Learnings and Recommendations from Four EU-ToxRisk Case Studies on Applying New Approach Methodologies Data to Support Read-Across

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Conflict of Interest Statement

- No conflicts of interest

Overview/Objectives

- To provide examples on how New Approach Methodologies (NAM) can be used in read-across
- To provide learnings and recommendations on
  - Reporting
  - Performance of NAMs
  - Weight of Evidence

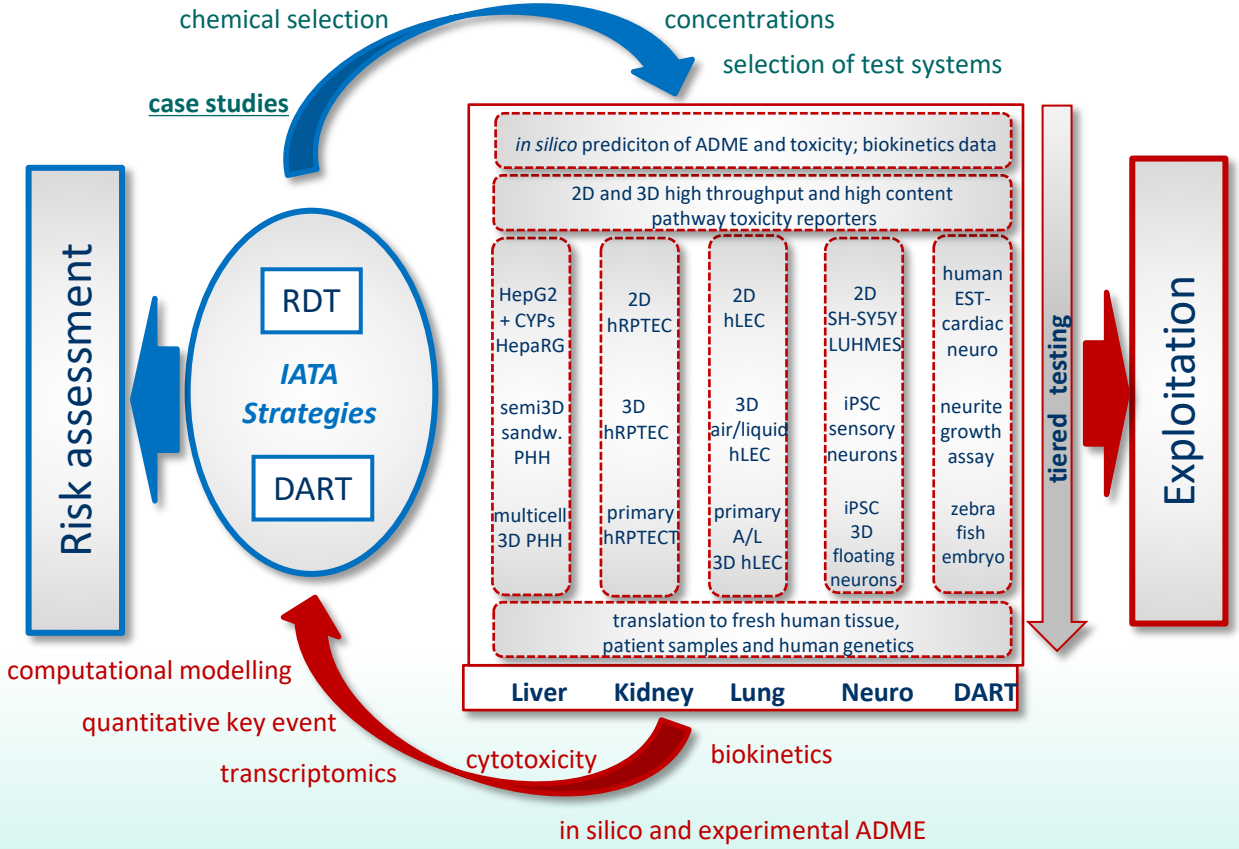
EU-ToxRisk

An Integrated **EU**ropean “Flagship” Program Driving Mechanism-based **Tox**icity Testing and **Risk** Assessment for the 21st Century

