Using next generation risk assessment to make safety decisions for cosmetic ingredients under regulatory scrutiny

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Background

- In 2019, the European Commission established a list of chemicals that were thought to have endocrine activity and therefore required further safety assessment by the Scientific Committee on Consumer Safety (SCCS). A priority list A and list B were compiled consisting of 28 materials in total, including UV filters and preservatives used in cosmetics[a].
- Cosmetics Europe's Long Range Science Strategy (LRSS) initiated a series of systemic toxicity case studies to practically implement, test and refine non-animal-based workflows in applied safety assessments using these priority list chemicals as examples of the application of Next-Generation Risk Assessment.
- Next Generation Risk Assessments (NGRA) should be exposure-led, hypothesis driven and designed to prevent harm. Published ab initio systemic toxicity case studies such as phenoxyethanol (ENV/CBC/MONO(2021)35) and coumarin (Baltazar et al, 2020) followed these principles in comparing estimates of internal exposure to *in vitro* measures of bioactivity to determine bioactivity:exposure ratios (BERs) to understand the likelihood of systemic bioactivity occurring at consumer relevant concentrations.
- Here we have performed *ab initio* style assessments for chemicals from these priority lists and comparators to benchmark the outputs in a Tier 1 assessment.

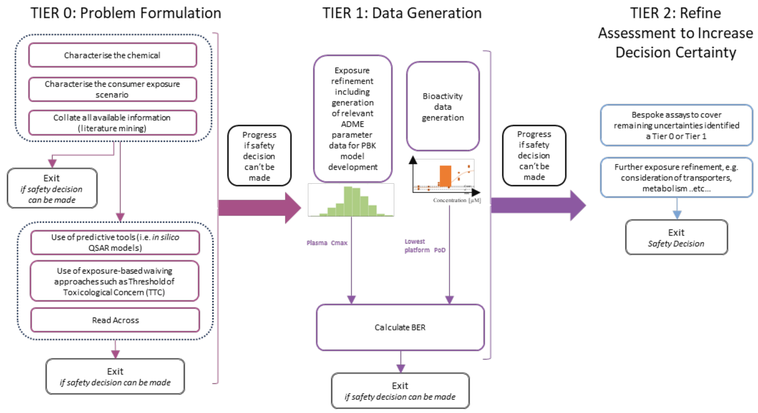

Fig. 1: Risk assessment framework demonstrating where a Tier 1 assessment would be performed after an initial problem formulation tier that could not reach a safety decision with the available information. It also shows where higher tier testing might be implemented to increase confidence in a decision or if a safety decision can't be reached after Tier 1 data generation.

Methods

- In order to evaluate the decision-making performance of a non-animal method (NAM)-based workflow, use scenarios and corresponding risk classifications were identified for test chemicals and comparators based on traditional toxicological studies or authoritative scientific or regulatory opinions.
- Internal exposure estimates were generated for all use scenarios by building physiologically based kinetic (PBK) models parameterised with *in silico* only (L1), *in vitro* data (L2) or calibrated against human clinical data (L3). Data were generated for all test chemicals to include a minimum set of *in vitro* parameters for fraction unbound, hepatic intrinsic clearance and blood:plasma ratio.
- NAM bioactivity data were generated in 3 different platforms: a high-content imaging cellular stress assay in HepG2 cells; whole genome high-throughput transcriptomics in HepG2, HepaRG and MCF7 cells; in vitro pharmacological profiling of clinically significant protein interactions (83 targets in total, e.g. enzyme inhibition, receptor binding).
- Points of departure (PoDs) were calculated from bioactivity dose-response data (IC_{50} for pharmacological profiling, Bayesian approach for cell stress (BIFROST method) and BIFROST and pathway-based benchmark dose modelling for transcriptomics[b,c].
- PoDs were compared to the plasma C_{max} estimates to give a BER for each chemical-use scenario.

