals outside the categories, but which were nonetheless structural analogues, supported the argumentation that the category members would be metabolised through same metabolic pathway such as hydrolysis or oxidation. This contributed to the increase in the reliability of the metabolic assessment for the category members without empirical data. In addition, it was explained by using the metabolic data of a structurally similar chemical why 5 structurally similar chemicals were excluded from the category. The metabolic data suggested that these five chemicals are probably metabolised through a different metabolic pathway. This provides transparency as to how to build the category and helps with reducing uncertainties relevant to the toxicokinetic similarity.

Impact of structural variation of analogues when smaller core structure is moiety of toxicological interest

In the considerations document of the second review cycle, it was described that five case studies (2015-2, 2015-3, 2016-1, 2016-3 and 2016-4) addressed how to explain the impact of structural differences on toxicity (OECD, 2017a). The differences in substituents, chain length and degree of branching impact on kinetics (e.g. bioavailability and reactivity), mode of action (e.g. transcriptomic profiles) and toxicokinetics (e.g. hydrolysis). As shown in Table 6, the four case studies discussed here highlighted the impact of structural variation of analogues on bioavailability, toxicokinetics and toxicity.

Table 6. Impact of structural variation of analogues

Case study	Structural variation of analogues	Possible impact of structural variation
2015-1 (Canada)	Substituent including one or more sulfonate sodium or lithium salt	• Bioavailability Key physico-chemical properties (pKa, LogD, water solubility) • Toxicokinetics
2015-3 (Japan)	Chain length and degree of branch	• Toxicokinetics (Hydrolysis rate) • Toxicity (Differences in toxicity levels)
2017-4 (ICAPO)	Chain length and degree of branch	• Toxicokinetics (Hydrolysis rate) • Toxicity (Differences in toxicity levels)
2018-1 (Japan)	Substituent (varied ester or ethers)	• Bioavailability • Toxicokinetics • Toxicity (other endpoints)

In general, substituent, chain length and degree of branch impact on the relevant physico-chemical properties for bioavailability, such as logKow and molecular weight. The impact on bioavailability may have an effect on the toxicity level or the target organ. In this case, in order to confirm that the structurally varied analogues are bioavailable, it is important to confirm the relevant physico-chemical properties. In Case Study 2018-1, all chemical category members are rationalised to be bioavailable because the physico-chemical parameters of all chemicals in the category fall within certain range (log Pow: -1.0-1.5 and MW: 76-262).

In addition, structural variation can impact on the quantitative toxicokinetics to produce the key metabolites. This may have an effect on the toxicity level. For example, as described in Case Study 2017-4, the rate of hydrolysis is affected by the degree of branching and the carbon chain length. However, it is difficult to estimate the key metabolites quantitatively.

These examples illustrate the importance of considering the impact of structural variation beyond the moiety of interest and questioning if it could contribute to changes in the toxicological profile of the substance for the endpoint of interest. These considerations might lead to a decision not to include the substance in a particular read-across category.

How to consider other toxicological endpoints of the metabolites other than target endpoints

A principle of conducting read-across is that it should be endpoint specific (OECD, 2014a). However, information on non-target endpoints which are related to the target endpoint can also be helpful for category definition and support toxicological similarity assessment. Table 7shows other toxicological endpoints noted in the case study that were induced by the key metabolites in the four case studies. Although other toxicological endpoints were identified in the two case studies (2015-3 and 2018-1), they were not considered because they were not relevant to the target endpoints. For example, in Case Study 2018-1, testicular toxicity was defined based on weight loss, atrophy and degeneration of testis caused by key metabolites. It was suggested that data on mutagenicity could be considered as supportive information for the testicular toxicity.