1. Improved **toxicological knowledge** and **quantitative understanding** of concentration- and time-response relationships for key **RDT and DART adverse outcomes** to be annotated in semi-q**AOP** and some qAOP.
2. **New robust Read-across procedures** incorporating toxicokinetics data and similarity evaluation on the level of KE activation, and **a complementary KE screen battery** to easily **fill data gaps of the Read-across procedure** and meet regulatory needs.
3. A validated set of **tiered IATAs** for ***ab initio*** RDT and DART assessment for regulatory use.
4. Novel validated ***in silico* tools** and ***in vitro* test systems** ready

….and more, see http://www.eu-toxrisk.eu/

Matrix


chemical selection
concentrations
case studies
selection of test systems
in silico prediciton of ADME and toxicity; biokinetics data
2D and 3D high throughput and high content pathway toxicity reporters
HepG2 + CYPs HepaRG
semi3D sandw. PHH
multicell 3D PHH
2D hRPTEC
3D hRPTEC
primary hRPTECT
2D hLEC
3D air/liquid hLEC
primary A/L 3D hLEC
2D SH-SY5Y LUHMES
iPSC sensory neurons
iPSC 3D floating neurons
human EST-cardiac neuro
neurite growth assay
zebra fish embryo
translation to fresh human tissue, patient samples and human genetics
Liver
Kidney
Lung
Neuro
DART
tiered testing
Exploitation
Risk assessment
RDT
IATA Strategies
DART
computational modelling
quantitative key event
transcriptomics
cytotoxicity
biokinetics
in silico and experimental ADME

Read-Across: Current Limitations

Read Across rarely accepted by regulatory authorities

- Based often only on structural & physicochemical data
  → no biological proof for similarity
- Lack of sufficient evidence to substantiate read-across justifications- fail to demonstrate toxicokinetic
- and toxicodynamic similarities
  → Including lack of data on analogues provided in dossier
-
- Lack of scientific plausibility
  → Disagreement with hypothesis, data not supportive of arguments presented, high uncertainty
  → coupled with lack of evidence

t4 report*

Toward Good Read-Across Practice (GRAP) Guidance

Nicholas Ball[1§], Mark T. D. Cronin[2§], Jie Shen[3§], Karen Blackburn[4], Ewan D. Booth[5], Mounir Bouhifd[6], Elizabeth Donley[7], Laura Egnash[7], Charles Hastings[8], Daland R. Juberg[1], Andre Kleensang[6], Nicole Kleinstreuer[9], E. Dinant Kroese[10], Adam C. Lee[11], Thomas Luechtefeld[6], Alexandra Maertens[6], Sue Marty[1], Jorge M. Naciff[4], Jessica Palmer[7], David Pamies[6], Mike Penman[12], Andrea-Nicole Richarz[2], Daniel P. Russo[13], Sharon B. Stuard[4], Grace Patlewicz[14], Bennard van Ravenzwaay[10], Shengde Wu[4], Hao Zhu[13] and Thomas Hartung[6,15]

How can NAM data fill the gap?

Basis for Learnings: Case Studies

Prediction of a 90 day Repeated Dose Toxicity Study for 2-Ethylbuturic acid using a read-across approach to other branched carboxylic acids

Read-across based filling of DART data-gap for methyl hexanoic acid using NAM

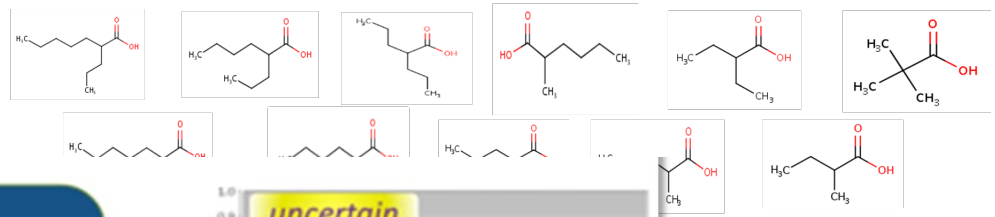
Mitochondrial complex-III-mediated neurotoxicity of azoxystrobin? Read-across to other strobilurins

