7th German Pharm-Tox Summit – Combined Online Advanced Course
of the Working Groups „Regulatory Toxicology“ and „Computational Toxicology“
of the Society of Toxicology (GT)

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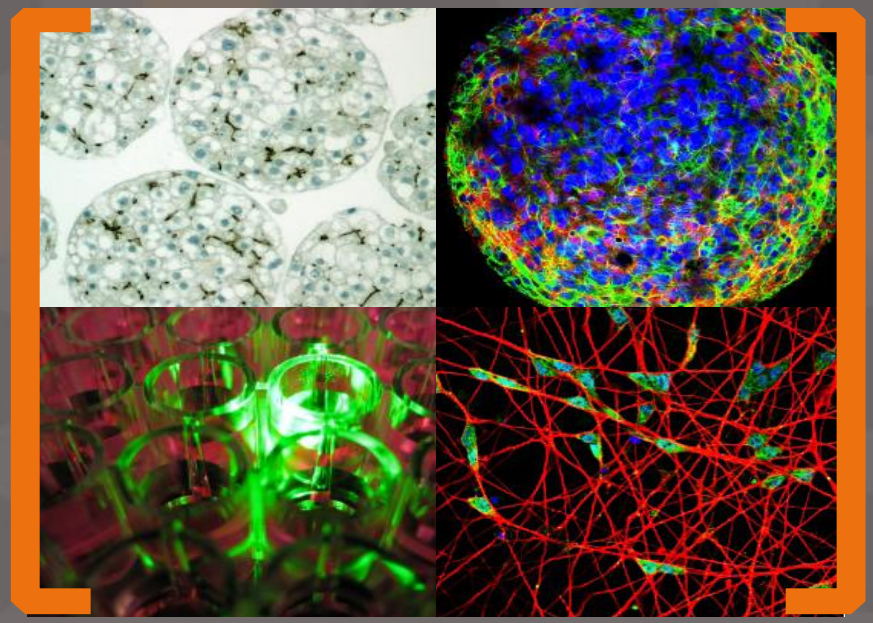
Application of AOPs to support read-across assessments



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Fraunhofer Institute for Toxicology and Experimental Medicine

Fraunhofer ITEM

08.03.2022

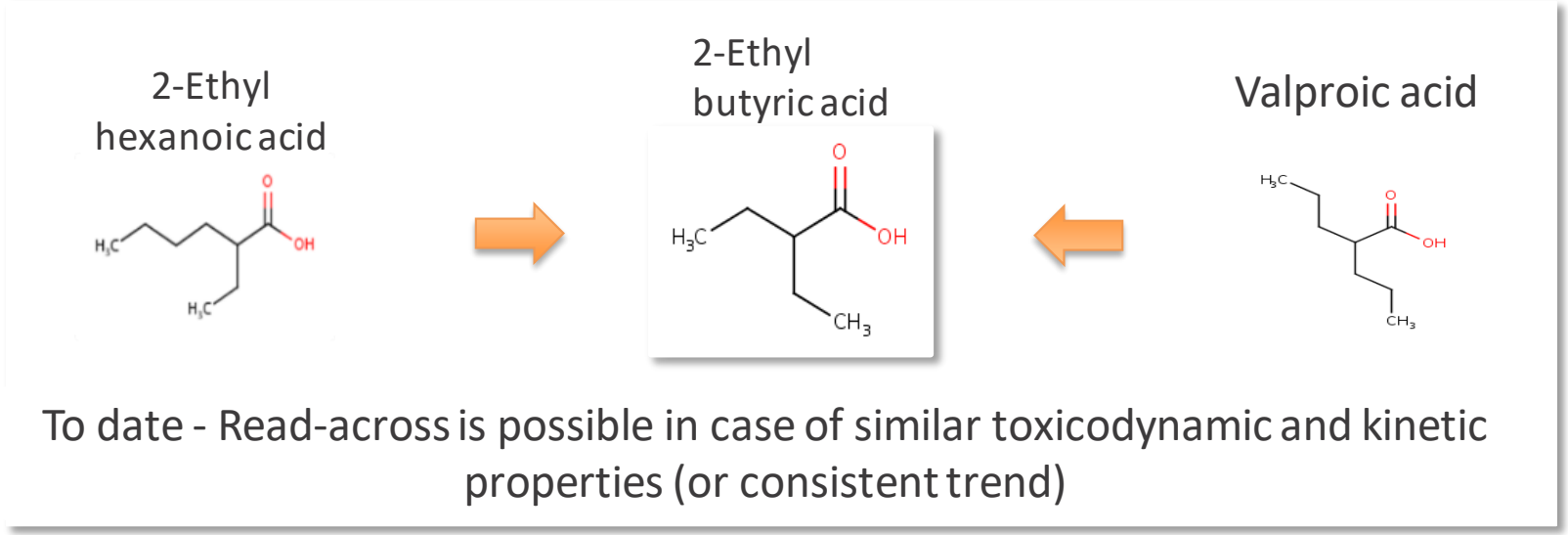
Case study - > AIM of EU ToxRisk-project

- Learn how to apply NAM in a human risk assessment for mainly two endpoints repeated dose toxicity and reprotoxicity

- Develop case studies as tool to gain experience for different aspects of regulatory decision-making

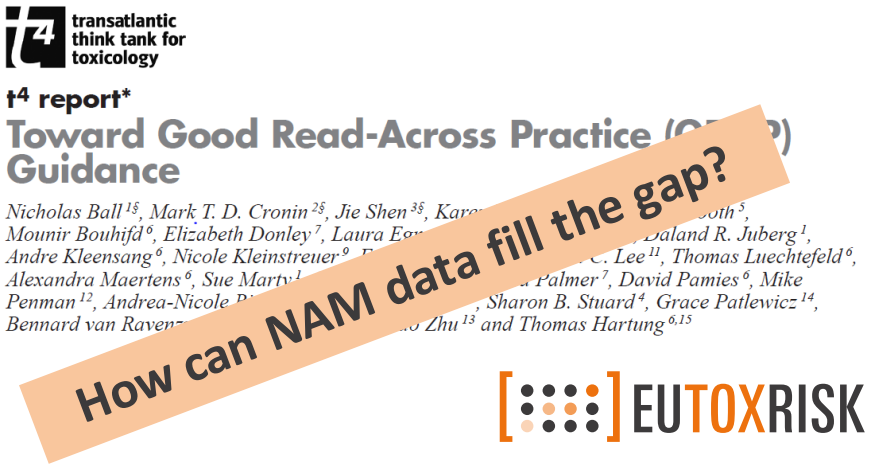
EUTOXRISK Read-Across case studies – Why?

(1) Why Read-Across Case Studies - the Regulatory Need



Read-Across rarely accepted by regulatory authorities

- Based often on structural & physicochemical data
- Lack of sufficient evidence to substantiate read-across justifications- **fail to demonstrate toxicokinetic and toxicodynamic similarities**
  → Including lack of endpoint 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



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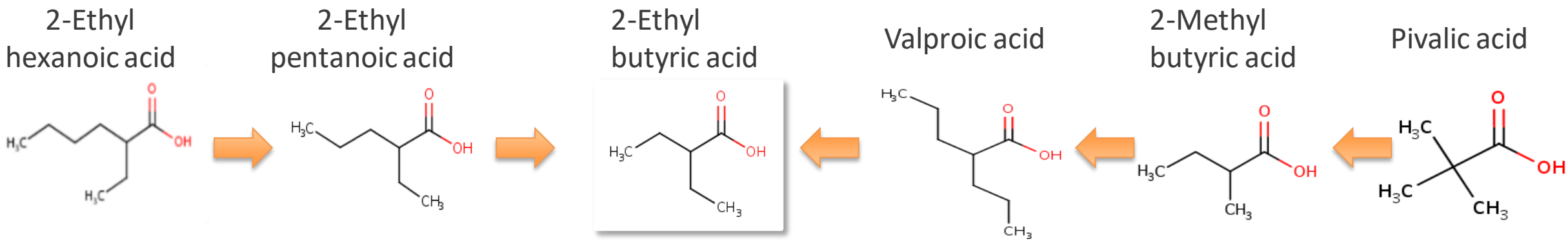
(2) Why Read-across? Compare New Data to Traditional in vivo Data



NAM - Opportunities

- testing of a series of potential analogues, not limited to analogue with in vivo endpoint data
- trends can be investigated more comprehensively
- test human models
- **provide mechanistic information**
- help to understand kinetic properties and (dis)similarities

NAM - Challenges

- scope of in vitro testing + in silico prediction? What is good enough?
- integration of different types of information
- resulting relevance and predictivity of result
- uncertainty assessment

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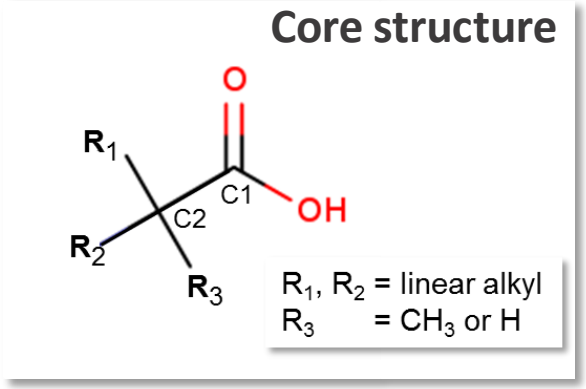
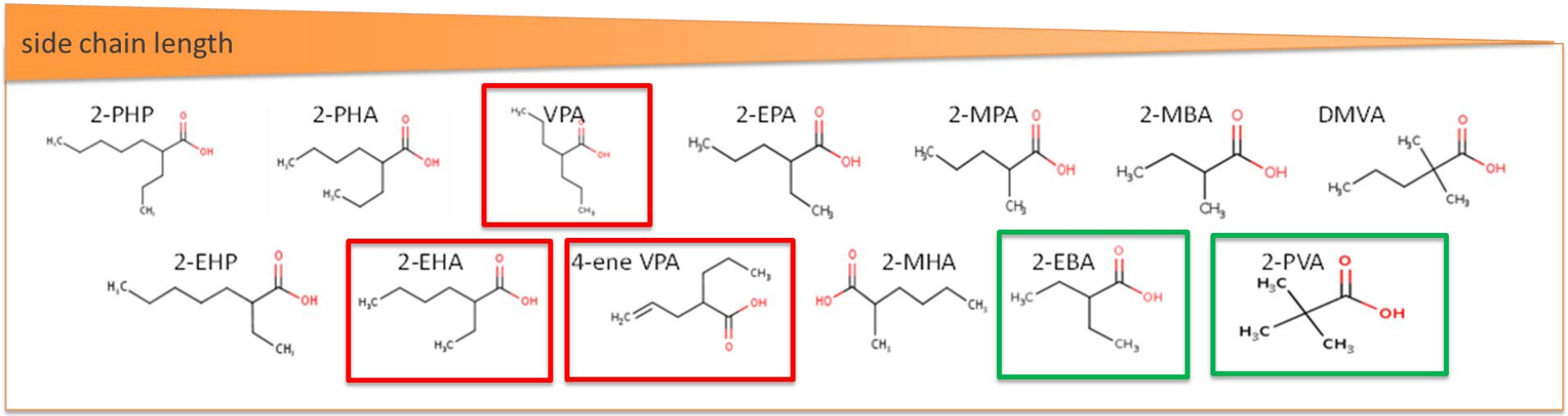
Case study 1– RAX hypothesis for systemic toxicity, lead effect liver steatosis





