Safety & Environmental Assurance Centre

A Next Generation Risk Assessment Case Study for Coumarin in Hypothetical Cosmetic Products

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1. Introduction

Next Generation Risk Assessment (NGRA) is an exposure-led, hypothesis-driven risk assessment approach that integrates New Approach Methodologies (NAMs) to assure safety without the use of animal testing. Over recent years several theoretical frameworks depicting a tiered and iterative approach to conducting a NGRA have been published [Berggren et al, 2017; Dent et al, 2018], although there is a lack of examples of implementation of these frameworks.

In this study we conducted a hypothetical safety assessment of 0.1% coumarin in a face cream and body lotion using only NAMs to inform a safety decision, focusing on the potential for systemic toxicity

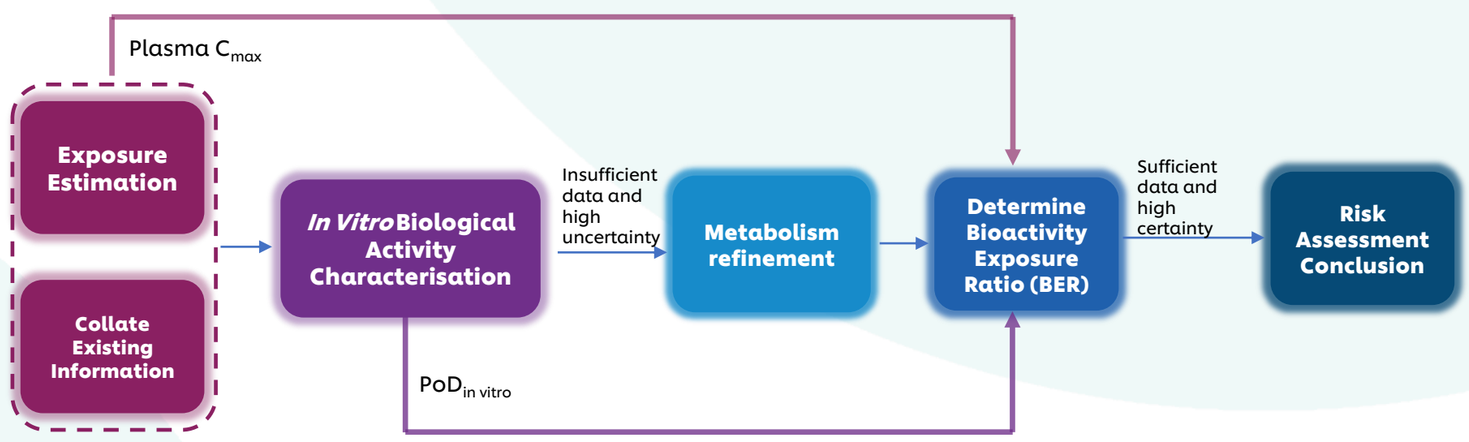


Figure 1. Example framework implemented for the hypothetical risk assessment of coumarin in face cream and body lotion using NAMs.

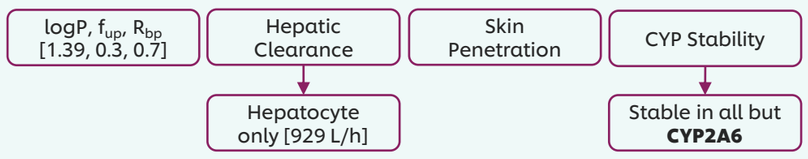
2. Exposure Estimation

Local and systemic exposure estimates

Exposure Estimation

- Use scenario
- Consumer Habits and Practices
- Applied Dose
- ADME parameters
- Internal Exposure (PBK)

Applied dose estimates can be calculated using representative usage amounts for each product type and typical physiological data for consumers. However to facilitate comparison to in vitro points of departure (PoDs) an internal consumer exposure can be estimated using Physiologically-based Kinetics (PBK) Modelling. ADME parameters were identified either from literature or from experimental data to support the creation of a PBK model for coumarin.



In this case, distributions of C_{max} values were determined for both face cream and body lotion use scenarios and can be seen in Fig.2. The final output for coumarin shows possible distributions at two different clearance rate (*in silico* and *in vitro*) to visualise the impact this parameter can have on the predicted C_{max} and standard deviation.