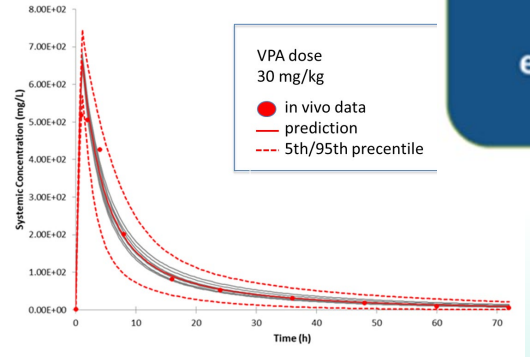
Identification and characterisation of parkinsonian hazard liability of deguelin by an AOP-based testing and read across approach

Case Studies – Examples of NAMs

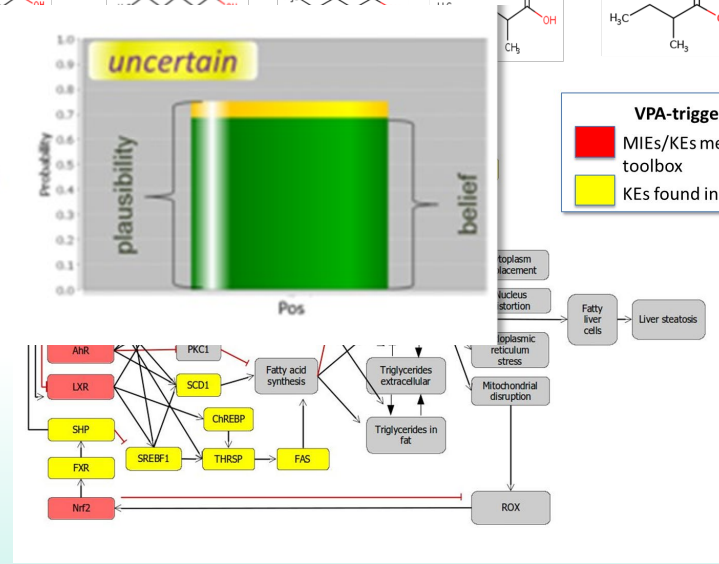
Prediction of a 90 day Repeated Dose Toxicity Study for 2-Ethylbuturic acid using a read-across approach to other branched carboxylic acids

2-Propyl heptanoic acid	2-Ethyl heptanoic acid	**2-Propyl hexanoic acid**	2-Ethyl hexanoic acid	Valproic acid	2-Ethylpentanoic acid	2-Methyl-hexanoic acid	2-Methyl-pentanoic acid	**2-Ethylbutyric acid**	2-Methylbutyric acid	Pivalic acid
2-PHP	2-EHP	**2-PHA**	2-EHA	VPA	2-EPA	2-MHA	2-MPA	**2-EBA**	2-MBA	PVA



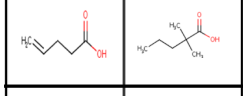
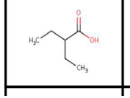
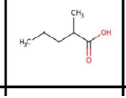
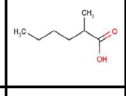
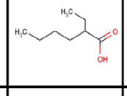
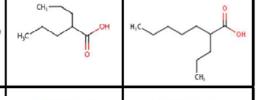
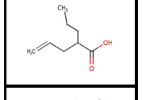


Combination of evidence



Case Studies – Examples of NAMs

Read-across based filling of DART data-gap for methyl hexanoic (MHA) [illegible] NAM

PA	DMPA	EBA	MPA	MHA	EHA	VPA	PHA	4-ene-VPA
								
						source	source	control
						+++	+++	++

Data integration statistical models

Dempster-Schaefer Theory approach

Bayesian Automatic Classification approach

Biological Fingerprint Classification approach

In vivo Ora

Assay	EBA				
In vivo	-				
ZET	61				
OED	*490-1060*				
UKN1	>4476				
OED	*>40-48*				
mEST	>2910				
OED	*>26-32*	*>25-30*	*2.7-2.8*	*3.8-4.1*	*0.21-0.27*
CALUX	1	1	1	9	8