Table 7. Case Studies whose metabolites induced the target endpoints

Case study	Target endpoint	Metabolites	Other toxicity (Reason not be considered as target effect)
2015-1 (Canada)	Mutagenicity	DMOB	-
2015-3 (Japan)	Hepatotoxicity	Allyl alcohol	Forestomach hyperplasia (non-systemic toxicity)
2017-4 (ICAPO)	Reduced body weight gain	Phenyl alkyl alcohol Saturated carboxylic acids	-
2018-1 (Japan)	Testicular toxicity	Key metabolites EGME, EGEE, Methoxy- or ethoxyacetic acid	-
		Other metabolites alpha–beta unsaturated alkyl carbonic acid (Group 1)	Repeated dose toxicity in liver, kidney or spleen (No toxic effect in reproductive organ)

In the considerations document in the second review cycle (OECD, 2017a), it was described how to define the target effect as well as why the other observed effects were not considered as target effects in the case studies (2015-2, 2015-3, 2016-1, 2016-3 and 2016-4). The reasons why they were not considered as target effects included rationales for being secondary effects or non-systemic toxicity. In discussions on Case Study 2018-1 it was raised as to what other toxicological effects might arise from the resulting metabolites or if they also could contribute to the target effect, which would alter the quantitative read-across. To support the case study, data for alpha–beta unsaturated alkyl carbonic acid, which were a hydrolysis products of some target chemicals, illustrated that it induced repeated dose toxicity in liver, kidney or spleen but not reproductive toxicity. This information was used to support that non-key metabolites were not contributing the reproductive toxicity in this case.

4.3.2. How to use information from an AOP under development (status on AOP-wiki)

So far, there have been seven case studies using MOA/AOP information in the IATA Case Studies Project (Table 8). As described in the considerations documents in the first and second review cycles (OECD, 2016a and 2017a), MOA/AOP information is useful for building a hypothesis, forming a category and clarifying uncertainties of mechanistic key events. Case Study 2016-2 and 2016-5 reported that the information from an AOP under development was used to inform cumulative risk assessment and targeted *in vitro* testing, respectively.

In Case Study 2018-1, there are three potential mechanisms of testicular toxicity of methoxyacetic acid. For one mechanism, there is an AOP under development within the AOP-wiki. Although these mechanisms were not crucial to the read-across based on a common metabolite, they do provide support for the hypothesis for the grouping. The mechanistic information was useful to understand how the metabolites induce the target toxicity even if these AOPs have not been published.

Although information from AOPs under development may include some extent of uncertainty, available toxicological information for multiple group members, including data from *in vitro* and *in silico*, can increase the confidence in the use of an incomplete AOPs.

