



SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Read-Across with Computational and *In vitro* Data

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Systems Toxicology Unit & EURL ECVAM

Conflict of Interest Statement

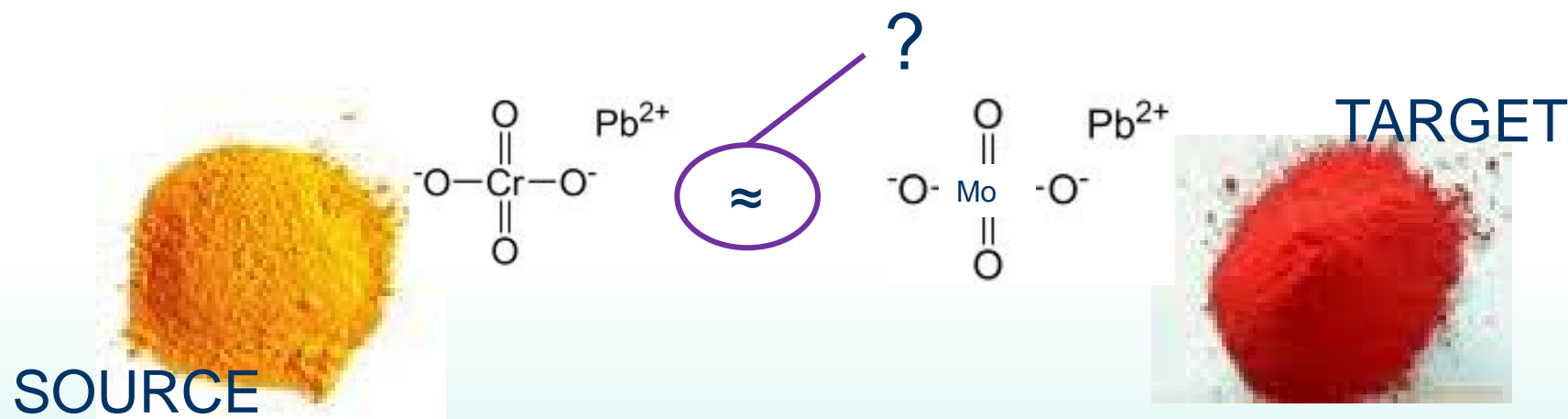
The SEURAT-1 research initiative as referred to in the presentation has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 267044, and has also received financing from Cosmetics Europe.



What is Read-Across?

Read-across is

- when the already available data of a data-rich substance (the source)
- is used for a data-poor substance (the target), which is considered similar enough to the source substance to use the same data as a basis for the safety assessment.



“Structured Expert Judgment” in read-across assessment framework

	TYPE	DESCRIPTION
Analogue approach	Identical toxicants through biotransformation	Chemical or biological transformation results in exposure to the same toxicants, and subsequently the same effects
	Different ultimate toxicants	Source and target are known to belong to a group of substances that cause effects by means of an identical mode of action with identical tox endpoints. Identical interactions or endpoints imply predictability of effects.
Category approach	Trend in the property to read across	A plot of the property under consideration on another property shows a clear trend for a group of substances, this trend alone may suffice for prediction
	Trend in the property to read across plus a mechanistic explanation	A plot of the property under consideration on another property shows a trend for a group of substances; moreover, there is a mechanistic explanation why group membership goes with predictive power
	Trend in other properties	Trends observed for other properties than the property under consideration go with possibilities to predict effects.

de Raat K, Netzeva T. 2012.



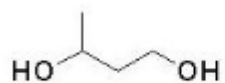
Why reading across?

- To avoid additional animal testing
- To save time and costs
- To use human data, if available, for one compound but not possible to produce for another
- To cover more substances within one safety assessment

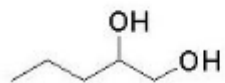


Reading across in a category

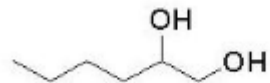
High confidence: a series of structurally-related **sources** with robust data and the unknown (**target**) "in the middle"



SOURCE



SOURCE

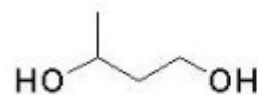


TARGET

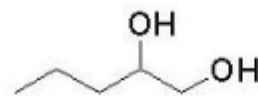


SOURCE

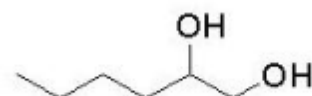
More often the case:



