The Intersection of Predictive Toxicology Roadmaps - Tox21, FDA’s Predictive Tox Roadmap, and ToxCast

Gertrude-Emilia Costin, Ph.D., M.B.A.

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Modernizing Predictive Toxicology for Regulatory Decisions: Influx of Modern Non-animal Testing Technologies and Strategies
Institute for In Vitro Sciences (IIVS)
Who we are and what we do

Founded as a **non-profit** laboratory in 1997 to use and promote non-animal methods for toxicology

- “Non-profit” means **no owners or shareholders**
- Supported by laboratory services and contributions
- Additional revenues earned are reinvested into programs at the end of the year

This allows IIVS to be a “**neutral**” party when **choosing the best in vitro assays**, working with regulatory agencies, or interacting with animal welfare organizations.
Science
We conduct *in vitro* contract testing daily

We have supplied *in vitro* testing to hundreds of companies for thousands of finished products and ingredients using a wide variety of methods with multiple levels of complexity.
Education and Outreach
We teach the methods: global approach to training

Informational Workshops for:
- Industry
- Government
- Animal Welfare Organizations
- High School and College Students

Hands-On Training Sessions
- General or Corporate-Specific
- International, e.g. workshops in/for China, Brazil, Russia, Vietnam for industry scientists and regulators

Expert Users Workshops
- Assay / System specific groups
- Regulatory and non-regulatory applications
Perspectives, challenges, common goals and working together
Current regulatory climate – global acceptance of *in vitro* methods
Drivers of *in vitro* methods advancement

Ongoing evolution on so many levels

- Improve scientific basis for testing using human-derived test models
- Reduce the number of animals for testing
- Increase predictivity
- Reduce time, price
- Harmonize requirements and prediction models

The reductionist concept of *in vitro* models

**1940s**
- Whole animal (Rabbit)
- **Organ - Eyeball** (Enucleated chicken or rabbit eye)
- **Tissue - Cornea** (Resected bovine cornea)
- **Cell culture** (Statens Seruminstitut Rabbit cornea cells)

**1990s**
- Whole animal (Rabbit)
- **Organ - Eyeball** (Enucleated chicken or rabbit eye)
- **Tissue - Cornea** (Resected bovine cornea)
- **Cell culture** (Statens Seruminstitut Rabbit cornea cells)

**“Less is more”**

**2010s**
- **Body-on-a-chip** (Human organotypic microtissues)
- **Organ-on-a-chip** (Human retina)
- **Tissue construct** (Human EpiCorneal™ model)
- **Cell culture** (Normal human corneal epithelial cells)

**2000s**
- **Body-on-a-chip** (Human organotypic microtissues)
- **Organ-on-a-chip** (Human retina)
- **Tissue construct** (Human EpiCorneal™ model)
- **Cell culture** (Normal human corneal epithelial cells)


Challenges

How are classification and labeling predictions communicated to the regulatory community using the non-animal paradigm?

1. What information is acceptable?
2. Can an ingredient or a formulation be classified without testing?
3. What assays or endpoints are accepted?
4. Can they stand-alone?
5. Is there a hierarchy to follow?
6. How are data gaps addressed?

Must meet global expectations of OECD members
Mutual Acceptance of Data
Further challenges

How can the best method be selected?
How are data interpreted?

1. By target tissue (eye, skin, systemic toxicity, etc.)?
2. By endpoint? (category specific?)
3. By test system type? (*in chemico*, cellular 2D, 3D, *ex vivo*)?
4. By relevance to the test material?
   (chemicals, formulations, solubility issues)
5. By regulatory acceptance only?
   (can non-regulatory assays be used in WofE – Weight of Evidence?)
Plenty of assays to choose from

Four major groups of non-animal test methods used in research and regulatory safety testing of chemicals and products

1. *In chemico* test systems
   - Skin Corrosion: Membrane Barrier Test Method Corrositex™ (OECD TG 435)
   - Eye Irritation: “Irritection” Test (draft OECD TG)
   - Skin Sensitization: Direct Peptide Reactivity Assay (DPRA) (OECD TG 442C)

2. *In vitro* monolayer cell culture systems
   - Skin Phototoxicity: Phototoxicity Test (OECD TG 432)
   - Ocular Irritation: Cytosensor Microphysiometer (US EPA AMCP and draft TG) Short-Term Exposure (STE) Assay (OECD TG 491)
   - Skin Sensitization: KeratinoSens (OECD TG 442D)
     - hCLAT (OECD TG 442E)