Table 8. Case Studies Using Information from an MOA/AOP

Case Study	MoA/AOP information	Status AOP-wiki	How to use AOP
2015-1 (Canada)	Released DMOB can be metabolically activated to electrophilic nitrenium and carbonium ions. The electrophilic metabolites can covalently bind to nucleophilic sites on DNA, through an SN1 substitution reaction. DNA binding is associated with DNA mutation (direct DNA acting mutagens).	No relevant AOP on the AOP-wiki	Hypothesis for the category
2015-3 (Japan)	Following the hydrolysis of allyl esters, allyl alcohol is readily oxidised to acrolein by ADH in the liver. Acrolein is a highly reactive substance that appears to cause hepatotoxicity. It readily forms an acrolein-GSH adduct, leading to GSH depletion, oxygen radical formation, and lipid peroxidation. Acrolein is also capable of reacting with cellular macromolecules nonenzymatically via Michael additions. Reactions with critical intracellular proteins and subsequent adduct formation are proposed as one component of the cytotoxicity of acrolein. Additionally, it has been proposed that oxidative stress subsequent to the loss of GSH may be related to mitochondrial dysfunction. These biochemical events caused by acrolein are believed to be associated with hepatocellular damage and death.	Endorsed by WPHA/WNT as AOP 38*1 and published as OECD Series on Adverse Outcome Pathway No.2: Adverse Outcome Pathway on Protein Alkylation Leading to Liver Fibrosis (AOP)*2	Hypothesis for the category
2016-2 (US)	OPs share the ability to bind and to phosphorylate the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems. When acetylcholinesterase is inhibited, acetylcholine accumulates and cholinergic toxicity results due to continuous stimulation of cholinergic receptors throughout the central and peripheral nervous systems which innervate virtually every organ in the body. Some OPs are active as the parent compound but some require activation to the oxon metabolite and both are considered in the cumulative risk assessment.	Under development as AOP 16: Acetylcholinesterase inhibition leading to acute mortality*3	Hypothesis for the category
2016-5 (JRC/BIAC)	The MIEs include PPARγ (peroxisome proliferator-activated receptor) activation and covalent protein binding, respectively. In the case of piperonyl butoxide (PBO), the broad screening results from *in silico* or *in vitro* HTS assays pointed to MIEs and effects within the steatosis AOP, i.e. binding of PPARγ, PXR (pregnane X receptor), activation of a subset of cytochrome P450s and biotransformation pathways, leading to potential liver toxicity. The fibrosis AOP was also considered as covering potential liver toxicity effects, supported for example by alerts for protein binding for PBO metabolites.	Steatosis AOP: Under development as AOP 34: LXR activation leading to hepatic steatosis*4 Fibrosis AOP: Endorsed by WPHA/WNT as AOP 38*1 and published as OECD Series on Adverse Outcome Pathway No.2: Adverse Outcome Pathway on Protein Alkylation Leading to Liver Fibrosis (AOP)*2	Confirmation of hypothesis based on collected *in vitro* test.
2017-1 (Canada/US)	The ER is a ligand-activated nuclear receptor, which means that it can translocate to the nucleus and modify the expression of a variety of genes when it is bound to estrogen. Since it is small and lipophilic, estrogen can enter the cell passively through the plasma membrane. Once inside the cell, estrogen binds to the ER in a specific binding pocket, thereby activating the receptor. Two activated ERs will then combine to form a dimer that will translocate into the cell's nucleus. Once in the nucleus, it will scan the DNA until it finds an estrogen response element (ERE; which is a specific sequence in the DNA to which the estrogen-bound ER dimer can bind). The ERE is associated with an estrogen-responsive gene, whose expression will be altered (typically increased) upon binding of the ER. These changes in gene expression will have a variety of effects on the immediate biology of the cell that can lead to effects in the organism as a whole.	No relevant AOP on the AOP-wiki	Formulating the approach
2018-1 (Japan)	Lactate is produced in Sertoli cells and utilised as the main energy substrate by developing germ cells through lactate-carrying monocarboxylate transporters (MCTs). Methoxyacetic acid is associated with inhibition of lactate production in Sertoli cell and transport to spermatocyte through lactate-carrying MCTs. Energy shortage results in spermatocyte disorders.	No relevant AOP on the AOP-wiki	Hypothesis for the category
	Methoxyacetic acid inhibits sarcosine dehydrogenase activity, leading to a decrease in the level of 5,10-methylenetetrahydrofolate pentaglutamate (5,10-	No relevant AOP on the AOP-wiki	

	CH_2-THF). Defective supply of 5,10-CH_2-THF in testis is also depicted as a possible cause of the disorders. Decreased nucleic acid synthesis coincide with developmental-phase selective appearances of methoxyacetic acid toxicity at pachytene spermatocytes.		
	Methoxyacetic acid inhibits histone deacetylase (HDAC) activity in spermatocytes, leading to histone hyperacetylation. Cell cycle disorder is induced by up-regulation of p21 expression in rapidly dividing germ cells, followed by apoptosis.	Under development as AOP 212: entitled histone deacetylase inhibition leading to testicular toxicity*5	
2018-2 (US)	Endocrine Receptor (ER) Pathway was developed based on the known biology of the estrogen receptor signaling pathway including reproductive, developmental and other health effects. The pathway includes molecular initiating event (i.e., receptor binding), and several key events (e.g., receptor dimerisation, DNA binding, Cofactor recruitment and cell proliferation)	Nonspecific AOP	Hypothesis for performing the approach

*1: https://aopwiki.org/aops/38
*2: https://www.oecd-ilibrary.org/environment/adverse-outcome-pathway-on-protein-alkylation-leading-to-liver-fibrosis_5jlsvwl6g7r5-en
*3: https://aopwiki.org/aops/16
*4: https://aopwiki.org/aops/34
*5: https://aopwiki.org/aops/212

4.3.3. Use of combinations of high-throughput screening assays and computational models in Defined Approach/IATA, including documentation

Case Study 2018-2 demonstrated the use of combinations of 16 high-throughput screening (HTS) assays and a computational model in the IATA for identifying the estrogen receptor active chemicals. 16 HTS assays were used to measure the MIE (i.e., receptor binding), and several KEs (e.g., receptor dimerisation, DNA binding, transactivation, gene expression, and cell proliferation) for potential ER activation. The concentration−response curves for all 16 assays were included in a computational ER pathway model.

