इंटरनेट मानक

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Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

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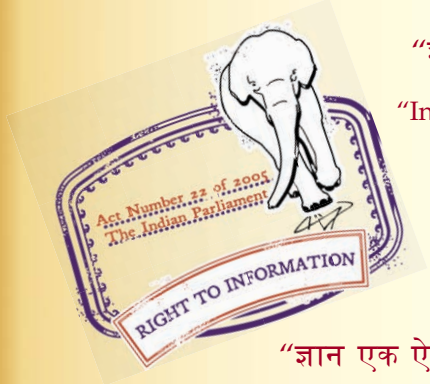
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

IS 11601 (2002): Methods of Safety Evaluation of Synthetic Detergents - Tests for Skin Irritation and Sensitization Potential of Synthetic Detergents [CHD 25: Soaps and other Surface Active Agents]



"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

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भारतीय मानक

संश्लिष्ट डिटरजेंटों के सुरक्षा मूल्यांकन की पद्धतियाँ — संश्लिष्ट डिटरजेन्टों से त्वचा में होने वाली जलन और सुग्रहिता संभावनाओं का परीक्षण

(पहला पुनरीक्षण)

Indian Standard

METHODS OF SAFETY EVALUATION OF SYNTHETIC DETERGENTS — TESTS FOR SKIN IRRITATION AND SENSITIZATION POTENTIAL OF SYNTHETIC DETERGENTS

(First Revision)

ICS 71.060.50,71.100.40

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

September 2002

Price Group 4

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Soaps and Other Surface Agents Sectional Committee had been approved by the Chemical Division Council.

Detergents are used almost universally on regular basis. Their safety aspect has been the subject of numerous investigations in the past. The committee responsible for formulation of this standard felt the need for specifying test methods which can evaluate the safety aspects of synthetic detergents used by the consumers.

Detergent products which come in contact with skin should be free from skin sensitizing/skin allergy producing components. Such components even when present in minute levels (ppm—part per million concentration) in the formulation, would cause itching, redness, swelling and blister formation on the skin. The onset of such episodes is usually a delayed response from the time of first contact with the product which makes it difficult to identify the incriminating product/ingredient, even by an experienced medical expert. The detection involves cumbersome procedure of patch testing on skin with all suspect materials on people who are susceptible to such skin allergy. The intractable nature of this skin condition may cause unnecessary hardships to the consumers. Hence, it is essential that manufacturers of detergent products make sure that their products are free from such hazard, by subjecting their products/ingredients to a predictive skin sensitization test using a sensitive animal model. The allergic skin reaction described above is not caused by toxic or irritant properties of the substance (which need to be tested separately) but is due to the increased sensitivity and harmful immunological response to these substances caused by repeated contact. Identification of such allergenic ingredients/products is possible through the Guinea Pig Maximization Test (GPMT).

The rationale of the choice of guinea pigs for skin sensitization test are:

a) Guinea pigs are the most sensitive species for assessing skin sensitization; and

b) Ethical considerations prevent testing of new formulations directly on human subjects, unless one is sure of the safety of formulations. If the formulation happens to be a sensitizer, it would cause irreparable damage and misery to the volunteer, unlike the short duration skin irritancy test. The latter would only have a reversible transient effect. Skin sensitization test can be performed by any one of the two methods, namely, Buehler Test or Magnusson and Kligman Guinea Pig Maximization Test. Even though Buehler Test is not as sensitive as the Magnusson Kligman Guinea Pig Maximization Test, regulatory agencies, such as US FDA, OECD, EPA, etc, have approved of this test for the purpose of substantiation of safety of chemicals from the point of sensitization.

This standard was initially published in two parts, namely, IS 11601 'Methods of safety evaluation of synthetic detergents : Part 1 Method of test for irritant potential of synthetic detergents, and Part 2 Method of test for skin sensitization potential of synthetic detergents (Guinea Pig Maximization Test)'.