Chemical	Use Scenario	Risk Classification
Octocrylene	10% in sunscreen body lotion as a UV filter	Low risk under the use conditions as concluded in SCCS/1627/21
Octylmethoxycinnamate	10% in sunscreen body lotion as a UV filter	Low risk under the use conditions as listed on Annex VI of the EU Cosmetics regulation.
Butylated Hydroxytoluene	0.8% in body lotion as an anti-oxidant	Low risk under the use conditions as concluded in SCCS/1636/21
Climbazole	0.2% face cream as a preservative	Low risk under the use conditions as listed on Annex V of the EU Cosmetics Regulation. SCCS/1506/13
4-Methylbenzylidene camphor	4% in sunscreen body lotion as a UV filter	*Comparator.* High Risk from a systemic perspective and also with sufficient evidence for estrogen and thyroid system effects. SCCS/1640/21
Diethylstilbestrol	0.1 g/day oral medicinal use as a synthetic estrogen	*Comparator.* High risk from a systemic perspective and known endocrine disrupting chemical[d]
Prochloraz	0.01 mg/kg bw/day oral residue consumption following use as a fungicide	*Comparator.* Low risk from systemic perspective as concluded by EFSA (2011); known to affect the estrogen and androgen systems.[e]
Prochloraz	10 ml oral ingestion in poisoning overdose	*Comparator.* High risk from systemic perspective, known to affect the estrogen and androgen systems.[e]
Aminoglutethimide	1000 mg/day oral medicinal use for endocrine disorders (including cancers)	*Comparator.* High risk from systemic perspective, known to affect the estrogen and androgen systems[f]

Table 1. Use scenarios for test chemicals and comparators that were identified from literature or from regulatory opinions, along with the corresponding risk classification from a systemic toxicity perspective.

References:
[a]Dent et al., 2018 Aug 7; 20-26
[b]Hatherell et al., 2020 Jul 1;176(1):11-33
[c]Middleton et al., 2022 Aug 25;189(1):124-147
[d]Diamanti-Kandarakis et al., 2009 Jun; 30(4):293-342
[e]Mnif et al., 2011 Jun; 8(6): 2265-2303
[f]Samojlik et al., 1980 Mar; 65(3): 602-612

Chemical	Plasma C_{max} (μM) [PBK level]	Lowest NAM Bioactivity PoD (μM) [assay]
Octocrylene	0.027 [L3]	0.16 – Cholescystokinin receptor, pregnane X receptor, Progesterone receptor
Octylmethoxycinnamate	0.032 [L3]	0.032 – MCF7 HTTr probe level
Butylated Hydroxytoluene	0.064 [L3]	0.55 – HepaRG HTTr probe level
Climbazole	0.0034 [L2]	0.073 – Aromatase enzyme
4-Methylbenzylidene camphor	1.46 [L3]	0.12 – Progesterone receptor
Diethylstilbestrol	0.11 [L3]	0.00038 – MCF7 HTTr probe level. N.B. <0.001 for Estrogen receptor
Prochloraz (residue)	0.003 [L2]	0.0029 – MCF7 HTTr probe level N.B. 0.021 for Aromatase enzyme
Prochloraz (poisoning)	5.96 [L2]	0.0029 – MCF7 HTTr probe level N.B. 0.021 for Aromatase enzyme
Aminoglutethimide	25.5 [L3]	0.46 – Aromatase enzyme

Table 2. Overview of predicted plasma C_{max} values and the leading PoDs from the NAM bioactivity assays.

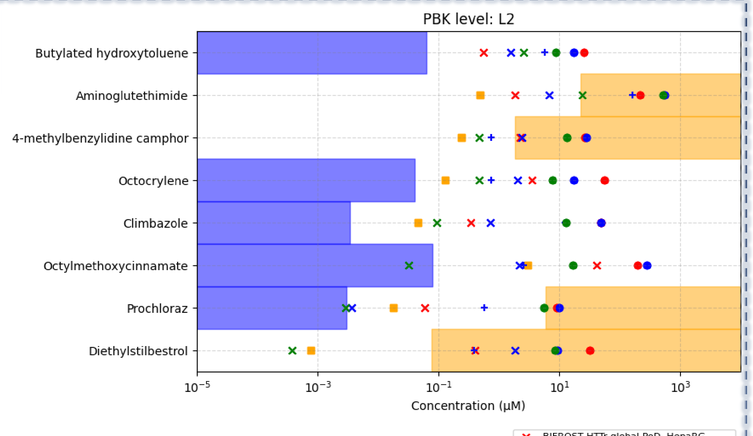

Fig. 2. Overview plot of PoDs and exposures per chemical. Blue regions represent the exposure estimates for a low risk scenario, whilst orange regions represent the exposure estimates for a high risk scenario. PoDs are described in the key to the right and are plotted for all chemicals. BIFROST HTTr global PoDs refer to the probe level PoD from the transcriptomics; CSP refers to the cellular stress assay and IPP refers to the in vitro pharmacological profiling. Where PoDs are to the left of the plotted exposure, the NAM assays detect activity at levels below the predicted exposures.