)

ial differentiation) (ZET Reporter)

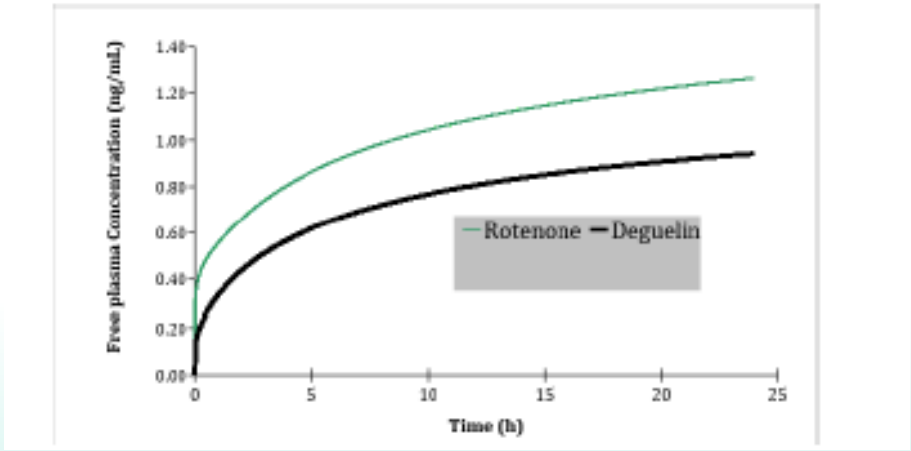
entiation model (UKN1)

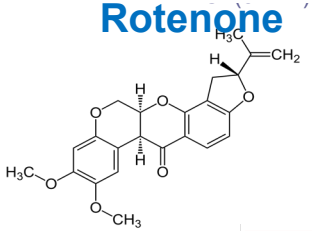
ifferentiation model (mEST)

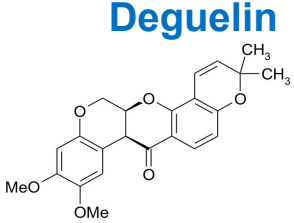
(CALUX)

Case Studies – Examples of NAMs

Identification and characterisation of parkinsonian hazard liability of deguelin by an AOP-based testing and read across approach







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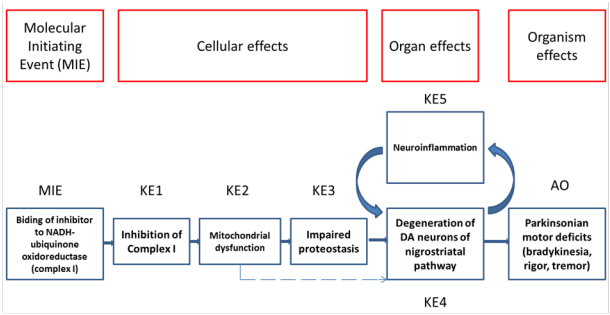
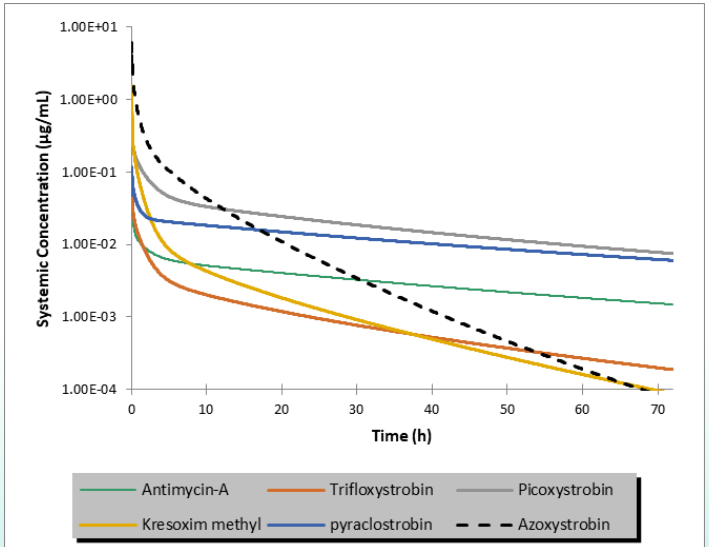


