Preliminary Evaluation of the Underprediction Rate of the In Vivo Dermal Irritation Test Method
Part I: Introduction

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• Data Source
Outline

• Introduction
  – Background
  – Current Testing Procedures
  – Prior Analyses
  – Study Objectives
  – Database
  – Future Plans

• Data Analysis
Background

- Draize rabbit skin test method
  - Used since the 1940’s to identify skin irritants and corrosives

- Skin corrosion: the production of irreversible damage to skin following application of a test substance for up to 4 hrs

- Skin irritation: the production of reversible damage to skin following application of a test substance for up to 4 hours
Background

• 2003 Globally Harmonized System of Classification and Labelling of Chemicals (GHS)
  – Tiered testing approach incorporating the use of valid and accepted in vitro methods for dermal irritation should be considered

• Non-animal alternative methods proposed for assessing dermal irritation
  – EPISKIN™, EpiDerm™, and SIFT
  – ECVAM validation in progress

• Estimates of the underprediction likely in an animal would assist with interpreting the usefulness and limitations of in vitro test methods
Tiered-Testing Strategy

Valid and accepted *in vitro* dermal corrosion test

- Negative Response or no data

Valid and accepted *in vitro* dermal irritation test*

- Negative response or no data

*In vivo* dermal corrosion (1 animal)

- Negative response

*In vivo* dermal irritation test (3 animals total)

- Negative response

When is it ethical to perform human patch testing?

- Not as above

Positive Response → Classify as corrosive

Positive Response → Classify as irritant

Corrosive response → Classify as corrosive

Irritant response → Classify as irritant

No further testing → Classify as non-irritant

Irritant response → Classify as irritant

Non-irritant response → No further testing

*Must be capable of detecting false negative chemicals from an *in vitro* corrosivity test.
Current Testing Procedures

- Draize rabbit skin test method

- Current test guideline procedures since 1981 (OECD TG 404)

- Test method protocol
  - 0.5 mL or 0.5 g of test substance applied to intact skin with patch for 4 hours
    - Originally 6 animals; reduced to 1-3 animals in 1992
    - Test substance removed after 4 hr exposure period
  - Erythema and edema scored at 24, 48, and 72 hours
  - Observation for 14 days to determine persistence or delayed effects
Dermal Irritation Scoring

• Erythema
  1 = Very slight (barely perceptible)
  2 = Well defined
  3 = Moderate to severe
  4 = Severe erythema (beefy redness) to eschar formation preventing grading of erythema

• Edema Scores
  1 = Very slight (barely perceptible)
  2 = Slight (edges of area well defined by definite raising)
  3 = Moderate (raised approximately 1 mm)
  4 = Severe (raised more than 1 mm and extending beyond area of exposure)
Hazard Classification for Dermal Irritation

• UN Globally Harmonized System (GHS), 2003

• Classification Scheme
  – **Irritant**
    - At least 2 animals have an average erythema or edema score that is greater than 2.3
  – **Mild irritant**
    - At least 2 animals have an average erythema or edema score that is between 1.5 and 2.3
  – **Nonirritant**
    - If no more than 1 animal has an average erythema or edema score that is greater than 1.5
Prior Analysis of the Reproducibility of the Rabbit Dermal Irritation Test

• Weil and Scala (1971)
  – Evaluated the reproducibility of the Draize rabbit skin test method within and among 24 laboratories for 10 substances
• This study is the only formal evaluation of the reproducibility of the Draize rabbit skin test method
• Conclusions
  – Moderate intra-laboratory reproducibility
  – Low inter-laboratory reproducibility
  – Primary reasons for the low inter-laboratory reproducibility attributed to the subjective nature of the visual observations and variations in procedures among labs

Limitations of the Weil and Scala Analysis

- The standard protocol used was different from the current Draize \textit{in vivo} rabbit skin test method protocol in use since 1981
  - The Weil and Scala studies used a 24-hour exposure period versus the current maximum 4-hour exposure
  - Prolonged exposure likely responsible for corrosive lesions observed for several irritants

- Good Laboratory Practice (GLP) Guidelines had not yet been established
  - Impact unknown
Study Objectives

• Evaluate ECETOC Chemical Data Bank to estimate the likelihood of underpredicting:
  – An irritant as a mild irritant
  – An irritant as a non-irritant
  – A mild irritant as a non-irritant

