INTEGRATED TESTING STRATEGY
FOR IN VITRO SKIN CORROSION AND SKIN IRRITATION
USING SKINETHIC RHE TEST METHODS

2nd PAN AMERICAN CONFERENCE
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ACUTE SKIN IRRITATION AND SKIN CORROSION
WHAT DIFFERENCES?

**Irritation**

Local and *reversible* inflammatory response to skin injury caused by direct contact with a substance.

**Clinical signs:**
- Oedema
- Erythema
- Itching, Pain

**Corrosion**

Potential of a substance to induce *irreversible* damage to skin.

**Clinical signs:**
- Necrosis through the epidermis and the dermis
- *Idem* irritation signs in surrounding areas

- Protein and lipids modification
- Dehydration
- Membrane modifications
- Release mediators pool
- mRNA transcription
- Protein synthesis (mediators)
- Interaction with other cells
- Increased lipid synthesis
- Abnormal differentiation
- Hyper-proliferation
- Irreversible membrane damage
- Cell death

*Boelsma & Ponec: Dermatopharmacology of topical preparations. Springer, 2000, p.37*

*T. Welss et al., Toxicology in Vitro 18 (2004) 231-243*
**UN GHS/ EU CLP/ TDG CLASSIFICATIONS**

<table>
<thead>
<tr>
<th>EU DSD</th>
<th>No label</th>
<th>R38</th>
<th>Cause burns (R34)</th>
<th>Cause severe burns (R35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN GHS / EU CLP</td>
<td>No Category</td>
<td>Cat. 3</td>
<td>Cat.2</td>
<td>Cat.1C</td>
</tr>
<tr>
<td>Regulations Dangerous Goods</td>
<td></td>
<td></td>
<td>Class 8 PG III</td>
<td>Class 8 PG II</td>
</tr>
<tr>
<td></td>
<td>Not classified</td>
<td>Irritant</td>
<td>Corrosive</td>
<td></td>
</tr>
</tbody>
</table>

P.I.I. = Primary Irritation Index

OECD TG404: Max reaction score = 4.0 - Cat.2 and 3: Mean score (24, 48 and 72hrs) for at least 2/3 tested animals – Cat.1: at least 1 animal

Corrosive substances and mixtures: Class 8 Packaging Groups – PG I: very dangerous; PG II: medium danger; PG III: minor danger

TDG: transportation of dangerous Goods
Stand alone test methods using Human Reconstructed Epidermis (RhE)

SKIN CORROSION & SKIN IRRITATION
Human Reconstructed Epidermis (RhE)

IN VITRO RECONSTRUCTION PROCESS

MAIN STEPS

Industrialization is a must have
Each test chemical was applied topically onto tissues. At the end of the treatment period, cytotoxicity was determined by the MTT conversion test.

Adapted controls were added for MTT reducers and coloring/tissue staining test substances.

**TEST METHODS**

- **Corrosion**
  - Exposure time: 3 min
  - Post-treatment incubation: 60 min
  - Viability: MTT test
  - Room Temperature: none

- **Skin irritation**
  - Exposure time: 42 minutes
  - Post-treatment incubation: 42 hours
  - Viability: MTT test
  - Room Temperature: 37°
Prediction Models

### Skin Corrosion

<table>
<thead>
<tr>
<th>Corrosive</th>
<th>3 min exposure</th>
<th>1 h exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. 1A</td>
<td>&lt; 15%</td>
<td>(independent of results obtained at 1 h)</td>
</tr>
<tr>
<td>Cat. 1B-and-1C</td>
<td>≥15%</td>
<td>&lt;15%</td>
</tr>
<tr>
<td>Non Corrosive</td>
<td>≥15%</td>
<td>≥15%</td>
</tr>
</tbody>
</table>

### Skin Irritation

- Irritant (Category 2): viability ≤ 50%
- Non-Irritant: viability > 50%

Performances

<table>
<thead>
<tr>
<th></th>
<th>Skin Corrosion (n=84)</th>
<th>Skin irritation (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REPRODUCIBILITY</td>
<td>93%</td>
<td>98%</td>
</tr>
<tr>
<td>SENSITIVITY</td>
<td>94%</td>
<td>90%</td>
</tr>
<tr>
<td>SPECIFICITY</td>
<td>74%</td>
<td>80%</td>
</tr>
<tr>
<td>ACCURACY</td>
<td>85% (2 classes)</td>
<td>85%</td>
</tr>
<tr>
<td></td>
<td>71% (3 classes)</td>
<td></td>
</tr>
</tbody>
</table>
Broad application to chemicals, pesticides, drugs, cosmetics, medical devices, …
FROM a stand alone test method
TO Integrated approach on testing
And Assessment (IATA)

SKIN CORROSION & SKIN IRRITATION
Guidance Document # 203 on an Integrated Approach on Testing and Assessment (IATA) for skin corrosion and irritation.

Provide guidance on how to integrate all existing and newly generated information into a modular approach for Classification and Labelling.
If the WoE analysis in the IATA is inconclusive and prospective testing is required, a Top-Down or Bottom-Up Sequential *in vitro* testing strategy is followed. **8 modules**

**Part 1:**
Collection and evaluation of existing data covering human, *in vivo* and *in vitro* data, physico-chemical properties and non-testing methods (i.e., Read-across, QSAR, grouping)

**Part 2:**
A Weight-of-evidence (WoE) analysis, for decision-making on C&L

**If the WoE analysis in the IATA is inconclusive and prospective testing is required**, a Top-Down or Bottom-Up Sequential *in vitro* testing strategy is followed.

**OECD TG 431, 439**

*skin irritation / corrosion Using SkinEthic RHE test methods*
A total of 83 chemicals (~ 680 dataset) with *in vivo* data according to the OECD GD 203 were tested using the formally adopted *in vitro* SkinEthic™ RHE test methods for skin irritation (OECD TG 439) and skin corrosion (OECD TG 431).
High similar predictive capacities in both the bottom-up and top-down testing sequences.
FROM Integrated approach on testing And Assessment (IATA) TO Application

SKIN CORROSION & SKIN IRRITATION
**Physicochemical data**
- Physicochemical properties
  - Lipid solving
  - Surfactant
  - Acid/base

**In silico data**
- Structure activity
- Chemicals

**In vitro data**
- Biological events
  - Inflammation
  - Cell death

**Statistical Analysis**
- No Danger / Danger
  - % of confidence

**TO BUILD AN INTEGRATED (& INTELLIGENT) TESTING STRATEGY**
✓ SkinEthic™ RHE tissues are produced in Brazil ([www.episkin.com](http://www.episkin.com))

✓ No Intellectual Property Rights nor license fee applied on the test methods: skin irritation/corrosion


✓ High safety standards demonstrated: Reliable and relevant to identify chemicals according to UN GHS classification

✓ Applicable to the testing of all types of chemicals

✓ Adopted for regulatory purposes
  • in the OECD Test Guidelines TG 431 and 439 for testing
  • in the OECD Guidance no. 203 on integrated approaches to testing and assessment

CONCLUSIONS

The SkinEthic™ RHE model showed to provide accurate and safe assessments of skin irritation and corrosion hazards, ensuring consumer safety.