ICCVAM Workshop Series on Best Practices for Regulatory Safety Testing

Assessing the Potential for Chemically Induced Eye Injuries

New Models in the Validation Pipeline for Ocular Safety Testing

Short Time Exposure Test: STE test

Hitoshi Sakaguchi, Kao Corporation
Development of a new *in vitro* eye irritation test

**Focus of development**

- **Corneal injury**
- **Conjunctival injury**

**Tissue (Cornea)**

**Cells on the surface**

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**New in vitro test: Identifying minimal to moderate eye irritation potential**

- **Short contact**
  - Eliminate time when a drop of 50mL placed in the eye (Motose et al., 1984)
  - Human: 1-2 min, Rabbit: 3-4 min (80% elimination)
  - Retention time in eye is short (around 5 min)

- **Contact with the surface cells of cornea**
  - Cytotoxicity → Onset of eye irritation

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**Development a short time exposure cytotoxicity test designed for the actual exposure condition**

*Short Time Exposure (STE)* test
Procedure of Short Time Exposure (STE) test

**Cell culture**

SIRC cells

rabbit corneal cell line

Seeded into 96-well plate at 3.0 x 10^3 cells/well

Pre-incubation (37°C, 5% CO₂) for 5 days

**Exposure**

5 minutes

Test concentration: 5% and 0.05%

Vehicle:
Saline, 5% DMSO in saline, or mineral oil

(Select vehicle based on solubility)

**Measurement of viability**

Measurement of Formazan formation (MTT assay)

Mean cell viability (%)

\[
\frac{OD_{570} \text{ (Test Sample)}}{OD_{570} \text{ (Vehicle Control)}} \times 100
\]

For each sample concentration, three wells were used to obtain viability

Takahashi Y. et al., Toxicology in Vitro, 22 (2008)
• **Category classification**
Based on viability at 5% test conc.

Criteria for classifying

<table>
<thead>
<tr>
<th>Non irritant</th>
<th>&gt; 70 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irritant</td>
<td>≤ 70 %</td>
</tr>
</tbody>
</table>

• **Rank classification**
Based on total score obtained from viabilities at 5% and 0.05% test conc.

Scoring of 5% and 0.05% test conc.

<table>
<thead>
<tr>
<th>5% score</th>
<th>0.05% score</th>
</tr>
</thead>
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<tr>
<td>viability &gt; 70</td>
<td>viability &gt; 70</td>
</tr>
<tr>
<td>viability ≤ 70</td>
<td>viability ≤ 70</td>
</tr>
</tbody>
</table>

5% score + 0.05% score = **STE rank**

- 1: Minimal irritant
- 2: Moderate irritant
- 3: Severe irritant
Today’s presentation

• **Predictive capacity**
  – Evaluation of 109 chemicals by the STE test to compare with GHS classification

• **1st validation study data**
  – Transferability, Inter-laboratory reproducibility, Predictive capacity
  – 25 blinded chemicals were evaluated by 5 laboratories

• **2nd validation study data**
  – Predictive capacity
  – 40 blinded chemicals were evaluated by 3 laboratories
# Evaluation for 109 chemicals

<table>
<thead>
<tr>
<th>Test chemicals</th>
<th>GHS Class</th>
<th>Category</th>
<th>5% con. visibility (%)</th>
<th>0.05% con. visibility (%)</th>
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<tbody>
<tr>
<td>Methoxyleryl acetate</td>
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<td>I</td>
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<td>Butyl cellulose</td>
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<tr>
<th>Test chemicals</th>
<th>GHS Class</th>
<th>Category</th>
<th>5% con. visibility (%)</th>
<th>0.05% con. visibility (%)</th>
<th>Rank</th>
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<td>Acetone</td>
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<td>0.1</td>
<td>12.8</td>
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</tr>
</tbody>
</table>

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- : Irritant by GHS (category 1 or 2) or STE test
- : Non irritant by GHS or STE test
**Predictive capacity of 109 chemicals data**

(Correspondence between STE eye irritation and GHS)

### Category classification

<table>
<thead>
<tr>
<th>STE test</th>
<th>Not irritant</th>
<th>Irritant</th>
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<tbody>
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<td>GHS</td>
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<td>54</td>
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<tr>
<td>Not irritant</td>
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<td>47</td>
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<tr>
<td>Irritant</td>
<td>56</td>
<td>8 *</td>
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</tbody>
</table>

*: 3 alcohols and others

- Sensitivity: 86% (48/56)
- Specificity: 89% (47/53)
- Positive predictivity: 89% (48/54)
- Negative predictivity: 85% (47/55)

### Rank classification

<table>
<thead>
<tr>
<th>GHS rank</th>
<th>STE rank</th>
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<td>Cat 2</td>
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<tr>
<td></td>
<td>12</td>
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</table>

