Safety Evaluation Ultimately Replacing Animal Testing

The ab Initio safety assessment case study for daily exposure to an active ingredient in a body-lotion

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The data and models used within the poster have been kindly provided by partners across the SEURAT-1 cluster projects and the US EPA, and we kindly thank all involved including (non-exhaustive list) Frederic Bois, Andrea Richarz, Leo van Grunsven, Sofia Leite, Marjan Vracko, Emilio Benfenati, Simona Kovarich, Christof Schwab, Vessela Vitcheva, Ivanka Tsakovska, Fozia Noor, Elmar Heinzle, Thomas Exner, Lucian Farcal, Barry Hardy, Alfonso Lostia, Tomislav Horvat, Elisabeth Joossens, Richard Judson with support from other US EPA colleagues.

TIER 0: IDENTIFY USE SCENARIO, CHEMICAL OF CONCERN AND COLLECT EXISTING INFORMATION

1. IDENTIFY USE SCENARIO
2. IDENTIFY MOLECULAR STRUCTURE
3. COLLECT EXISTING DATA
4. IDENTIFY ANALOGUES, SUITABILITY ASSESSMENT AND EXITING DATA
5. SYSTEMIC BIOAVAILABILITY (TARGET ORGANS, INTERNAL CONCENTRATION)

TIER 1: HYPOTHESIS FORMULATION FOR AB INITIO APPROACH

6. MOA HYPOTHESIS GENERATION (WEIGHT OF EVIDENCE BASED ON AVAILABLE TOOLS)

TIER 2: APPLICATION OF AB INITIO APPROACH

7A. TARGETED TESTING

7B. BIOKINETIC REFINEMENT (IN VIVO CLEARANCE, POPULATION, IN VITRO STABILITY, PARTITION)

8. POINTS OF DEPARTURE, IN VITRO IN VIVO EXTRAPOLATION, UNCERTAINTY ESTIMATION, MARGIN OF SAFETY

9. FINAL RISK ASSESSMENT OR SUMMARY ON INSUFFICIENT INFORMATION APPROACH

Objective and Case Study scenario

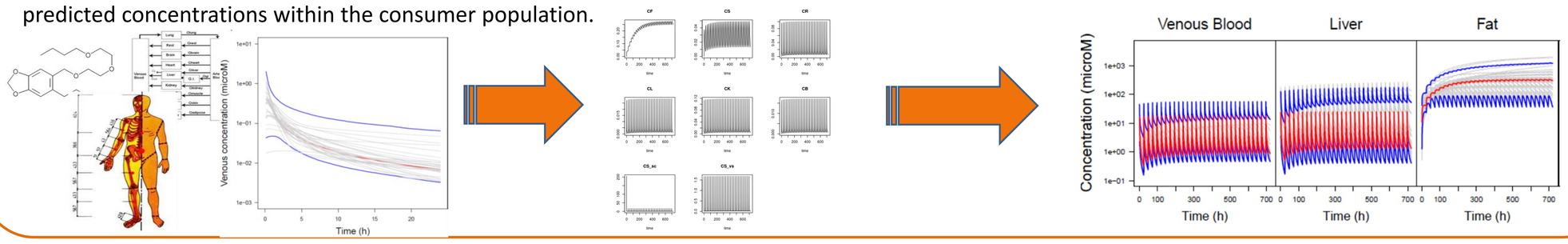
The ab initio case study attempts to structure in silico knowledge & predictions alongside in vitro data in a logic decision workflow. Key components developed across some of the SEURAT-1 projects and related initiatives have been integrated to build a weight of evidence without animal. We demonstrate that this could be the basis for an integrated risk assessment relying only on alternative methods, while also identifying remaining weaknesses and knowledge gaps to further advance alternative assessment approaches.