Some data



Some other data



TARGET No data



No data

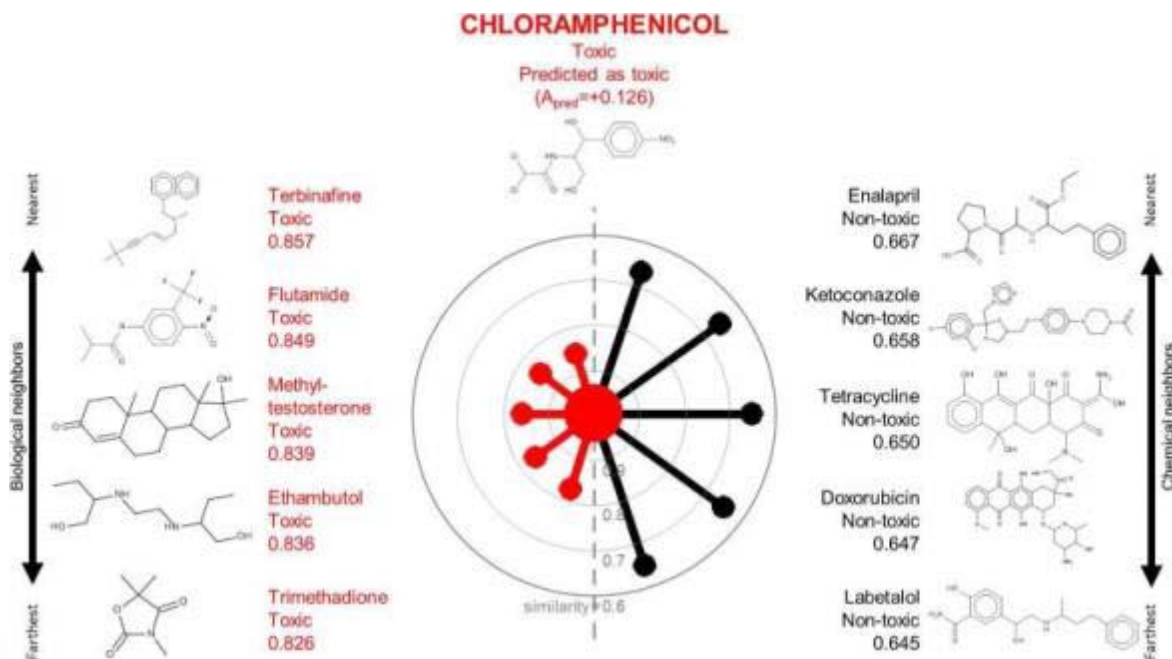


Chemical and biological similarity

Structurally dissimilar chemicals can have similar toxicological properties and there might be reasons to start from evaluation of biological similarities rather than being limited to the chemical structure alone (Low et al. 2013) .*

Biological similarity can be based on Mode-of-Action knowledge and *in vitro* data profiles.

Biological similarity can provide better confidence in chemically structure-based read-across.



* Low Y, Sedykh A, Fourches D, Golbraikh A, Whelan M, Rusyn I et al. 2013. *Chem. Res. Integrative Chemical-Biological Read-Across Approach for Chemical Hazard Classification. Toxicol.* 26:1199–1208.



Application of read-across in regulatory context

- 75% of all registration dossiers under REACH* include read-across or categorisation reasoning
- The highest rate of read-across was for repeated dose toxicity endpoint.
- This does not mean that all read-across predictions satisfy the regulatory requirements.
- It rather shows a request to improve the methodology on read-across and categorisation for regulatory acceptance



http://echa.europa.eu/documents/10162/13628/read_across_introductory_note_en.pdf

* Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)



Successful example of EU accepted read-across in the CLP Regulation*

In 2006 hazard classification of 118 nickel compounds was agreed based on data provided for 5 nickel compounds subject to risk assessment, and already classified some years earlier, and the solubility of the individual nickel compounds.

Basis for read across:

- The nickel ion is responsible for the effects to be assessed.
- The concentration of the ion at the site of action is the most important factor determining the toxicity of the compound.
- The bioavailability depends on various characteristics of the individual nickel compounds of which solubility in water is considered as being most important for the release of the nickel ion.
- Solubility in water was therefore used as an approximation of systemic bioavailability of the nickel ion and the ultimate hazard.



* Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures

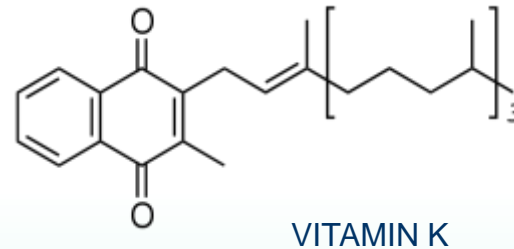
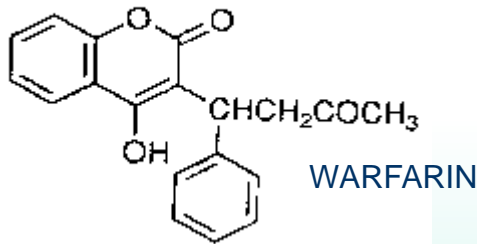


Difficult case of read-across

Anticoagulant rodenticides

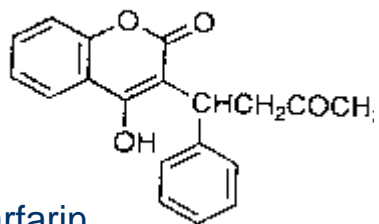
The anticoagulants are often called antivitamin K substances and they are all assumed to have the same mode of action as Warfarin.

