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, safrrole and 1'-hydroxy safrrole, PBO lacks the aliphatic 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.

**TIER 1:** HYPOTHESIS FORMULATION FOR AB INITIO APPROACH

<table>
<thead>
<tr>
<th>1. Identify Use Scenario</th>
<th>2. Identify Molecular Structure</th>
<th>3. Collect Existing Data</th>
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**TIER 2:** APPLICATION OF AB INITIO APPROACH

<table>
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<th>4. Identify Analogues, Suitability Assessment and Existing Data</th>
<th>5. Systemic Bioavailability (Target Organs, Internal Concentration)</th>
<th>6. MoA Hypothesis Generation (Weight of Evidence Based on Available Tools)</th>
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**TIER 0:** IDENTIFY USE SCENARIO, CHEMICAL OF CONCERN AND COLLECT EXISTING INFORMATION

|----------------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------|

**Objective and Case Study scenario**

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 characterisation.

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

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 (180μM & 540μM) 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.

**Safety Evaluation Ultimately Replacing Animal Testing**

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

Elisabeth Berggren1, Alicia Paini2, Gladys Ouedraogo2, Andrea Richarz2, Andrew White3 and Catherine Mahony4, 1. JRC, 2. Ciba® KBL, 3. Unilever SEAC, 4. P&G

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