In this standard both the parts have been merged to make it user friendly as well as a complete standard. There is a strong movement the world over, to reduce the usage of animals for experimental purposes. The existing version is primarily based on animal experimentation to assess the irritancy potential of fabric washing synthetic detergent products. With a view to reduce such animal experimentation, an alternative test involving human subjects — the species of concern — is incorporated in this revision. For assessing skin sensitization potential, a single, stringent but most sensitive test (Magnusson and Kligman) was given. However in this revision in addition to the above test a simpler test (Buehler) involving only topical application is prescribed.

In line with the other Indian Standards, that is, IS 4011 : 1997 'Methods of test for safety evaluation of cosmetics (*second revision*)', it is recommended that formulations are made conforming to the updated versions of IS 4707 (Parts 1) : 2001 'Classification for cosmetic raw materials and adjuncts : Part 1 Dyes, colours and pigments (*second revision*)' and IS 4707 (Part 2) : 2001 'Classification for cosmetic raw materials and adjuncts : Part 2 List or raw materials generally not recognized as safe for use in cosmetics (*second revision*)', guidelines of CTFA (Cosmetics Toiletries and Fragrance Association), EEC (European Economic Community), Directive and

(*Continued on third cover*)

Indian Standard

METHODS OF SAFETY EVALUATION OF SYNTHETIC DETERGENTS — TESTS FOR SKIN IRRITATION AND SENSITIZATION POTENTIAL OF SYNTHETIC DETERGENTS

(First Revision)

1 SCOPE

This standard prescribes methods of test for skin irritation and skin sensitization potential of synthetic detergents.

2 REFERENCE

IS 7597 : 2001 'Glossary of terms relating to surface active agents', is a necessary adjunct to this standard.

3 TERMINOLOGY

For the purpose of this standard, the following definitions along with the definitions given in IS 7597 shall apply.

3.1 Allergic Reactions — Symptoms/signs caused by exposure of an allergic individual to the corresponding substance.

3.2 Dermatitis — Inflammation of the skin.

3.3 Erythema — Redness of the skin due to dilatation of the blood vessels.

3.4 Hypersensitivity — A state where an individual is capable of developing an allergic reaction due to some external agent.

3.5 Occlusion Area — An area of skin which is cut off from the environment.

3.6 Repeated Insult Irritant — An agent which causes an irritant reaction only after repeated applications to the surface of the skin.

3.7 Sensitization — A process in which an individual develops the capability of reacting in an allergic (abnormal) manner to a particular substance.

4 SKIN IRRITANCY TEST ON HUMAN SUBJECTS — HAND IMMERSION TEST

4.1 Outline of the Method

Human volunteers immerse one of their hands in the solution of the detergent product to be tested while the other hand, simultaneously in a solution of a positive control for 10 min, twice daily for 4 consecutive days under supervision. Monitor the hands for any adverse skin responses prior to and after each immersion and finally on the morning after the last immersion. If the skin responses with the product tested are very similar to that manifested by the positive control, the product could be judged to have an unacceptable level of skin irritancy potential.

4.2 Procedure

4.2.1 *Volunteers*

Select 24 healthy adult volunteers (except pregnant ladies and breast feeding mothers) between the age of 18 and 55 years, with no known acute or chronic diseases or no adverse skin responses to cosmetics, personal wash or fabric wash products and no skin blemishes on their hands and forearms. They should not be under any medication, particularly anti-inflammatory drugs. Inform the volunteers about the study in detail including the likely hazards and obtain a signed informed consent from each of them.

4.2.2 *Products*

Carry out the test on newly formulated fabric washing synthetic detergent products, the skin irritancy potential of which is in doubt, prior to marketing. Store samples for the test properly to prevent contamination or spoilage. Do not use spoiled or contaminated sample. Preserve adequate quantity of the sample which is used for testing for at least 2 years for any re-testing. Identify each sample with an unique code number and make appropriate entry in the records. Ingredients used in the formulation should be of appropriate quality and have sufficient data to support safety at the level which is used in the product. In order to have a realistic approach for product dosing in the test, use the dosage regime given on the pack, however, in the event of not having such recommendation on the pack, a dosage of 7, 10 or 14 g/l is recommended depending on the BIS grade of the product, namely, 1, 2 or 3 as these are the levels commonly found in wash liquor with these grades.