Warfarin inhibits the regeneration of vitamin K by enzyme blocking at two phases in the vitamin K cycle. (Active vitamin K acts as a co-factor in the process leading to formation of blood clotting factors and in the bone metabolism. The name vitamin K comes from "Koagulations-Vitamin" in German).

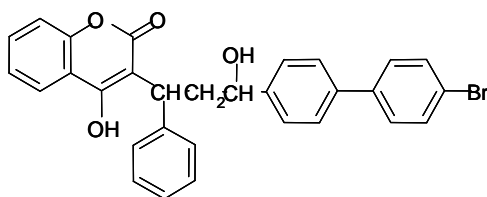
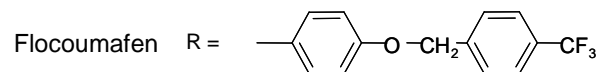
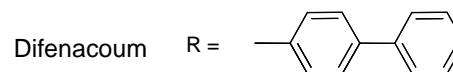
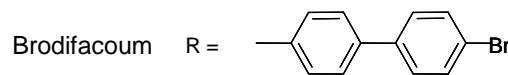
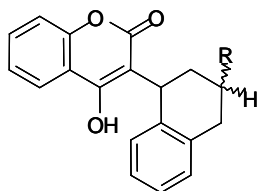


Structural similarity of the anti-coagulants

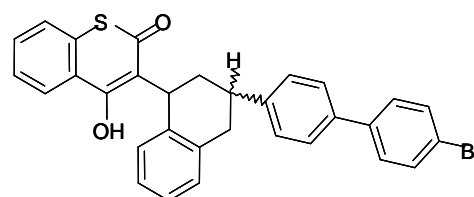
The interference with the enzymatic pathway in the regeneration process of vitamin K seems to be related to the shape of the molecule.



Warfarin



Bromadiolone



Difethialone

No evidence that other anticoagulants could not pass the placenta.



Contradictive animal data

For the anticoagulants tested, no developmental effects were seen, but it was discussed whether in the case of the anticoagulant rodenticides, the conventional rat and rabbit developmental studies (including OECD guideline test 414) were reliable.

Warfarin is used as a rodenticide and human pharmaceutical.

Warfarin is a substance known to cause developmental toxicity in humans, on the basis of human data. (It is classified for Reproductive toxicity in Category 1A.)



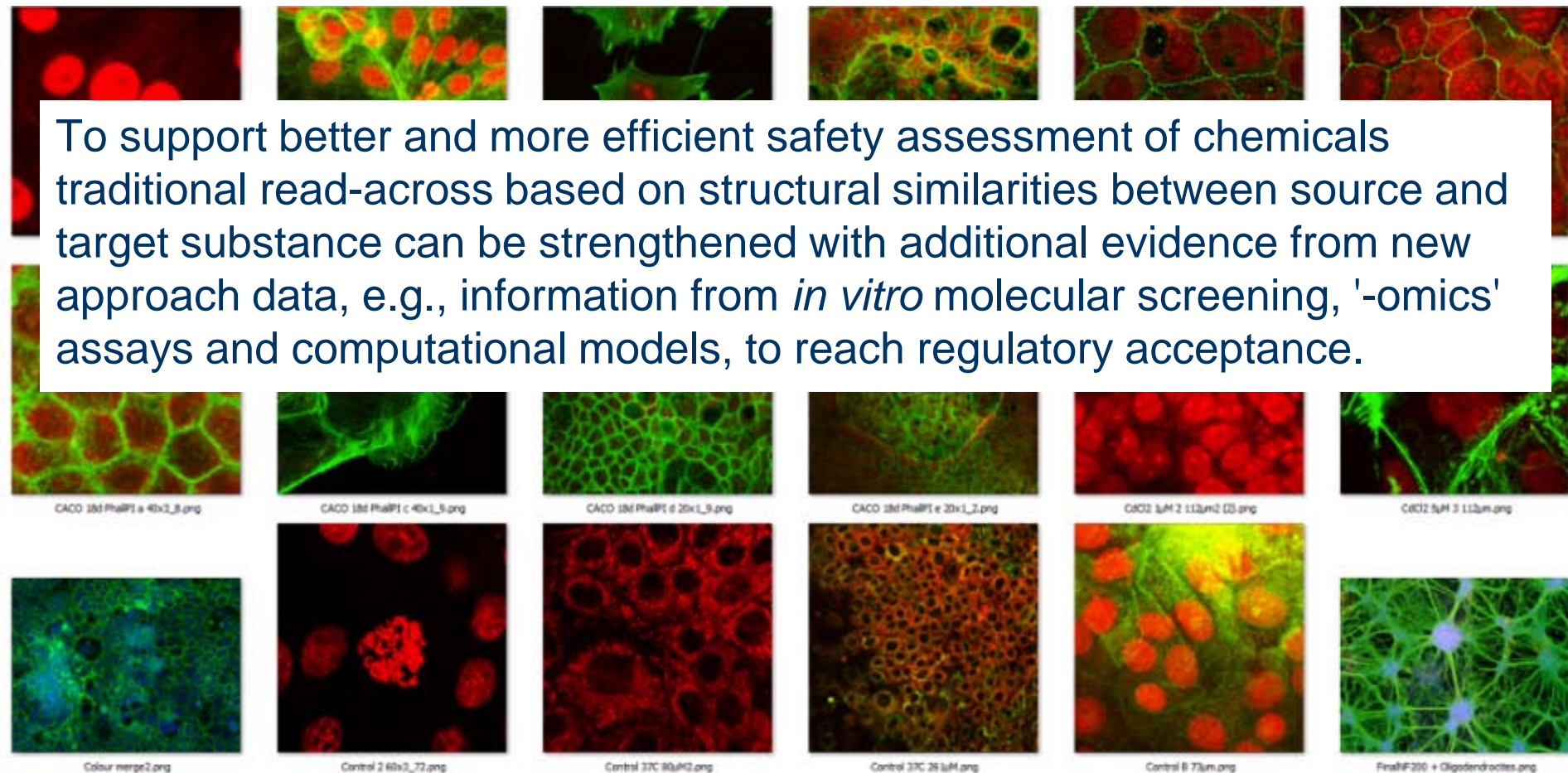
Warfarin was never tested in rats. (We do not know what should be the outcome of such a test.)