Results

- Clinical exposure data were available for 6 of the 8 chemicals enabling an L3 PBK model to be built. Clinical data were not available for Prochloraz and Climbazole and so an L2 C_{max} estimate is the highest available for these chemicals and their use scenarios.
- For 5 chemicals the lowest PoD came from the in vitro pharmacological profiling (IPP), for 3 chemicals the lowest PoD came from the MCF7 transcriptomics and for 1 chemical the lowest PoD came from the HepaRG transcriptomics data.
- For the chosen comparators, their known mechanism of endocrine activity was detected in the pharmacological profiling apart from 4-methylbenzylidene camphor that did not produce estrogen or androgen activity in the *in vitro* systems tested. Prochloraz and Aminoglutethimide are known to cause steroidogenic effects and both inhibited this enzyme, along with Climbazole *in vitro,* with varying potencies.
- BERs were calculated for all exposure scenarios using the plasma C_{max} and the lowest *in vitro* NAM PoD ranging from 0.4 (10% OMC in a sunscreen) to 21 (0.2% Climbazole in a face cream) for the test chemicals; and 0.00002 (Ingestion of 2.5 mg Prochloraz) to 0.08 (4% 4-MBC in a sunscreen) for the high-risk comparator chemical use scenarios. Fig. 4 below shows the result of plotting the BERs calculated for all the use scenarios, where "PBK level: highest" uses L3 predictions where possible but combines L2 for Prochloraz and Climbazole.

Conclusions

Using NAM-based bioactivity data in a risk assessment workflow results in full separation of low and high-risk benchmark chemical use scenarios in accordance with safety opinions published by authorities. The UV filter and preservative test chemicals are all active *in vitro* with BERs as low as 0.4 calculated, where a BER of 1 conceptually represents a scenario where *in vitro* activity is happening at concentrations equivalent to consumer exposures. Higher tier testing could be useful to determine the in vivo significance of the in vitro results and the likelihood of adverse effects from scenarios resulting in a BER <1.
These results build confidence that a low-tier NGRA can distinguish high risk and low risk exposures.

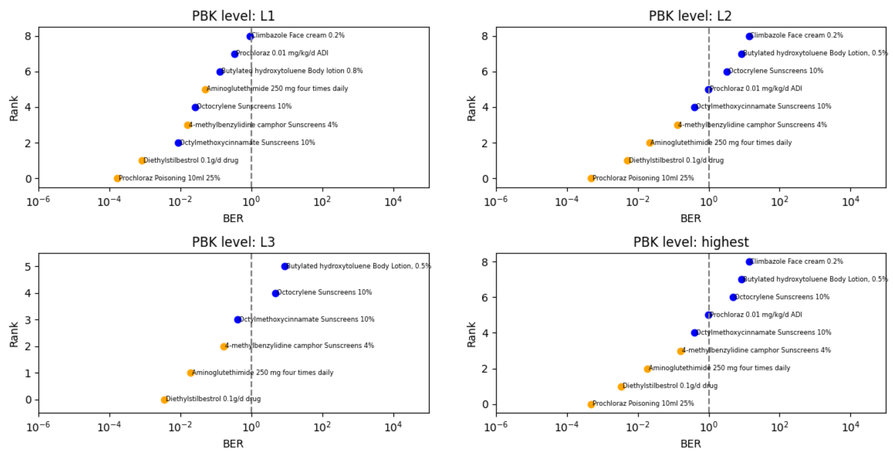

Fig. 3 BER plots for all chemical use scenarios at all PBK levels. Blue dots represent low risk scenarios, orange dots represent high risk scenarios and the dotted line is plotted at BER = 1. Conceptually a BER < 1 could indicate low risk, although more detail on an evaluation activity to benchmark this can be found in the Middleton et al., poster.

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