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DRDE REPORT

Primary Skin Irritation Test of Samples of "Stored Toxic Wastes at the former UCIL Plant Site at Bhopal" in Rabbits



June, 2008

Performing Laboratory

Defence Research and Development Establishment  
Ministry of Defence, Government of India  
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CONFIDENTIAL

### MANAGEMENT STATEMENT

This is to certify that the research entitled "Primary Skin Irritation Test of Samples of 'Stored Toxic Wastes at the former UCIL Plant Site at Bhopal' in Rabbits" was carried out by Defence Research and Development Establishment, Jhansi Road, Gwalior, M.P., India. This report contains 17 pages including front page and tables.

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## SUMMARY

This study was conducted to assess the safety with special reference to the primary skin irritation, if any, of six samples (Excavated waste, Lime sludge, Naphthol tar (or Napthol tar), Reactor residue, Semi processed pesticide and Sevin tar) collected from 'Stored Toxic Wastes at the former UCIL Plant Site at Bhopal'. All the six coarse samples were separately collected in polythene bags and brought to DRDE, Gwalior. The samples were grinded, homogenized and filtered using 40 mesh sieve and stored in glass bottles at room temperature. Thereafter, administered as such percutaneously or dermally (on intact but hair clipped non-abraded and abraded skin) for a period of four hours in male rabbits.

Skin irritation testing in laboratory animals has not changed significantly since the method of Draize *et al.* (1944) even though several modifications of the basic procedures have been developed. The technique described by Draize *et al.* is successfully used to evaluate primary skin irritation properties of consumer products, drugs etc. The rabbits were selected as a test system because it is a readily available laboratory animal and also recommended for such type of studies. It has been historically shown to be a suitable model for primary skin irritation assessment. The percutaneous or dermal application (administration) represents the inadvertent route of administration/ contact of the samples in humans. The results of the study are believed to be of value in predicting the irritation of the samples in humans.

Briefly, each of the sample as such (0.5 g on each site) was applied on closely hair clipped non-abraded and abraded dorsal skin sites of rabbits. Thereafter, the animals were restrained for 4 hours so as to keep the sample(s) in direct contact with the skin. After the aforesaid duration the skin sites were carefully cleaned with moist cotton and after drying the sites were examined in clear day light following standard scoring procedure. The data was analyzed to determine Primary Skin Irritation Index (PSII).

Results: All the six samples did not induce the sign of irritation on the skin sites. Further, no noticeable change in clinical signs and gross motor activity was observed in any of the rabbits either during the period of treatment with the sample(s) or up to fourteen days. Body weight gain, feed and water consumption of all the animals grossly observed for a period of 14 days post treatment and compared to that of normal animals, did not show significant change.

### **The important finding of the study:**

The percutaneous (dermal) application of any of the six samples to hair clipped skin sites (non-abraded and abraded) of rabbits for a duration of 4 hours did not cause change compared to that of control skin sites. Further, no treatment related alterations were observed in gross locomotor activity, feed intake, water intake and body weight up to 14 days.

## CONCLUSION

Based on the primary skin irritation test, all the six samples (Excavated waste, Lime sludge, Naphthol tar, Reactor residue, Semi processed pesticide and Sevin tar) collected from 'Stored Toxic Wastes at the former UCIL Plant Site at Bhopal' were found to be not-irritant to the rabbit's skin.

## INTRODUCTION

This study was conducted to assess the safety with special reference to the primary skin irritation (if any) of samples collected from 'Stored Toxic Wastes at the former UCIL Plant Site at Bhopal'. The samples collected were as follow:

- (i) Excavated waste,
- (ii) Lime sludge,
- (iii) Naphthol tar (or Napthol tar),
- (iv) Reactor residue,
- (v) Semi processed pesticide and
- (vi) Sevin tar

All the six coarse samples were separately collected in polythene bags and brought to Defence Research and Development Establishment (DRDE), Gwalior for the aforesaid study.

### Significance of Primary skin irritation test

Virtually most of the chemicals have a tendency to produce some reaction when they come into close contact with the skin. The response may be caused by physical abrasion by suspended particulate matters, which is transitory in nature and mild in degree. Or it may be due to chemical interaction of the foreign substance, liquid or solid, with the skin. This may result in varying degrees of redness and swelling at the site of contact.