Based on the available negative animal data, the read-across was not accepted.



Requested: improved read-across strategies

To support better and more efficient safety assessment of chemicals traditional read-across based on structural similarities between source and target substance can be strengthened with additional evidence from new approach data, e.g., information from *in vitro* molecular screening, '-omics' assays and computational models, to reach regulatory acceptance.



Opportunities for incorporating *in vitro/in silico* data into read-across

1. Replacing an animal study/endpoint with an “apical endpoint-relevant” non-animal alternative method

- Providing additional “biological” plausibility to the chemical structure-based similarity argument using relevant alternative method(s)

2. Reducing uncertainty in a read-across argument in a regulatory submission:

- Using *in vitro/in silico* data to confirm the similarity in the mechanism of action within a category and/or between the “target” and “source” compounds
- Confirming or refuting a hypothesis that proposed analogues may have “other” effects
- Assessing the relative “potency” of the analogues



The SEURAT-1 research initiative

Towards replacement of *in vivo* repeated dose systemic toxicity testing

- Cluster of seven collaborative projects
- 50 million Euro investment
- Co-financed by EC and Cosmetics Europe
- Over 70 research partners
- 16 countries plus EC
- 6 year programme



<http://www.seurat-1.eu/>



The SEURAT strategy

The SEURAT strategy is to adopt a toxicological **mode-of-action framework** to describe how any substance may adversely affect human health, and to use this knowledge to develop complementary theoretical, computational and experimental (in vitro) models that predict quantitative points of departure needed for safety assessment.

Adverse Outcome Pathway reasoning:



SEURAT-1 Proof-of-Concept

Level 3, APPLICATION:

Predictive systems to support regulatory safety assessment

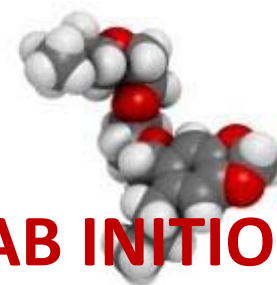
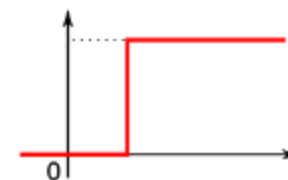
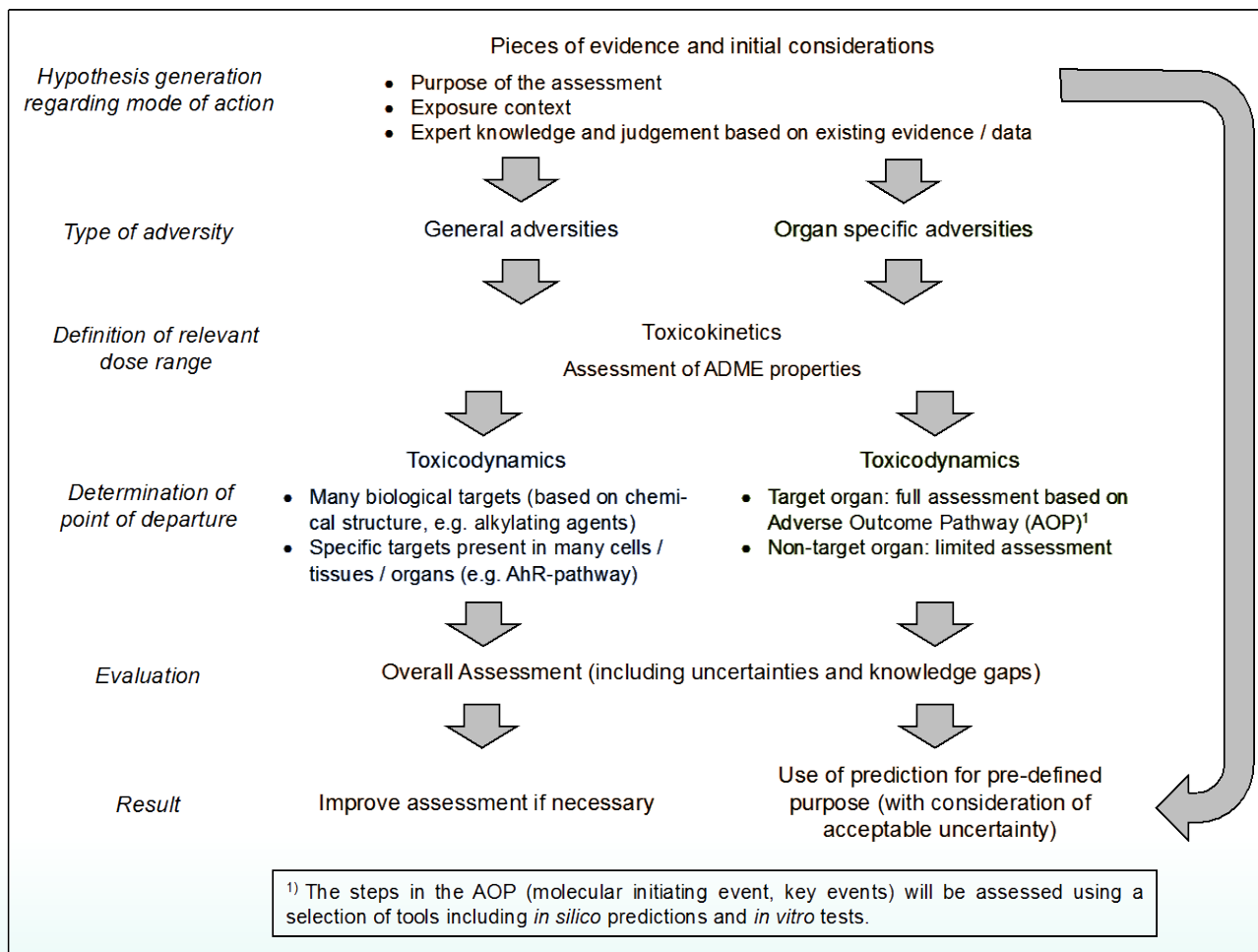
Level 2, PREDICTION: Integrated systems including *in vitro* and computational methods to predict toxicity

Level 1, KNOWLEDGE:

Adverse Outcome Pathway (AOP) constructs



The SEURAT conceptual framework for safety assessment



Four read-across scenarios

- I. Chemical similarity of compounds that do not require metabolic transformation to exert a potential adverse human health effect
- II. Chemical similarity involving metabolic transformation resulting in exposure to the same/similar proximal toxicant
- III. Chemicals with general low or no toxicity
- IV. Distinguishing chemicals in a structurally similar category with variable toxicities based on Mode of Action hypothesis

A read-across strategy

- Describe the rationale for the similarity between the source and target chemical in a transparent manner.
- Document the logic and data leading to the read-across prediction so that, if required, it can subsequently be recreated.
- Describe the uncertainties in the prediction; specifically separating the uncertainties in data and definition of similarity from procedural uncertainty.
- Clarify the roles of any endpoint specific and/or endpoint non-specific factors affecting the assessment.



Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

Regulatory Toxicology and Pharmacology 72 (2015) 586–601






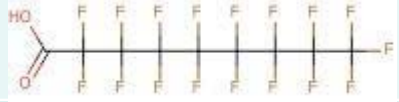
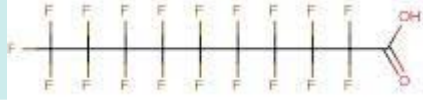

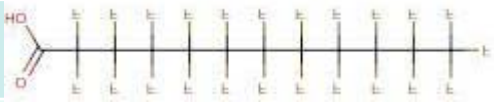
A strategy for structuring and reporting a read-across prediction of toxicity [☆]

T.W. Schultz^a, P. Amcoff^b, E. Berggren^c, F. Gautier^d, M. Klaric^b, D.J. Knight^e, C. Mahony^f, M. Schwarz^g,
A. White^h, M.T.D. Cronin^{l,*}

Example (Scenario I) : Perfluoroalkyl acids (PFAAs)

1

Is it possible to read-across a result from a 90-day oral repeated-dose toxicity from perfluorooctanoic acid (PFOA) to other compounds in this category?