The details of 16 HTS assays were described including the biological target. In addition, technology-specific interference that can be mistakenly interpreted as ER-specific activation was documented (e.g., chemicals that denature the receptor protein, are luminescent, are cytotoxic, etc.). Although each HTS assay may have technical interference, the ER pathway model can address this interference by deploying "orthogonal" assays which are used in combination to distinguish activity towards the intended target or pathway from non-specific activities.

The ER pathway model was described including the process for evaluation of the chemicals and reporting of results. The performance of the ER pathway model was evaluated by using the 40 *in vitro* reference chemicals (an overall balanced accuracy of 93%) and 43 *in vivo* reference chemicals (an overall balanced accuracy of 86%.).

Case Study 2018-2 demonstrated the importance of a DIP to integrate the HTS assays into one computational model. In addition, technical limitations in each HTS assays and the applicability domain of the computational model should be described in the documentation in order to understand what type of chemical can be applied to the model.

4.3.4. The analysis of uncertainties in the read-across case studies in the IATA Case Studies Project

Six selected case studies were analysed in order to review the issues of uncertainty for read-across for a complex health endpoint, report all sources and types of uncertainty previously identified in the cases studies and use the knowledge to assist in the development of

guidance on assessing uncertainty in read-across (Schultz T.W. et al, 2019). Six case studies with complex endpoint (for example 90day repeated dose toxicity studies) were chosen as shown in Table 9: four published OECD case studies, two other case studies from outside the OECD IATA Case Studies Project.

Table 9. Case Studies Analysed for Uncertainties in the Read-Across

Case Study	Title	Considered compound	Reference
2016-3 (ICAPO)	Case Study on the Use of Integrated Approaches for Testing and Assessment of 90-Day Rat Oral Repeated-Dose Toxicity for Selected n-Alkanols: Read-Across	n-Alkanols	OECD, 2017d
2016-4 (ICAPO)	Case Study on the Use of Integrated Approaches for Testing and Assessment of 90-Day Rat Oral Repeated-Dose Toxicity for Selected 2-Alkyl-1-alkanols: Read-Across	2-Alkyl-1-alkanols	OECD, 2017e
2017-4 (ICAPO)	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	Arylalcohol alkyl carboxylic esters	OECD, 2018e
2015-3 (Japan)	Case Study on the Use of an Integrated Approach to Testing And Assessment for Hepatotoxicity of Allyl Esters	Allylesters	OECD, 2016d
Outside project	Read-across for rat oral gavage repeated-dose toxicity for short-chain mono-alkylphenols	Short-chain mono-alkylphenols	Mellor CL et al. (2017)
Outside project	Read-across of 90-day rat oral repeated-dose toxicity: A case study for selected β-olefinicalcohols	β-Olefinicalcohols	Przybylak KR et al. (2017)

In the analysis, the definition of uncertainty used was that of the European Food Safety Authority (EFSA): "all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question" (EFSA, 2018). Twelve types of uncertainty in read-across were identified as follows, which were categorised into four main sources of uncertainty:

- Uncertainty related to the regulatory use i.e., the impact of the regulatory scenario
    1. Context of, and relevance to, the regulatory use
- Uncertainty related to the data for the endpoint under consideration for the source compound(s).
    2. Quality of the apical endpoint data
    3. The consistency and concordance in the effects and their severity
- Uncertainty related to the argumentation of the read-across including, but not limited to, data quality
    4. Hypothesis
    5. Mechanistic plausibility
    6. Strength or robustness of the supporting data sets
    7. Weight-of-Evidence
    8. Documentation and written evidence
- Uncertainty related to the justification of similarity between the target and source compounds
    9. Type of category / group

10. Toxicodynamic similarity
11. Similarity in chemistry
12. Toxicokinetic and ADME (absorption, distribution, metabolism and excretion) similarity

The most important areas of uncertainty identified in the case studies were regarded to be mechanistic plausibility, consistency and concordance in the effects and their severity, along with toxicokinetic similarity of analogues. It was mentioned that the context of, and relevance to, the regulatory use will influence the level of acceptance of the overall uncertainty related to application of specific read-across approaches.

