Next Generation Risk Assessment (NGRA) Decision-Making for Skin Allergy

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Assessing ingredient & product safety without animal testing

Next Generation Risk Assessment (NGRA)

Is it safe to include x% of chemical y in product z?
Main overriding principles:
» The overall goal is a human safety risk assessment
» The assessment is exposure led
» The assessment is hypothesis driven
» The assessment is designed to prevent harm

Principles describe how a NGRA should be conducted:
» Following an appropriate appraisal of existing information
» Using a tiered and iterative approach
» Using robust and relevant methods and strategies

Principles for documenting NGRA:
» Sources of uncertainty should be characterized and documented
» The logic of the approach should be transparently and documented

Dent et al (2018), Computational Toxicology, 7, 20-26: https://doi.org/10.1016/j.comtox.2018.06.001
Next Generation Risk Assessment for Skin Allergy

Tier 0
Identify use scenario, chemical of concern and existing information

1. Identify use scenario
- Applied dose μg/cm² skin
- Single product / aggregate exposure

2. Identify molecular structure
- Chemical(s) of concern
- Analysis of specification and impurities

3. Identify existing hazard information
- In silico predictions
- In vitro / In chemico data (OECD TG or non-OECD TG)
- Historical In vivo data (animal or human)

4. Identify analogues / suitability assessment and existing data
- Read across

Tier 1
Hypothesis generation; how will data be used in risk assessment?

5. Hypothesis generation
- WoE prediction (S / NS) based upon T0 information
- Choice of DA in WoE
- Use of analogues in WoE

Tier 2
Risk assessment

6. Targeted testing
- Refinement of exposure estimate
- Bioactivation / metabolism data
- In vitro hazard data (OECD TG or non-OECD TG)

7. Point of Departure, uncertainty analysis, Margin of safety and final risk assessment
- POD determination
- Characterise uncertainty
- Compare reference dose to consumer exposure

Skin Sensitisation AOP

Key Event 1 (KE1) -> KE2 -> KE3 -> KE4 -> Adverse Outcome (AO)

Electrophilic Chemicals

Covalent Binding to Skin Proteins -> Keratinocyte Activation -> Dendritic Cell Activation -> T-cell Activation and Proliferation -> Skin Sensitisation

Chemical Structure/Properties

MIE

Cellular Level

Organ Level

Predictive Chemistry

For example:
- DEREK-NEXUS
- OECD QSAR Toolbox
- TIMES
- ToxTree

Protein Reactivity

OECD TG 442C
- ADRA
- DPRA

Keratinocyte Activation

OECD TG 442D
- KeratinoSens™
- LuSens

DC Activation

OECD TG 442E
- h-CLAT
- IL-8 Luc Assay
- U-Sens™

T Cell Proliferation

For Example:
- Human T cell proliferation assays (hTCPA)

Skin Sensitisation

OECD TG 429: mouse local lymph node assay (LLNA) & variants TG442A & 442B

OECD TG 406: Buehler & Guinea Pig Maximisation Test (GPMT)

Human evidence
e.g. Human Repeat Insult Patch Test (HRRIPT)

in silico NAM

in chemico/vitro NAM

in vivo evidence
Our NGRA framework for skin allergy is based upon the ICCR principles (Dent et al 2018) and the previously published NGRA frameworks for systemic tox {SEURAT-1} (Amaral et al 2018) and skin allergy {Cosmetic Europe} (Gilmour et al 2020).

- Designed to use a WoE based upon all available information, accommodate range of consumer product exposure scenarios and provide a quantitative point of departure and risk metric → SARA Model.
The SARA model uses Bayesian statistics to infer a probability that a consumer exposure to some chemical can be considered low risk, to inform risk assessment decisions.

The SARA Model uses a database of public NAM data covering AOP KEs 1-3, and historic LLNA and HRIPT data for the AOP AO.
The point of departure (PoD) metric is a dose with a 1% chance of human skin sensitisation (termed ED$_{01}$).

The SARA dataset contains 81 chemicals.

The model accounts for variability in the DPRA, KeratinoSens™, h-CLAT and U-Sens™ and the in vivo data.

The model has been expanded to incorporate benchmark exposure information.
Use of consumer exposure information and clinical evidence to develop skin allergy risk benchmarks

• Traditional risk assessment approaches for skin allergy use safety factors to rescale PoDs to market-equivalent safe doses for comparison against consumer exposure estimates.

• For NGRA, publicly available benchmark exposure information can be used to establish that an exposure is low risk and can be considered safe.