Skin irritation testing in laboratory animals has not changed significantly since the method of Draize *et al.* (1944) even though several modifications of the basic procedures have been developed. The technique described by Draize *et al.* is successfully used to evaluate skin irritation properties of consumer products, drugs etc.

The rabbits were selected as a test system because it is a readily available laboratory animal and also recommended for such type of studies. It has been historically shown to be a suitable model for primary skin irritation assessment. The percutaneous or dermal application (administration) represents the inadvertent route of administration/ contact of the samples in humans. The results of the study are believed to be of value in predicting the irritation of the samples in humans.

The study was performed at Animal Facility Division, Defence Research & Development Establishment, Gwalior (M.P.).

## EXPERIMENTAL PROCEDURE

### Test Substance

Six samples i.e. Excavated waste, Lime sludge, Naphthol tar, Reactor residue, Semi processed pesticide and Sevin tar were collected from 'Stored Toxic Wastes at the former UCIL

**Table 1: Evaluation of skin response by Draize technique**

| Response   | Score    |
|--|----------|
| <b>Erythema and eschar formation</b>                                       |          |
| No erythema  | 0        |
| Very slight erythema (barely perceptible)                                  | 1        |
| Well-defined erythema  | 2        |
| Moderate to severe erythema  | 3        |
| Severe erythema (Beet red) to slight eschar formation (injury in-depth)    | 4        |
| <b>Edema formation</b>   |          |
| No edema   | 0        |
| Very slight edema (barely perceptible)                                     | 1        |
| Well-defined edema (edges of area well defined by raising)                 | 2        |
| Moderate to severe edema (raised approx. 1 mm)                             | 3        |
| Severe edema (raised more than 1 mm and extending beyond application site) | 4        |
| <b>Maximum erythema plus edema</b>   | <b>8</b> |

The primary skin irritation index (PSII) i.e. the average sum of erythema and edema for all sites on all rabbits was computed as per following formula:

$$\text{PSII} = \frac{\text{Total of averages}}{\text{Types of skin sites} \times \text{Number (time) of readings}}$$

**BIBLIOGRAPHY**

- Draize, JH, Woodard, G and Calvery, HO (1944). Method for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Therp.* 82: 377-389.
- OECD Guidelines for the Testing of Chemicals (1993). Volume - 1, page 411-1 to 9. OECD Publications Service, 2 rue Andre Pascal, 75775 PARIS Cedex 16, France.



Table 2: Computation of Primary Skin Irritation Index (PSII) after application of "Excavated waste" in Rabbits – After 4 hours exposure

| Rabbit   | Site | Non-abraded Skin Site |   |   |   |    |              |   |   |   |    | Abraded Skin Site |   |   |   |    |              |   |   |   |    |
|----------|------|-----------------------|---|---|---|----|--------------|---|---|---|----|-------------------|---|---|---|----|--------------|---|---|---|----|
|          |      | Erythema (days)       |   |   |   |    | Edema (days) |   |   |   |    | Erythema (days)   |   |   |   |    | Edema (days) |   |   |   |    |
|          |      | 1*                    | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 | 1*                | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 |
| # 1      | 1    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 2    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | 3    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 4    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| # 2      | 1    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 2    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | 3    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 4    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | TA   | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| PSII = 0 |      |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |

TA = Total of averages (treated sites). 1 & 2 are left skin sites (control) and 3 & 4 are right skin sites (treatment site).  
 PSII = Total of averages / Types of skin sites (2) x Number (time) of readings (3).

\* First scoring was done after 4 hours.

For computation of PSII, three times of readings i.e. after 4 hours (day 1), day 2 and day 3 were taken.