A series of 30 questions was developed relating to the uncertainties identified (Table 10). They will help provide a greater understanding of uncertainties and guide read-across developers and assessors through their assessment.

Table 10. A Series of Questions to be Addressed on Evaluating Uncertainties in Read-Across (adapted from Schultz T.W. et al, 2019)[10]

Uncertainty in Read-Across	Questions that Need to be Addressed Regarding Uncertainty
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)?

[10] Schultz T.W., Richardz A.-N. and Cronin M. T.D. (2019) Assessing uncertainty in read-across: Questions to evaluate toxicity predictions based on knowledge gained from case studies. Computational Toxicology 9 (2019) 1-11. https://doi.org/10.1016/j.comtox.2018.10.003

5. Conclusion

Two case studies were reviewed in the fourth review cycle of the project in 2018. One of the case studies addressed read-across based on the metabolites which induced the target toxicological effect (i.e. testicular toxicity) and the other case study describes an IATA with elements similar to a defined approach for the identification of estrogen receptor active chemicals. The lessons and learnings gained from the review experience of the case studies by the project team demonstrate new possibilities for the use of IATA in various objectives and promote the application of IATA in the regulatory contexts of member countries.

Based on the review experience of the two case studies, new areas for further developing guidance were identified. Although the guidance for evaluating the reliability/robustness of data including TK/TD data has been already identified in previous review cycles, Case Study 2018-1 demonstrated the importance of such a similarity assessment of the metabolic pathway including how structural variation impacts on the metabolic pathway. Case Study 2018-2 addressed the area of using elements of a defined approach for the IATA. Therefore, elements of guidance related to an IATA based on defined approach was newly identified as area for further developing guidance.

Three consideration topics from the fourth review cycle were discussed: Use of metabolic information, information from an AOP under development and combinations of HTS assays and computational models. In addition, the analysis of uncertainties in the read-across case studies in the IATA Case Studies Project is described. It will provide a greater understanding of uncertainties and guide read-across developers and assessors through their assessment.

In summary, although there were only two case studies in the fourth review cycle, the considerations obtained from the case studies provided new knowledge on the application of the IATA concept. This knowledge will promote the acceptance of the IATA in the regulatory context of the member countries.

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

Annex 1. 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"

(N.B. The following template should not be viewed as a strict structure, but rather identifies the information that should be included in this type of case study. Depending on the specific case study additional information/(sub)section(s) may be required or particular subsections may not apply. The order of the (sub)sections of the template can be changed and two or more (sub)sections of the template can be merged, as necessary. The titles of a (sub)section can be changed as necessary. The template will be revised based on experience with use. A case study based on the template is expected to be assessed as stand-alone, thus needs to contain all necessary information and appropriate links for a detailed assessment.)

Abstract / Synopsis / Executive summary

(This section should provide a brief overview of the case study, including the objectives, concepts, methodologies, outcomes and conclusion in about 300 words.)

Introduction

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

Table of Contents

A. Purpose

a. Purpose of use

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

b. Target chemical(s)/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 (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.
- It is recommended to specify the analogues and justification for data gap filling, used for each addressed endpoint, in order to identify 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.
- For the complete list of parameters and more information on grouping of nanomaterials please, see "ECHA (2017), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals", 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: https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf

B . 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)]

- For an analogue approach, describe the characteristics 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].

- 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
    - Physical-chemical properties and other molecular description
    - Kinetics: Absorption, distribution, metabolism and excretion
    - Mode/Mechanism of action or adverse outcome pathways (MOA/AOP)
    - Chemical / biological interaction
    - Toxicological and epidemiological information, along with information from new approach methodologies (NAMs)
    - 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. And, 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.
- Describe how a data gap is intended to be filled for the purpose of read-across (the prediction model used - worst case scenario, regression etc.). Here it could also be justified as to why read-across is sufficient, and why further testing is not needed.

Tip

- Hypothesis needs to be described as a testable format.
- For the hypothesis that metabolite induces target effect, the effects induced by other metabolites other than the toxicant need to be considered (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):
    - 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 (2017), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals", 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:

  https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf

C. 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 2, 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 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.

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.

D. 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 the methods used for gathering the data for target and source chemicals/category members (e.g. selection criteria of the data, data source).