• To apply this concept, we established **62 low or high risk benchmark exposures** using 10 human skin allergens (e.g. MCI/MI) with an established history of use in 7 cosmetic product types.

<table>
<thead>
<tr>
<th>Material</th>
<th>Product type</th>
<th>Use level (ppm)</th>
<th>Consumer exposure to benchmark product (ng cm⁻²)</th>
<th>Induction risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCI/MI</td>
<td>Deo</td>
<td>30</td>
<td>350</td>
<td>HIGH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>87.8</td>
<td>HIGH</td>
</tr>
<tr>
<td></td>
<td>Face cream</td>
<td>30</td>
<td>100</td>
<td>HIGH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>25</td>
<td>HIGH</td>
</tr>
<tr>
<td></td>
<td>Body lotion</td>
<td>30</td>
<td>18</td>
<td>HIGH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>4</td>
<td>HIGH</td>
</tr>
<tr>
<td></td>
<td>Liquid hand soap</td>
<td>15</td>
<td>7.3</td>
<td>LOW</td>
</tr>
<tr>
<td></td>
<td>Shampoo</td>
<td>15</td>
<td>1.1</td>
<td>LOW</td>
</tr>
<tr>
<td></td>
<td>Shower gel</td>
<td>15</td>
<td>0.2</td>
<td>LOW</td>
</tr>
</tbody>
</table>
Expansion of SARA model to use benchmark exposure information

- The SARA model was expanded to incorporate benchmark exposure information as an additional input alongside historic in vivo and NAM data.

- After fitting the model, and given some exposure scenario of interest, the model can calculate the SARA risk metric, defined as the probability that the exposure is low risk for human skin sensitisation induction.
Case Study

A hypothetical skin sensitisation next generation risk assessment for coumarin in cosmetic products

Reynolds et al., 2021
Application of NGRA framework for Skin Allergy

This NGRA framework is applied to a hypothetical skin allergy assessment of a consumer product at two exposures - 0.1% coumarin in a face cream and 1% in a non-spray deodorant.

For the purposes of the case study, *in vivo* data and read-across were not used, and the use of dermal sensitisation threshold (DST) was not appropriate.
<table>
<thead>
<tr>
<th>Product type</th>
<th>Face cream</th>
<th>Deodorant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product used per day (90th percentile) (g/day)</td>
<td>1.54</td>
<td>1.5</td>
</tr>
<tr>
<td>Ingredient inclusion level (%)</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Skin surface area (face / axilla) (cm²)</td>
<td>565</td>
<td>200</td>
</tr>
<tr>
<td>Leave-on or Rinse-off</td>
<td>Leave-on</td>
<td>Leave-on</td>
</tr>
<tr>
<td>Local dermal exposure (µg/cm²)</td>
<td>2.7</td>
<td>75</td>
</tr>
</tbody>
</table>
In silico predictive chemistry:

- TIMES-SS reported coumarin to be a non-sensitiser
- DEREK Nexus predicted coumarin to be a weak sensitiser
- ToxTree and OECD QSAR Toolbox predicted coumarin to have skin sensitisation potential (protein binding alerts).

Predictions were also made for coumarin’s major metabolites as found in in vitro liver metabolism studies (Baltazar et al., 2020).

- TIMES-SS predicted that these metabolites found in the liver could also be formed in skin.
- The in silico tools predicted a number of coumarin’s metabolites to have sensitising potential, including the major stable metabolite (7-OH coumarin) and the minor reactive metabolites o-Hydroxyphenylacetaldehyde and (3R, 4R)-3, 4-Dihydroxy-2-chromanone (coumarin 3,4 epoxide as precursor).
Problem Formulation

Hypothesis: Coumarin has the potential to be a sensitiser and can act as either a hapten or pro-hapten.

1. Generate DPRA, KeratinoSens™, h-CLAT, U-SENS™ and peptide reactivity profiling data for coumarin to evaluate its skin sensitisation potential, potency, and a risk prediction for the given exposure scenarios.

To address areas of uncertainty regarding pro-haptens:
1. Replicate the testing strategy for coumarin for the major metabolite, 7-OH coumarin
2. Ex vivo skin cultures were used to determine if the 7-OH coumarin formation pathway was relevant/significant in human skin
3. Reactive metabolites were investigated through an experiment designed to trap reactive chemicals with GSH
### Data Generation

<table>
<thead>
<tr>
<th></th>
<th>DPRA (TG442C)</th>
<th>KeratinoSens™ (TG 442D)</th>
<th>h-CLAT (TG 442E)</th>
<th>U-SENS™ (TG 442E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%cys depl.</td>
<td>%lys depl.</td>
<td>EC1.5 (µM)</td>
<td>CD86 (EC200 µg/mL)</td>
</tr>
<tr>
<td>Coumarin</td>
<td>1.3</td>
<td>0</td>
<td>187.5</td>
<td>&lt;178</td>
</tr>
<tr>
<td>7-OH Coumarin</td>
<td>0*</td>
<td>0</td>
<td>&gt;2000</td>
<td>&gt;566</td>
</tr>
</tbody>
</table>

- **Coumarin was positive in all tests, except for DPRA** where peptide depletion was too low to meet positive threshold. No adducts were observed in the peptide profiling assay.