4.2.3 *Positive Control*

A 3 percent (*w/v*) solution of sodium lauryl sulphate (SLS) with 99.9 percent purity (analytical grade) in water is used as positive control.

4.2.4 *Method*

Prepare fresh solution of the product and the positive control at the concentration mentioned above (*see* **4.2.2** and **4.2.3** respectively) using warm (37°C) municipal water on each of the immersion days. Determine the *p*H and buffering capacity of the detergent solution and *p*H of SLS solution on all the treatment (immersion) days. Transfer 500 ml of the respective solution into a suitable beaker/container for each volunteer for every immersion. Organize the seating arrangements of volunteers for hand immersion in a convenient/comfortable manner. 50 percent of the volunteers in a test immerse their left hand in the test product/sample coded as 'A' and right hand in the positive control coded 'B', the other 50 percent, *vice versa*. Each volunteer immerses his/her respective hand up to the wrist in the solution with constant movement of the fingers for agitating the liquid for 10 minutes twice a day (one in the morning and the other in the afternoon with a gap of about 4 h between two immersions) for 4 consecutive days. After each immersion, the volunteers wash the hands thoroughly with tap water and gently wipe with a dry towel.

4.2.5 *Assessment and Scoring*

Assess the skin reactions and score them (Double blind) as per the scale given in Table 1, before each immersion under a constant light source by a trained assessor. The assessor should not know which hand received what product treatment. The final assessment and scoring shall be carried out on the morning after the last treatment. Add up the scores obtained at each assessment (total 9 assessments) and the mean of this score (total score divided by 9) is used for evaluating the product. Examine carefully the back of the hand, palm and the webs for scaling and glaze. Any sensation felt by the volunteer either during or after the immersion, and any other comments shall be taken into consideration in evaluating the product. Skin *p*H, hydration and trans epidermal water loss (TEWL) may also be monitored (purely optional) and recorded, to support the clinical observation.

4.2.6 *Analysis of Data and Conclusion*

Analyse the final score using student's paired *t*-test for determining the significance between the product and the positive control. If the product tested is found to cause adverse skin responses very similar to that manifested by the positive control, such a product could be judged to have unacceptable level of skin irritancy potential.

Table 1 Scale for Assessing the Skin Reaction

(*Clause* 4.2.5)

Sl No.	Skin Reactions	Score
(1)	(2)	(3)
i)	No reaction	0
ii)	Very slight reaction (barely perceptible dryness and scales)	1
iii)	Slight reaction (perceptible dryness and scales usually in a small area)	2
iv)	Moderate reaction (perceptible dryness and scales in a major or whole area)	3
v)	Severe reaction (severe dryness and scales in the whole area with or without cracking)	4

4.2.7 *Reporting*

The report shall contain the following information:

a) Location of the study and date,
b) Identification of the sample,
c) Number of volunteers used,
d) Procedure followed and any deviation,
e) Result of the test in tabular form,
f) Any unusual findings,
g) Conclusion, and
h) Name of the investigator.

5 SKIN SENSITIZATION TEST ON GUINEA PIGS — BUEHLER TEST

5.1 Outline of the Method

Apply the material at a suitable concentration topically on the clipped skin of guinea pigs under covered patch for 6 h once a week, for 3 consecutive weeks for inducing sensitization. Challenge the animals topically 2 weeks later with the highest non-irritant concentration of the test material in an identical manner as was done for sensitization induction. Assess the resulting skin reactions 24 h and 48 h after removal of the patch and compare them with the reactions produced on the control group of animals treated simultaneously with vehicle.

5.2 Procedure

The test is carried out in two phases:

a) *Preliminary irritancy test* — To determine the suitable concentrations of the test material for sensitization induction and challenge treatments.
b) *Main test* — Carry out induction and challenge treatments with the test substance using the

concentrations determined in the preliminary irritancy test.