liver steatosis, in peclinical rodent studies

no liver effect observed up to highest in vivo tested dose

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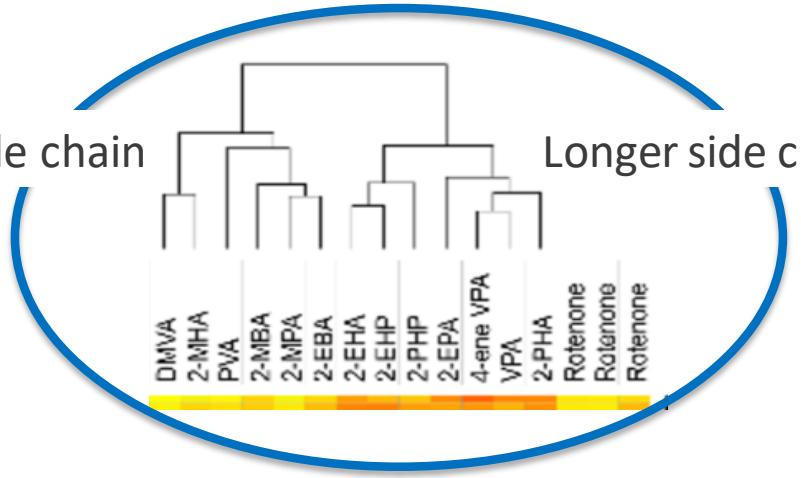
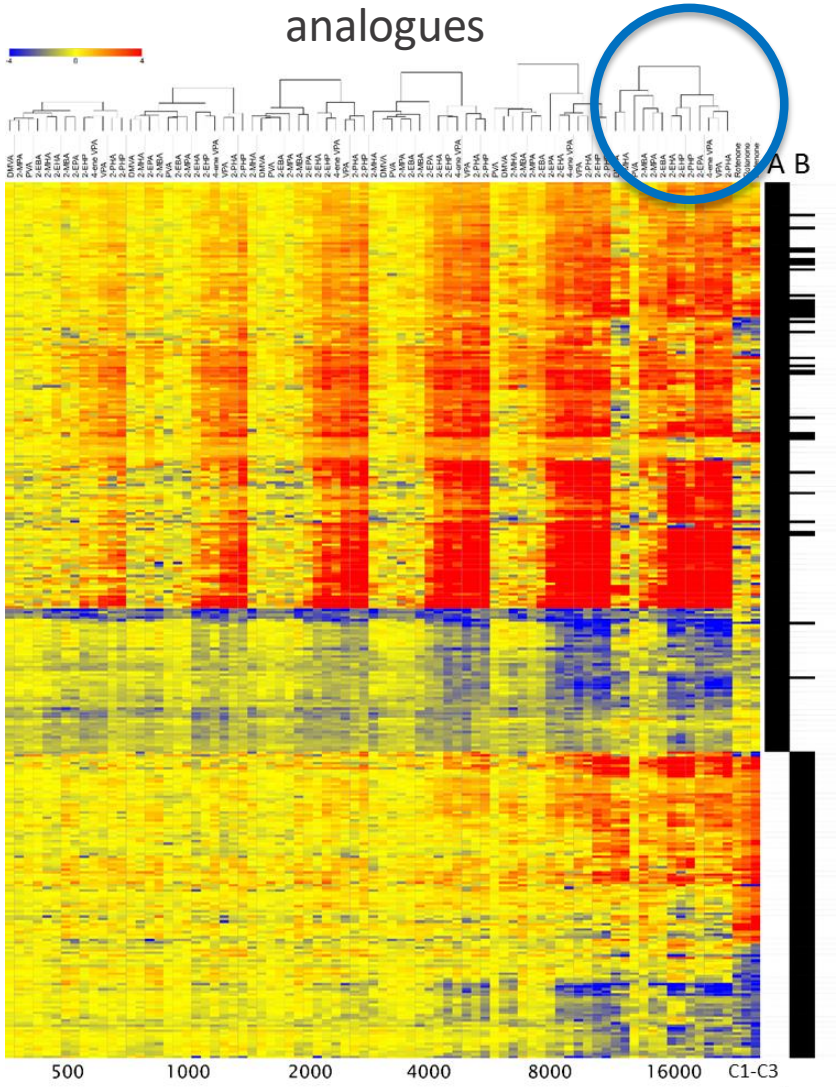
Analysis of DEGs - first indication of biological similarity

- Dose dependent testing of HepG2 cells
- TempOseq 1500+ panel (about 3500 genes)

- Expression profile of carboxylix acids differ from rotenone (michondrial complex I inhibitor)

- Analogues with longer side chain are more active and cluster together





A- DEGs of carboxylic acids

B- DEGs of rotenone

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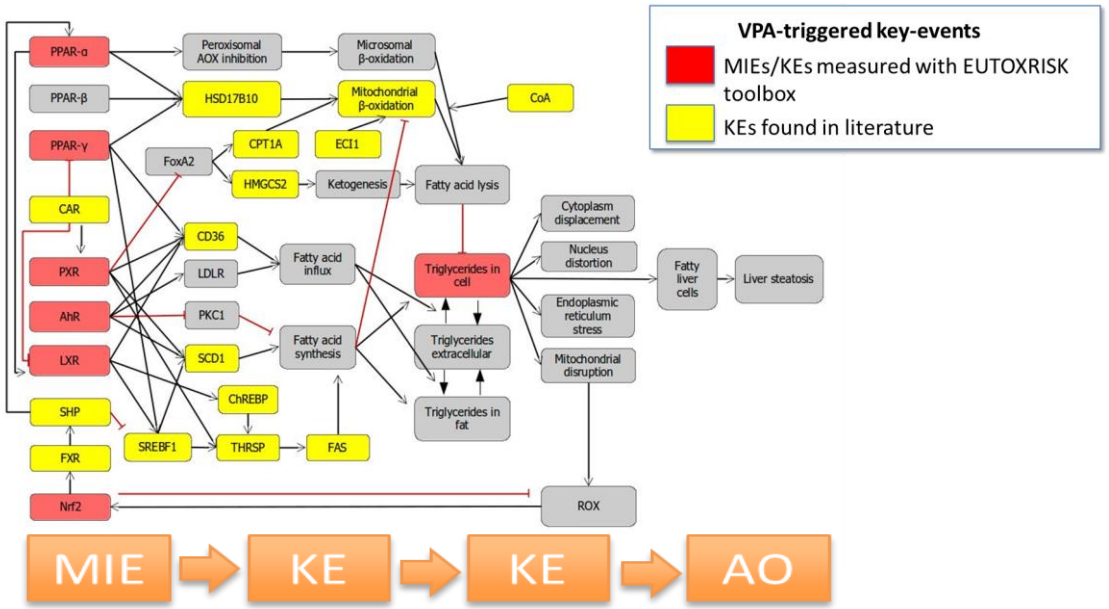
Learn to use AOPs …. AOP network for liver steatosis



AOP Network

AOP network

- comprise evidence from 55 different AOPs

- Coloured boxes – Evidence known for VPA
- Red- MIEs and KE tested in EUTOXRISK in vitro toolbox

- is used to infrom the testing strategy

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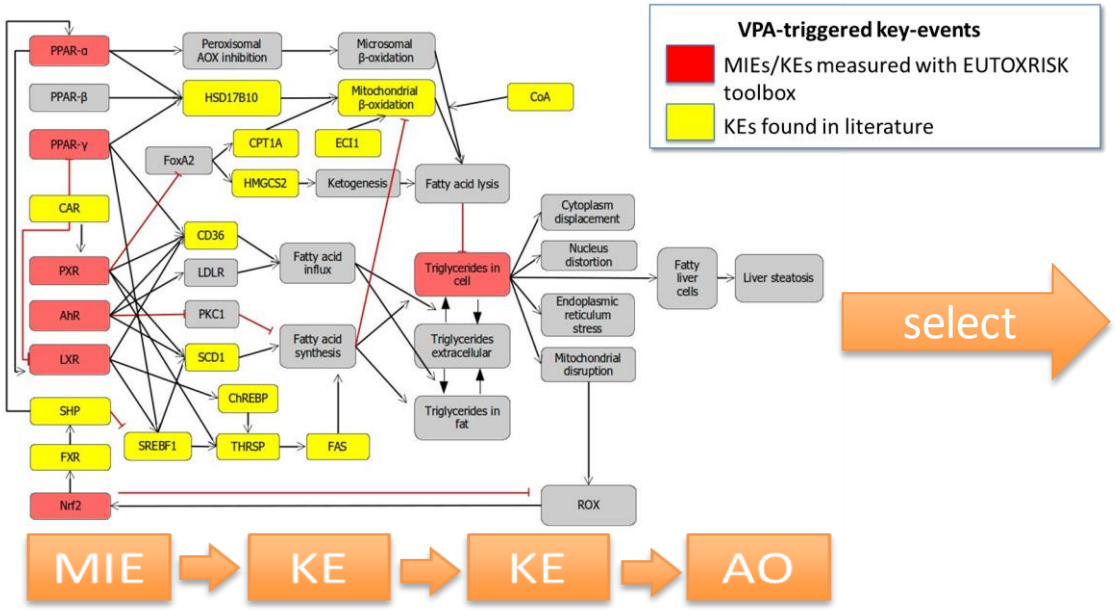
Learn to use AOPs …. AOP network for liver steatosis



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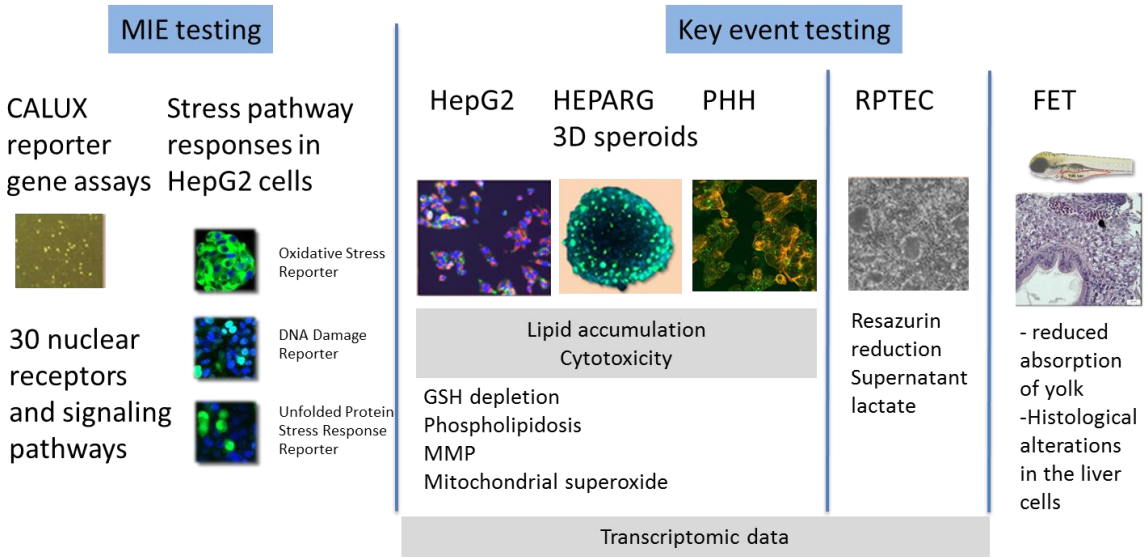

