Occupational Next Generation Risk Assessment (NGRA) on an exclusive use cosmetic ingredient for EU REACH: A Case Study on C12-15 Alkyl Benzoate

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Background

Next-Generation Risk Assessment (NGRA) is as an exposure-led, hypothesis-driven approach that integrates new approach methodologies (NAMs) to assure safety without animal testing. There are numerous examples in the scientific literature highlighting NGRA for **consumer safety** of cosmetic ingredients.[1,2]

In this specific case study, NGRA was applied in an **occupational safety** context for an exclusive use cosmetic ingredient (INCI: C12-15 Alkyl Benzoate; C12-15 AB) to avoid animal testing in accordance with EU REACH.

Benzoic acid, C12-15-alkyl esters
INCI: C12-15 Alkyl Benzoate

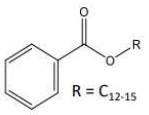


Figure. 1. Structure of C12-15 AB

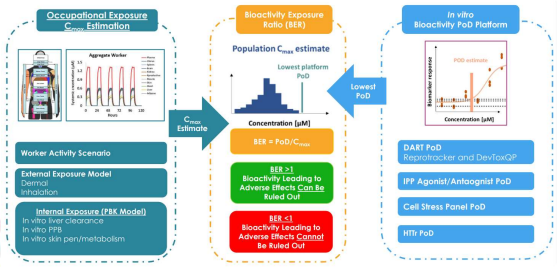


Figure. 2. Overview of the Occupational NGRA Workflow. Figure adapted from [3]

Methods: Exposure Estimation

A comprehensive assessment was performed to cover all potential instances and routes of exposure during the formulation of cosmetic products containing C12-15 Alkyl Benzoate (C12-15 AB). Factory-specific process and handling data was used within a suite of models (CHESAR, Advanced REACH Tool (ART), Stoffenmanager® and RISKOFDERM) to estimate the external dermal and inhalation worker exposure during formulation. These estimated levels of external exposure based on realistic conditions of use, in combination with newly generated *in vitro* ADME data were used as inputs for the highly conservative physiologically based kinetic (PBK) modelling using GastroPlus 9.8 (Simulation Plus, Lancaster, CA, USA), in order to estimate worst-case internal concentrations (plasma C_{max}).

External Exposure Modelling

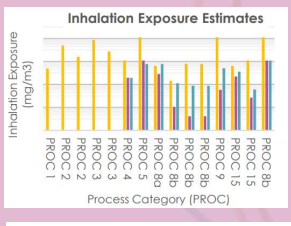


Figure 3. Inhalation exposure estimates for C12-15 AB use scenarios.

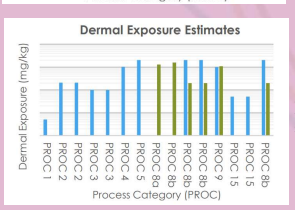


Figure 4. Dermal exposure estimates for C12-15 AB use scenarios.

In vitro ADME input to PBK Model

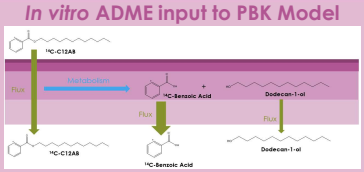


Figure 5. Skin Absorption

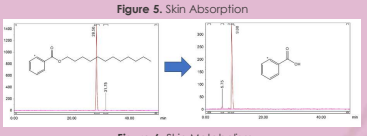


Figure 6. Skin Metabolism

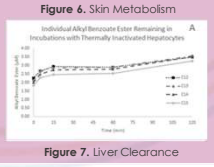


Figure 7. Liver Clearance

Internal Exposure (PBK Population Model)

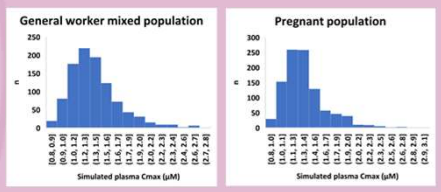


Figure 8. Probabilistic distribution for the predicted plasma Cmax of C12-15 AB for aggregated scenario of Workers, distribution for general population with mixed genders (50% female) and pregnant population.

Population type (n=1000)	Median (µM)	5th %ile [µM]	Mean [µM]	95th %ile [µM]
General population	1.32	0.97	1.38	1.98
Pregnant population	1.29	0.98	1.34	1.90

Table 1. The median, 5th percentile, mean and the 95th percentile of the probabilistic distribution for the predicted plasma Cmax of C12-15 AB.

Methods: Bioactivity Assessment

Systemic toxicity potential was assessed using a suite of *in vitro* NAMs to identify bioactivity points of departure (PoDs). The platforms included were Cell Stress Panel (CSP), *in vitro* pharmacological profiling (IPP) and high throughput transcriptomics (HTTr), which provide wide biological coverage. These bioactivity platforms were complemented by two additional assays (ReproTracker and DevTox QuickPredict) which are human induced pluripotent stem cell (hiPSC) models, in order to provide important additional screening for DART endpoints.

