EC3 > 2% (11/27) or were negative in the LLNA (2/27). Of the 11 strong human sensitizers with an EC3 > 2%, ICCVAM evaluated the LLNA as a stand-alone test method to determine potency categorization of substances as strong sensitizers when EC3 ≤ 2%, a criterion recently adopted by the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (GHS) to minimize false negatives. In vitro allergic sensitization potency categories for chemicals are intended to serve as a starting point for further studies to improve the accuracy of the LLNA for identifying strong sensitizers. To improve the accuracy of the LLNA for identifying strong sensitizers, ICCVAM recommends the development and evaluation of integrated decision strategies that consider other types of assay results. This would encourage the development and evaluation of integrated decision strategies that consider other types of assay results.

Validation Status of the LLNA to Classify Substances into Skin Sensitization Potency Categories

### Figure 1

**Timeline of Evaluation of Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans**

- 2001: Methods for classifying chemicals using murine local lymph node assay (LLNA) for skin sensitization were developed.
- 2008: Peer Review Panel meeting evaluated LLNA compared to the Human DSA05 (500 µg/cm²) threshold.
- 2009: Revised LLNA classification criteria to include subclassification of substances.

### Figure 2

**LLNA EC3 and Human DSA05 by GHS Potency Category for 130 Substances**

<table>
<thead>
<tr>
<th>GHS Potency Category</th>
<th>LLNA EC3</th>
<th>Human DSA05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong (1A)</td>
<td>≤ 2%</td>
<td>500 µg/cm²</td>
</tr>
<tr>
<td>Weak (1B)</td>
<td>2% - 10%</td>
<td>200 µg/cm²</td>
</tr>
<tr>
<td>Very weak (2A)</td>
<td>10% - 50%</td>
<td>100 µg/cm²</td>
</tr>
<tr>
<td>Undetermined (2B)</td>
<td>50% - 100%</td>
<td>50 µg/cm²</td>
</tr>
<tr>
<td>Negative (3)</td>
<td>&gt; 100%</td>
<td>20 µg/cm²</td>
</tr>
</tbody>
</table>

### Figure 3

**LLNA EC3 Classification of 27 Strong Human Skin Sensitizers**

- 48% (13/27) of strong human skin sensitizers were underclassified as other than strong skin sensitizers.
- 11 with negative LLNA results
- 3 with LLNA EC3 ≤ 2%
- 2 with negative LLNA results
- 11 with LLNA EC3 > 2%
- This accounts for 76% (10/13) of the strong human skin sensitizers underclassified by the LLNA.

### Figure 4

**LLNA Test Method Protocol**

1. **Preparation of Test Material**
   - Ensure stability and homogeneity of test material.
   - Prepare test material at the appropriate concentration for in vivo application.
2. **Application**
   - Apply test material to the shaved flank of the mouse.
   - Ensure proper application technique to minimize variability.
3. **Assessment of Response**
   - Observe for any signs of adverse effects post-application.
   - Collect lymph node material for analysis.
4. **Data Analysis**
   - Use geometric mean values to calculate EC3.
   - Compare EC3 to the GHS classification criteria.

### ICCVAM Recommendations:

- **Future Studies**
  - Emphasis should be placed on identifying substances that are classified as strong skin sensitizers in the LLNA.
  - This would encourage the development and evaluation of integrated decision strategies that consider other types of assay results.
- **Test Method Protocol**
  - Additional analyses might improve the correlation between the LLNA EC3 values and the human threshold values.
- **Test Method Usefulness and Limitations**
  - The LLNA is limited by the inability to predict the potency of very weak or negative substances.
  - Further studies are needed to improve the accuracy of the LLNA for identifying strong sensitizers.

**ICCVAM Interagency Immunotoxicity Working Group (IWG)**

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**ICCVAM Recommendations**

- **Test Method Protocol**
  - Additional analyses might improve the correlation between the LLNA EC3 values and the human threshold values.
  - Additional studies are needed to improve the accuracy of the LLNA for identifying strong sensitizers.

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