AOP Network

Targeted in vitro testing battery

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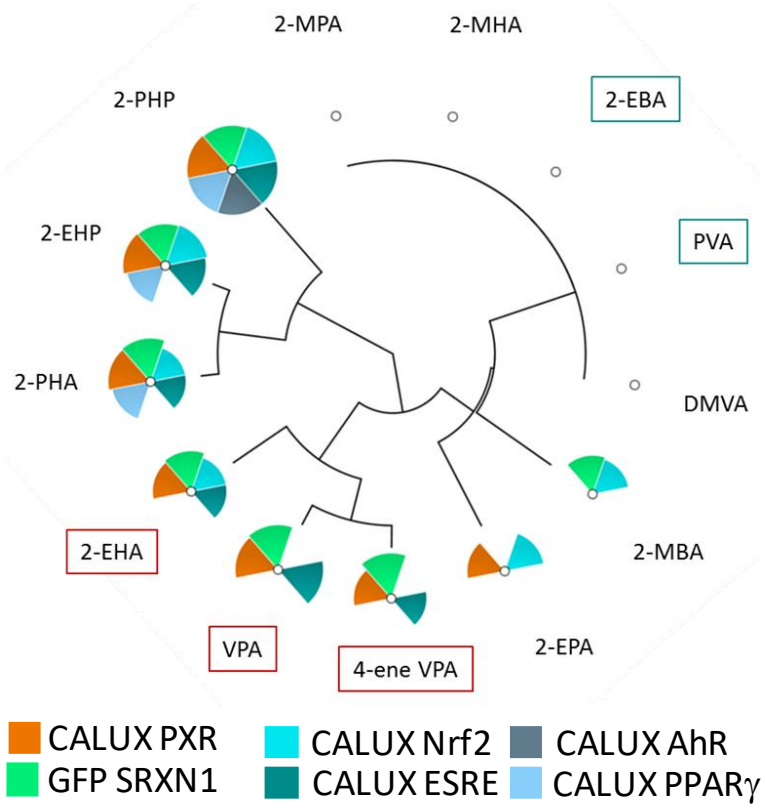
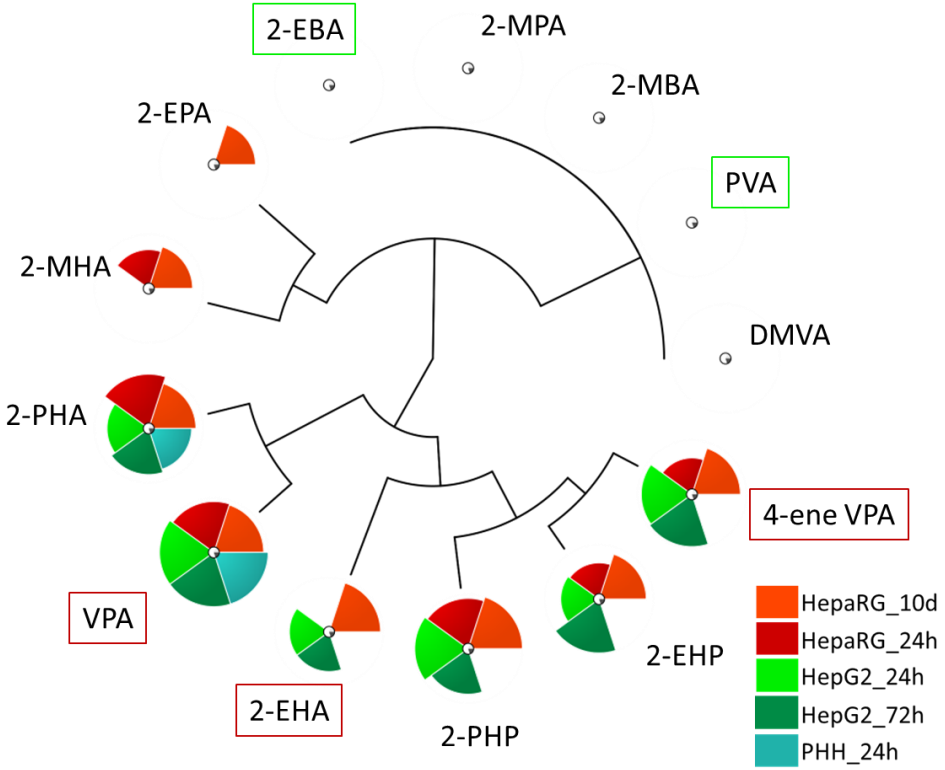
Learn to use AOPs in Read-Across Context

MIE/early KEs - reporter gene assays



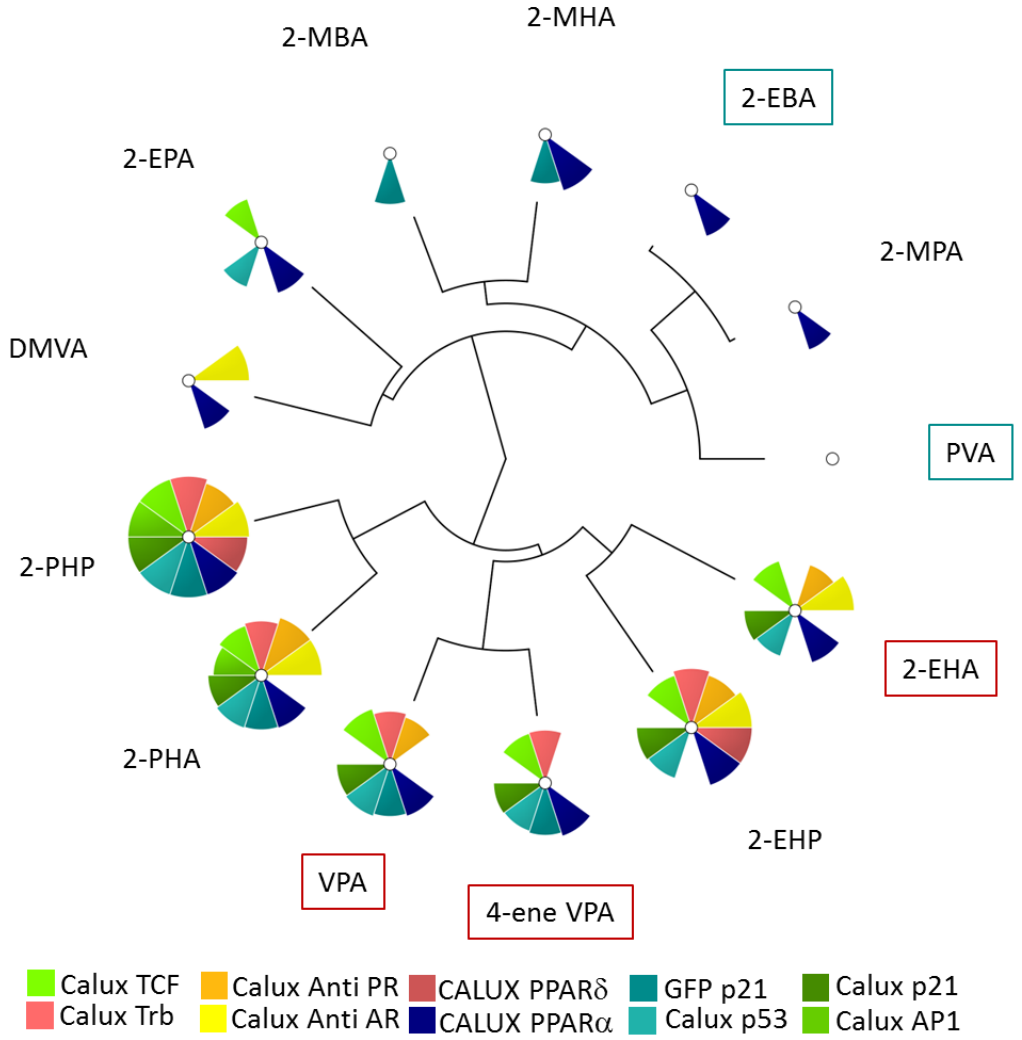
Late KE – lipid accumulation



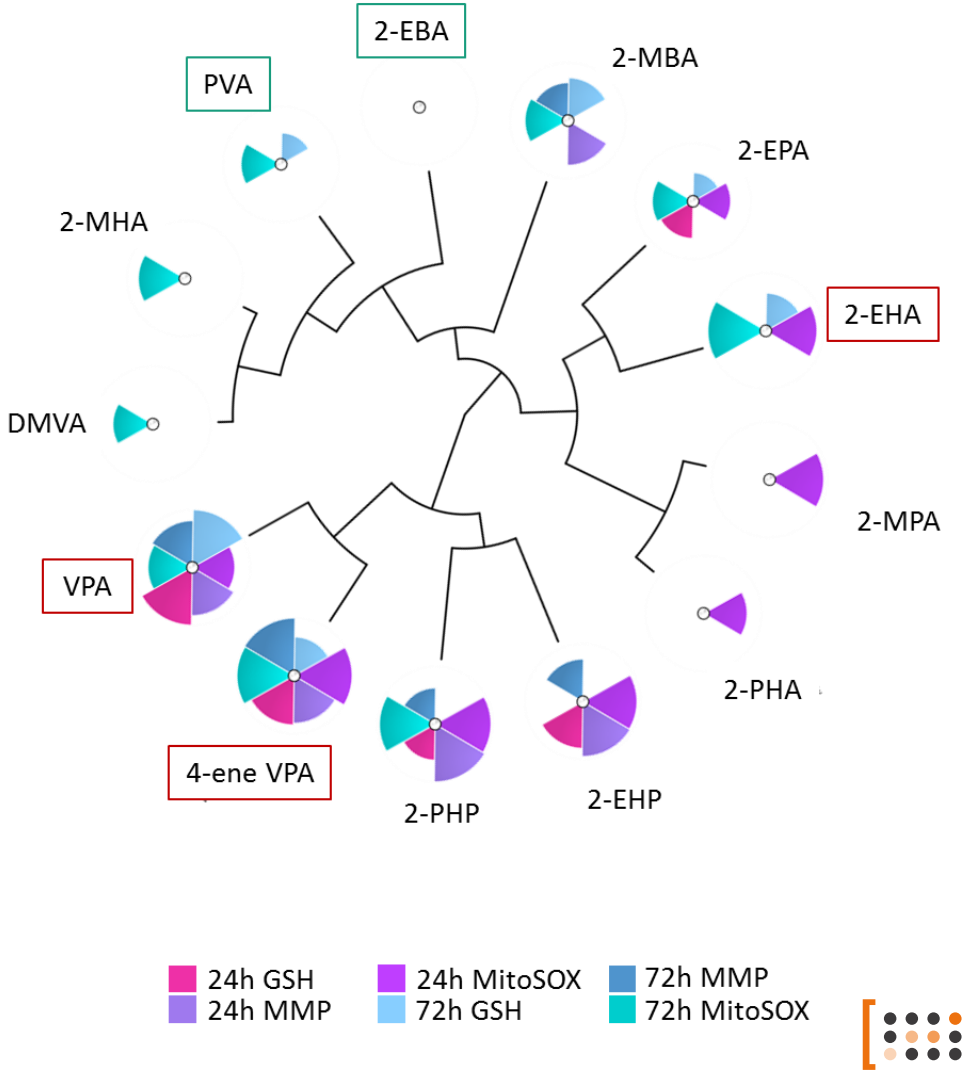
MIEs and Lipid accumulation – increased activity with increasing side chain length; in vivo pos. and neg. compounds predicted correctly