Name	C-atom Chain Length	Structure	90-day rodent study NOAEL
Perfluorohexanoic acid	6		20- 200
Perfluoroheptanoic acid	7		
Perfluorooctanoic acid (PFOA)	8		0.3-76
Perfluorononanoic acid	9		
Perfluorodecanoic acid	10		
Perfluoroundecanoic acid	11		0.1
Perfluorododecanoic acid	12		

Similar compounds not requiring metabolic activation to exert toxicity

Example (Scenario I) : Perfluoroalkyl acids (PFAAs)

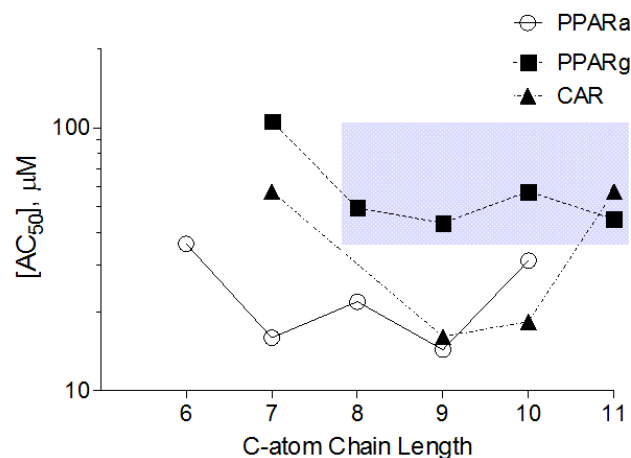
2

Question: Can *in vitro/in silico* data be used to confirm the similarity in the mechanism of action within a category and/or between the “target” and “source” compounds?

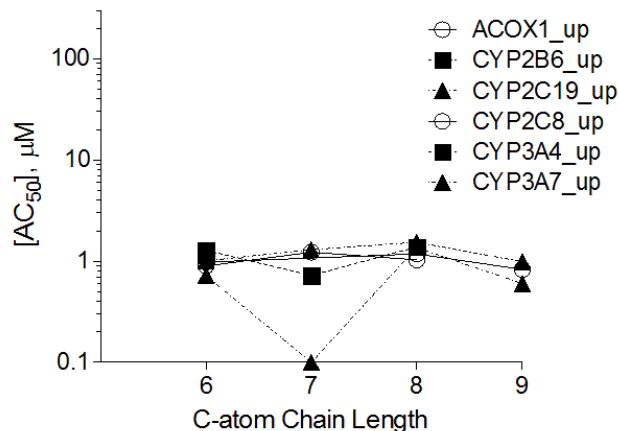
Answering with
in vitro
ToxCast™*
data:

*ToxCast™ Data, National Center for Computational Toxicology. Available: <http://www.epa.gov/ncct/toxcast/data.html>

Nuclear
receptor
activation



Enzyme
induction



➔ YES

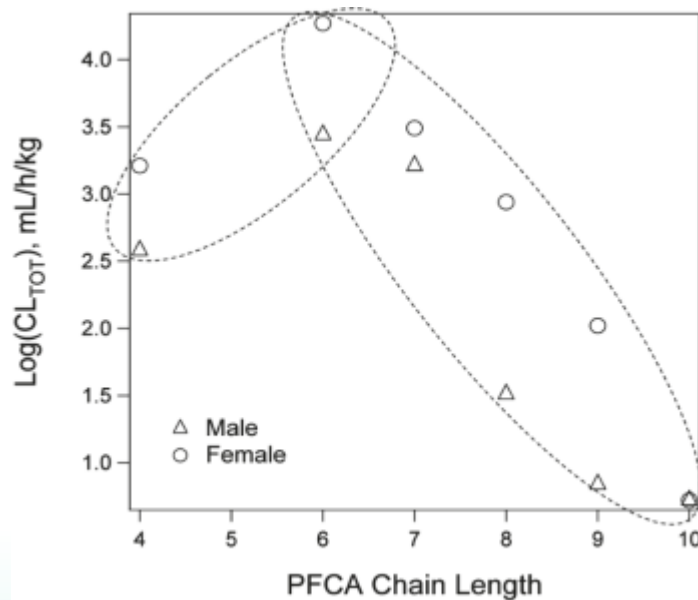
Example (Scenario I) : Perfluoroalkyl acids (PFAAs)

3

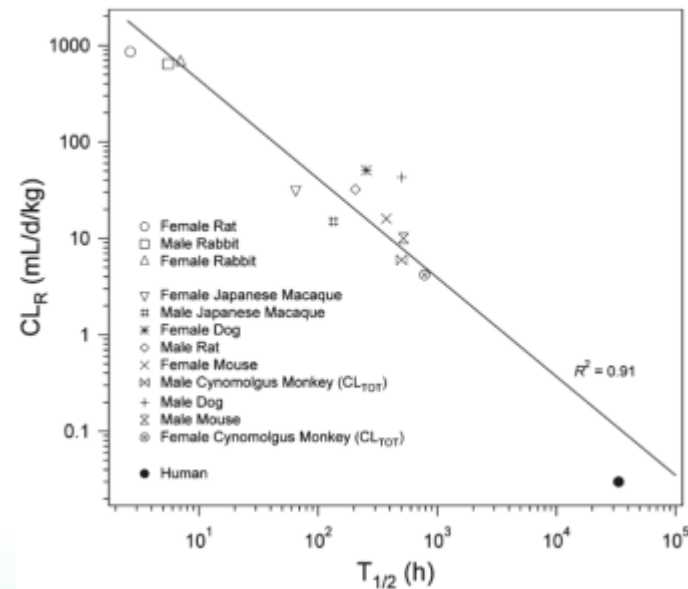


Further toxico-kinetics evidence is needed...