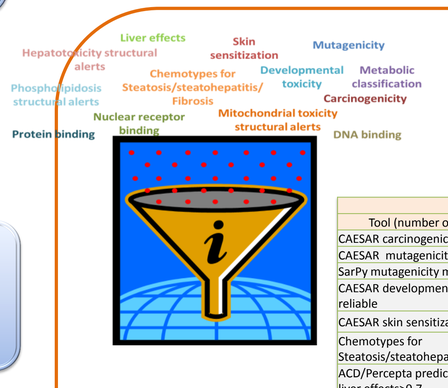
Piperonyl butoxide (PBO) is not a cosmetic ingredient but fits within a chemical space relevant to cosmetics and has prior use in medicated shampoos. The hypothetical question to the risk assessor is based on the assumption that this is a novel chemical entity with no prior animal data and is stated as

"Can we safely use 12.5% PBO in a daily body lotion?"

- **TTC?** Based on predicted exposure levels a TTC led exposure waiving is considered in the absence of compound-specific data, but is not applicable in this case as TTC is applicable only to low exposures. Using TTC we could support 0.0493% in body lotion.
- **Read Across?** Based on structural similarity a read across approach is considered, but is not applicable in this case as there were no suitable analogues with sufficiently high similarity to read across confidently from. Whilst there are some structural similarity between PBO, safrole and 1'-hydroxysafrole, PBO lacks the allyl group which is important for the metabolic activation needed to cause genotoxicity and it has a PEG-based side chain which will give additional differences in potential metabolic pathways.

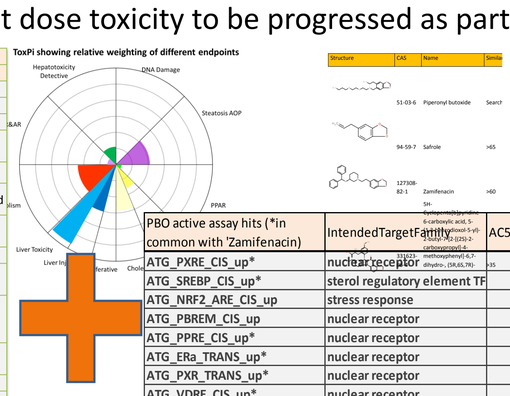
A **6 compartment PBPK model** was built for PBO incorporating metabolism and skin as the route of exposure. Systemic concentrations of the PBO compound were generated for repeat dose exposures based on the expected consumer use of body lotion. **Monte Carlo analysis** shows 95% confidence intervals for predicted concentrations within the consumer population.



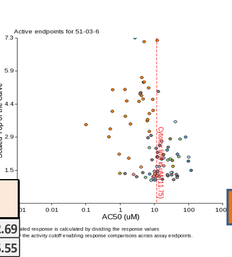


Broad characterization of hazard: *In* silico and *in* vitro tools are essential for progressing in the use of non-animal approaches in defining the relevant key MoAs for human adversity from the predicted or observed perturbations at relevant doses for the exposure scenario. This initial filter enables subsequent lower throughput assays of more in vivo relevance for repeat dose toxicity to be progressed as part of the hazard characterization.

