Taking the sting out of mildness testing

COSMETICS BUSINESS REGULATORY SUMMIT

Dr Carol Treasure
8th October 2019
XCellR8’s mission

To accelerate the world’s transition to 100% animal-free testing through our scientifically advanced and ethical approach.
Satisfy consumer demand for a more ethical supply chain

• We are the only regulator-approved GLP lab globally to make all of our tests 100% animal-product-free, or vegan
  • Our long-standing approach for many years
• We don’t use serum, tissues or antibodies extracted from animals
• This provides a better model of human physiology and higher reproducibility (synthetic components)
• Vegan Society accreditation expected Autumn 2019
What I’ll cover today

1. Why the industry needs a new model to predict mildness to skin
2. What existing methods are available and their limitations
3. How we optimised them to create a new model
4. Correlation between in vitro and in vivo results
5. Real world applications of the model
6. Comparison data between soaps and facial cleansers
Why we need a new method to predict mildness (I)

- World Health Organisation (WHO) has described stress as the “health epidemic of the 21st Century”
- Stress puts our inflammatory reactions on alert and lowers the threshold to elicit a reaction
- Stress makes skin more likely to react and contributes to increased incidence of skin reactions
- Other contributory factors: air pollution; air conditioning; extreme weather; poor diet; chemical exposure (e.g., household products); frequent washing; hormonal changes; underlying skin diseases; occupational exposure
- Skin disorders affect self-esteem, quality of life, physical and mental health

*Mildness is a safety and efficacy issue:*

Relevant to the scale between stress and wellbeing
Why we need a new method to predict mildness (II)

- Study of 12,377 individuals in Europe*
- Incidence of skin reactions lasting more than 3 days:
  - 19.3% within the last month
  - 31.8% within the last year
  - 51.7% within a lifetime
- Avoidance of daily life consumer products due to skin reactions:
  - 37.0% for skincare
  - 17.7% for “household or functional” products

Why we need a new method to predict mildness (III)

- Increasing demand from consumers for ever milder products, that they feel confident using even when their skin is feeling extra sensitive
- Increasing demand from marketing teams for differentiating claims
- New research project started in 2017, funded by Innovate UK
- Research aims:
  - Optimising \textit{in vitro} and human \textit{in vivo} test methods for maximum sensitivity
  - Assess predictive capacity
  - Real world applications
Existing methods: *In vitro* irritation testing

- 3D human skin models, grown at the air-liquid interface
- Suitable for testing ingredients and finished products
- Applied directly to the tissue surface – good model of “real life” exposure
- Standard regulatory method (OECD TG 439) measures a single exposure time to classify irritants vs non-irritants for hazard identification and labelling purposes
- Validated against historical animal data (Draize test)
- A more sensitive approach is required for today’s mild cosmetic ingredients and formulations beyond a yes/no answer – *how* mild is the test item?
The ET50 method

- Measures cell damage over a time course
- Classifies as Severe, Moderate, Mild or Minimal / Non-Irritant
- ET50 = time taken to reduce the viability of the skin model to 50% compared with untreated controls
- ET50 values allow rank order of irritation to be determined in comparison with other formulations / competitor and market leading products
- Standard methodology limited to 18 hours
How we optimised the test methods *in vitro*

- Development of an extended timepoint *in vitro* 3D model to look at the irritancy potential of ultra-mild test items over 48 hours
- Determination of ET$_{50}$ values for known surfactant controls with a range of irritation potentials
- Development of a prediction model linking the *in vitro* skin irritation ET$_{50}$ method with an *in vivo* human skin patch test model for ultra-mild surfactants
- Creation of a database of industry leading ingredients and formulations to be used as benchmarks in future tests for client companies
# How we optimised the test methods *in vitro*

| Test Items       | Surfactants: SLS, SLES, CAPB, a novel “mild” surfactant  
|                 | Applied to the skin model surface and incubated for 1, 5, 18, 24 and 48 hours |
| Controls         | Negative control: not treated  
|                 | Positive control: Triton X-100 (non-ionic surfactant): 1% solution |
| Measurement      | Metabolic activity (conversion of MTT) as an indicator of cell damage |
| Output           | ET50 value (time taken to reduce the viability of the cells to 50% compared with the untreated negative control) |
Our testing partner is Cutest, a human volunteer CRO based in Cardiff, UK.