MIEs and KEs that do not belong to the AOP show no trend

MIE/early KEs not in AOP



Mitochondrial dysfunction (HepG2 cells)



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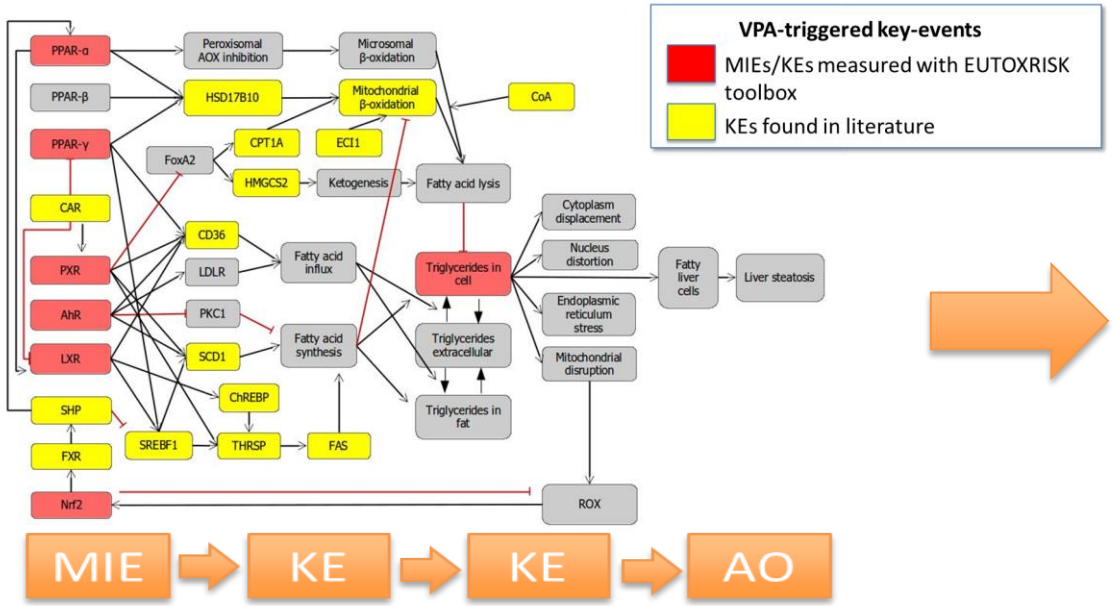
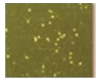
Learn to use AOPs …. AOP network for liver steatosis



MIE testing

CALUX reporter gene assays

Stress pathway responses in HepG2 cells



Oxidative Stress Reporter

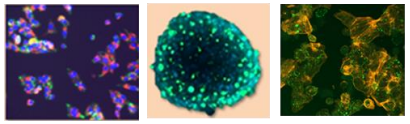
DNA Damage Reporter

Unfolded Protein Stress Response Reporter

30 nuclear receptors and signaling pathways

Key event testing

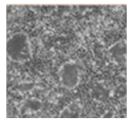
HepG2 HEPARG PHH 3D speroids



Lipid accumulation
Cytotoxicity

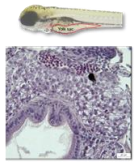
GSH depletion
Phospholipidosis
MMP
Mitochondrial superoxide

RPTEC



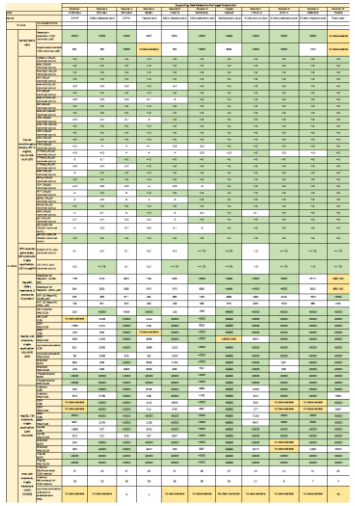
Resazurin reduction
Supernatant lactate

FET

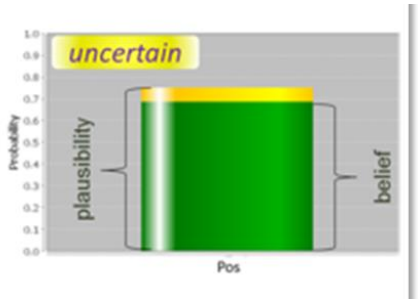


- reduced absorption of yolk
-Histological alterations in the liver cells

Transcriptomic data



Combination of evidence



Dempster Shafer

Minimal scope of NAM testing

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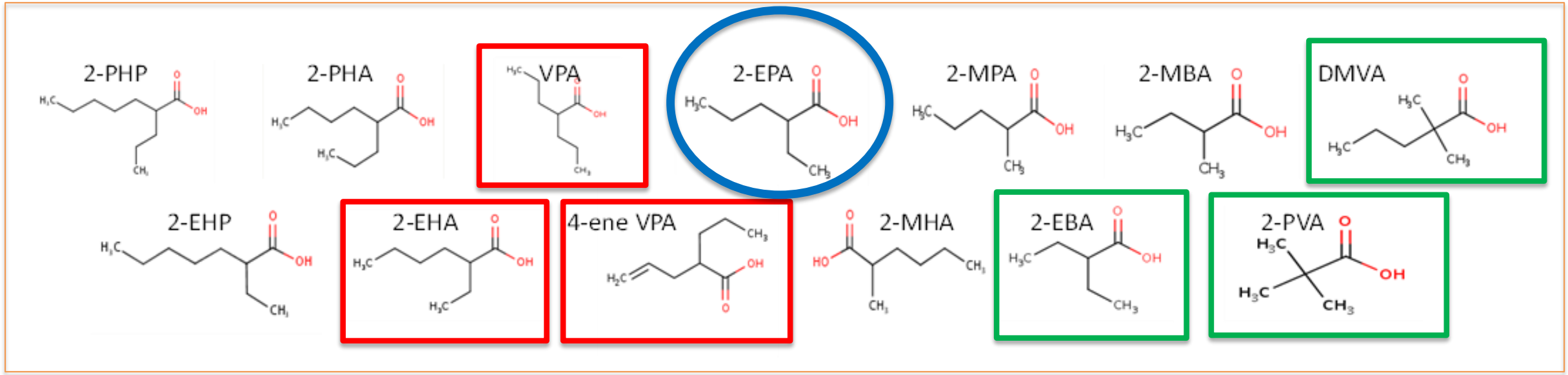
Read-Across Supported by NAMs -Toxikodynamics



NAMs used to illustrate **shared mode of action**

- 2-EPA show activation of MIEs and KEs belonging to AOP, induces late KE „lipid accumulation“ in different liver cells
- Early MIEs/KEs can be used to prove similar mode of action

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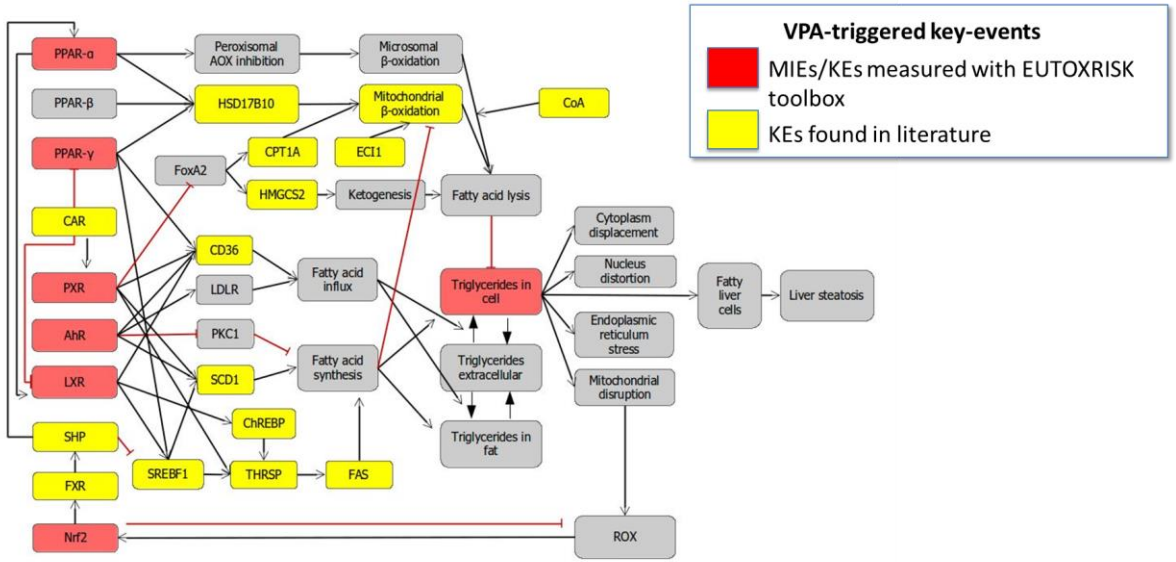
First learnings – application of AOPs in reg risk assessment

1. AOP-based testing strategy
   - AOP-network -> no need to test all MIEs/KEs; test shared toxicological profile
   - include KE close to apical endpoint
   - data integration might be challenging

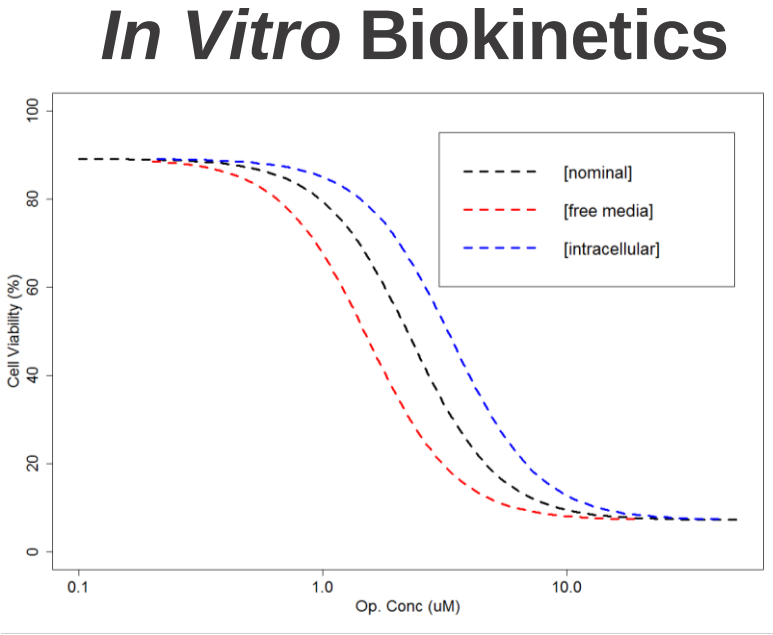
2. If no AOP available/ AOP weak: Describe the scientific rational of the testing in detail



Next step – In Vitro to In Vivo Extrapolation

What is the free concentration of the test compound in the cell?