3. *In vitro* reconstructed tissue models systems
   - Skin Corrosion: Reconstructed human EpiDermis (RhE) Corrosion Assay (OECD TG 431)
   - Skin Irritation: RhE Skin Irritation Test (SIT) (OECD TG 439)
   - Eye Irritation: Eye Irritation Test (EIT) (OECD TG 492)

4. *Ex vivo* tissues and organ systems
   - Ocular irritation: Bovine Corneal Opacity and Permeability Assay (OECD TG 437 and US EPA AMCP)
     - Isolated Chicken Eye Test (OECD TG 438)
   - Skin Absorption: *In vitro* Skin Absorption (OECD TG 428)
   - Skin Corrosion: Rat Skin Transcutaneous Electrical Resistance Test (OECD TG 430)
1. *In chemico test systems*

**General considerations**

- Do not require cell culture facility or cell culture expertise
- May be relatively inexpensive to conduct
- Standardized manufacturing or processes ensure standard testing platforms
- Some allow exposures as *in vivo*
- Some test methods may require specialized equipment (DPRA: HPLC)

**Limitations**

- Reliance on a limited number of manufacturers for specific commercial platforms
- Lack complex biological responses
  - *Are metabolism, inflammatory mechanisms included?*
- Assay may require further information or testing
  - *Endpoint may be simplistic*
  - *May only model chemical initiating event*
## Membrane barrier test method (Corrositex®) (OECD 435)

### Brief overview and current regulatory status

<table>
<thead>
<tr>
<th>Test system:</th>
<th>Artificial membrane designed to respond to corrosive substances in a manner similar to animal skin <em>in situ</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay endpoint:</td>
<td>The time (in minutes) required for a test substance to penetrate through the Corrositex™ BioBarrier Membrane and produce a color change in the Chemical Detection System (CDS)</td>
</tr>
<tr>
<td>Assay controls:</td>
<td>Negative (10% citric acid, 5% propionic acid); Positive (sodium hydroxide)</td>
</tr>
<tr>
<td>Applicability:</td>
<td>Assigns UN Packing Group to corrosives or verifies if a test substance is non-corrosive</td>
</tr>
<tr>
<td>Limitations:</td>
<td>Materials with a pH of $\geq 4.5$ and $\leq 8.5$ generally fail to qualify for testing based on the CDS used in the kit provided by In Vitro International</td>
</tr>
</tbody>
</table>
Biobarrier Preparation

To prepare the biobarrier membranes, the biobarrier matrix powder is completely solubilized. The solubilized collagen matrix is then added to a membrane disc containing a porous cell membrane and placed onto a vial containing CDS.

Biobarrier Placement

Break Through Observations

Corrositex®: typical protocol

- Test substance is added to a tube containing Chemical Detection System (CDS).
- Materials with a pH of $\geq 4.5$ and $\leq 8.5$ generally fail to qualify for testing.
- The test substance is added to two test tubes to determine the appropriate timetable for Packing Group Assignment.
- A Category 1 test substance will be evaluated for up to 4 hr; a Category 2 test substance will be evaluated for up to 1 hr.

Prediction Model

<table>
<thead>
<tr>
<th>Category I</th>
<th>Category II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Time to Produce a Change in Chemical Detection System</td>
<td>Packing Group</td>
</tr>
<tr>
<td>$\leq$ 3 Minutes</td>
<td>I</td>
</tr>
<tr>
<td>$&gt;3$ Minutes - 1 Hour</td>
<td>II</td>
</tr>
<tr>
<td>$&gt;1$ - 4 Hours</td>
<td>III</td>
</tr>
<tr>
<td>$&gt;4$ Hours</td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>False negative rate</th>
<th>False positive rate</th>
<th>Packing Group Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>89%</td>
<td>75%</td>
<td>11%</td>
<td>25%</td>
<td>96%</td>
</tr>
</tbody>
</table>

Each test substance is added onto four replicate biobarrier membranes and the CDS vial is continuously monitored for the first 10 min. The vials are observed until a color change (i.e., break through) occurs. When a color change occurs in each vial, the break through times are recorded.
**Classification examples:**