In vitro Pharmacological Panel (IPP)

IPP platform contains 73 targets, 44 of the targets have been associated with *in vivo* adverse drug reactions, a further 29 targets implicated in DART were added. C12-15 AB did not exhibit inhibiting or activating effects in any of the assays at the limit screening concentration of 10 µM.[5]

High Throughput Transcriptomics (HTTr)

Whole genome transcriptomics assay was performed to provide a non-targeted approach to capture biological effects. HepG2, HepaRG, and MCF-7 were included to increase biological coverage. BIFROST model and minimum benchmark dose lower confidence limit (BMDL) obtained using BMDexpress2 were used to analyse the transcriptomics data.[4]

Cell Stress Panel (CSP)

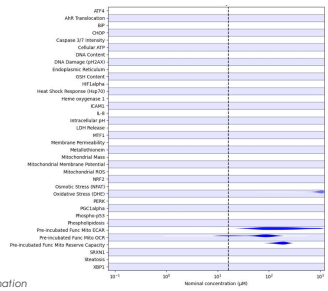
CSP in HepG2 cells consisting of 36 biomarkers across 10 different stress pathways measured using high content imaging. C12-15 AB showed very limited activity following 24 hours incubation.[6]

Table 2. PoDs (µM) from all bioactivity platforms

Bioactivity Platform	Cell Line/Type	Nominal PoD (µM)
Cell Stress Panel	Human HepG2	16 (6.4*)
IPP	Various	>10
HTTr (BIFROST)	Human MCF7	1400
HTTr (BIFROST)	Human HepG2	77
HTTr (BIFROST)	Human HepaRG	2200
HTTr (BMDExpress)	Human MCF7	617
HTTr (BMDExpress)	Human HepG2	155
HTTr (BMDExpress)	Human HepaRG	>5000
DevTox QP	Human iPSC	>30
Reprotracker	Human iPSC	>30

*Lowest Nominal PoD adjusted based on analytical dose confirmation

Figure 9. Summary of cell stress panel bioactivity for C12-15 AB.



Dose Confirmation

Factors such as media solubility, plastic binding and instability can result in the dose to which cells are exposed being significantly lower than the nominal dose.[7] Nominal and measured concentrations were compared and where experiments showed deviations, final PoD's were adjusted accordingly.

DevTox QuickPredict

hiPSCs are exposed to a range of concentrations of the test item and the ornithine and cysteine concentration in the media are measured. A decrease of the ornithine and cysteine ratio (o/c ratio) indicates developmental toxicity potential. At all exposure levels evaluated, C12-15 AB showed no response for cell viability and o/c ratio compared to control. [8]

ReproTracker

Assay monitors the differentiation of hiPSCs towards cardiomyocytes, hepatocytes and neural rosettes. By assessing the expression of specific biomarkers, the progress of differentiation and whether a compound interferes with embryonic development can be evaluated. Incubation with C12-15 AB did not impact differentiation of cells up to the limit concentration of 30uM.[9]

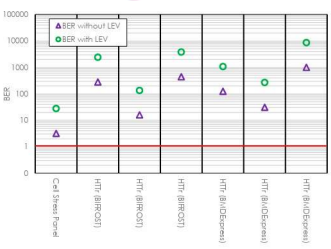
Risk Assessment

To estimate a Bioactivity Exposure Ratio (BER), the worst-case Plasma C_{max} is compared with the lowest bioactivity PoD for all exposure scenarios. BER values >1 are associated with low-risk exposures.[3]

Lowest **bioactivity PoD of 6.4 µM** was compared with the **highest C_{max}, 1.98 µM** (representing 95th percentile general population simulation) resulting in **BER of 3.2.**

As external exposure estimates are conservative (especially inhalation) and worst-case parameters were selected for PBK modelling, the resulting BER is considered to be highly conservative.

Figure 10. Plot visualising BER's for C12-15 AB (red line depicts BER =1). LEV = Local Exhaust Ventilation



Occupational BER >1 for All Activities
Includes Aggregate Exposure from Multiple Tasks During Typical 8h Shift
Conservative Inhalation Exposure Predominant Driver of Potential Risk/Low BER's

Conclusion

- ✓ Occupational NGRA successfully executed in EU REACH context
- ✓ BER >1 for all worker activities - no bioactivity is expected as a result of exposure from registered uses of C12-15 AB.
- ✓ Confidently assign a low-risk conclusion using conservative human-relevant exposure and bioactivity based approach.
- ✓ Animal testing to demonstrate worker safety is not justified from both a scientific and ethical standpoint.
- ✓ Upheld last resort principle as per Articles 13 and 25 EU REACH.
- ✓ Innospec REACH dossier submitted May 2023. No feedback from ECHA yet.

References

[1] Dent et al. Computational Toxicology 7 (2018): 20-26. [2] Baltazar, et al. Toxicological Sciences 176.1 (2020): 236-252 [3] Middleton et al., 2022, Toxicological Sciences, 189 (1): 124–147 [4] BioClavis., TempO-Seq Gene Expression Data Generation Benzoic acid, C12-15 alkyl esters, 2022. [5] Eurofins., In Vitro Pharmacology and ADME-Tox, 2021. [6] Cyprotex., CYP742 R30 Cell Stress Panel, 2022. [7] Groothuis et al. Toxicology 332 (2015): 30-40. [8] Stemina., devTOXqP-iPS Cell Assay Results for Study 1ul: Benzoic Acid, C12-15 Alkyl Esters., 2022 [9] Toxys., The ReproTracker assay., 2023.