*In vivo* irritation testing (patch testing) uses the principle of maximising exposure of products to the skin under occlusion for multiple days.

- Highly sensitive methodology to detect weakly irritant products.
- The method ensures products in critical applications are unlikely to cause irritation under normal use.
Determining the correlation between *in vitro* and *in vivo* results – some examples

1. SURFACTANTS
2. SURFACTANT BLENDS
3. SURFACTANT FORMULATIONS
4. FACE MASKS
**In vitro** irritation potential of 4 surfactants

Test items (0.3%, pH 4.7) at 1, 5, 18, 24, 48hrs

Irritancy classification:

- **C** = SLS: Moderate to Mild
- **A** = SLES: Moderate to Mild
- **B** = CAPB: Non-Irritant
- **D** = Novel surfactant: Non-Irritant

Rank order of irritancy using linear extrapolation and logic equation

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET50</td>
<td>9.37</td>
<td>10.25</td>
<td>29.4</td>
<td>38.08</td>
</tr>
</tbody>
</table>

Irritancy classification:

- C = SLS: Moderate to Mild
- A = SLES: Moderate to Mild
- B = CAPB: Non-Irritant
- D = Novel surfactant: Non-Irritant
Using same 4 surfactants to determine the correlation with *in vivo*

<table>
<thead>
<tr>
<th>Rank order of irritancy</th>
<th>Cumulative irritation scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stepanol WA (SLS) ®</td>
<td>16</td>
</tr>
<tr>
<td>SLS 70%</td>
<td>14</td>
</tr>
<tr>
<td>SLES 70%</td>
<td>9</td>
</tr>
<tr>
<td>Cocamidopropyl Betaine</td>
<td>4</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
</tr>
<tr>
<td>Novel Surfactant</td>
<td>0</td>
</tr>
</tbody>
</table>

- 3 cohorts of volunteers
- Expert clinical scoring of erythema by nurses
- Clinical scoring matches *in vitro* predictions
In vitro irritation potential of surfactant blends commonly used in personal care products

ET50 determination of surfactant blends

Rank order of irritancy using linear extrapolation and logic equation

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<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET50</td>
<td>1.82</td>
<td>3.85</td>
<td>5.59</td>
<td>9.01</td>
</tr>
</tbody>
</table>

IRRITANCY CLASSIFICATION

C = SLES / CAPB blend 3: Moderate
A = SLES / CAPB blend 1: Moderate
B = SLES / CAPB blend 2: Moderate to Mild
D = SLES / CAPB blend 4: Moderate to Mild
**Clinical scoring matches *in vitro* predictions**

### In vivo irritation potential of surfactant blends (SLES / CAPB)

<table>
<thead>
<tr>
<th>Rank order of irritancy</th>
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</tr>
</thead>
<tbody>
<tr>
<td>E (SLS 70%)</td>
<td>12</td>
</tr>
<tr>
<td>C (SLES/CAPB blend 3)</td>
<td>4</td>
</tr>
<tr>
<td>A (SLES/CAPB blend 1)</td>
<td>3</td>
</tr>
<tr>
<td>B (SLES/CAPB blend 2)</td>
<td>0</td>
</tr>
<tr>
<td>D (SLES/CAPB blend 3)</td>
<td>0</td>
</tr>
<tr>
<td>Control (E45 Cream)</td>
<td>0</td>
</tr>
</tbody>
</table>
Mild surfactant formulations (shampoos) *in vitro*

**ET50 determination of TA1-4**

**Rank order of irritancy using linear extrapolation and logic equation**

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>C</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET50</td>
<td>1.65</td>
<td>8.33</td>
<td>8.57</td>
<td>8.94</td>
</tr>
</tbody>
</table>