<table>
<thead>
<tr>
<th>Product</th>
<th>Solvent (% Active)</th>
<th>pH</th>
<th>Alkaline Reserve</th>
<th>In Vivo</th>
<th>Corrositex®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product 7</td>
<td>20</td>
<td>13.7</td>
<td>2.83</td>
<td>Corrosive</td>
<td>Not tested</td>
</tr>
<tr>
<td>Product 8</td>
<td>1.5</td>
<td>12.95</td>
<td>0.91</td>
<td>Corrosive</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Product 9</td>
<td>15</td>
<td>11.41</td>
<td>1.35</td>
<td>Non-corrosive</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Product 10</td>
<td>0</td>
<td>13.5</td>
<td>2.36</td>
<td>Non-corrosive</td>
<td>Non-Corrosive</td>
</tr>
<tr>
<td>Product 11</td>
<td>32.7</td>
<td>12.6</td>
<td>0.36</td>
<td>Non-corrosive</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Product 12</td>
<td>3</td>
<td>12.15</td>
<td>0.02</td>
<td>Non-corrosive</td>
<td>Not tested</td>
</tr>
<tr>
<td>Product 13</td>
<td>3</td>
<td>12.16</td>
<td>0.10</td>
<td>Non-corrosive</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Product 14</td>
<td>10</td>
<td>12.76</td>
<td>0.91</td>
<td>Corrosive</td>
<td>Not tested</td>
</tr>
<tr>
<td>Product 15</td>
<td>23.8</td>
<td>12.15</td>
<td>2.51</td>
<td>Corrosive</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Product 16</td>
<td>0</td>
<td>12.5</td>
<td>0.47</td>
<td>Non-Corrosive</td>
<td>Not tested</td>
</tr>
<tr>
<td>Product 31</td>
<td>27</td>
<td>11</td>
<td>1.36</td>
<td>Non-Corrosive</td>
<td>Not tested</td>
</tr>
<tr>
<td>Product 32</td>
<td>34.5</td>
<td>11</td>
<td>1.38</td>
<td>Non-Corrosive</td>
<td>Not tested</td>
</tr>
<tr>
<td>Product 33</td>
<td>15</td>
<td>11.9</td>
<td>Not recorded</td>
<td>Non-Corrosive</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Product 39</td>
<td>0</td>
<td>13.2</td>
<td>Not recorded</td>
<td>Non-Corrosive</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

- 3/7 products tested using the Corrositex® assay predicted the same skin classification when compared to the *in vivo* data. The remaining 4 formulas over-predicted the skin classification. There were no under-classifications.

- **Formulas with high levels of solvent (≥15%)** may result in a more conservative classification when using this *in vitro* assay.

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2. *In vitro monolayer cell culture systems*

**General considerations**

- Generally easy to conduct – cell lines
- Quite **rapid** to execute
- Cost effective with batches of test materials – **HTP** – robotics
- Mechanistic modes of action
- **Machine scored** endpoints
- Identify potential **hazards**
- Evaluate individual chemicals (**ingredients**) rather than formulations

**Limitations**

- **Dilution effects** which mask toxicity of the neat material
- **Buffering** effects of the vehicle, and **reaction** of the chemical
- **Solubility issues**
- **Pharmacokinetics** poorly modeled
- **No tissue barrier function** modeled
- Typically lack realistic cell-cell contact: may impact cellular responses
Adverse Outcome Pathway (AOP) for skin sensitization

**INDUCTION**
- Molecular properties
- Electrophilic reactivity
- Covalent interaction with proteins
- Expression of cell surface markers and cytokines
- Proliferation of T-cells in lymph nodes

**ELICITATION**
- Penetration into the viable epidermis
- Molecular Initiating Event
- Cellular Response
- Organ Response
- Organism Response
- Dermal inflammation (after challenge)

**Biological event**
- Penetration into the viable epidermis
- Electrophilic reactivity
- Covalent interaction with proteins
- Expression of cell surface markers and cytokines
- Proliferation of T-cells in lymph nodes

**AOP phase**
- Molecular properties
- Molecular Initiating Event
- Cellular Response
- Organ Response
- Organism Response

**Test method**
- In silico
- In chemico
- In vitro
- In vivo
- Clinical

**Molecular properties**
- Penetration into the viable epidermis
- Electrophilic reactivity
- Covalent interaction with proteins
- Expression of cell surface markers and cytokines
- Proliferation of T-cells in lymph nodes

**Molecular Initiating Event**
- Expression of cell surface markers and cytokines
- Proliferation of T-cells in lymph nodes