Scoring scale:

| PSII      | Irritation category |
|-----------|---------------------|
| 0.0       | not-irritant        |
| > 0.0-0.5 | negligible irritant |
| > 0.5-2.0 | mild irritant       |
| > 2.0-5.0 | moderate irritant   |
| > 5.0-8.0 | severe irritant     |



Table 3: Computation of Primary Skin Irritation Index (PSII) after application of "Lime sludge" in Rabbits – After 4 hours exposure

| Rabbit   | Site | Non-abraded Skin Site |   |   |   |    |              |   |   |   |    | Abraded Skin Site |   |   |   |    |              |   |   |   |    |
|----------|------|-----------------------|---|---|---|----|--------------|---|---|---|----|-------------------|---|---|---|----|--------------|---|---|---|----|
|          |      | Erythema (days)       |   |   |   |    | Edema (days) |   |   |   |    | Erythema (days)   |   |   |   |    | Edema (days) |   |   |   |    |
|          |      | 1*                    | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 | 1*                | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 |
|          |      |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |
| #1       | 1    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 2    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | 3    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 4    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| #2       | 1    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 2    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | 3    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 4    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| TA       | 0    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 |    |
| PSII = 0 |      |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |

TA = Total of averages (treated sites). 1 & 2 are left skin sites (control) and 3 & 4 are right skin sites (treatment site).  
 PSII = Total of averages / Types of skin sites (2) x Number (time) of readings (3).  
 \* First scoring was done after 4 hours.  
 For computation of PSII, three times of readings i.e. after 4 hours (day 1), day 2 and day 3 were taken.

Scoring scale:

| PII       | Irritation category |
|-----------|---------------------|
| 0.0       | not-irritant        |
| > 0.0-0.5 | negligible irritant |
| > 0.5-2.0 | mild irritant       |
| > 2.0-5.0 | moderate irritant   |
| > 5.0-8.0 | severe irritant     |

Table 4: Computation of Primary Skin Irritation Index (PSII) after application of "Naphthol tar" in Rabbits – After 4 hours exposure

| R<br>a<br>b<br>b<br>i<br>t<br>s | S<br>i<br>t<br>e<br>s | Non-abraded Skin Site |   |   |   |    |              |   |   |   |    | Abraded Skin Site |   |   |   |    |              |   |   |   |    |
|---------------------------------|-----------------------|-----------------------|---|---|---|----|--------------|---|---|---|----|-------------------|---|---|---|----|--------------|---|---|---|----|
|                                 |                       | Erythema (days)       |   |   |   |    | Edema (days) |   |   |   |    | Erythema (days)   |   |   |   |    | Edema (days) |   |   |   |    |
|                                 |                       | 1*                    | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 | 1*                | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 |
| #<br>1                          | 1                     | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|                                 | 2                     |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|                                 | 3                     | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|                                 | 4                     |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| #<br>2                          | 1                     | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|                                 | 2                     |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|                                 | 3                     | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|                                 | 4                     |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| TA                              | 0                     | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 |    |
| PSII = 0                        |                       |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |

TA = Total of averages (treated sites). 1 & 2 are left skin sites (control) and 3 & 4 are right skin sites (treatment site).  
 PSII = Total of averages / Types of skin sites (2) x Number (time) of readings (3).

\* First scoring was done after 4 hours.

For computation of PSII, three times of readings i.e. after 4 hours (day 1), day 2 and day 3 were taken.

Scoring scale:

| PII       | Irritation category |
|-----------|---------------------|
| 0.0       | not-irritant        |
| > 0.0-0.5 | negligible irritant |
| > 0.5-2.0 | mild irritant       |
| > 2.0-5.0 | moderate irritant   |
| > 5.0-8.0 | severe irritant     |

Table 5: Computation of Primary Skin Irritation Index (PSII) after application of "Reactor residue" in Rabbits - After 4 hours exposure

| Rabbit   | Site | Non-abraded Skin Site |   |   |   |    |              |   |   |   |    | Abraded Skin Site |   |   |   |    |              |   |   |   |    |
|----------|------|-----------------------|---|---|---|----|--------------|---|---|---|----|-------------------|---|---|---|----|--------------|---|---|---|----|
|          |      | Erythema (days)       |   |   |   |    | Edema (days) |   |   |   |    | Erythema (days)   |   |   |   |    | Edema (days) |   |   |   |    |
|          |      | 1*                    | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 | 1*                | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 |
| #1       | 1    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | 2    |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |
|          | 3    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 4    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| #2       | 1    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 2    |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |
|          | 3    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 4    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| TA       | 0    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 |    |
| PSII = 0 |      |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |

TA = Total of averages (treated sites). 1 & 2 are left skin sites (control) and 3 & 4 are right skin sites (treatment site).  
 PSII = Total of averages / Types of skin sites (2) x Number (time) of readings (3).  
 \* First scoring was done after 4 hours.  
 For computation of PSII, three times of readings i.e. after 4 hours (day 1), day 2 and day 3 were taken.