- Is the effective concentration in vitro translated correctly to the in vivo concentration?
- Do we have confounding factors? Like volatility? Binding properties (to protein/serum; to plastics…)?
- **AD**ME properties are depenedent on ionization status, pH of medium.
- RAX- do we have large differences between the grouped compounds?



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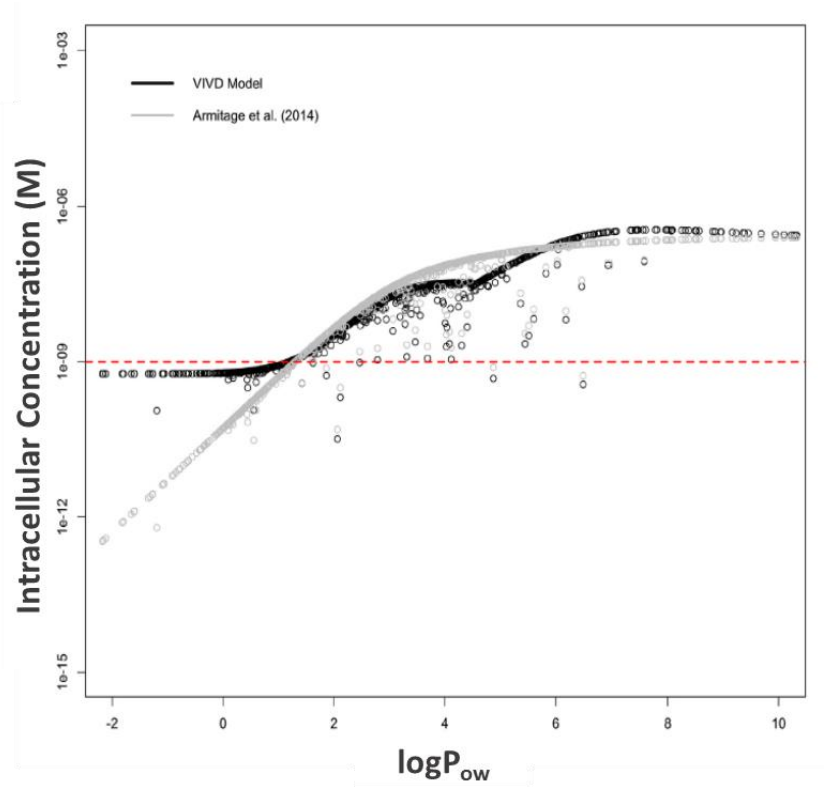
Predicting *In Vitro* Distribution – VIVD Model

$$C_{media,dissolved,u} = \frac{C_{nominal} \cdot fu_{diluted} \cdot V_{media} \cdot 1e^{-3}}{V_{bulk} + k_{air} f_{ui} V_{air} + k_{cell,u} V_{cell} + k_{plastic} SA_{media} \cdot 1e^{3}}$$



Toxicology in Vitro 58 (2019) 42–50

Contents lists available at ScienceDirect

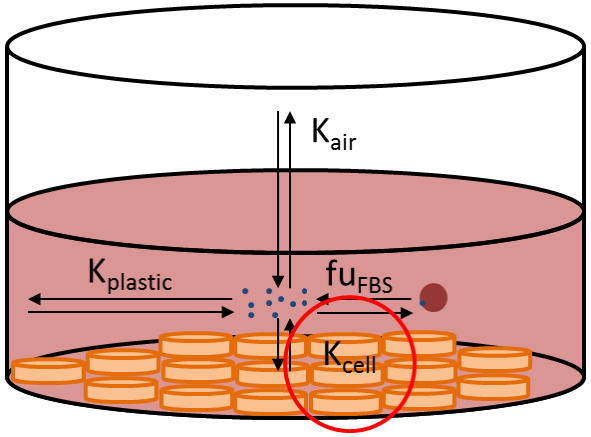
Toxicology in Vitro

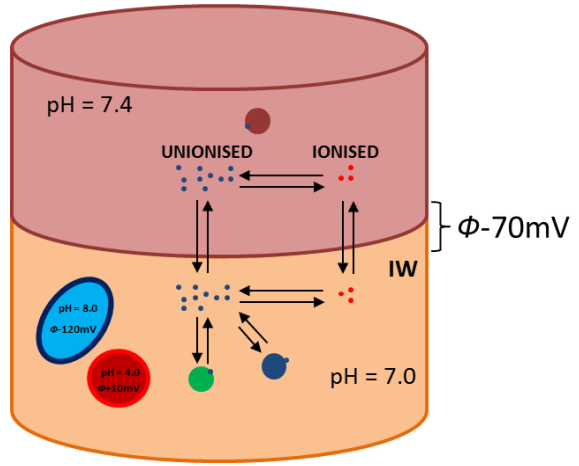
journal homepage: www.elsevier.com/locate/toxinvit

VIVD: Virtual *in vitro* distribution model for the mechanistic prediction of intracellular concentrations of chemicals in *in vitro* toxicity assays

Fisher C.[a,*], Siméon S.[b], Jamei M.[a], Gardner I.[a], Bois Y.F.[b]

[a] Certara UK Limited, Simcyp Division, Acero, 1 Concourse Way, Sheffield S1 2BJ, UK
[b] INERIS, METO Unit, Verneuil en Halatte, France





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Fisher et al. (2019) Toxicology In Vitro. 58:42-50

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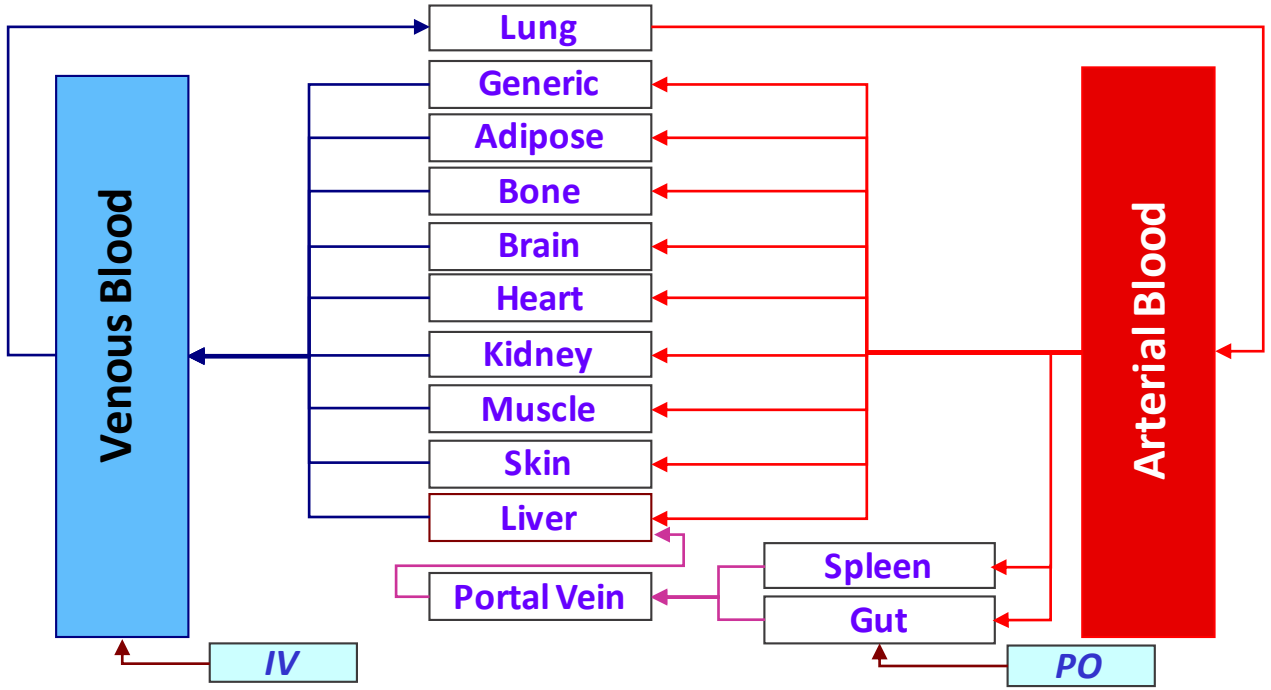
Physiologically-based Pharmacokinetic Modelling

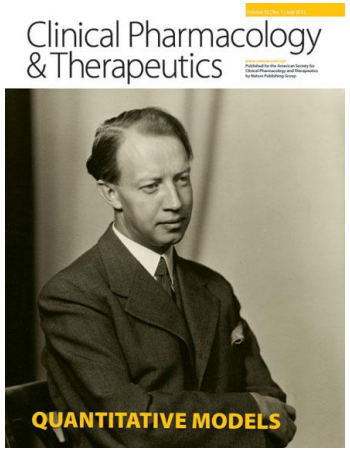


- octanol:water partition coefficient ($logP_{ow}$)
- pKa
- PSA(Å^2), HBD
- blood to plasma ratio (B:P)
- plasma protein binding (fu)
- fraction absorbed (fa)
- first-order absorption rate constant (Ka)
- steady-state volume of distribution (V_{ss})
- intrinsic hepatic clearance ($CL_{int,Hep}$)

Nothing New:

Teorell, T. Studies on the diffusion effect upon ionic distribution: II. experiments on ionic accumulation. J. Gen. Physiol. 21, 107–122

(1937)



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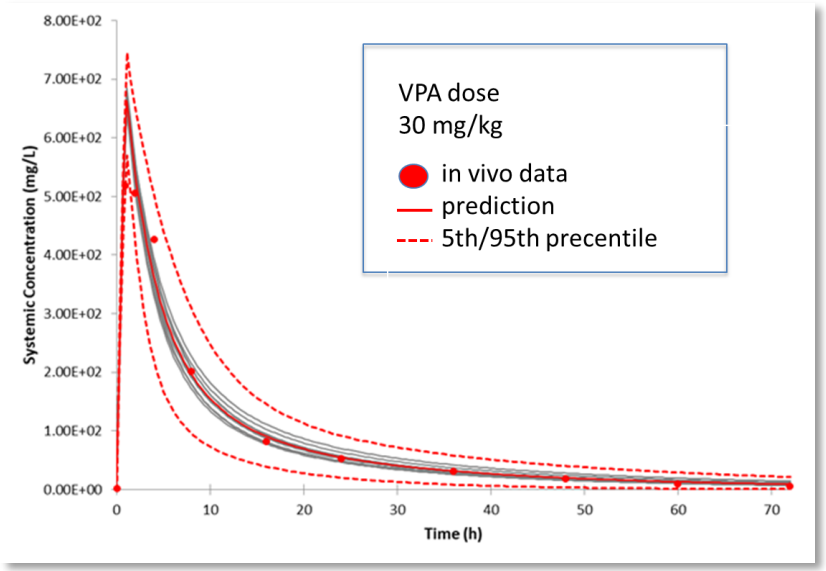
VPA – data rich compound with in vivo ADME data