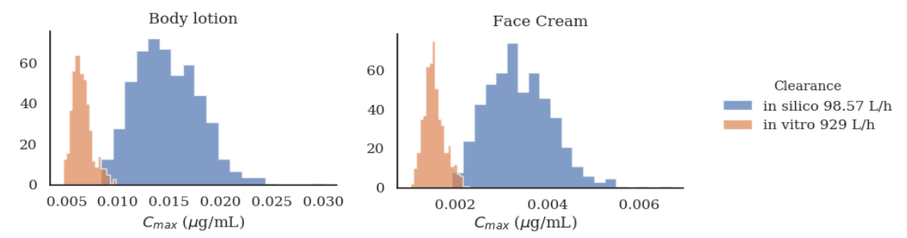


Figure 2. Cmax distributions from PBK modelling of 0.1% coumarin in body lotion and face cream

Table 2. Internal Exposures From Use of 0.1% Coumarin in Face Cream and Body Lotion Following the Exposure Scenario Outlined in Table 1

Total Plasma C_{max} (μM)	Mean	Median	90th Percentile	95th Percentile	97.5th Percentile	99th Percentile
Body lotion	0.01	0.01	0.018	0.019	0.02	0.022
Face cream	0.0022	0.0021	0.004	0.0043	0.0046	0.005

Collate Existing Information

- *Problem Formulation*
- Molecular Structure
- *In silico* predictions
- Literature

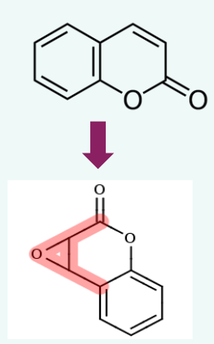
QSAR TOOLBOX · OECD · Meteor nexus · SOT Society of Toxicology · TaxSci 20 Years · Derek nexus · UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events

Timothy E. H. Allen,* Jonathan M. Goodman,*,‡ Steve Gutsell,† and Paul J. Russell†

Tox21/ToxCast ~700 HTS Biological Pathways Assays

EPA iCSS ToxCast Dashboard



In silico tools predicted:

- Protein binding
- DNA binding
- Reactive metabolites (e.g. epoxides) predicted to be formed.
- No binding alerts for the 39 targets in MIE atlas (pharmacologically relevant receptor binding predictions)

Pubchem and ToxCast databases showed:

- Coumarin was only 'Active' in very few assays of the ~5000 present
- Coumarin inhibited both Monoamine oxidases and carbonic anhydrases at concentrations between 3 μM – 40 μM
- The AC50 values from the dose response curves of the 'Active' assays were used as PoDs for the MoS calculation.

3. In Vitro Biological Activity Characterisation

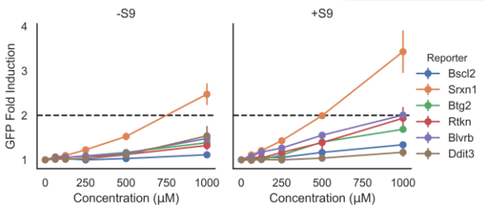
In Vitro Biological Activity Characterisation

Initial PoD Identification

ToxTracker

In vitro genotoxicity screen using 6 GFP reporter mouse embryonic stem (mES) cells spanning DNA damage, p53 activation, oxidative stress and protein damage biomarkers.

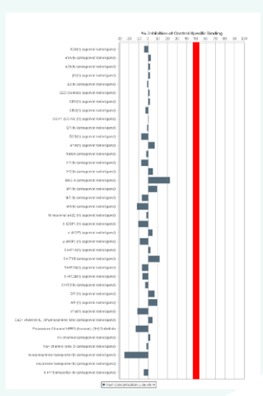
Coumarin was negative in ToxTracker, but reactive metabolite(s) could induce DNA lesions secondary to oxidative stress



SafetyScreen44©

In vitro binding and enzymatic assays in pharmacologically relevant targets (GPCRs, enzymes, nuclear receptors, ion channels, transporters)

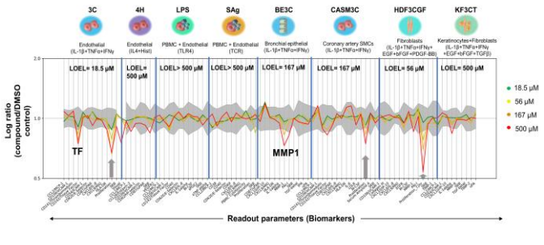
All binding and enzymatic assays were negative at the screening concentration of 10 μM.



BioMap© Diversity 8 Panel

In vitro assay in various stimulated immune-related cell lines to investigate possible effects on vascular inflammation, immune activation and tissue remodelling

Data suggested that coumarin has no immunomodulatory effects at relevant concentrations and is not an anti-inflammatory compound.



High-throughput transcriptomics – TempO-Seq

Full genome transcriptomic analysis was applied as a broad non-targeted screen in HepG2, HepaRG and MCF7 cell lines.

Concentration response analysis was performed on the results and multiple PoDs at both gene and pathway level were derived using several published methods [Farmahin et al. 2017].