**Cellular Response**
- Expression of cell surface markers and cytokines
- Proliferation of T-cells in lymph nodes

**Organ Response**
- Expression of cell surface markers and cytokines
- Proliferation of T-cells in lymph nodes

**Organism Response**
- Expression of cell surface markers and cytokines
- Proliferation of T-cells in lymph nodes

**Direct Peptide Reactivity Test (DPRA)**
- Keratinocyte activation
- LuSens
- Keratinosens
- LC activation
- h-CLAT

**Human Cell Line Activation Test (monocyte cell line THP-1)**
- LLNA
- GPMT
- HIRPT


KeratinoSens assay
(Nrf-2-electrophile sensing pathway)

Pre-testing:
solubility assessment

Cell dosing

Treatment termination

Addition of luciferase

Addition of MTT

Sensitization endpoint

Cytotoxicity endpoint
# KeratinoSens assay (OECD 442D)

## Brief overview and current regulatory status

<table>
<thead>
<tr>
<th><strong>Test system:</strong></th>
<th>HaCaT cells (immortalized keratinocytes containing a reporter construct with a copy of the Antioxidant Response Element (ARE) of the human AKR1C2 gene upstream of a luciferase gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay endpoints:</strong></td>
<td>Induction of luciferase activity, cytotoxicity</td>
</tr>
<tr>
<td><strong>Assay controls:</strong></td>
<td>Negative (Solvent: Assay Media containing 1% DMSO); Positive (cinnamic aldehyde)</td>
</tr>
<tr>
<td><strong>Applicability:</strong></td>
<td>Support the discrimination between skin sensitizers and non-sensitizers for the purpose of hazard classification and labeling as part of an IATA (Integrated Approaches to Testing and Assessment)</td>
</tr>
<tr>
<td><strong>Limitations:</strong></td>
<td>Since activation of the Keap1-Nrf2-ARE pathway addresses only the second key event of the skin sensitization AOP, information from test methods based on the activation of this pathway is unlikely to be sufficient when used on its own to conclude on the skin sensitization potential of chemicals. Solubility challenges</td>
</tr>
<tr>
<td><strong>Regulatory status:</strong></td>
<td>OECD Test Guideline 442D (TG 442D, adopted 2015)</td>
</tr>
</tbody>
</table>
KeratinoSens: data interpretation

Data calculation:
EC1.5 value: test substance concentration for induction 1.5 fold time above threshold
Imax: the largest average gene fold induction above 1.5 by the test substance
Ci max: the test substance concentration at which the largest average fold induction value is achieved

Prediction Model
A test substance will be considered to have sensitization potential if:

1) The EC1.5 value falls below 1000 µM (or 200 µg/mL) in at least 2 of 3 repetitions
2) At the lowest concentration with a gene induction above 1.5, cellular viability should be greater than 70%
3) An apparent overall dose response should be similar between repetitions.

Induction- dark blue; viability- pink
## Integrated Testing Strategies (ITS)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Bauch et al., 2012</th>
<th>Natsch et al., 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual assays</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPRA</td>
<td>87%</td>
<td>79%</td>
</tr>
<tr>
<td>ARE reporter gene assay</td>
<td>82%</td>
<td>81%</td>
</tr>
<tr>
<td>2 of 3 DPRA, ARE-based assay</td>
<td>94%</td>
<td>83%</td>
</tr>
</tbody>
</table>

The ITS is selected based on the goals of the testing:

- Screening (before animal/clinical testing)
- Stand-alone (internal)
- Submissions to regulatory agencies
- Timing and costs (sequential/parallel)
- Chemistries, risk (cosmetics/household/pharma)

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Defined Approaches (DA)


### Table 3. Skin sensitization potential predictivity of individual test methods and the mechanistic domains compared to both human and LLNA reference data, incl.