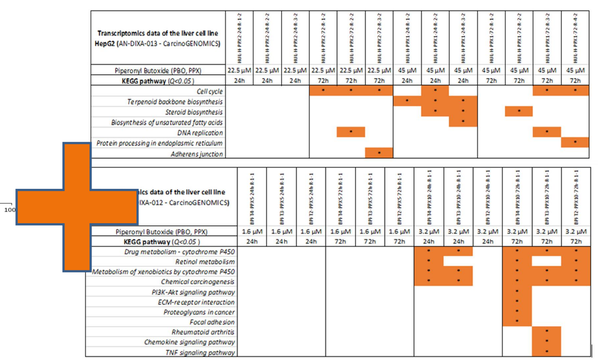
In silico predictions	
Tool (number of alerts considered)	Alert (prediction)
CAESAR carcinogenicity - highly reliable	carcinogen
CAESAR mutagenicity- highly reliable	Non mutagen
SarPy mutagenicity model- highly reliable	Non mutagen
CAESAR developmental toxicity - Maybe not reliable	predicted non-toxic
CAESAR skin sensitization - Maybe not reliable	sentisizer
Chemotypes for Steatosis/steatohepatitis/Fibrosis (9)	potential steatotic compound
ACD/Percepta prediction of probability of liver effects>0.7	No liver effect
Cosmos Hepatotoxicity structural alerts (16)	No hepatotoxicity
Cosmos phospholipidosis structural alerts (45)	No phospholipidosis
Cosmos Mitochondrial toxicity structural alerts (21 mechanistic alerts + 21 chemotypes)	No alert
Cosmos nuclear receptor binding alerts	7 alerts out of 12: AHR, AR, ER, GR, PR, THR, PXR
	PPARγ full agonist
	No LXR binding
Cosmos Protein binding (108)	No protein binding
Cosmos DNA binding (111)	No DNA binding
Molecular Network Public set of generic metabolic classes (25)	7 metabolic classes activated



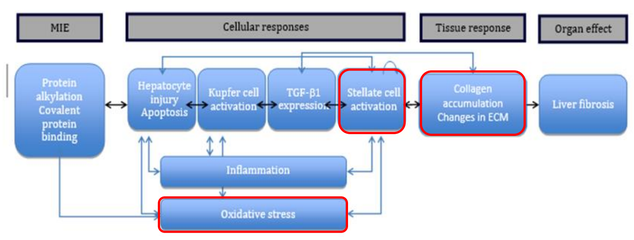
PBO active assay hits (*in common with 'Zamifenacin)	IntendedTargetFamily	AC50
ATG_PXRE_CIS_up*	nuclear receptor	2.69
ATG_SREBP_CIS_up*	sterol regulatory element TF	5.55
ATG_NRF2_ARE_CIS_up	stress response	7.11
ATG_PBREM_CIS_up	nuclear receptor	7.23
ATG_PPRE_CIS_up*	nuclear receptor	10.23
ATG_ERa_TRANS_up*	nuclear receptor	11.11
ATG_PXR_TRANS_up*	nuclear receptor	12.07
ATG_VDRE_CIS_up*	nuclear receptor	12.29
Cytotoxicity threshold		11.40
ATG_PPARg_TRANS_up*	nuclear receptor	14.17

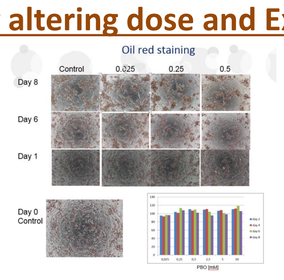


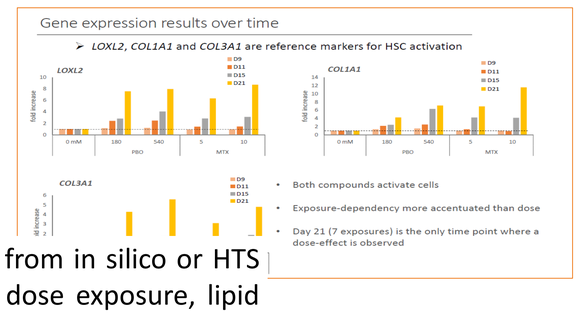
ToxCast data for PBO



Refinement on AO pathway altering dose and Exposure for based on in vitro repeat dosing exposures



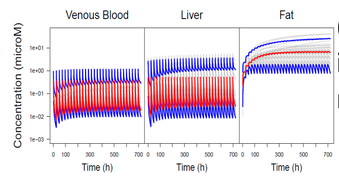




MIEs within the steatosis AOP (not including LXR activation), were identified from in silico or HTS results. In order to determine if these could lead to steatosis after repeat dose exposure, lipid accumulation was measured in metabolically competent SEURAT-1 test systems. Single dose and repeat dosing once /48 hr showed no increase up to 500uM. Saturation of the system using multiple dosing within a 24hr period indicated increased lipid accumulation at 10uM

–> unable to rule out potential steatosis under consumer use scenario.