**Accuracy: 73% (80/109)**

STE rank (1, 2 or 3) almost correlated with GHS rank respectively

STE irritation category (NI or I) was well correlated with GHS

**Good predictive capacity was confirmed**
1st validation study

- Organization by the Validation Committee of the Japanese Society for Alternative to Animal Experiments (JSAAE) (Validation Executive Committee)
- Test Laboratories: 5 labs. (Kanebo Cosmetics Inc., Kose Corp., POLA Chemical Industries Inc., Pias Corp., Lion Corp.)
- Duration: April, 2008 – March, 2009
- Purpose and experimental design
  1. Confirmation of transferability
     With 3 standard chemicals (Sodium lauryl sulfate, Calcium Thioglycollate, Tween 80)
  2. Evaluation of inter-laboratory reproducibility
     With 25 blinded test chemicals at each labs.
  3. Evaluation of predictive capacity
     With 25 blinded test chemicals at each labs.
     Evaluate the correspondence with Globally Harmonized System (GHS)
### Transferability

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Test conc.</th>
<th>Viability (%)</th>
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<tbody>
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<td>SLS</td>
<td>calcium thioglycollate</td>
<td>Tween 80</td>
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<tr>
<td>Lab 1</td>
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<td>2.2 ± 0.7</td>
<td>18.5 ± 6.8</td>
<td>110.3 ± 6.2</td>
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<tr>
<td></td>
<td>0.05%</td>
<td>3.1 ± 2.2</td>
<td>107.1 ± 10.5</td>
<td>103.8 ± 3.3</td>
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<td>Rank</td>
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<tr>
<td>Lab 2</td>
<td>5%</td>
<td>2.1 ± 0.9</td>
<td>12.8 ± 3.6</td>
<td>101.7 ± 1.9</td>
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<tr>
<td></td>
<td>0.05%</td>
<td>1.6 ± 0.3</td>
<td>106.8 ± 7.7</td>
<td>99.2 ± 3.6</td>
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<td>Rank</td>
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<td>1</td>
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<tr>
<td>Lab 3</td>
<td>5%</td>
<td>0.0 ± 0</td>
<td>13.7 ± 4.3</td>
<td>102.5 ± 15.1</td>
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<tr>
<td></td>
<td>0.05%</td>
<td>0.2 ± 0.4</td>
<td>99.0 ± 11.7</td>
<td>99.0 ± 5.4</td>
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<tr>
<td>Rank</td>
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<td>1</td>
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<td>Lab 4</td>
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<tr>
<td></td>
<td>0.05%</td>
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<tr>
<td></td>
<td>0.05%</td>
<td>6.3 ± 2.7</td>
<td>87.9 ± 2.0</td>
<td>95.7 ± 2.0</td>
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<td>Lead Lab. (Kao)</td>
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<tr>
<td></td>
<td>0.05%</td>
<td>0.4 ± 0.3</td>
<td>101.1 ± 5.2</td>
<td>98.7 ± 5.6</td>
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<tr>
<td>Rank</td>
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<td>1</td>
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</table>

Mean ± SD (n=3)  
1: minimal irritant  
2: moderate irritant  
3: severe irritant

- Cell viability of 3 standard chemicals at all labs and each conc were almost equivalent compared to that of lead lab.
- Ranking of all chemicals were completely-consistent between labs.

**Good transferability was confirmed**
Inter-laboratory reproducibility
(Consistency of category and rank classification)

<table>
<thead>
<tr>
<th>Test Chemicals</th>
<th>Eye irritation category</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Eye irritation rank</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>GHS Lab. 1</td>
<td>Lab. 2</td>
<td>Lab. 3</td>
<td>Lab. 4</td>
<td>Lab. 5</td>
<td>GHS Lab. 1</td>
<td>Lab. 2</td>
</tr>
<tr>
<td>A 3-Methoxy-1,2-propanediol</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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<tr>
<td>B Polyethylene glycol 400</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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<tr>
<td>C Glycerol</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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<tr>
<td>D Tween20</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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<tr>
<td>E Ethanol</td>
<td>I</td>
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<td>NI</td>
<td>NI</td>
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<tr>
<td>F Sodium hydroxide</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
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<tr>
<td>G Triton X-100</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
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<tr>
<td>H Cetylpyridinium bromide</td>
<td>I</td>
<td>I</td>
<td>I</td>
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<td>I</td>
<td>I</td>
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<tr>
<td>I Benzalkonium Chloride</td>
<td>I</td>
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<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
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<tr>
<td>J Methyl amyl ketone</td>
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<td>K 2-Methyl-1-pentanol</td>
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<tr>
<td>L n-Hexanol</td>
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<td>I</td>
<td>I</td>
<td>I</td>
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<tr>
<td>M 3,3-Dimethylpentane</td>
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<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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<tr>
<td>N Methyl cyclopentane</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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<tr>
<td>O Methyl isobutyl ketone</td>
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<td>NI</td>
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<td>P Toluene</td>
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<td>NI</td>
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<td>NI</td>
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<tr>
<td>Q 1-Octanol</td>
<td>I</td>
<td>I</td>
<td>I</td>
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<tr>
<td>R 2-Ethyl-1-hexanol</td>
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<tr>
<td>S Acetone</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>1</td>
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<tr>
<td>T Cyclohexanol</td>
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<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>1</td>
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<tr>
<td>U n,N-Dimethylguanidine sulfate</td>
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<td>NI</td>
<td>NI</td>
<td>NI</td>
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<td>NI</td>
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<tr>
<td>V 2-Ethylhexyl p-dimethyl amine benzolate</td>
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<td>NI</td>
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<td>NI</td>
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<td>W Guandactone</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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<tr>
<td>X Methyl ethyl ketone</td>
<td>I</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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<tr>
<td>Y Propylene glycol</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
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</table>