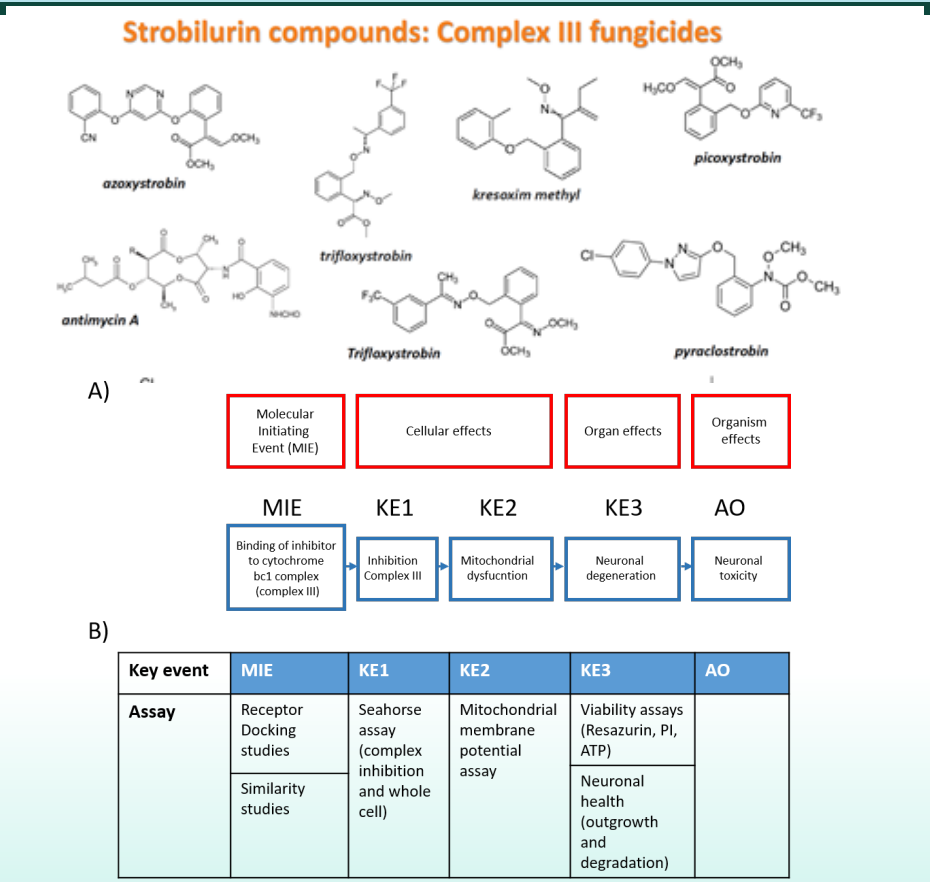
B)

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

Case Studies – Examples of NAMs

Mitochondrial complex-III-mediated neurotoxicity of azoxystrobin? Read-across to other strobilurins





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The EU Regulatory Advisory Board

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1. **Get the science right**
2. **Convince the regulators that you got the science right**
3. **Address the specific requirements of the regulatory process**

Aimed at Regulators

Template for Reporting Case Studies on Chemical Grouping (Read-across)

1 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.

2 Introduction

This should include a very short summary of the background/problem formulation, purpose, endpoints covered, as

Aimed at Scientist

Bench Marks!

Template for the Description of Cell-Based Toxicological Test Methods to Allow Evaluation and Regulatory Use of the Data

Alice Krebs[1,2], Tanja Waldmann[1], Martin F. Wilks[3], Barbara M. A. van Vugt-Lussenburg[4], Bart van der Burg[4], Andrea Terron[5], Thomas Steger-Hartmann[6], Joelle Ruegg[7], Costanza Rovida[8], Emma Pedersen[9], Giorgia Pallocca[1,8], Mirjam Luijten[10], Sofia B. Leite[11], Stefan Kustermann[12], Hennicke Kamp[14], Julia Hoeng[14], Philip Hewitt[15], Matthias Herzler[16], Jan G. Hengstler[17], Tuula Heinonen[18], Thomas Hartung[8,19], Barry Hardy[20], Florian Gantner[21], Ellen Fritsche[22], Kristina Fant[9], Janine Ezendam[10], Thomas Exner[20], Torsten Dunkern[23], Daniel R. Dietrich[24], Sandra Coecke[11], Francois Busquet[8,25], Albert Braeuning[26], Olesja Bondarenko[27], Susanne H. Bennekou[28], Mario Beilmann[29] and Marcel Leist[1,2,8]