Scoring scale:

| PII       | Irritation category |
|-----------|---------------------|
| 0.0       | not-irritant        |
| > 0.0-0.5 | negligible irritant |
| > 0.5-2.0 | mild irritant       |
| > 2.0-5.0 | moderate irritant   |
| > 5.0-8.0 | severe irritant     |

Table 6: Computation of Primary Skin Irritation Index (PSII) after application of "Semi processed pesticide" in Rabbits – After 4 hours exposure

| R<br>a<br>b<br>b<br>i<br>t | S<br>i<br>t<br>e | Non-abraded Skin Site |   |   |   |    |              |   |   |   |    | Abraded Skin Site |   |   |   |    |              |   |   |   |    |
|----------------------------|------------------|-----------------------|---|---|---|----|--------------|---|---|---|----|-------------------|---|---|---|----|--------------|---|---|---|----|
|                            |                  | Erythema (days)       |   |   |   |    | Edema (days) |   |   |   |    | Erythema (days)   |   |   |   |    | Edema (days) |   |   |   |    |
|                            |                  | 1*                    | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 | 1*                | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 |
| #<br>1                     | 1                | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|                            | 2                |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|                            | 3                | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|                            | 4                |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| #<br>2                     | 1                | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|                            | 2                |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|                            | 3                | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|                            | 4                |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| T<br>A                     | 0                | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 |    |
| PSII = 0                   |                  |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |

TA = Total of averages (treated sites). 1 & 2 are left skin sites (control) and 3 & 4 are right skin sites (treatment site).  
 PSII = Total of averages / Types of skin sites (2) x Number (time) of readings (3).

\* First scoring was done after 4 hours.

For computation of PSII, three times of readings i.e. after 4 hours (day 1), day 2 and day 3 were taken.

Scoring scale:

| PII       | Irritation category |
|-----------|---------------------|
| 0.0       | not-irritant        |
| > 0.0-0.5 | negligible irritant |
| > 0.5-2.0 | mild irritant       |
| > 2.0-5.0 | moderate irritant   |
| > 5.0-8.0 | severe irritant     |

Table 7: Computation of Primary Skin Irritation Index (PSII) after application of "Sevin tar" in Rabbits - After 4 hours exposure

| Rabbit   | Site | Non-abraded Skin Site |   |   |   |    |              |   |   |   |    | Abraded Skin Site |   |   |   |    |              |   |   |   |    |
|----------|------|-----------------------|---|---|---|----|--------------|---|---|---|----|-------------------|---|---|---|----|--------------|---|---|---|----|
|          |      | Erythema (days)       |   |   |   |    | Edema (days) |   |   |   |    | Erythema (days)   |   |   |   |    | Edema (days) |   |   |   |    |
|          |      | 1*                    | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 | 1*                | 2 | 3 | 7 | 14 | 1*           | 2 | 3 | 7 | 14 |
| # 1      | 1    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 2    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | 3    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 4    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| # 2      | 1    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 2    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | 3    | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |                   |   |   |   |    |              |   |   |   |    |
|          | 4    |                       |   |   |   |    |              |   |   |   |    | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
|          | TA   | 0                     | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  | 0                 | 0 | 0 | 0 | 0  | 0            | 0 | 0 | 0 | 0  |
| PSII = 0 |      |                       |   |   |   |    |              |   |   |   |    |                   |   |   |   |    |              |   |   |   |    |

TA = Total of averages (treated sites). 1 & 2 are left skin sites (control) and 3 & 4 are right skin sites (treatment site).  
 PSII = Total of averages / Types of skin sites (2) x Number (time) of readings (3).  
 \* First scoring was done after 4 hours.  
 For computation of PSII, three times of readings i.e. after 4 hours (day 1), day 2 and day 3 were taken.

Scoring scale:

| PII       | Irritation category |
|-----------|---------------------|
| 0.0       | not-irritant        |
| > 0.0-0.5 | negligible irritant |
| > 0.5-2.0 | mild irritant       |
| > 2.0-5.0 | moderate irritant   |
| > 5.0-8.0 | severe irritant     |