<table>
<thead>
<tr>
<th>Test method</th>
<th>Sample size</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Accuracy</th>
<th>Balanced accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLNA</td>
<td>128</td>
<td>50.0%</td>
<td>85.2%</td>
<td>74.2%</td>
<td>67.6%</td>
</tr>
<tr>
<td>DPRA</td>
<td>124*</td>
<td>74.4%</td>
<td>72.9%</td>
<td>73.4%</td>
<td>73.6%</td>
</tr>
<tr>
<td>KeratinoSens™</td>
<td>128</td>
<td>77.5%</td>
<td>75.0%</td>
<td>75.8%</td>
<td>76.3%</td>
</tr>
<tr>
<td>h-CLAT</td>
<td>127*</td>
<td>52.5%</td>
<td>89.7%</td>
<td>78.0%</td>
<td>71.1%</td>
</tr>
<tr>
<td>U-SENS™</td>
<td>105*</td>
<td>44.7%</td>
<td>95.5%</td>
<td>77.1%</td>
<td>70.1%</td>
</tr>
<tr>
<td>SENS-IS</td>
<td>126*</td>
<td>47.5%</td>
<td>93.0%</td>
<td>78.6%</td>
<td>70.3%</td>
</tr>
<tr>
<td>Mechanistic reaction domain</td>
<td>122**</td>
<td>75.0%</td>
<td>86.6%</td>
<td>82.8%</td>
<td>80.8%</td>
</tr>
<tr>
<td>LLNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
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<td>Sensitivity</td>
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<tr>
<td>Accuracy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balanced accuracy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Defined Approach (DA) performance in predicting human hazard (sensitizer/non-sensitizer).

<table>
<thead>
<tr>
<th>Defined Approach: BASF 2/3 (DKH)</th>
<th>Kao STS</th>
<th>Kao ITS</th>
<th>ICCVAM SVM (Human)</th>
<th>Shiseido ANN (D_hC)</th>
<th>Shiseido ANN (D_hC_KS)</th>
<th>P&amp;G BN ITS-3</th>
<th>LLNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>127</td>
<td>126</td>
<td>120</td>
<td>120</td>
<td>126</td>
<td>119</td>
<td>128</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>77.2</td>
<td>80.2</td>
<td>85.0</td>
<td>81.7</td>
<td>78.6</td>
<td>75.6</td>
<td>74.2</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>79.3</td>
<td>97.7</td>
<td>93.8</td>
<td>86.4</td>
<td>95.4</td>
<td>81.3</td>
<td>85.2</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>72.5</td>
<td>41.0</td>
<td>66.7</td>
<td>71.8</td>
<td>41.0</td>
<td>64.1</td>
<td>50.0</td>
</tr>
<tr>
<td>BA (%)</td>
<td>75.9</td>
<td>69.4</td>
<td>80.3</td>
<td>79.1</td>
<td>68.2</td>
<td>65.4</td>
<td>67.6</td>
</tr>
</tbody>
</table>

DPRA; hCLAT; DEREK

Not applicable to natural products
3. *In vitro reconstructed tissue models systems*

**General considerations**
- Higher order of complexity - Reconstructed tissues better model tissues of interest (relative to monolayer)
- Exposure to substances as *in vivo*
- Relevant mechanisms of action
- Endpoints may be machine scored
- Standardized manufacturing expected to ensure reproducibility

**Limitations**
- Tissue models tend to be costly
- Reliance on a small number of manufacturers
- Tissues differ slightly among manufacturers
- Still relatively simple models, and do not have support of whole body accessory functions

*How might this impact the toxicity predictions?*
- Care needs to be exercised not to over-interpret

*(just as in the case of animal models!)*
## RhE test method - skin corrosion assay (OECD TG 431)

### Brief overview and current regulatory status

<table>
<thead>
<tr>
<th>Test system:</th>
<th>RhE models [EpiDerm™ (EPI-200); EpiSkin™ (SM); SkinEthic™ RHE and epiCS®]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay endpoint:</td>
<td>Tissue viability (%) – assessed by reduction of the vital dye MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) by viable cells</td>
</tr>
<tr>
<td>Assay controls:</td>
<td>Negative (sterile, deionized water or NaCl solution 9g/L); Positive (8N KOH or glacial acetic acid – only for 4 hr exposure)</td>
</tr>
<tr>
<td>Applicability:</td>
<td>The results can be used for regulatory purposes for distinguishing corrosive from non-corrosive test substances. The method also allows for sub-categorization, i.e., 1A vs. 1B-and-1C vs. non-corrosive test substances.</td>
</tr>
<tr>
<td>Limitations:</td>
<td>The method does not allow discriminating between skin corrosive sub-categories 1B and 1C according to the UN GHS due to a limited set of well-known in vivo corrosive Category 1C chemicals.</td>
</tr>
<tr>
<td>Regulatory status:</td>
<td>OECD Test Guideline 431 (TG 431, updated 2016)</td>
</tr>
</tbody>
</table>
Tissue Receipt
Upon receipt, tissues are incubated for at least 1 hr in standard culture conditions (37±1°C in a humidified atmosphere of 5±1% CO₂ in air).