- Most estimated eye irritation category and ranking of test chemicals were consistent between the five labs (except J, R, W and X)

Inter-laboratory reproducibility is good
Category classification for 25 chemicals

<table>
<thead>
<tr>
<th>GHS</th>
<th>STE (5%)</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NI (CV&gt;70)</td>
<td>I (CV≤70)</td>
</tr>
<tr>
<td>NI</td>
<td>13 (12*)</td>
<td>0 (1*)</td>
</tr>
<tr>
<td>I (Cat. 1 and 2)</td>
<td>3 (2*)</td>
<td>9 (10*)</td>
</tr>
</tbody>
</table>

*1: Lab 3, 5    *2: Lab 3, 4

<table>
<thead>
<tr>
<th></th>
<th>Lab 1</th>
<th>Lab 2</th>
<th>Lab 3</th>
<th>Lab 4</th>
<th>Lab 5</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>9/12</td>
<td>9/12</td>
<td>10/12</td>
<td>10/12</td>
<td>9/12</td>
<td>(78.3)</td>
</tr>
<tr>
<td></td>
<td>(75.0)</td>
<td>(75.0)</td>
<td>(83.3)</td>
<td>(83.3)</td>
<td>(75.0)</td>
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</tr>
<tr>
<td>Specificity</td>
<td>13/13</td>
<td>13/13</td>
<td>12/13</td>
<td>13/13</td>
<td>12/13</td>
<td>(96.9)</td>
</tr>
<tr>
<td></td>
<td>(100.0)</td>
<td>(100.0)</td>
<td>(92.3)</td>
<td>(100.0)</td>
<td>(92.3)</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>22/25</td>
<td>22/25</td>
<td>22/25</td>
<td>23/25</td>
<td>21/25</td>
<td>(88.0)</td>
</tr>
<tr>
<td></td>
<td>(88.0)</td>
<td>(88.0)</td>
<td>(88.0)</td>
<td>(92.0)</td>
<td>(84.0)</td>
<td></td>
</tr>
</tbody>
</table>

- STE (5% data) irritation category (NI or I) showed good correlation with GHS category (NI or I: category 1 and 2)
- All most same results in all labs
**Rank classification for 25 chemicals**

<table>
<thead>
<tr>
<th>GHS Rank</th>
<th>STE Rank</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NI</td>
<td>13 (12(^1))</td>
<td>0 (1(^2))</td>
</tr>
<tr>
<td>Cat. 2</td>
<td>2 (3(^4))</td>
<td>5 (4(^4))</td>
</tr>
<tr>
<td>Cat. 1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^1\): Lab 3, 5, \(^2\): Lab 5, \(^3\): Lab 3 \(^4\): Lab 1, 2, 5

<table>
<thead>
<tr>
<th>Accuracy</th>
<th>Lab 1</th>
<th>Lab 2</th>
<th>Lab 3</th>
<th>Lab 4</th>
<th>Lab 5</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21/25</td>
<td>21/25</td>
<td>21/25</td>
<td>22/25</td>
<td>20/25</td>
<td>(84.0)</td>
</tr>
<tr>
<td></td>
<td>(84.0)</td>
<td>(84.0)</td>
<td>(84.0)</td>
<td>(88.0)</td>
<td>(80.0)</td>
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</tr>
</tbody>
</table>

- STE rank (1, 2 or 3) showed good correlation with GHS rank (NI, Cat. 2, Cat. 1)
- All most same results were obtained among all labs
Summary and the next step

- **Predictive capacity**
  - Accuracy: 87% (109 chemicals)
  - Good predictive capacity was confirmed

- **1st and 2nd validation study data**
  - Good transferability was confirmed
  - Inter-laboratory reproducibility was good
  - Good predictive capacity was confirmed
  - Some alcohols and organic salts were false negatives

- **The next step**: Make further progress toward peer review