1. Fulfills all requirements of GD211
2. Guides the user concerning the types of answers and detail of information required
3. Includes acceptance criteria for test elements
4. Defines the cells sufficiently and transparently

A database containing exemplary descriptions of more than 20 cell-based tests.

Template for the Description of Cell-Based Toxicological Test Methods to Allow Evaluation and Regulatory Use of the Data

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Regulatory Feedback

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OECD Home > Chemical safety and biosafety > Assessment of chemicals > Integrated Approaches to Testing and Assessment (IATA)

Integrated Approaches to Testing and Assessment (IATA)

WHAT'S NEW

The Integrated Approaches to Testing and Assessment (IATA) Case Studies Project allows countries to share and explore the use of novel methodologies in Integrated Approaches to Testing and Assessment within a regulatory context.

Two new cases have been published that focus on use of metabolism information to support read-across and use of a combination of novel methods to identify potential endocrine disruptors, respectively. In addition a considerations document includes lessons and learnings from analysis across case studies and an analysis of uncertainties in six read-across cases.

INTRODUCTION TO INTEGRATED APPROACHES TO TESTING AND ASSESSMENT (IATA)

EUTOXRISK

EU-ToxRisk workshop: New Approach Method (NAM)-supported Read-across: from Case Studies to Regulatory Guidance in Safety Assessment

May 21-22, 2019
Espoo, Finland

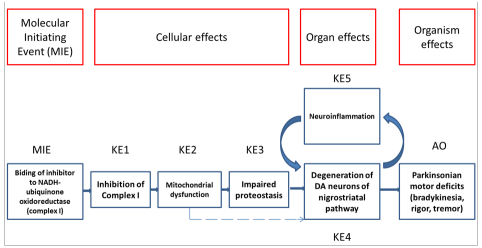
Co-organized with ECHA, EFSA, NTP, EPA, OECD, and SCCS

Learnings - AOPs

1. AOP-based testing strategy
   - Test all KEs?
   - Test KEs with different assays?

2. If no AOP available/AOP weak: Describe the scientific rational of the testing in more detail
3. Necessary to address other AOPs/MoAs relevant for the problem formulation

A)



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	Similarity studies			CHOP-GFP expression	Neuronal health (outgrowth and degeneration)	
Key event					KE5	
Assay					No assay available	