Tissue Treatment
Media is refreshed after the initial 1 hr incubation. Duplicate tissues are treated topically with control and test substances for 3 min / 1 hr (4 hr).

Tissue Rinsing
After exposure, tissues are rinsed to remove the control and test substances.

MTT Reduction
Individual tissues are placed into wells containing unreduced MTT solution and incubated at standard culture conditions for 3 hr.

Spectrophotometric Quantification
Optical density (OD) at 550 nm (OD₅₅₀) is determined using a 96-well plate reader. OD values are used to calculate relative viability values presented relative to negative control tissue values.

Isopropanol Extraction
The tissues are placed in isopropanol at room temperature for 2 hr to extract the reduced MTT. Extracted MTT is thoroughly mixed and transferred to a 96-well plate.
## Prediction Models

### EpiSkin™ (SM)

<table>
<thead>
<tr>
<th>Viability measured after exposure time points (3, 60 and 240 minutes)</th>
<th>Prediction to be considered UN GHS Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 35% after 3-minutes exposure</td>
<td>Corrosive: • Optional Sub-category 1A</td>
</tr>
<tr>
<td>≥ 35% after 3-minutes exposure AND &lt; 35% after 60-minutes exposure  OR ≥ 35% after 60-minutes exposure AND &lt; 35% after 240-minutes exposure</td>
<td>Corrosive: • A combination of optional Sub-categories 1B and 1C</td>
</tr>
<tr>
<td>≥ 35% after 240-minutes exposure</td>
<td>Non-corrosive</td>
</tr>
</tbody>
</table>

### EpiDerm™ (EPI-200)

### SkinEthic™ RHE epiCS®

<table>
<thead>
<tr>
<th>Viability measured after exposure time points (3- and 60-minutes)</th>
<th>Prediction to be considered UN GHS Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEP 1</td>
<td></td>
</tr>
<tr>
<td>&lt; 50% after 3-minutes exposure</td>
<td>Corrosive</td>
</tr>
<tr>
<td>≥ 50% after 3-minutes exposure AND &lt; 15% after 60-minutes exposure</td>
<td>Corrosive</td>
</tr>
<tr>
<td>≥ 50% after 3-minutes exposure AND ≥ 15% after 60-minutes exposure</td>
<td>Non-corrosive</td>
</tr>
<tr>
<td>STEP 2</td>
<td></td>
</tr>
<tr>
<td>&lt;25%; 18%; 15% after 3-minutes exposure</td>
<td>Optional Sub-category 1A</td>
</tr>
<tr>
<td>≥25%; 18%; 15% after 3-minutes exposure</td>
<td>A combination of optional Sub-categories 1B-and-1C</td>
</tr>
</tbody>
</table>

Classification examples: extreme pH mixtures (alkalis)

- Extreme pH can be a useful predictor of irritation but may lead to over-classifications in weakly buffered systems.
- 8/12 products tested using the RhE testing system predicted the same skin classification when compared to the *in vivo* data. The remaining 4 formulas **over-predicted** the skin classification. There were no under-classifications.
- **Formulas with high levels of solvent (>15%)** may result in a more conservative classification when using this *in vitro* assay.

**Integrated Approaches to Testing and Assessment (IATA)**

**Dermal corrosion and irritation (self-correcting)**

### Top-Down Strategy
- Test substance expected to be: **Corrosive**
- Assay to be used: *In Vitro Corrosion Assay(s)*
- *Is the test substance predicted as corrosive?*
  - **Y**: Labeling:
  - **N**: *In Vitro Skin Irritation Assay(s)*
  - *Is the test substance predicted as skin irritant?*
    - **Y**: Labeling:
    - **N**: Further testing

### Bottom-Up Strategy
- Test substance expected to be: **Non-Corrosive**
- Assay to be used: *In Vitro Skin Irritation Assay(s)*
- *Is the test substance predicted as skin irritant?*
  - **Y**: Labeling:
  - **N**: Further testing

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Scott L. et al., A proposed eye irritation testing strategy to reduce and replace in vivo studies using Bottom-Up and Top-Down approaches. Toxicol. In Vitro, 24, 1-9 (2010).