- 7-OH coumarin was negative in KeratinoSens™ & h-CLAT, positive in USENS™, inconclusive in DPRA. *Peptide profiling identified cysteine depletion to be caused by dimerization and therefore the DPRA value was considered a false positive, no other adducts were observed.

- *Ex vivo* skin data show that the formation of 7-OH coumarin is not a major pathway in human skin. The formation of reactive metabolites such as coumarin-3,4-epoxide could not be excluded nor confirmed in *ex vivo* skin.
Determine Point of Departure (PoD) using SARA Model

- The generated DPRA, KeratinoSens™, hCLAT and USens™ data were used as inputs into the SARA Model to define a human relevant PoD (ED$_{01}$ i.e. the 1% sensitising dose for a HRIPT population).

- For coumarin, with all NAM data, the expected SARA Model derived ED$_{01}$ is 11,000 µg cm$^{-2}$, whilst for 7-OH coumarin the expected ED$_{01}$ is 110,000 µg cm$^{-2}$ (not shown).

- Risk assessment based on coumarin potency data would be more conservative.

- Coumarin ED$_{01}$ 4200 µg cm$^{-2}$ for NAM data excluding DPRA.
Determine Margin of Exposure (MoE)

- The SARA risk metric is 0.90 for the face cream dermal exposure and 0.39 for the deodorant dermal exposure. Excluding DPRA, the SARA risk metric was 0.77 for the face cream dermal exposure and 0.25 for the deodorant dermal exposure.

- The lower SARA risk metrics are reflective of a decrease in the ED01 and increased uncertainty in the prediction due to removal of DPRA input data.

- For the face cream exposure, the SARA model predicted low risk as being the most likely classification. For the deodorant risk assessment, the high risk classification was more certain.
The goal of the project is to develop a version of the SARA model for different skin sensitisation hazard and risk assessment regulatory use-cases.

The project has a capability build phase and an evaluation phase.

Project proposal led by the US and UK submitted to OECD to be considered in April 2022.

Conclusions & Next Steps

- Significant progress has been made in the last decade to apply non-animal experimental data using Defined Approaches & tiered frameworks.
- Bayesian DAs enable experimental data variability to be modelled and uncertainty in PoDs & risk metrics to be factored into decision-making.
- NICEATM collaboration established to evaluate SARA, expand the approach and make it publicly available.
- In-house work ongoing to explore new SARA inputs & expand the database, including risk benchmarks.
Back-up
Discordance and bias across differing datasets

Additional Problem Formulation:

1. A metric of discordance between NAMs and *in vivo* risk estimates was computed to evaluate if the SARA Model performance is comparable for pro-haptens and direct acting sensitisers.
Determine Point of Departure (PoD) using SARA Model (incl. 7-OH)

- The generated DPRA, KeratinoSens™, hCLAT and USens™ data were used as inputs into the SARA Model to define a human relevant PoD (ED$_{01}$ i.e the 1% sensitising dose for a HRIPT population).

- For coumarin, the expected SARA Model derived ED$_{01}$ is 11,000µgcm$^{-2}$, whilst for 7-OH coumarin the expected ED$_{01}$ is 110,000µgcm$^{-2}$ i.e. 7-OH coumarin is estimated to be 10-fold less potent than coumarin).

- Therefore, a risk assessment based on coumarin potency data only would be conservative.
Determine Margin of Exposure (MoE)

- The MoE was calculated from the ED$_{01}$ for coumarin and the dermal exposures for each product type using the SARA Model.

- The median MoE for face cream exposure ranks with the low-risk benchmarks whilst the median MoE for the deodorant exposure ranks with the high-risk benchmarks.

- The SARA DA probability that the exposure is low risk is calculated to be 0.90 for the face cream dermal exposure and 0.39 for the deodorant dermal exposure.

- Coumarin exposure at 0.1% in a face cream is low risk for skin sensitisation whereas coumarin exposure at 1% in a deodorant is high risk.