This case study is an exposure-based next generation risk assessment (NGRA) case study for the preservative ingredient phenoxyethanol. It was guided by the SEURAT-2 assessment workflow (Bergman et al., 2017) and the International Cooperation on Cosmetics Regulation NGRA principles (Dent et al., 2018), with the aim of using only non-animal approaches to assure the systemic safety of the ingredient when present at an active level (1%) in a product with a high level of consumer use (body lotion). The overall strategy of the case study was to use in vitro/skin in vitro approaches instead of animal-based approaches for hazard identification in order to protect animal welfare. No animal data were therefore used in the assessment. Instead, the approach involved the generation of new approach methodology (NAM) data on biokinetics and biodynamics. In silico and in vitro approaches showed the major metabolite of phenoxyethanol to be phenoxycetic acid (PAA), and PBK modelling was used to predict the 95th percentile population exposures of both phenoxyethanol and PAA in blood and tissues. These internal exposures were compared with points of departure (PoDs) derived from in vitro bioactivity assays. These included published non-animal data and new in vitro pharmacological, stress, and transcriptional data. The PoDs exceeded the predicted internal exposure levels for both phenoxyethanol and PAA. This provided some assurance that in vitro bioactivity does not occur at consumer-relevant exposure levels. However, the margins of internal exposure for PAA were small (2 and 3 for C50 and AUC50, respectively), meaning that confidence in the risk assessment was low. This case study illustrates one possible approach to safety assess both a parent chemical and its major stable metabolite in non-animal systemic toxicity risk assessment.

Tier 0

1. Exposure estimates SCCS Notes of Guidance 90th percentile exposure to body lotion, ingredient present at 1% (as per SCCS Notes of Guidance, 10th Revision SCCS/162/18).
2. Phenoxethanol is a broadly acting antimicrobial and is safely used in both rinse-off and leave-on cosmetics at up to 1%.
3. Existing data harvested from PubChem and ToxCast, as the purpose of this case study was to test the current ability to make a safety decision without any in vivo data, any pre-existing animal data on the case study ingredient were discounted. No animal data considered in the evaluation.
4. Read across was not a feature of this case study.

Tier 1

5. PBK model developed using literature inputs: no in vitro data were generated in Tier 1. Possible metabolic products predicted in silico using Meteor.

Tier 2

6. In silico tools used to supplement existing in vitro data to try to identify any modes of action of concern: OECD QSAR Toolbox, Derek Nexus, COSMOS nuclear Receptors Binding profilers, MIL Atlas, CERAPP and ComPARY.

Phenoxyethanol was inactive in the in vitro pharmacological profiling assays and in all stress cell panel assays, in contrast to other test items known to cause adverse health effects and cellular stress (Hathaway et al., 2020) reproduced under Creative Commons CC-BY-NC license.

The formation of PAA was measured over time in one in cell systems used to provide the PoDs for the safety assessment (HepG2 and HepaRG cells). This information was used to calculate Cmax and AUC for the major stable metabolite under the same conditions as the transcriptional assays.

B. Comparisons were performed for both phenoxyethanol and its major stable metabolite PAA.

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**Tier 0**

**Step 1:** Identify exposure/assess scenarios for target chemical.

**Step 2:** Identify molecular structure of target chemical.

**Step 3:** Collate existing data.

**Step 4:** Identify Analogues.

**Tier 1**

**Step 5:** Systemic bioavailability (internal concentration).

**Step 6:** Metabolism hypothesis generation (weight of evidence based on available tools).

**Tier 2**

**Step 7a:** Bioactivity testing: High throughput transcriptionomics in HepG2, HepaRG and MCF-7 cells; cell stress panel in HepG2 cells, in vitro pharmacological profiling.

**Step 7b:** Biokinetic refinement. Population modelling, confirmatory in vitro clearance data, confirmatory in vitro metabolite characterization in primary hepatocytes and in cells used in targeted testing.

**Step 8:** Points of departure, IVIVE.

**Step 9:** Final Safety Assessment or summary of insufficient information.

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**Notes**

1. Dent, MP; (frymenska, A); Troutman, LA; Hewitt, NLP; Makombar, S; Houghton, P; White, A; Kukic, P; Nicod, B; Pirzadehs, K; Plechak, P; Crane, MD; Bellfield, S; Finnan, I; Closehill, I; HMP; Scheppa, A; Suzuki, P; Mukda, YP; Zucca, S; Kenne, GC; Vargani, AP; Mahony, C*1


**References**

Dent et al., 2018; Przybylak, K; Mahony, C; Nukada, K; Dent et al., 2018; Wetmore et al., 2012; Troutman et al, 2015; Mahony, C; Efremenko, A; Przybylak, K; Dent et al., 2018.