5.2.1 *Animals*

Use equal number of male and non-pregnant female healthy albino guinea pigs, weighing about 300-400 g each. Use 4 animals for the preliminary irritancy test and 30 animals (20 for the test substance and 10 as control) for the main test. Select a few more, two days prior to the treatment, to replace the ones which may be found unsuitable due to skin blemishes, at the start of the test. Keep the animals individually in cages located in a room maintained at 23 ± 3°C, relative humidity 40-70 percent, light dark cycle of 12 h in 24 h, with adequate ventilation and air changes. Animals should have access to standard feed and water all the time. Prior to each treatment remove hair from the flanks by clipping depending on the nature of the treatment. Assess sensitivity of the animal stock every 6 months by the use of any one of the positive control substances, for example, dinitro fluoro benzene (DNFB), dinitro chloro benzene (DNCB), hexyl cinnamic aldehyde (HC), etc, which are strong to moderate sensitizers. These compounds should give a response in 15 percent (for non-adjuvant tests) or more of the animals. For such confirmation use the same method described in this standard. In the event, the animals are sourced from an outside agency, where the sensitivity monitoring is not conducted, carry out sensitivity test using the same stock ideally before carrying out the actual test or in parallel with the test on another group of animals. A test carried out on an insensitive stock is invalid.

5.2.2 *Test Patches for Topical Treatment*

Fold 2.5 cm wide surgical cotton gauze, into 12 ply of 2.5 cm × 2.5 cm dimension. Place this on a 2cm × 2cm thin polythene sheet which is stuck at the centre of a 2.5-cm wide and 6-7 cm long surgical adhesive plaster.

5.2.3 *Samples*

Store, preserve and identify the samples as described under **4.2.2**.

5.2.4 *Preparation of sample*

Prepare solutions/suspension on a weight-to-weight basis using distilled water. For water insoluble material (some of the positive controls) use either refined groundnut oil or pharmaceutical grade paraffin oil or propylene glycol.

Prepare adequate quantity of the solution/suspension in a single batch to complete all treatments of the day. When a very low concentration is required, it is best to prepare a concentrated stock solution/suspension and dilute a quantity of the stock to the required concentration for treatment. Use a mechanical agitator and heat (not more than 38°C) to facilitate solution preparation, if required.

5.2.5 *Preliminary Irritancy Test*

Select four treatment sites, two on the right and two on the left flanks, each approximately 2.5 cm × 2.5 cm, from the clipped area of each animal. Take 0.5 ml each of 4 serial concentrations (for example 0.5, 1.0, 2.0, 5.0 percent and so on) of the test substance on 4 different patches supported by polythene sheets. Apply them on the four selected sites of each animal. Cover the patches and hold them tightly by the aid of cloth bandage and return the animals to their respective cages. After six hours, remove the patches and mark the four corners of each test sites with indelible ink. Assess the sites for skin irritancy response 24 h after the removal of the patches (30 h after the application of the patch). Select the concentration which produces a mild irritation (score 1 on a scale of 0 to 3 as described in **5.2.6.3**, based on the mean of readings from four animals) for topical induction, select the highest concentration (based on the mean of readings from four animals) which does not produce any irritancy response as the challenge concentration.

5.2.6 *Main Test*

In the main test induction and challenge treatments are carried out.

5.2.6.1 *Induction*

As mentioned in **5.2.1**, use 20 animals for inducing sensitization with the test sample and 10 as control. Induction treatment is given once a week for 3 weeks. Take 0.5 ml of the solution/suspension of the test sample at the selected concentration on a single patch (*see* **5.2.2**), apply on the left flank of the animal of the test group, bandage as described earlier (*see* **5.2.5**) and allow a contact period of 6 h. Give the control group a similar treatment but with vehicle (distilled water) in place of the test substance. At the end of the contact period remove the patches and any substance sticking to the treatment site with moistened tissue paper. Repeat the application 7 days and 14 days later on the very same treatment site on each animal.

5.2.6.2 *Challenge*

Fourteen days after the last induction treatment, give the untreated flank of all the animals (including that of the control group) a challenge treatment, using the highest non-irritant concentration of the test substance in the same manner as described earlier (*see* **5.2.5**). After 6 hours remove the patches, clean the area with moistened tissue paper and mark with indelible ink. After 21 hours of the removal of the patches, examine the area and clip the hair if necessary. Approximately 3 h later, assess the skin reactions (double blind) under a constant artificial day light

source and grade them as per Table 2. Assess the reaction again 24 h later that is, 48 h after the patch removal. If the response in the first challenge is not conclusive, then a second challenge treatment may be given one week later using a new set of control animals.