Overall Learnings

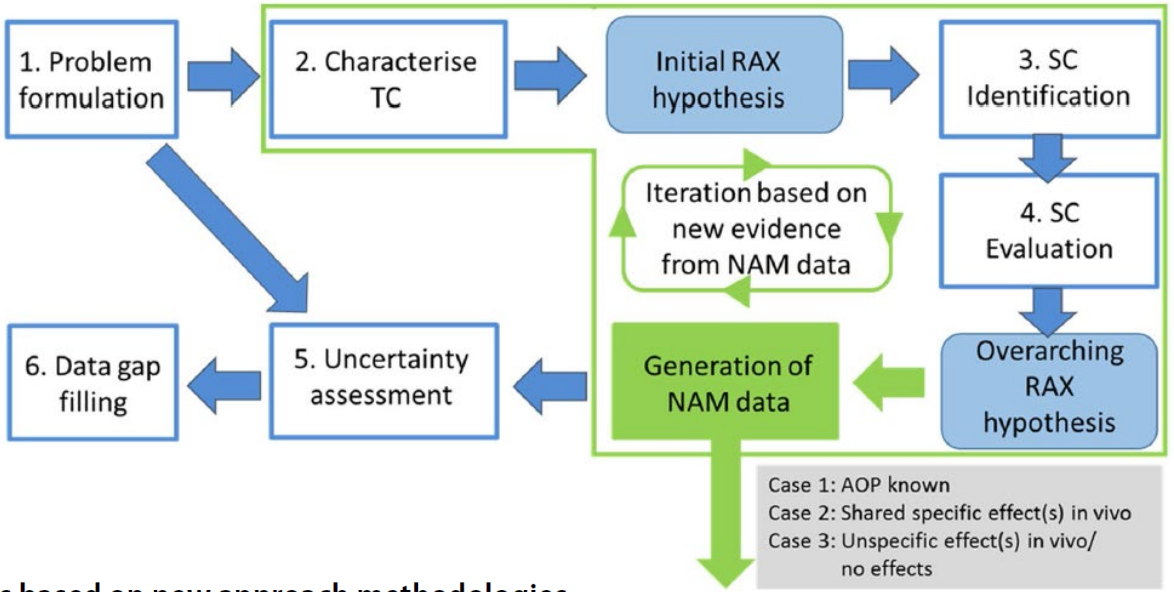
1. Helpful to include description of results with reference compounds even though they are not part of the Read-Across
2. PBPK modelling is useful – although there is still some skepticism
3. Useful to include compounds in a category approach which do not have *in vivo* data
4. An analogue approach based on one compound can be justified
5. Difficult to conclude that a category is adequate: structural similarity, number of source chemicals and their responses. In practice the number of source chemicals with *in vivo* data will be low
6. Case studies have shown some validity to biological read-across
7. Even with a reporting template tailored for the purpose – no consensus on the level of reporting
8. Situations where current *in vivo* models have limitations – easier to waive a study based on a clear mechanistic hypothesis
9. Validity of non-guideline test needs detailed description

Overall Learnings

Further Guidance would be helpful on

- On reporting of receptor docking/modelling
- How much evidence is needed to show low toxicity? -> A clear trigger can define space around a negative prediction,
- Data on reference compounds
- Does all KEs in an AOP need to be tested? Is It OK with just a few if they show a consistent pattern?
- Test method description, performance and validation – refer possibly to EU-ToxRisk Toxtemp (Krebs et al 2019) ?
- On how to integrate many lines of evidence? What integrating methodology to apply and when; When is a prediction from such methodology adequate?
- What does biologically RAX need to address?

