4488/ P362

Making Safety Decisions for a Sunscreen Active Ingredient Using Next-Generation Risk Assessment: Benzophenone-4 Case Study

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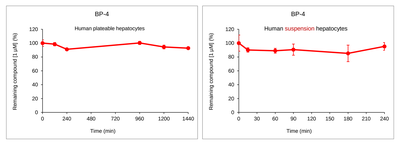
Cosmetics Europe
the personal care association

Introduction

The purpose of this work was to see if new approach methodologies (NAMs) could be used to evaluate the systemic safety of a UV filter present at a high level (up to 5%) in a sunscreen lotion. The exposure-led and hypothesis driven safety assessment was based on the International Cooperation on Cosmetics Regulation principles of Next Generation Risk Assessment and the Safety Evaluation Ultimately Replacing Animal Testing (SEURAT-1) *ab initio* safety assessment workflow. The overall hypothesis was that if biological activity measured using a broad suite of human-relevant test systems is not observed at concentrations experienced systemically by sunscreen users, there can be no adverse effects associated with product use. Different assays assessing bioactivity and exposure were used to test this hypothesis.

MODULE 1: Exposure NAMs

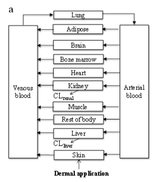


Primary human hepatocyte assay : Hepatic intrinsic clearance <2.5L/h (Below LOQ)

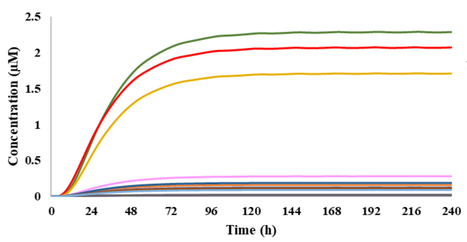
PAMPA assay: Very low permeability

Human liver S9 incubation: No metabolism of parent compound

BP-4 is not a substrate of metabolic enzymes and has very low permeability → Low clearance chemical → High confidence that liver clearance is negligible (set to 0 in PBK)