PFCA differences in ADME in rats



PFOA species differences in ADME



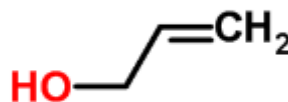
Data are from: Chem Res Toxicol (2012) 25, 35–46



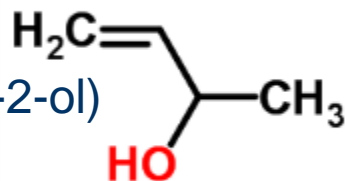
Example (Scenario II) : β -unsaturated alcohols

1

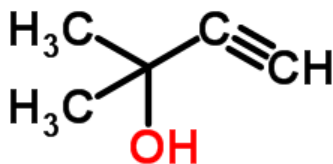
2-Propen-1-ol (allyl alcohol)



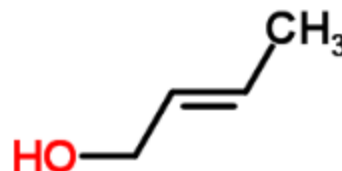
1-Buten-3-ol (3-buten-2-ol)



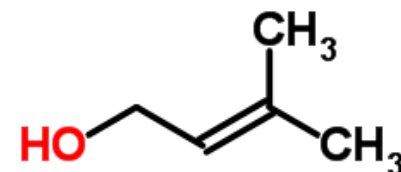
2-Methyl-3-butyn-2-ol



Crotyl alcohol

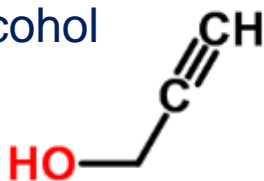


Allyl alcohol is an indirect-acting toxicant with metabolism via ADH, and is leading to necrosis and apoptosis and subsequent liver fibrosis