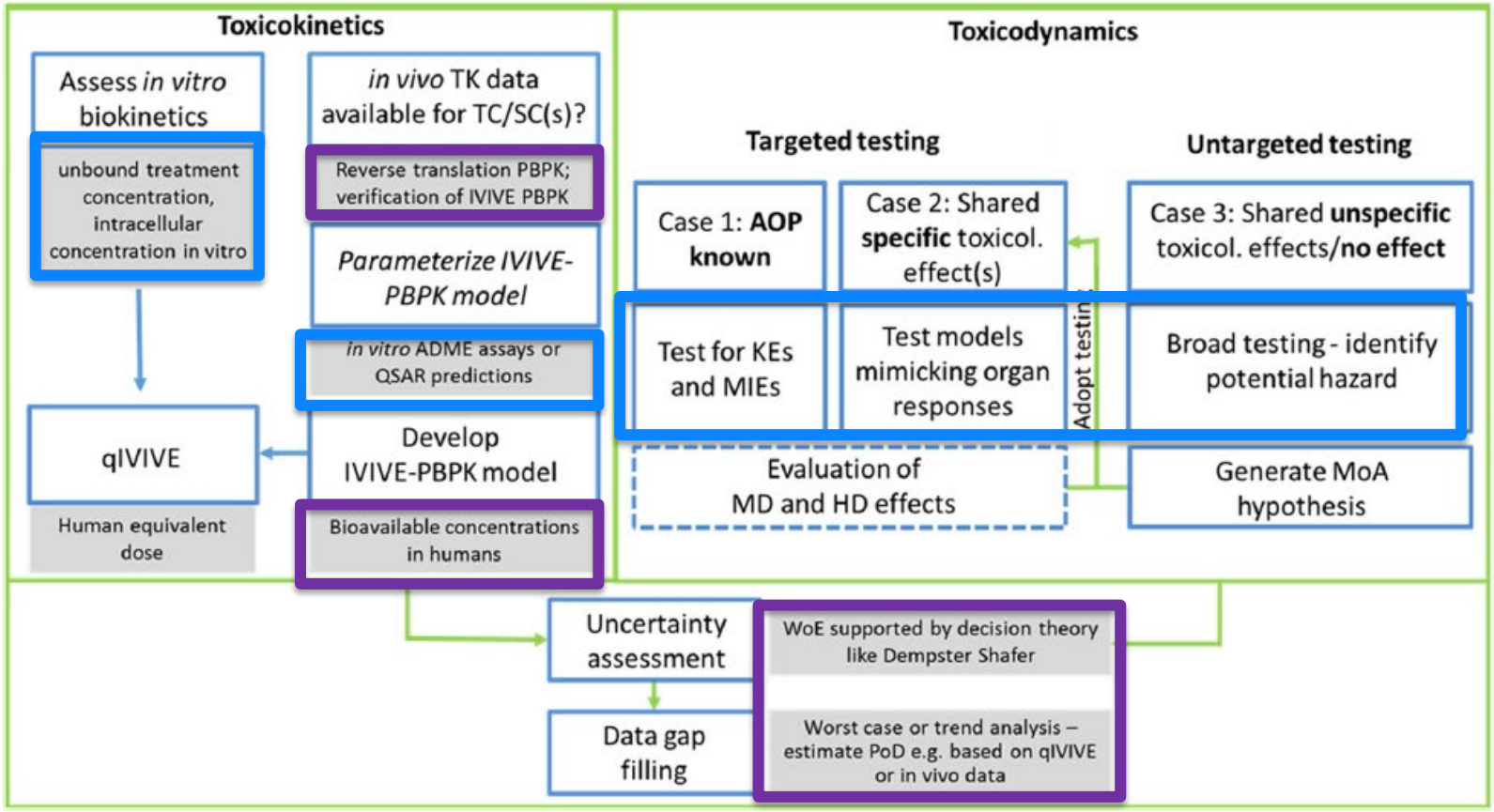
Read-Across Assessment Steps – the EUToxRisk Approach: Making use of New Approach Methodologies (NAMs)



Towards grouping concepts based on new approach methodologies in chemical hazard assessment: the read-across approach of the EU-ToxRisk project

Sylvia E. Escher[1] · Hennicke Kamp[2] · Susanne H. Bennekou[3] · Annette Bitsch[1] · Ciarán Fisher[4] · Rabea Graepel[5] · Jan G. Hengstler[6] · Matthias Herzler[7] · Derek Knight[8] · Marcel Leist[9] · Ulf Norinder[10] · Gladys Ouédraogo[11] · Manuel Pastor[12] · Sharon Stuard[13] · Andrew White[14] · Barbara Zdrazil[15] · Bob van de Water[5] · Dinant Kroese[16]

Read-Across Assessment Steps – the EU-ToxRisk Approach



Experimental NAM data

In silico tools for integration of NAM data

Summary

For a successful r

- (Currently) no r
  - Regulatory a
- For application
  - Ensure trans
  - Explain met
  - Justify the r
    evidence
  - Integrate ha

ECD guideline testing
ods
entation
od data and scientific
limitations

- Advisory Document on read-across based on data from new approach methodologies (NAMs)"
- Publication of case studies on OECD web-site

References

- Escher SE et al. **Towards grouping concepts based on new approach methodologies in chemical hazard assessment: the read-across approach of the EU-ToxRisk project.** Arch Toxicol. 2019 Dec;93(12):3643-3667. doi: 10.1007/s00204-019-02591-7
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- Ball N et al. **Toward Good Read-Across Practice (GRAP) guidance.** ALTEX. 2016;33(2):149-66. doi: 10.14573/altex.1601251.
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Acknowledgements

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- Regulatory Advisory Board of EU-ToxRisk

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- Bob van der Water, Leiden University, Netherlands
- Dinant Kroese, TNO, Netherlands
- Hennicke Kamp, BASF, Germany

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