PBPK model predicts in vivo human data for one analogue well

NAM derived – ppb (in silico) and intr. hep clearance in PHH (in vitro)

VPA model used for all analgues in RAX group

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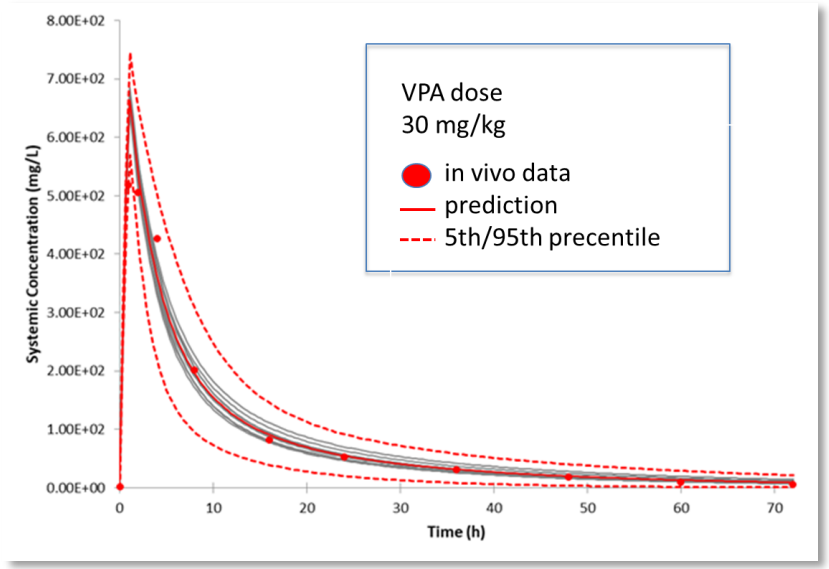
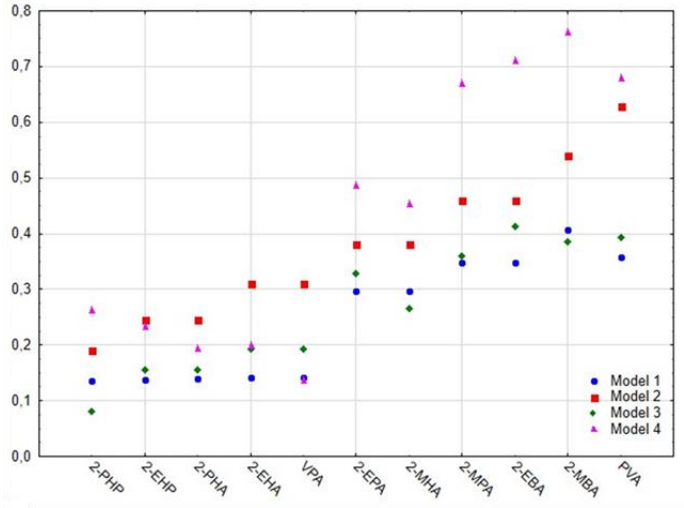
Toxikokinetic properties within grouped compounds



apply



fu increase with decreasing side chain length

Hepatic clearance decreases with side chain length

	99-66-1 VPA	149-57-5 2-EHA	20225-24-5 2-EPA	88-09-5 2-EBA	97-61-0 2-MPA	4536-23-6 2-MHA
$CL_{int,H}$ (µl/min/10^6; HµREL co-culture)	0.22	0.55	0.78	9.62	10.2	3.95

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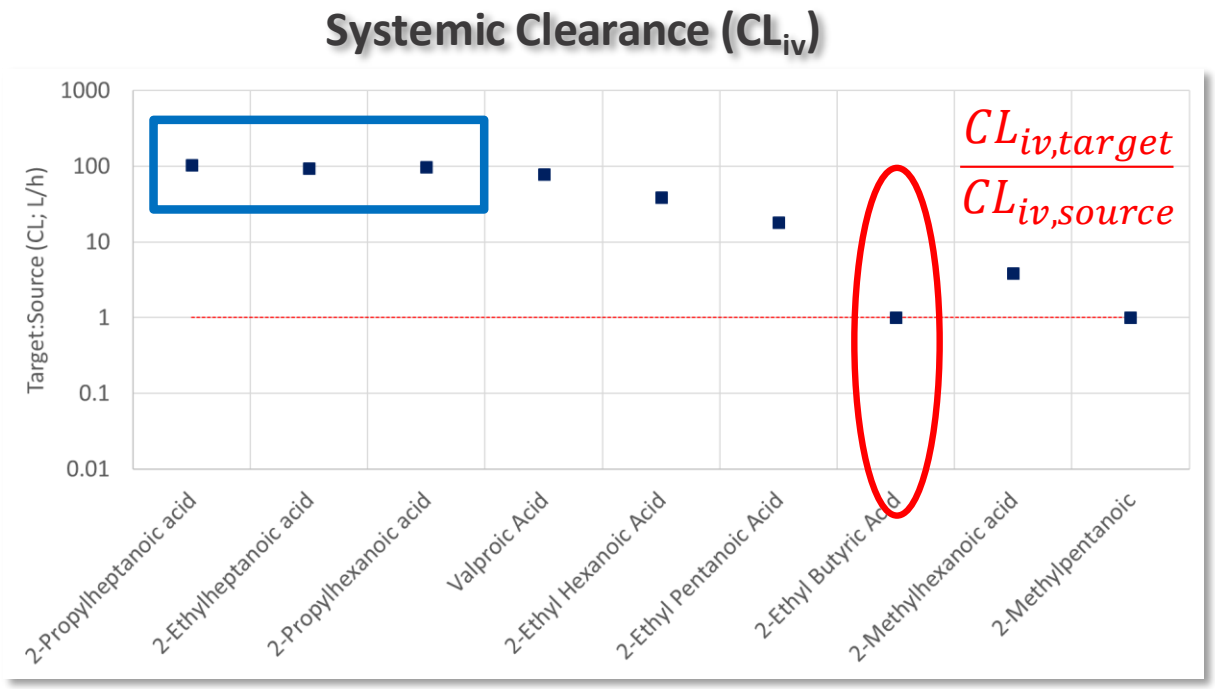
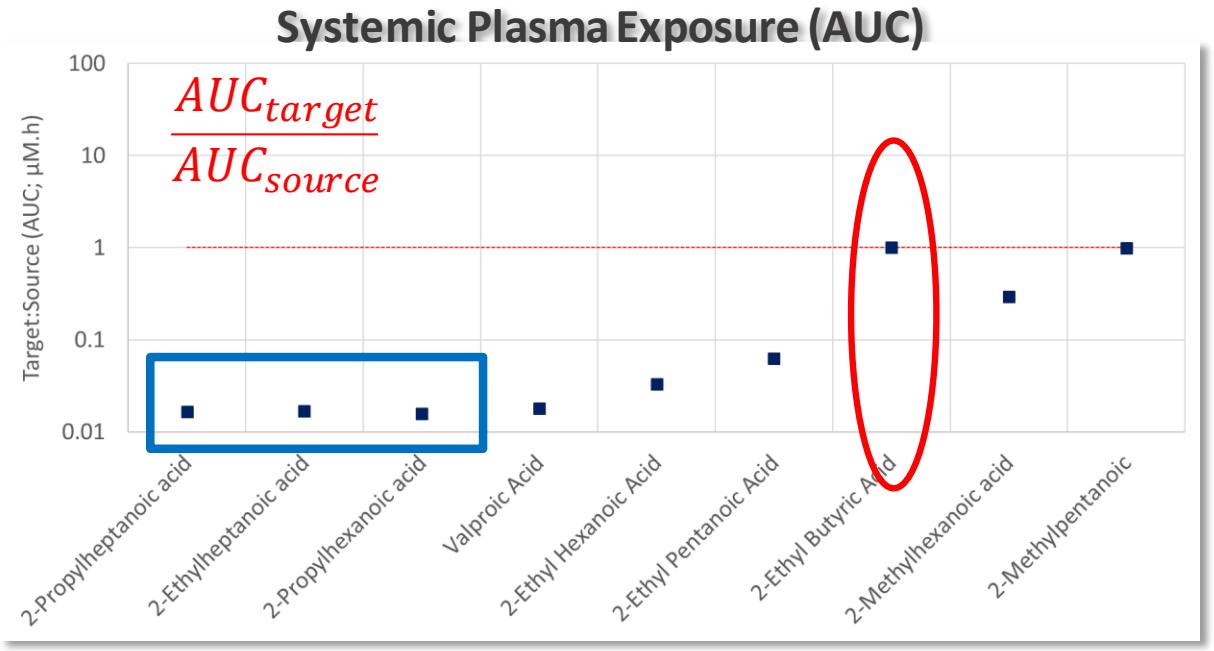
RAX Case Study - PBPK Predictions Across Analogues





- Three source compounds show intrinsic hepatic clearance (CL_{int}) below limit of detection of *in vitro* assay (Hurel co-culture system); CL_{int} (µl/min/10^6 hepatocytes) assumed to be 2-fold lower than VPA
- Trend observed – short chain target compound 2-EBA shows comparable predicted exposure and clearance to 2-MHA and 2-MPA