**IRRITANCY CLASSIFICATION**

- **A = new mild shampoo 1:** Moderate to Mild
- **B = new mild shampoo:** Moderate to Mild
- **C = new mild shampoo:** Moderate to Mild
- **D = best-selling standard shampoo:** Moderate
Mild surfactant formulations (shampoos) *in vivo*

**CLINICAL SCORES**

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<tbody>
<tr>
<td>D</td>
<td>21</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
</tr>
<tr>
<td>A</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
</tr>
<tr>
<td>E (E45 Cream)</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

Clinical scoring matches *in vitro* predictions
Face mask comparison \textit{in vitro}

**ET\textsubscript{50} determination of 3 face mask formulations**

- **Percentage of viability relative to untreated control**
- **Time (h)**

**Rank order of irritancy using linear extrapolation and logic equation**

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>&gt;</th>
<th>A</th>
<th>&gt;</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET\textsubscript{50}</td>
<td>12.86</td>
<td></td>
<td>14.42</td>
<td></td>
<td>&gt;48</td>
</tr>
</tbody>
</table>

**IRRITANCY CLASSIFICATION**

- **B = face mask 2:** Very mild
- **A = face mask 1:** Very mild
- **C = face mask 3:** Non-irritating

Face mask C is the mildest product using this method
### CLINICAL SCORES

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<tr>
<td>B</td>
<td>11</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
</tr>
<tr>
<td>D (E45 Cream)</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
</tr>
</tbody>
</table>

Clinical scoring matches *in vitro* predictions
Conclusions and future work

- *In vitro* data accurately predicted the rank order of human *in vivo* clinical scores in all cases and, for industry case studies, matched expectations of the manufacturers

- 2 peer reviewed papers in preparation
  - Method optimisation
  - Industry applications

- We now want to grow the *in vitro* database to a wider range and number of products and ingredients, expanding further on the benchmarking capacity of the test

- Widen the use of the model as a pre-screen for baby care products, as a prelude to human patch tests and dermatologist led clinical studies
Real world applications
Building an *in vitro* database

**BENCHMARK INGREDIENTS AND PRODUCTS**

- Highly sensitive results show micro differences in levels of mildness for the first time
- Mildness of soaps can be compared to facial cleansers, to reassure consumers who want less plastic packaging without affecting performance
- Mass market best-sellers can be compared to luxury brands
- Mildness of soap vs facial cleanser within the same brand family can be compared
- Similar study recently completed on baby care products

Soaps

Facial cleansers

This product is 8 times more expensive than its neighbour, but is equally ultra-mild

Products above this line are classed as Non-Irritant, the mildest classification available

These 2 products are from the same brand but the soap is notably milder

This media favourite claims its low pH levels make it milder than other soaps

ET-50
A variety of applications

- **Ingredients:**
  - Assessment of novel biosurfactants and other ingredients to assess mildness compared with other manufacturers and traditional materials

- **Formulations:**
  - *In vitro* benchmarking of new products against other brands or in-house formulations in development
  - Growing database for benchmark values currently includes:
    - ✓ Facial soaps
    - ✓ Facial cleansers
    - ✓ Face masks
    - ✓ Moisturisers
    - ✓ Body soaps
    - ✓ Shower gels
    - ✓ Sunscreens
    - ✓ Deodorants
    - ✓ Baby care products (oils, lotions, shampoos, bubble baths)
Why use *in vitro*?

- Lower cost
- Faster turnaround
- Standardised conditions
- Strong database of reference values for benchmarking the mildness of ingredients and formulations both within and between brands
- Ethical advantages: limits human exposure, whether used as stand-alone test or pre-screen to clinical studies
- Marketing / consumer appeal: lab data, vegan-compliant “cruelty-free”*
- Brand differentiation: positive message using latest test methods. Moves beyond “not tested on animals”* to using latest technologies to demonstrate product safety and efficacy, going beyond the minimum requirements

* *In vitro* XtraMild test now available from XCellR8

*Use of these phrases now limited by new EU claims guidance. But “not tested on animals” should never mean “not tested at all”*
Thank you to the following companies who have participated in this research, and to Innovate UK for funding the work.
Thank you!

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