WC11
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Poster

Non-Animal Safety Assessment Case Study of Phenoxyethanol in Cosmetics

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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-1 assessment workflow (Berggren 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 this 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 is one where *in vitro/in silico* approaches instead of animal-based approaches for hazard identification are used in the risk assessment. 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 phenoxyacetic 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 profiling, cell stress, and transcriptomics 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 C_{max} and AUC_{24} 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.

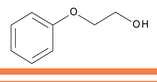
Tier 0

Step 1: Identify exposure/use scenarios for target chemical

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/1602/18)

Step 2: Identify molecular structure of target chemical

2. Phenoxyethanol is a broadly acting anti-microbial and is safely used in both rinse-off and leave-on cosmetics at up to 1%.



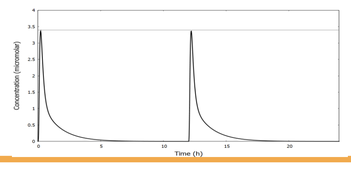
Step 3: Collate existing data

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 using any in vivo data, any pre-existing animal data on the case study ingredient were discounted. no animal data considered in the evaluation.

Step 4: Identify Analogues

4. Read across was not a feature of this case study

Tier 1



Step 5: Systemic bioavailability (internal concentration)

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

Step 6: MoA hypothesis generation (weight of evidence based on available tools

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, MIE Atlas, CERAPP and CoMPARA.

ToxCast assay name	Expert interpretation of hit
BSK_CASM3C_SAA_up	Only top concentration above the cut-off (the threshold indicating a positive response); hit potentially confounded by overfitting. The curve fit and resultant AC_{50} (16.6 µM) are reasonable.
ATG_AP_1_CIS_dn	Borderline hit with a single replicate; single concentration just above the cut-off (the threshold indicating a response) at 100 µM.
NVS_ADME_hCYP2C19_Activator	Borderline hit at a single dose at the cut-off and predicts an AC_{50} at a concentration where the activity was not seen above the cut-off. Further, the NVS_ assays in the Activator direction are not likely to be biologically meaningful (increased activity in an enzyme inhibition assay).
ACEA_AR_agonist_80hr	Of the 4 replicates at each concentration, 2 replicates were completely negative and 2 were positive. Since the overall AR model score was negative (Kleinstreuer et al., 2017), phenoxyethanol was considered to not exhibit any meaningful AR-related activity.
TOX21_RT_HEPG2_FLO_08hr_viability	These data represent a single replicate at each concentration, and only one concentration in the middle of the range is marginally higher than the cut-off. It does not make biological sense for a viability assay, so this result was considered to be due to biological variation.
TOX21_p53_BLA_p5_ch2	A single point drives a gain-loss fit. This is likely assay interference because it does not make biological sense for p53 activity to increase and decrease precipitously for one point in the curve.
TOX21_p53_BLA_p5_ratio	A single point drives a gain-loss fit. This is likely assay interference because it does not make biological sense for p53 activity to increase and decrease precipitously for one point in the curve.
TOX21_ERR_Antagonist	A lot of variability within the data and divergence between replicates.

Toxcast hits for phenoxyethanol (8/785 assays) – with thanks to Richard Judson and colleagues at US EPA for help with expert interpretation

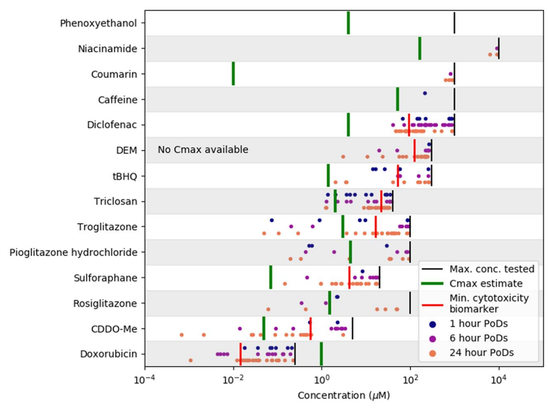
	Value	Units	Source
Consumer use of body lotion	123	mg/kg/day	SCCS Notes of Guidance, 10th Edition (SCCS, 2018)
Applied dose of phenoxyethanol	1.23	mg/kg/day	Assumed that ingredient present at 1%
Dermal Penetration (K_p)	0.0025	cm/hour	Human skin in vitro (Roper et al., 1997).
Dermal Evaporation (K_{loss})	0.0025	cm/hour	
Hepatic Clearance	20	µL/min/million cells	Primary human hepatocytes (Wetmore et al., 2012)
Fraction Unbound (Fup)	0.56	no units	Average of 3 QSAR estimates (ChemSilico v1.6.1 (fup=0.71), ACD/Percepta v14.0.0 (fup=0.37), ADMET Predictor v6.5.0013 (fup=0.59))
Dermal Dose	0.616	mg/kg/application	
Body Surface Area	15760	cm^2	Troutman et al, 2015
Dosing interval	12	Hours	