3-Methyl-2-buten-1-ol

Propargyl alcohol

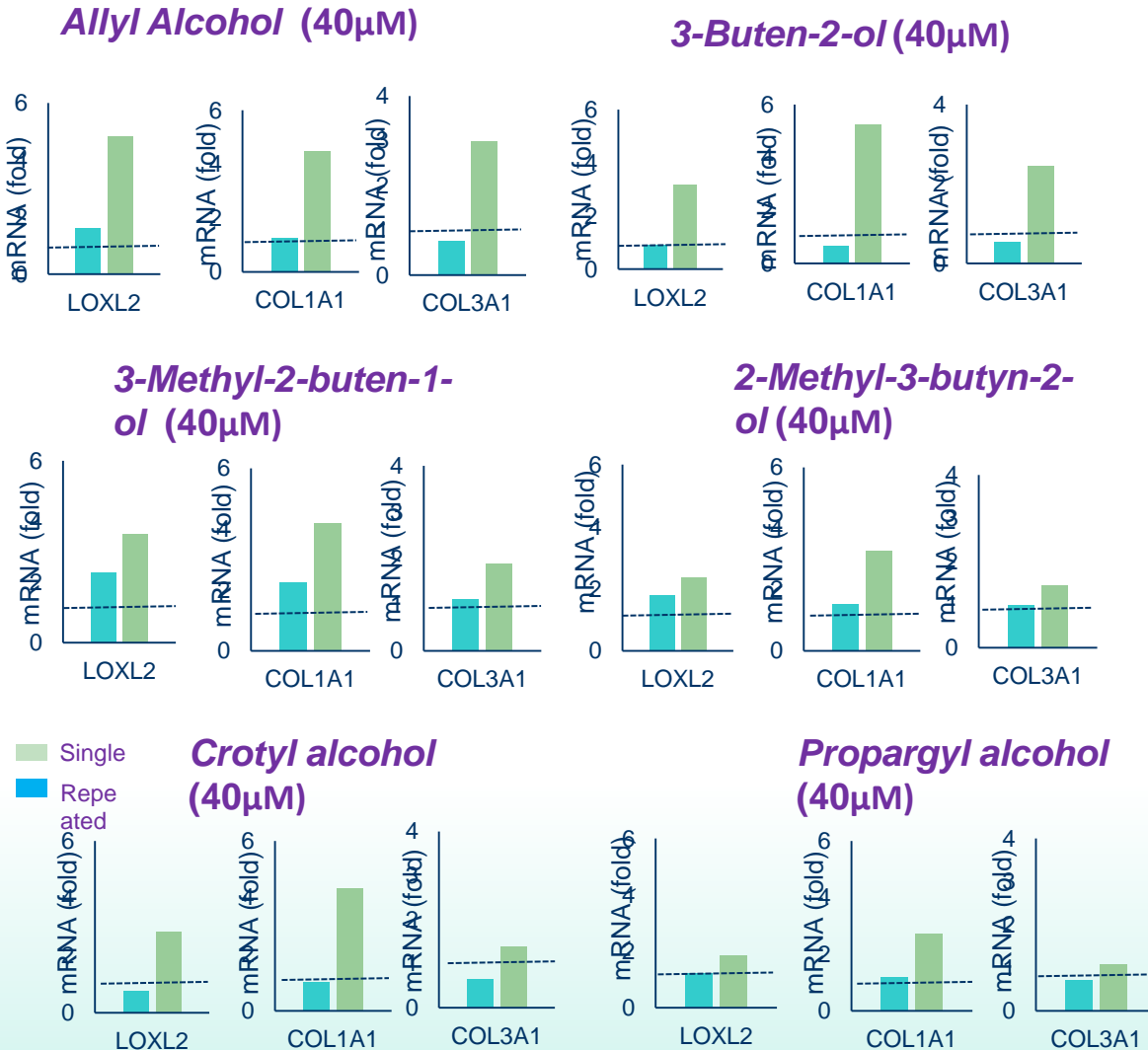


Hypothesis: β -unsaturated alcohols of short chain length (C3 to C6) are indirect-acting toxicants (metabolism critical to toxicity) with the same covalent mechanism of action (i.e., Michael addition electrophilicity) and similar reactive potency.

Example (Scenario II) : β -unsaturated alcohols

3

HSC activation in 3D HepaRG/HSC:



Conclusions:

- HSC activation by unsaturated alcohols is independent of the “toxicity” trend.
- Allyl Alcohol, 3-Buten-2-ol, 3-methyl-2-buten-1-ol, Crotyl alcohol
- 3-methyl-3-buten-2-ol and Propargyl alcohol are not pro-fibrotic

Additional categories under investigation

The largest challenge: Scenario III – Low or no toxicity

- Primary Alcohols
- Propylene glycol ethers

Scenario IV - Distinguishing chemicals in a structurally similar category with variable toxicities based on Mode of Action hypothesis

- Alkyl phenols
- Short-chain carboxylic acids (SCCAs)

Special thanks to Terry Schultz contracted by Cosmetics Europe and the other experts that contributed and still contributes to the exercise.



Additional *in vitro/in silico* data can:

- improve confidence in the “traditional” read-across,
- suggest that smaller sub-categories, or more refined selection of analogues, may be more appropriate (better frame the category),
- suggest what additional studies may need to be performed to strengthen read-across and/or reduce uncertainty
- discourage reliance on chemical structure similarity-based read across



Future activities on read-across 1

Final reporting:
You are all welcome!

Register at: <http://www.seurat-1.eu/>



SEURAT-1 Symposium

Painting the future animal-free safety assessment of
chemical substances: Achievements of SEURAT-1

4 December 2015, Brussels, Belgium

Arch Toxicol
DOI 10.1007/s00204-014-1421-5 November 2014

RESEARCH ARTICLE

SEURAT: Safety Evaluation Ultimately Replacing Animal Testing—Recommendations for future research in the field of predictive toxicology

George Daston · Derek J. Knight · Michael Schwarz ·
Tilman Gocht · Russell S. Thomas · Catherine Mahony ·
Maurice Whelan

Horizon 2020 project: EUToxRisk21
Starting this autumn will continue what
SEURAT-1 started.

ECHA
EUROPEAN CHEMICALS
AGENCY

Read-Across Assessment
Framework (RAAF)



Future activities on read-across 2

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Topical Scientific Workshop - New Approach Methodologies in Regulatory Science

19-20 April 2016 | Helsinki, Finland

Topical scientific workshops of the European Chemicals Agency (ECHA) aim to foster discussion among academia, regulators, industry and other stakeholders on the possible regulatory impacts of the latest scientific developments. An anticipated outcome of these workshops is the emergence of new or improved approaches which may be applied to the implementation of the REACH, CLP and biocides regulations.

Aim of the workshop

The Topical Scientific Workshop on New Approach Methodologies in Regulatory Science will explore the potential regulatory benefits arising from fundamental change in scientific thinking. Complex toxicological apical endpoints cannot be predicted by a single non-standard test. Instead, it is necessary to combine multiple lines of evidence (including '-omics' and high-throughput screening methods) to predict the hazardous property with tools to facilitate this integration of evidence.

Two motivating drivers for the workshop are:

- ▶ A better understanding of the underlying biology behind how chemicals cause adverse effects to human health; and
- ▶ New tools and techniques that provide a huge amount of data to be used in solving regulatory issues.

The workshop draws inspiration from the EU research programme SEURAT-1 and the US Tox21 initiative, but also takes into account general progress from the scientific field.





SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Thanks for your attention!

Elisabet Berggren

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Toxicology Unit & EURL ECVAM

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