• Data may assist in decisions on acceptable false-negative rate for irritant effects for *in vitro* test methods proposed as complete replacements for the rabbit skin test
  – i.e., those tests where no *animal* testing would be performed and *in vitro* results would serve as the basis for hazard classification and labeling
**In Vivo Dermal Irritation Database**

- ECETOC Reference Chemicals Data Bank
  - 164 chemicals in 197 studies
  - Represent a wide range of chemical classes
  - Studies were performed according to OECD TG 404 and GLPs
  - 23 chemicals were tested in multiple studies
  - Most chemicals tested in 3-6 animals

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of Animals Used per Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>ECETOC(^1)</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^1\)European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Skin Irritation and Corrosion: Reference Chemicals Data Bank. Technical Report No. 66. Belgium. (All studies followed OECD TG 404 and GLP Guidelines)
Future Analysis Plans

• Continue to seek high quality test data to add to the database:
  – Federal Register Notice (July 16, 2003)
    ◊ Requested in vivo dermal data for chemicals that could be considered for reference chemicals
  – EPA TSCATS database
    ◊ Current collaboration with EPA OPPTS to obtain reports for ~2400 commercially available chemicals with dermal test results
    ◊ 638 reports reviewed to date, but:
      o Limited individual animal data provided
      o Many studies were conducted prior to 1981 (exposure of 24 hr vs. 4 hr)

• Perform reanalysis when EPA data review completed
Preliminary Evaluation of the Underprediction Rate of In Vivo Dermal Irritation Test Method
Part II: Data Analysis

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October 20, 2004
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Definition of Underprediction Rate

- The under-prediction rate of an irritation test is defined as the probability that an irritant substance will not be classified as an irritant when subjected to the test
  - e.g., it will produce responses that classify an irritant as a non-irritant in the rabbit model
- The underprediction rate depends on
  - the distribution of animal responses for substances assigned to a specific classification category
  - the strategy that is used to assign a test substance to a classification category
### Classification of Potential Outcomes

<table>
<thead>
<tr>
<th>Erythema or Edema Score</th>
<th>Classification</th>
<th>Probability Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.5</td>
<td>Negative</td>
<td>((P_N)^3)</td>
</tr>
<tr>
<td>1.5-2.3</td>
<td>Negative</td>
<td>(3P_N^2P_M)</td>
</tr>
<tr>
<td>&gt;2.3</td>
<td>Negative</td>
<td>(3P_N^2P_I)</td>
</tr>
<tr>
<td></td>
<td>Mild Irritant</td>
<td>(6P_NP_M^2P_I)</td>
</tr>
<tr>
<td></td>
<td>Mild Irritant</td>
<td>(3P_M^2P_N)</td>
</tr>
<tr>
<td></td>
<td>Mild Irritant</td>
<td>((P_M)^3)</td>
</tr>
<tr>
<td></td>
<td>Mild Irritant</td>
<td>(3P_M^2P_I)</td>
</tr>
<tr>
<td></td>
<td>Irritant</td>
<td>(3P_I^2P_N)</td>
</tr>
<tr>
<td></td>
<td>Irritant</td>
<td>(3P_I^2P_M)</td>
</tr>
<tr>
<td></td>
<td>Irritant</td>
<td>((P_I)^3)</td>
</tr>
</tbody>
</table>

\(P_N\): probability that erythema/edema score < 1.5; \(P_M\): score = 1.5-2.3, \(P_I\): score > 2.3
Calculation of the Underprediction Rate

• The distribution of animal responses for each irritancy class (i.e., irritant, mild irritant, nonirritant) was calculated.

• Using this distribution and the possible outcomes provided in the previous table, response probabilities were calculated for each outcome for a specific irritancy classification.

• For each irritancy classification, these probabilities were then summed to provide an overall classification likelihood.

• 2 approaches were used:
  1) All substances in the database were used, OR
  2) Only substances tested multiple times were used
### Distribution of Animal Scores (Approach 1)

<table>
<thead>
<tr>
<th>Estimated Probability of … (No. animals)</th>
<th>True Classification of Test Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonirritant</td>
</tr>
<tr>
<td>An animal scoring &lt; 1.5</td>
<td>95.7% (222)</td>
</tr>
<tr>
<td>An animal scoring 1.5 - 2.3</td>
<td>3.9% (9)</td>
</tr>
<tr>
<td>An animal scoring &gt; 2.3</td>
<td>0.4% (1)</td>
</tr>
</tbody>
</table>