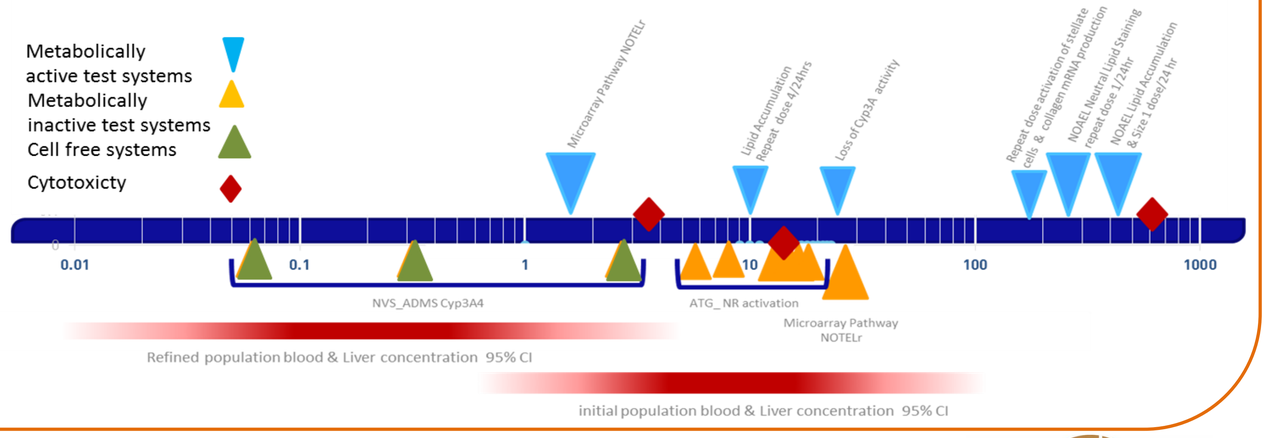
An assessment of fibrotic potential was performed using the SEURAT-1 multicellular assays developed based on flags in the HTS and omics data. Comparison to the positive control suggests upregulation of stellate cell activation and collagen deposition at both doses tested (180uM & 540uM) with increasing activation following repeated exposure.



Biokinetic and in vitro refinement:

Penetration data from human skin (published) reports reduced skin penetration for systemic exposure of 2.1% of applied dose.

- This case study highlights the challenge in integration and visualisation of multiple data streams for safety assessment decisions.
- We show progress in inferring mode of action using a combination of in silico, high throughput & high content data streams. The use of biological and chemical sub structure similarity screens can provide some anchoring to build confidence and give clues as to organismal outcomes.
- Where available, higher order assays aid the characterisation of dose relevance – ruling out certain AOPs. This highlights the continuing need to build mode of action ontologies and develop quantitative AOPs.
- Even with the remaining variability and uncertainty it appears there is not an adequate margin of safety for a use scenario of 12.5% PBO in a daily body lotion using the new approach data.
- Given the PPARγ response, its high expression in adipose tissue and the prediction of fat accumulation this would be a key area to be addressed to understand potential of effects such as on adipocytes differentiation and hormone levels.
- Further work would be needed to refine the human systemic exposure estimates. Including addressing uncertainties in plasma protein binding, liver metabolism, clearance and renal excretion. Similarly the in vitro exposure scenario needs to be characterised to enable comparison to the human systemic exposure, and ultimately to be able to convert the in vitro concentration to an amount applied to the skin.
- So, it appears we are going in the right direction but still have ways to travel…



The projects constituting the SEURAT-1 research initiative have received funding from the European Union Seventh Framework Programme (FP7/2007-2013). The projects constituting the SEURAT-1 research initiative have received financing from Cosmetics Europe.

European Commission

Cosmetics Europe the personal care association