Cell Stress Panel

In vitro assay measuring 36 biomarkers across 10 different stress pathways using high content image analysis to characterise non-specific biological activity. Data were generated in NHEK, HepG2 and HepaRG cell lines at 3 timepoints and for 8 concentrations [Hatherell et al, 2020].

Concentration response analysis of coumarin showed low bioactivity in the cell stress panel.

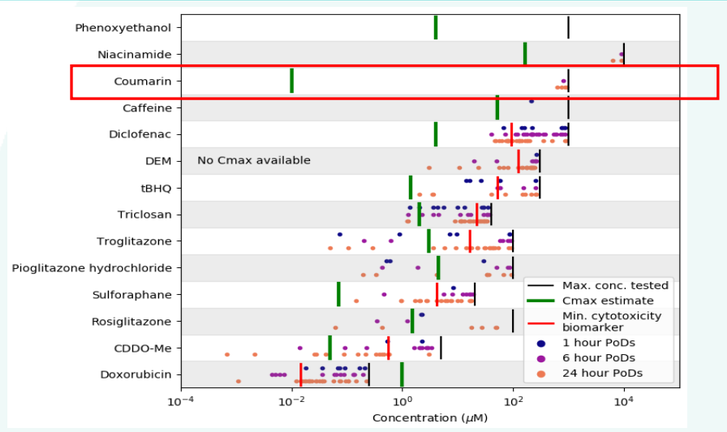


Fig.3 Plot comparing the PoDs determined from the cells tress panel assays and the Cmax estimates for various compounds and exposure scenarios; coumarin is highlighted in red.

Metabolism refinement

Increased certainty in PoD and In Vitro to In Vivo Extrapolation

Metabolite Identification

A human in vitro study with metabolite identification performed which showed that coumarin is preferentially detoxified to 7-hydroxycoumarin and that the epoxide is only formed at very high levels not relevant to consumer exposure

3D Models

Cell Stress Panel and Transcriptomics data were generated in 2D and 3D HepaRG cell models at longer incubation periods which confirmed the low bioactivity of coumarin even in cell models with higher metabolic competence.

4. Conclusions

Determine Bioactivity Exposure Ratio

Bioactivity Exposure Ratios compare the distribution of the exposure estimates with the distribution of the calculated PoDs. The data below show that the 5th percentile of the BER distribution ranged between 158 and 967387.

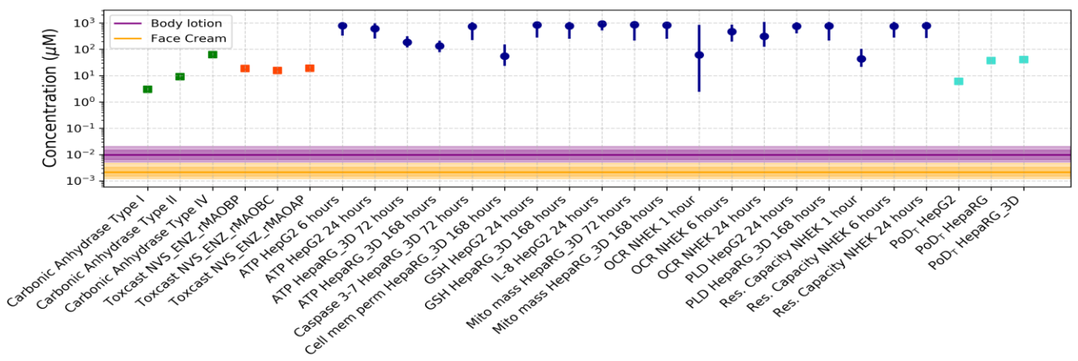


Figure. 4

Comparison of the literature and in vitro PoDs with the exposure estimates for 0.1% coumarin in a face cream (yellow) and 0.1% coumarin in a body lotion (purple). Where possible, the distribution of both the C_{max} predictions and PoD has been plotted.

Risk Assessment Conclusion

From the data presented above it can be concluded that Coumarin is not genotoxic, does not bind to any of the 44 SafetyScreen targets, shows low bioactivity in the test systems and does not show any immunomodulatory effects at consumer relevant exposures.

Whilst there is not yet agreement on how large a BER should be to assure human safety, the predicted Cmax values for face cream and body lotion were all at least 100 times lower than all the recorded PoDs. In conclusion, the weight of evidence suggests that the inclusion of 0.1% coumarin in these products would be low risk to a consumer.

References

Baltazar, et al. Toxicological Sciences 176.1 (2020): 236-252.

Dent et al. Computational Toxicology 7 (2018): 20-26.[2]

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