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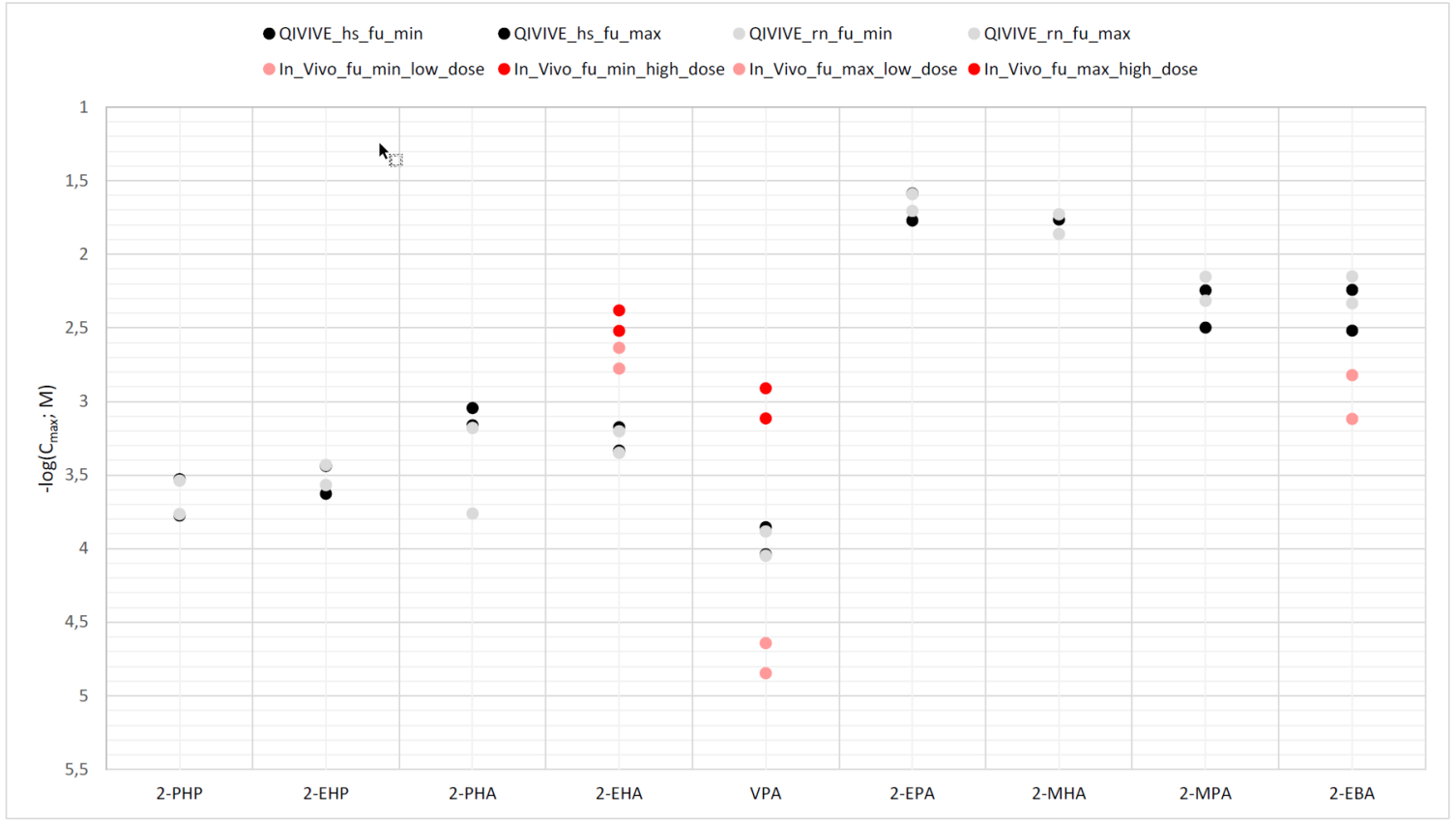
Estimate human equivalant dose –bridge back to AOP



Comparison of max. plasma concentration

- QIVIVE (red)
- Reverse dosimetry from rodent study (black)

Late KE „triglyceride accumulation corresponded best to in vivo situation“

MIEs/early KE – lower heD

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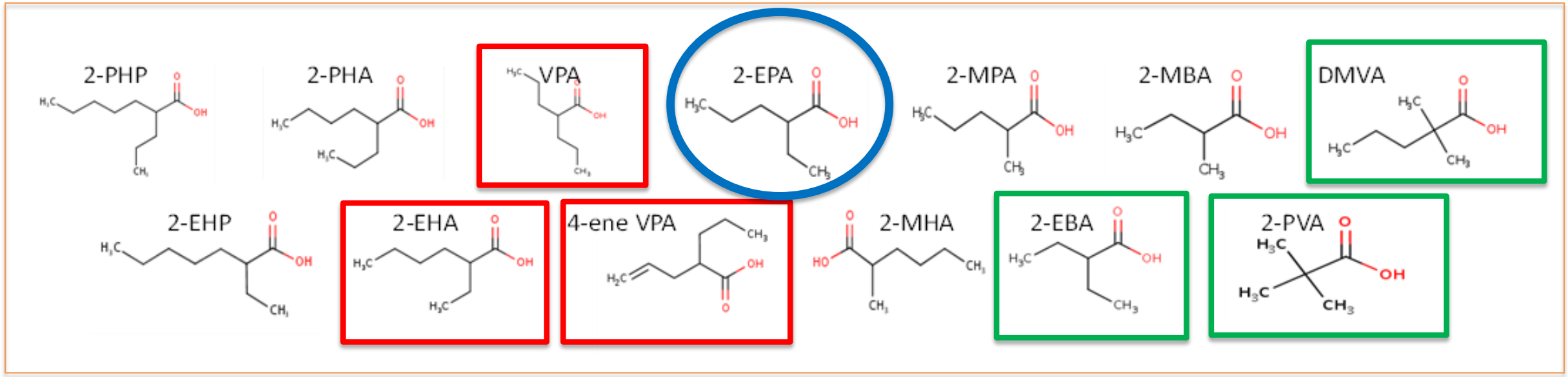
Read-Across Supported by NAMs –Toxikodynamics + Toxikokinetics



AOP used to illustrate **shared mode of action**

- 2-EPA show activation of MIEs and KEs belonging to AOP, induces late KE „lipid accumulation“ in different liver cells
- Early MIEs/KEs can be used to prove similar mode of action

In vitro ADME assays as well as PBK modelling used to show **trend in toxikokinetics**

- PBK simulations for all analogues identify a trend for increasing clearance and so decreasing systemic exposure with decreasing side chain length
- **Late KE** shows best predictivity compared to early MIEs and KEs

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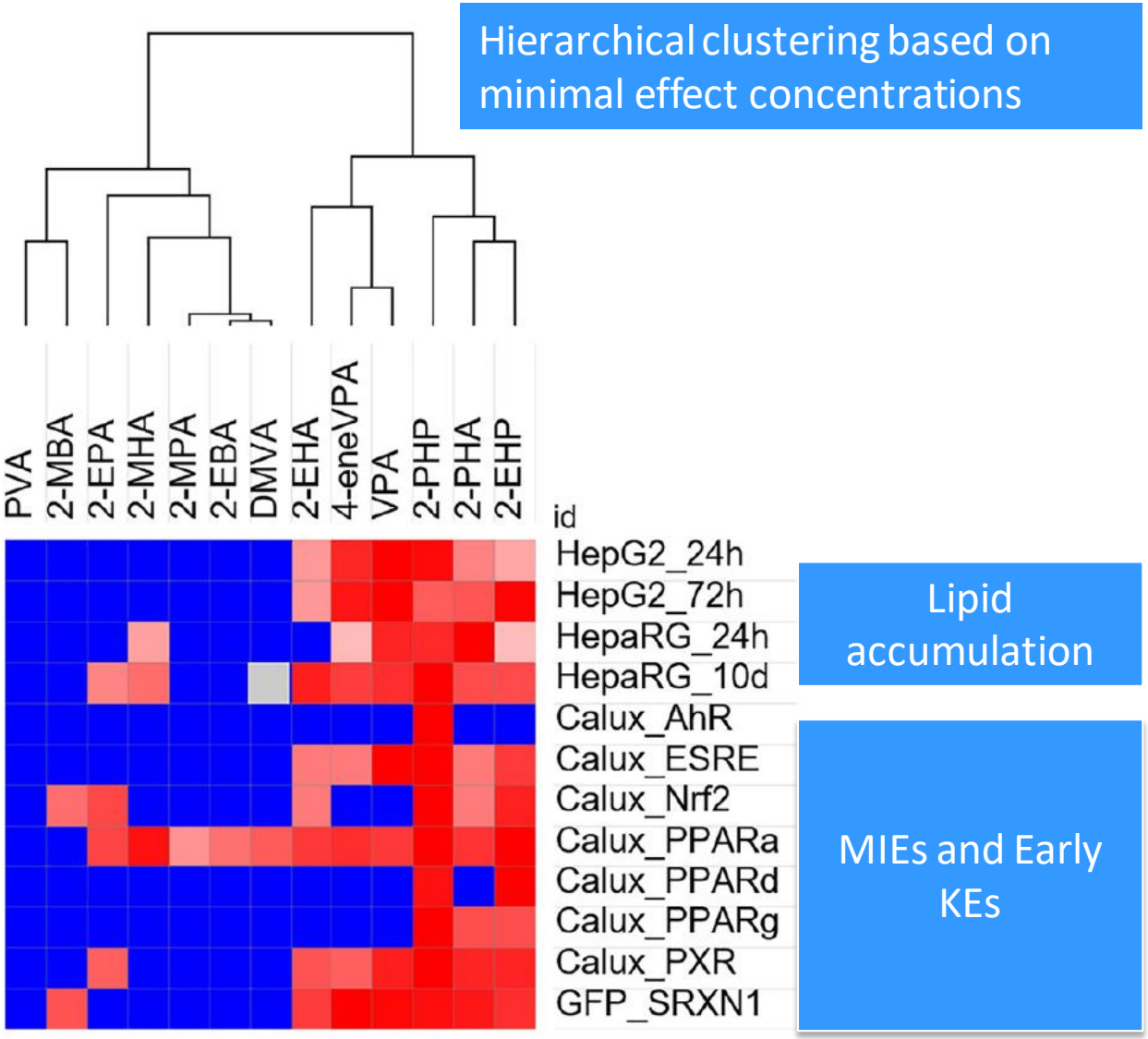
Read-across - options



Option 1

- NAM can be used to idnetify nearest neighbours; in vivo data from them to read-across

Option 2

- QIVIVE can be used to directly predict the human threshold

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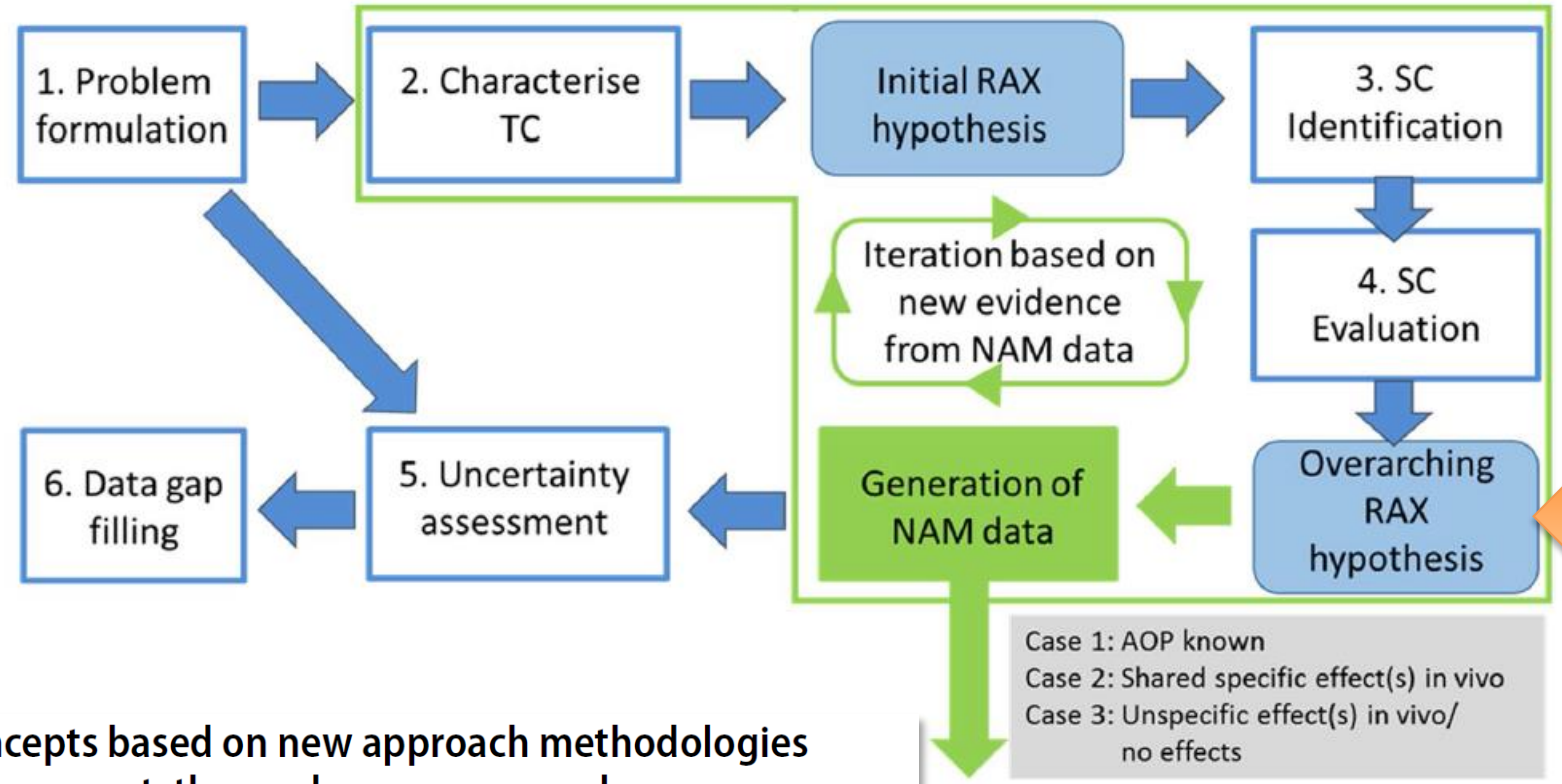
Result used to develope a Read-Across Assessment Schema