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", 5.2.4 "Step 3: Evaluate available data for adequacy." and 5.2.5 "Step 4: Construct a matrix of data availability" 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 detailed data as necessary (in case that the detailed data are used for the justification of the hypothesis). The appropriate degree of detail of the data should be considered in the context of the purpose of case study. Examples of reports of detailed data can be found in past IATA case studies[11].
- If data from non-guideline test methods are included, provide descriptions of the methods or links to sources that summarise the methods. The appropriate degree of detail of the description should be considered in the context of the purpose of the case study. A template for the description is available in an OECD guidance document (OECD, 2014c). Examples of description using the template can found in JRC EURL ECVAM Database service on Alternative Methods to animal experimentation (DB-ALM)[12] and U.S. EPA Toxicity ForeCaster (ToxCast™) Data[13].
- If QSAR data are included, provide the name, version, owner of the models used for deriving QSAR estimation data. QSAR models if not already described elsewhere should be reported using the QSAR Model Reporting Format (QMRF)[14], and individual predictions, if applicable, should be reported using the QSAR Prediction Reporting Format (QPRF)[15]. 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[16].

[11] http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm#casestudies

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

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

[14] https://community.oecd.org/docs/DOC-144256

[15] https://community.oecd.org/docs/DOC-144257

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

- If data derived from defined approaches of IATA are included, provide the descriptions of the defined approaches. A template for the description and case study examples are available in OECD guidance documents (OECD, 2016f; 2016g).
- Provide all available information for suitability regarding the defined purpose, including the data from *in silico*, *in vitro* and *in vivo*. 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 2.
- 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 2.
- Identify which elements drive the toxicity/endpoint.
- For category approach, describe the set of inclusion and/or exclusion rules that identify the boundaries within which reliable estimations can be made for category members. A broader consideration including mechanistic information, profiling computational methods, screening with non-standard *in vitro* tests should be given. Clearly indicate the boundaries of the category and for which substances the category does not hold 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 QSAR and alternative methods should be discussed based on the consistency between the estimation data and the experimental data of the source chemical(s)/category members.

Tip

- Reliability of each QSAR prediction result needs to be described in terms of the applicability domain of QSARs. For example, it can be discussed by the coverage of the fragments in the training sets (OECD, 2016e).
- It is recommended that every approach be described separately, e.g. if read-across, QSAR 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 explanations, 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 experimental information before considering QSAR 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

- It is recommended to describe which methodologies for measurements of the relevant
- Describe methods used for measuring the endpoints
- 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 (2017), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals", 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: https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf

E. 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 the data (e.g. applicability domain, type and quality) and assumptions used to develop the similarity rationale of the analogues/category members and uncertainty and confidence associated with the underlying data used for read-across from the source chemicals.
- The following is an example of reporting uncertainty (Please modify as appropriate and also it is recommended to describe what is not addressed.): Examples of the modified templates, which were used for past case studies, are shown in Appendix 1, 2 and 3. Also, refer to the case studies published in the past[17].

[17] http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm#casestudies

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

Tip

- When 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 the nanomaterials:
  - 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 the complete list of parameters and more information on grouping of nanomaterials please, see "ECHA (2017), Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals", 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: https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf

b. Integrated conclusion

- Provide the strategy used to fill the data gap and integrated conclusion of data gap filling, including description how the data gap is actually filled (e.g. average, most sensitive, similarity weighted, qualitative) In case of category approach, indicate proposed conclusion/value for each data gap. If prediction models were used, please describe the satisfaction with parameters related to the prediction.

- Give discussion of remaining uncertainties and how they might be addressed.
- Finally, provide a short conclusion wrapping up the outcome of the evaluation with linking to the given purpose.

References

Annex

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.

- Blackburn, K. and S.B. Stuard (2014) A Framework to Facilitate Consistent Characterisation of Read Across Uncertainty. Regulatory Toxicology and Pharmacology. Vol. 68, Issue 3, pp 353-62.

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

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

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

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

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

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

e This table is based on the framework by Blackburn and Stuard (2014).
f Analogue data set characteristics used for evaluating overall uncertainty of read across prediction.
g Description of the evaluation results of the analogue data set characteristics obtained by answering the questionnaire of the framework.
h Rank of overall uncertainty of read across prediction derived from the evaluation results of analogue data set characteristics (Low, Low to Moderate, Moderate, High) with the description of the reason.