**No. Studies Evaluated**

|                | 66 | 88 | 43 |

The table above illustrates the distribution of animal scores for different classifications of test substances. The estimated probabilities and the number of animals for each classification are provided.
### Example Calculation of Probability - Likelihood of a Nonirritant being Classified as a Nonirritant

<table>
<thead>
<tr>
<th>Erythema or Edema Score</th>
<th>Classification</th>
<th>Probability Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.5</td>
<td>3</td>
<td>(P_N)^3</td>
</tr>
<tr>
<td>1.5 - 2.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 2.3</td>
<td>0</td>
<td>Negative</td>
</tr>
</tbody>
</table>

#### Probabilities:

\[
(P_N)^3 + 3P_N^2P_M + 3P_N^2P_I = (0.957)^3 + [3(0.957)^2(0.039)] + [3(0.957)^2(0.004)] = 0.995 = 99.5\%
\]
## Estimated Probabilities of Classification (Approach 1)

<table>
<thead>
<tr>
<th>Our Classification of Test Substance</th>
<th>True Classification of Test Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative 99.5%</td>
</tr>
<tr>
<td>Mild Irritant</td>
<td>Negative 0.5%</td>
</tr>
<tr>
<td>Irritant</td>
<td>Irritant &lt;0.01%</td>
</tr>
</tbody>
</table>
## Distribution of Animal Scores (Approach 2)

<table>
<thead>
<tr>
<th>Estimated Probability of ... (No. animals)</th>
<th>True Classification of Test Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonirritant</td>
</tr>
<tr>
<td>An animal scoring &lt; 1.5</td>
<td>91.7% (55)</td>
</tr>
<tr>
<td>An animal scoring 1.5 - 2.3</td>
<td>8.3% (5)</td>
</tr>
<tr>
<td>An animal scoring &gt; 2.3</td>
<td>0% (0)</td>
</tr>
</tbody>
</table>

| No. Chemicals Evaluated                  | 8           | 12           | 3             |
## Estimated Probabilities of Classification (Approach 2)

<table>
<thead>
<tr>
<th>True Classification of Test Substance</th>
<th>Negative</th>
<th>Mild Irritant</th>
<th>Irritant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>98.0%</td>
<td>3.7%</td>
<td>0%</td>
</tr>
<tr>
<td>Mild Irritant</td>
<td>2.0%</td>
<td>94.0%</td>
<td>38.7%*</td>
</tr>
<tr>
<td>Irritant</td>
<td>0%</td>
<td>2.2%</td>
<td>61.3%</td>
</tr>
</tbody>
</table>

*Database includes only 3 irritants*
## Estimated Underprediction Rates of the *In Vivo* Dermal Irritation Test Method

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Approach 1*</th>
<th>Approach 2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underprediction of Irritant as Mild Irritant</td>
<td>10.3%</td>
<td>38.7%**</td>
</tr>
<tr>
<td>Underprediction of Irritant as Negative</td>
<td>0.01%</td>
<td>0%</td>
</tr>
<tr>
<td>Underprediction of Mild Irritant as Negative</td>
<td>5.5%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Underprediction of Irritant and Mild Irritant as Negative</td>
<td>5.5%</td>
<td>3.7%</td>
</tr>
</tbody>
</table>

*Approach 1 = All chemicals used; Approach 2 = Only multiply-tested chemicals

**Database includes only 3 irritants
## Mean Scores for the 3 Multiply Tested Skin Irritants

<table>
<thead>
<tr>
<th>Chemical (Study No.)</th>
<th>Mean Erythema</th>
<th>Mean Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>An. 1</td>
<td>An. 2</td>
</tr>
<tr>
<td>Alpha-terpineol (1)</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Alpha-terpineol (2)</td>
<td>2.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Alpha-terpineol (3)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cyclamen aldehyde (1)</td>
<td>2.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Cyclamen aldehyde (2)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cyclamen aldehyde (3)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cyclamen aldehyde (4)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Lilestralis/Lilial (1)</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Lilestralis/Lilial (2)</td>
<td>2.0</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Conclusions

• Within the limits of the assumptions, the under-prediction of:
  – an irritant as a mild irritant ranged from 10.3% to 38.7%*
  – an irritant as a nonirritant ranged from 0% to 0.01%
  – a mild irritant as a nonirritant ranged from 3.7% to 5.5%

• Based on these data, the likelihood that an irritant would be misclassified as a nonirritant is less than 0.01%.

• The relatively small number of irritants among the multiply-tested substances may impact the reliability of the estimated underprediction rate.

*The 38.7% underprediction rate is based on only 3 irritants