From several case studies



read-across hypothesis based on in vivo data

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

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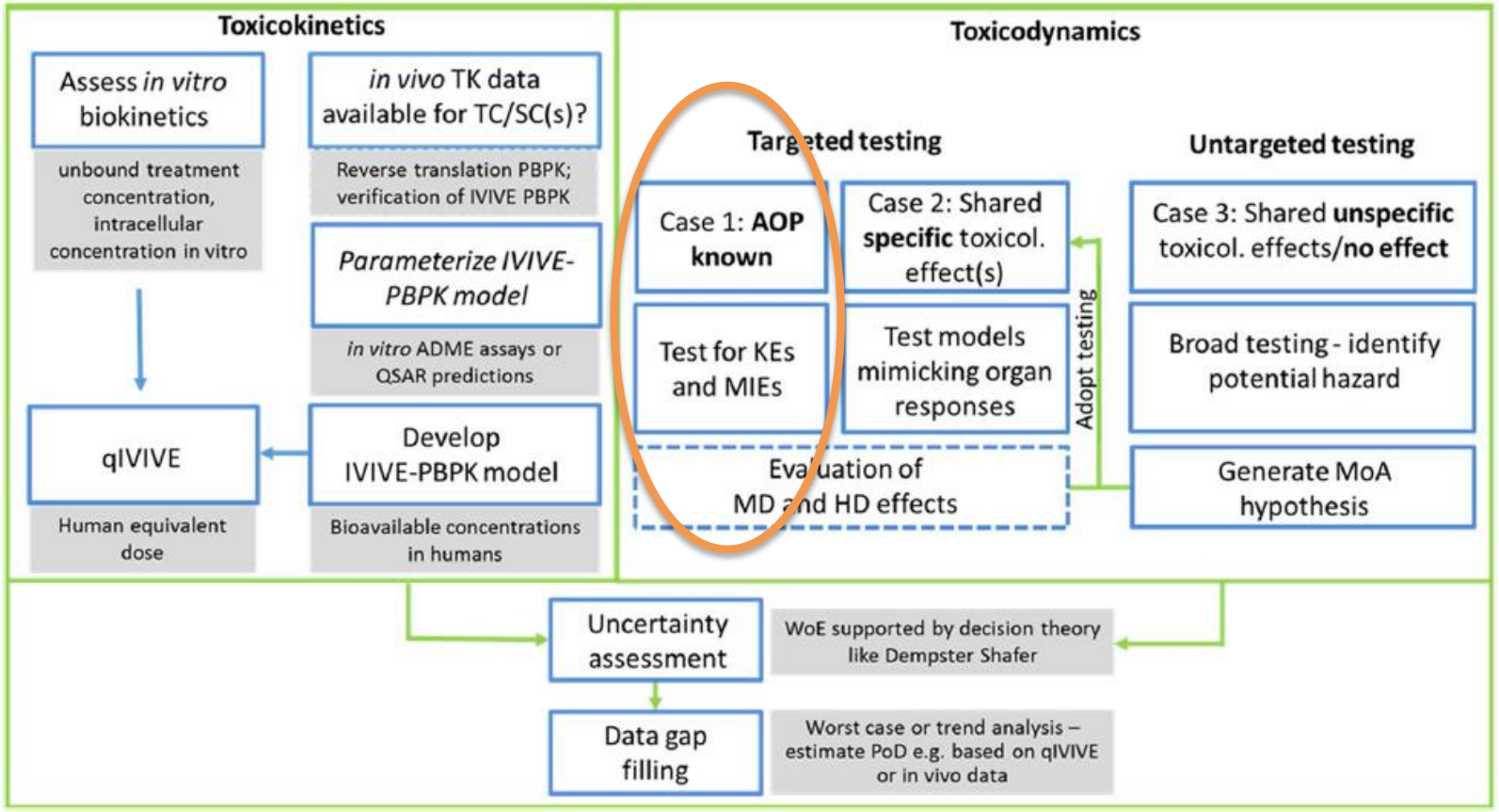
Generation of NAMs based on Read-Across Hypothesis –

NAM used as supporting evidence to proof shared toxicodynamic and kinetic properties



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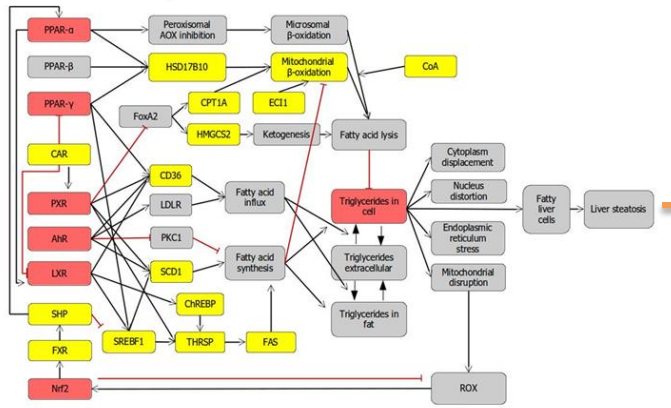
Summary

- Read across case studies can be used to gain confidence in the **use of non-formally standardised new approachs** such as **AOPs.**
- **In RAX -** in vivo endpoint data compared to the NAM based predictions –> **in situ validation** of formally non standardised NAM
- AOPs are extremely useful tools to inform the testing strategy
- Not all MIEs and KEs need to be tested
- In our case - late KE was most appropriate to predict the human equivalent dose

Read-across team

AOP



Vrijenhoek NG et al. (2022) ALTEX, doi:10.14573/altex.2107261
Escher SE et al. (2022) Toxicology in Vitro 79, 105269
Kamp et al. Read across advisory document, under preparation
Fischer C et al., qIVIVE, under preparation

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End of story?

RISK HUNT3R

Objectives

- Include metabolism in barrrier organs and better models for in vitro ADME
- Complete AOP landscape
- Move towards quantitative AOPs
- ….

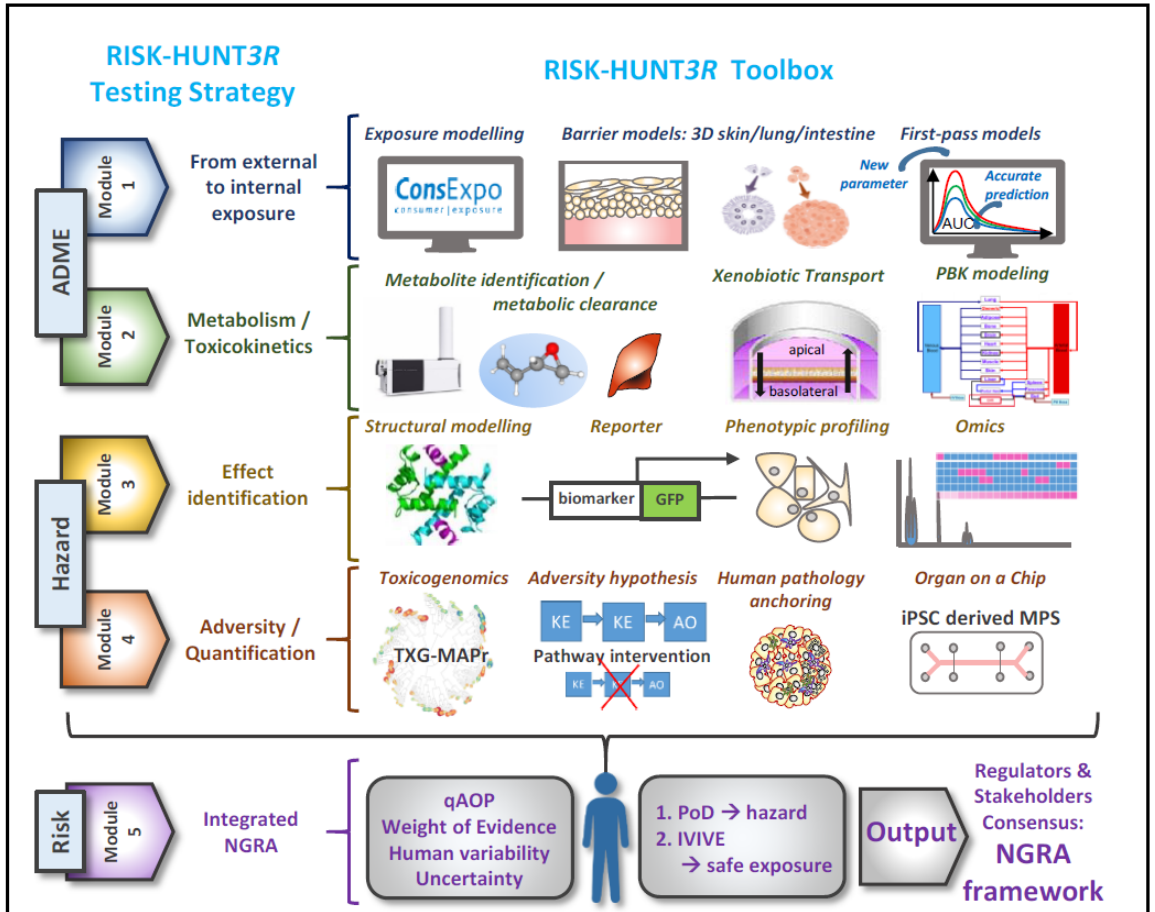

Figure 2: RISK-HUNT*3R* vision for an NGRA testing strategy and essential RISK-HUNT*3R* NGRA toolbox components.

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Case study 1 team

Thanks for your attention