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

The template was developed in the following framework and was used for the case studies 3&4 in 2016 of the project (OECD 2017d; 2017e).

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

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

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

Justification Parameter [a]	Data Uncertainty [b]	Strength of Evidence [c]	Comment [d]
Structural Similarity	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 for reporting from RAAF[18]

Assessment Elements for Scenario 1 (read-across based on hypothesis for (bio)transformation to common products

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

Assessment Elements for Scenario 1 (read-across based on hypothesis that different compounds have the same type of effects)

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

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

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 2. 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)"

(N.B. The following template should not be viewed as a strict structure, but rather identifies the information that should be included in this type of case study. Depending on the specific case study additional information/(sub)section(s) may be required or particular 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 change as necessary. The template will be revised based on experience with use).

Abstract / Synopsis / Executive summary

(This section should provide a brief overview of the case study, including the objectives, concepts, methodologies, outcomes and conclusion in about 300 words.)

Introduction

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

Table of Contents

A. Purpose

a. Purpose of use

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

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

b. Target chemical(s)

Provide the chemical descriptor common identifiers (including CAS number, name and composition including impurities) and chemical structure(s) of the target substance(s). In some case studies, target chemicals may be entire chemical classes or the IATA illustrated may be generic (SEE Canada ERC Case study 2017-2). Or if there are no specific chemicals, example chemicals can be used to illustrate the IATA (SEE Canada ERC Case study 2017-2).

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.*

B. Hypothesis for performing IATA

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

C. Approaches used (Potential Blocks for Inclusion)

- **AOP/MOA**: Description of potential mechanism(s) for the target chemicals to induce target endpoint toxicity. In particular, the graphical representation of the AOP would be helpful for the reader and key references. The tools in the AOP-KB should be referred to as appropriate (e.g. AOP wiki[19], Effectopedia[20] etc.). For AOPs that are not documented, consider the "Users' Handbook supplement to the Guidance Document for developing and accessing Adverse Outcome Pathways" (OECD, 2016h) - although an entire AOP description is not the purpose here.
- **Defined Approach**: If a defined approach is included use the template of "Guidance Document on the Reporting of Defined Approaches to be used within Integrated Approaches to Testing and Assessment" (OECD, 2016f).
- **Workflow:** If an IATA workflow is included, provide a schematic and explanation of the elements of the workflow including input, decision and exit points. If prioritisation is the goal of IATA workflow, provide an explanation of how to classify the hazard and exposure profiling and potential risk classification.
- **Read-across**: If a read-across is included, use elements of the template for IATA case studies on Read-Across.

D. Data/Information gathering

a. Data/Information

- Provide the methods used for gathering the data for target chemical(s) (e.g. selection criteria of the data, data source).
- Provide the data gathered using appropriate reporting format. The levels details for reporting the data should be considered depending on the purpose of the IATA.
- If data from non-guideline test methods are included, provide descriptions of the methods or links to sources that summarise the methods. The appropriate degree of detail of the description should be considered in the context of the purpose of the case study. A template for the description is available

[19] AOP Wiki. https://aopwiki.org/
[20] Effectopedia. https://www.effectopedia.org/

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)[21] and U.S. EPA Toxicity ForeCaster (ToxCast™) Data[22].

- If QSAR data are included, provide the name, version, owner of the models used for deriving QSAR estimation data. QSAR models if not already described elsewhere should be reported using the QSAR Model Reporting Format (QMRF)[23], and individual predictions, if applicable, should be reported using the QSAR Prediction Reporting Format (QPRF)[24]. A QMRF inventory is maintained by JRC that can be utilised as a resource of QMRFs and its reference number can be referred to the JRC QSAR Model Database[25].
- If the exposure elements are included, provide the methods used for the data generation (e.g. data source, exposure models/tools.)

b. Analogue chemicals.

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

E. Application of IATA

a. Summary of data

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

b. Application of IATA

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

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

[23] https://community.oecd.org/docs/DOC-144256

[24] https://community.oecd.org/docs/DOC-144257

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

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

c. Uncertainty

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

d. Strategy and integrated conclusion

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

References

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

Annex