1. INTRODUCTORY INFORMATION

- Prerequisites
  - Solid or liquid test substance
  - Chemical identification of test substance
  - Purity (impurities) of test substance
  - Solubility characteristics
  - pH (where appropriate)
  - Melting point/boiling point

- Standard documents
  There are no relevant international standards.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant or corrosive effects on skin of mammals is an important initial step. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the skin to the test substance.

- Definitions
  Dermal irritation is the production of reversible inflammatory changes in the skin following the application of a test substance.
  Dermal corrosion is the production of irreversible tissue damage in the skin following the application of a test substance.

- Principle of the test method
  The substance to be tested is applied in a single dose to the skin of several experimental animals, each animal serving as its own control. The degree of irritation is read and scored at specified intervals and is further described to provide a complete evaluation of the effects. The duration of the study should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed.

Users of this Test Guideline should consult the Preface, in particular paragraphs 3, 4, 7 and 8.
B. DESCRIPTION OF THE TEST PROCEDURE

Preparations

Approximately 24 hours before the test, fur should be removed by clipping or shaving from the dorsal area of the trunk of the animals. Care should be taken to avoid abrading the skin. Only animals with healthy intact skin should be used.

When testing solids (which may be pulverised if considered necessary) the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle, to ensure good contact with the skin. When vehicles are used, the influence of the vehicle on irritation of skin by the test substance should be taken into account. Liquid test substances are generally used undiluted.

Strongly acidic or alkaline substances, for example with a demonstrated pH of 2 or less or 11.5 or greater, need not be tested for primary dermal irritation, owing to their predictable corrosive properties. The testing of materials which have been shown to be highly toxic by the dermal route is unnecessary.

Experimental animals

Selection of species

Although several mammalian species may be used, the albino rabbit is recommended as the preferred species.

Number of animals

At least 3 healthy adult animals should be used. Additional animals may be required to clarify equivocal responses.

Housing and feeding conditions

Animals should be individually housed. The temperature of the experimental animal room should be 22°C (± 3°C) for rodents, 20°C (± 3°C) for rabbits, and the relative humidity 30 to 70 per cent. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. Conventional laboratory diets are suitable for feeding and an unrestricted supply of drinking water should be available.
Test conditions

Dose level
A dose of 0.5 ml of liquid or 0.5 g of solid or semi-solid is applied to the test site. Separate animals are not required for an untreated control group. Adjacent areas of untreated skin of each animal serve as control for the test.

Observation period
The duration of the observation period should not be fixed rigidly but should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed. It need not normally exceed 14 days after application.

Procedure
The test substance should be applied to a small area (approximately 6 cm²) of skin and covered with a gauze patch, which is held in place with non-irritating tape. In the case of liquids or some pastes it may be necessary to apply the test substance to the gauze patch and then apply that to the skin. The patch should be loosely held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of the exposure period. However, the use of occlusive dressing may be considered appropriate in some cases. Access by the animal to the patch and resultant ingestion/inhalation of the test substance should be prevented.

Exposure duration is four hours. Longer exposures may be indicated under certain conditions, e.g. expected pattern of human use and exposure. At the end of the exposure period, residual test substance should be removed, where practicable, using water or an appropriate solvent, without altering the existing response or the integrity of the epidermis.

Clinical observations and scoring
Animals should be examined for signs of erythema and oedema and the responses scored at 30-60 minutes, and then at 24, 48 and 72 hours after patch removal.

Dermal irritation is scored and recorded according to the grades in the Table 1, below. Further observations may be needed, as necessary, to establish reversibility. In addition to the observation of irritation, any serious lesions and other toxic effects should be fully described.
TABLE 1: EVALUATION OF SKIN REACTION

<table>
<thead>
<tr>
<th>Erythema and Eschar Formation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No erythema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight erythema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Well-defined erythema</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to severe erythema</td>
<td>3</td>
</tr>
<tr>
<td>Severe erythema (heat redness) to slight eschar formation (injuries in depth)</td>
<td>4</td>
</tr>
</tbody>
</table>

Maximum possible = 4

<table>
<thead>
<tr>
<th>Oedema Formation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No oedema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight oedema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Slight oedema (edges of area well defined by definite raising)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate oedema (raised approximately 1 millimetre)</td>
<td>3</td>
</tr>
<tr>
<td>Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)</td>
<td>4</td>
</tr>
</tbody>
</table>

Maximum possible = 4

3. DATA AND REPORTING

- **Treatment of results**

Data may be summarised in tabular form, showing for each individual animal the irritation scores for erythema and oedema at 30-60 minutes, 24, 48 and 72 hours after patch removal, any serious lesions, a description of the degree and nature of irritation, corrosion or reversibility, and any other toxic effects observed.

- **Evaluation of results**

The dermal irritation scores should be evaluated in conjunction with the nature and reversibility or otherwise of the responses observed. The individual scores do not represent an absolute standard for the irritant properties of a material, and they should be viewed as reference values which are only meaningful when supported by a full description and evaluation of the observation(s). The use of an occlusive dressing is a severe test and the results are relevant to very few likely human exposure conditions.
Test report

The test report must include the following information:

- species/strain used;
- physical nature and, where appropriate, concentration and pH value for the test substance;
- tabulation of irritation response data for each individual animal for each observation time period (e.g. 30-60 minutes, 24, 48 and 72 hours after patch removal);
- description of any serious lesions observed;
- narrative description of the degree and nature of irritation observed; and
- description of any toxic effects other than dermal irritation.

Interpretation of the results

Extrapolation of the results of dermal irritancy/corrosivity studies in animals to man is valid only to a limited degree. The albino rabbit is more sensitive than man to irritant substances in most cases. The finding of similar results in tests on other animal species may give more weight to extrapolation from animal studies to man.

4. Literature