Input to Tier 1 PBK model

Tier 2

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

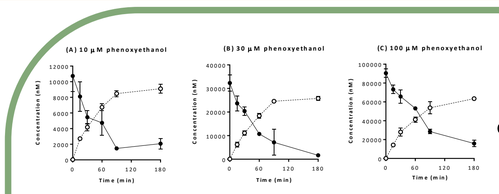
Step 7b: Biokinetic refinement: Population modelling, confirmatory *in vitro* clearance data, confirmatory *in vitro* metabolite characterization in primary hepatocytes and in cells used in targeted testing.



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

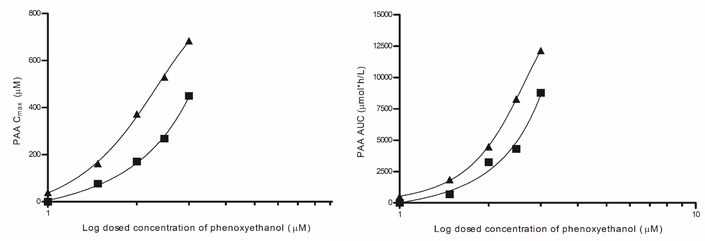
Pathway Tests	HepaRG	MCF-7	HepG2
BMD_{10} of pathway with the lowest BMD_{10} (µM)	552.90	760.33	232.00
$BMDL_{10}$	220.92	512.84	171.25
$BMDU_{10}$	911.72	1648.51	557.20

Phenoxyethanol showed very little transcriptomic activity in 3 cell lines using the Temp-o-seq Biospyder platform



In silico metabolism predictions were confirmed *in vitro*, and the PBK model was refined

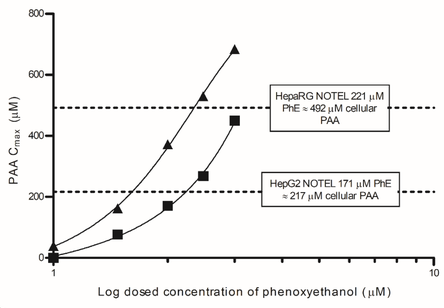
	Blood Phenoxyethanol		Blood PAA		Kidney PAA	
	C_{max}	AUC_{24}	C_{max}	AUC_{24}	C_{max}	AUC_{24}
	µM	µmol*h/L	µM	µmol*h/L	µM	µmol*h/L
Mean	3.7	7.3	10.5	230	36	789
SD	1.4	4.2	4.9	115	17	401
5th %ile	1.8	3.3	4.5	93	15	312
95th %ile	6.2	15	20	453	69	1569



The formation of PAA was measured over time in the cell systems used to provide the critical 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 transcriptomics assays.

Step 8: Points of departure, IVIVE

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



Chemical	Scenario	Human Exposure		PoD		Bioactivity: exposure ratio	
		AUC_{24}	C_{max}	AUC_{24}	C_{max}	AUC_{24}	C_{max}
		µmol*h/L	µM	µmol*h/L	µM		
Phenoxyethanol	Worst case (BMDL/P95 Exposure)	15	6.2	3215	171	214	28
Phenoxyethanol	Mean (BMD/Mean Exposure)	7.3	3.7	4381	232	600	63
Phenoxyethanol	Best case (BMDU/P05 Exposure)	3.3	1.8	10708	557	3245	309
PAA	Worst case (BMDL/P95 Exposure)	1569	69	3550	217	2	3
PAA	Mean (BMD/Mean Exposure)	789	36	4206	249	5	7
PAA	Best case (BMDU/P05 Exposure)	312	15	6573	359	21	24

Step 9: Final Safety Assessment or summary on insufficient information

9. This case study illustrates an ab initio risk assessment of a cosmetic ingredient based on the tools and approaches currently available, and provides a possible approach to evaluating major metabolite. Although the calculated BERs were above 1, which indicated that in vitro bioactivity was not seen at consumer-relevant concentrations, there were several uncertainties in the risk assessment which need to be addressed in future work. More case studies on both high and low risk substance exposures using these tools and approaches will further help to put the BER values obtained into context, and further embed the application of NGRA to cosmetics.

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