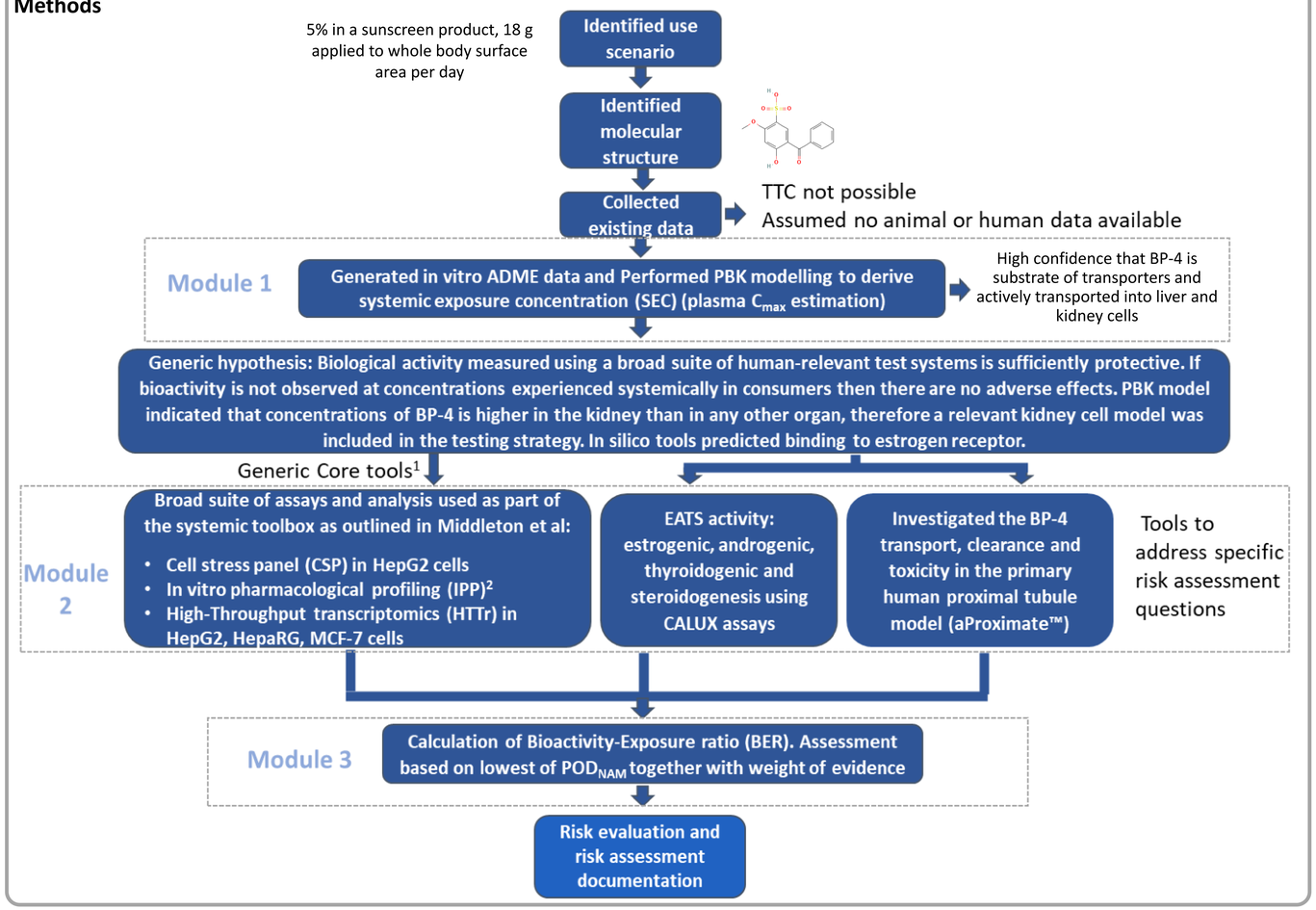


BP4-Systemic Exposure-repeat



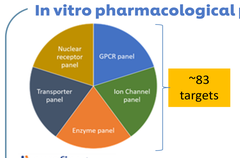
Calculated plasma exposure to a model consumer following daily use of a body lotion (18g/day). Low permeability and renal transport means that the plasma and the kidney are compartments with the greatest exposure

Methods

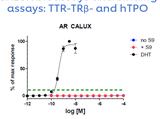


MODULE 2: Bioactivity NAMs and dose-response modelling

In vitro pharmacological profiling



CALUX bioassays and binding assays: TTR-TRβ- and hTPO

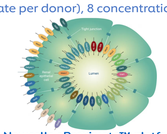


Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22
Sonneveld et al. 2005. Toxicol Sci 83(1): 136-48

Renal Toxicity

Nephrotoxicity (3 donors, duplicate per donor), 8 concentrations, 24h and 72h timepoints:

- KIM-1
- NGAL
- Clusterin
- TEER (Day 0 and Day 3)
- ATP
- LDH

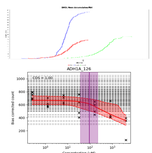


Newcells aProximate™ platform

Piyush Bajaj et al. 2020. Toxicology. 442, 152535

High-Throughput transcriptomics (HTTr)

- TempO-seek technology – full gene panel
- 24hr exposure
- 7 concentrations
- 4 cell models: HepG2, MCF7, HepaRG and aProximate cells
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

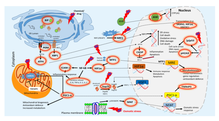
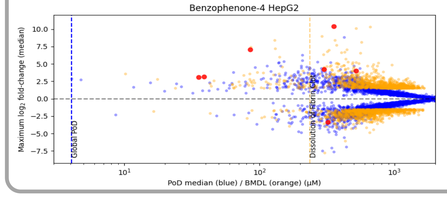


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

Benzophenone-4 HepG2

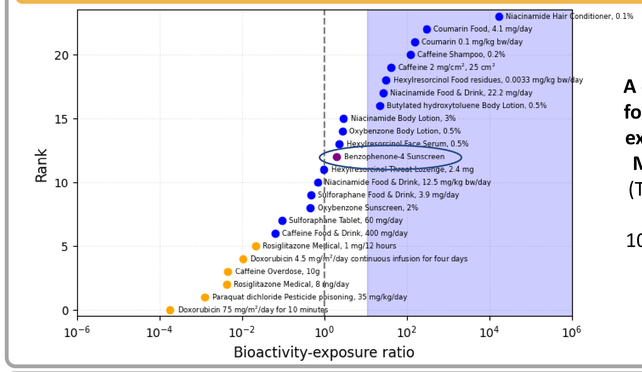


Max fold-change in expression against probe-level median POD (BIFROST, blue) and probe-level BMDLs (BMDExpress, orange) in HepG2 cells. Gene level POD (blue dotted line) minimum pathway BMDL (orange dotted line) Red circles are probe-level BMDLs contributing to the lowest pathway average.

MODULE 3: Bioactivity:Exposure Ratios (BERs)

NAM	Cell type	PODNAM Type	PODNAM Value (µM)	BER (using Cmax of 2.1 µM)	BER from individual Cmax (µM)	
					Median (95% interval)	Prob. BER>1
Cell stress panel	HepG2	Global PoD	140	67	110 (11, 1200)	1.0
HTTr	HepG2	Global PoD	4.2	2	3.4 (0.32, 35)	0.85
HTTr	HepaRG	Global PoD	52	25	42 (4, 430)	1.0
HTTr	MCF7	Global PoD	5.5	2.6	4.4 (0.42, 45)	0.90
HTTr	HepG2	Pathway BMDL	240	114	190 (18, 2000)	1.0
HTTr	HepaRG	Pathway BMDL	530	252	430 (41, 4400)	1.0
HTTr	MCF7	Pathway BMDL	330	157	260 (25, 2700)	1.0
hTPO-inhibition	-	LOEC	300	143	240 (23, 2500)	1.0
T4 binding to TTR	-	LOEC	630	300	510 (48, 5200)	1.0
Renal biomarkers	PTC	Global PoD	>1000	NA	NA	NA
HTTr (renal cells) (24 h)	PTC	Global PoD	320	152	260 (25, 2600)	1.0

Is the assessment protective?



A comparison with BERs for benchmark chemical exposures calculated by Middleton *et al.*, 2022 (Toxicol Sci. 189(1):124-147. doi: 10.1093/toxsci/kfac068)

Conclusion

Using highly conservative approaches in the calculation of BERs, the systemic exposure concentration is not likely to lead to perturbations in bioactivity that could lead to an adverse outcome in the human body when benzophenone-4 is used at 5% in a sunscreen body lotion product.

Acknowledgements

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