5.2.6.3 *Scale for Evaluation*

Scale for evaluation is given in Table 2.

Table 2 Scale for Evaluation

(*Clauses* 5.2.6.2, 5.2.6.3 *and* 6.2.7)

Sl No.	Observation	Reaction	Scale
(1)	(2)	(3)	(4)
i)	No visible change	No reaction	0
ii)	Discrete or patchy erythema	Mild reaction	1
iii)	Moderate and confluent erythema	Moderate reaction	2
iv)	Intense erythema and swelling	Severe reaction	3

5.2.7 *Conclusion*

Consider a reaction in a test animal as positive if it is significantly greater than the response on the control animals. When there is no reaction on the control animals, a reaction score of 1 or more in any of the test animal is considered to be a positive sensitization response. Fabric washing synthetic detergent product producing a positive sensitization in 15 percent of the animals in this test poses a risk to the consumer.

5.2.8 *Reporting*

The test report shall include the following information:

a) Location of the study and date;

b) Identification of the sample;

c) Guinea pigs: Strain, source, number, age, sex, housing conditions, diet. Date and results of the last sensitivity check including the substance and vehicle used. Individual weight of animals at the start and at the conclusion of the test;

d) Procedure followed and any deviation;

e) Result of the preliminary irritancy study with conclusion on induction and challenge concentration to be used in the test;

f) Result of the main test should be summarized in tabular form, giving skin reactions of each animal at each observation along with any narrative description of the nature and degree of effects observed;

g) Any unusual findings;

h) Conclusion; and

j) Name of investigators.

6 SKIN SENSITIZATION TEST ON GUINEA PIGS — MAGNUSSON AND KLIGMAN GUINEA PIG MAXIMIZATION TEST

6.1 Outline of the Method

Inject the test material along with Freund's Complete Adjuvant (FCA) intradermally for inducing sensitization in guinea pigs. FCA enhances the sensitivity of the immune system of the animals. Boost the induction further with the help of a closed patch application of the test material on the injected sites after a week. This is followed by topical challenge treatments with the test material a fortnight later to finally assess the allergenicity potential. Necessary concentrations of the test materials for the above mentioned treatments are determined through preliminary irritancy studies as described below.

6.2 Procedure

Carry out the test in two phases:

a) *Preliminary irritancy test* — To determine suitable concentrations of the test material for sensitization induction and challenge treatments.

b) *Main test* — Induction, boosting and challenge treatments with the test substance using the concentrations determined in the preliminary irritancy test.

6.2.1 *Animals*

Use a total of 26 albino guinea pigs (8 for preliminary irritancy test, 10 for the test material, 4 for treated control and 4 for untreated control) of either sex bred from a stock which is disease-free as well as showing positive sensitization response with a well known skin sensitizers. Select a few more, 2 days prior to the treatment, to replace the ones which may be found unsuitable due to skin blemishes, at the start of the test. House the animals and look after them as described earlier (*see* **5.2.1**). Follow the currently described procedure to assess the sensitivity of the animal stock and 30 percent (for adjuvant test) or more animals should show a positive response in the sensitivity test. Prior to each treatment hair from the flanks/ shoulder is removed by clipping depending on the nature of the treatment.

6.2.2 *Samples*

Store, preserve and identify the samples as described under **4.2.2**.

6.2.3 *Preparation of Sample*

Prepare solutions/suspension on a weight to weight basis using distilled water/normal saline. Prepare adequate quantity of the solution/suspension in a single batch to complete all treatments of the day.

When a very low concentration is required, it is best to prepare a concentrated stock solution/suspension and dilute a quantity of the stock to the required concentration for treatment. Use a mechanical agitator and heat (not more than 38°C) to facilitate solution preparation, if required.

6.2.4 *Preliminary Irritancy Test*

6.2.4.1 *Intradermal irritancy test*

This test is done to find out the suitable intradermal induction injection concentration which will be used for sensitizing the animals. Inject intradermally (at least 1 cm apart on the clipped and shaved flank of 4 guinea pigs of the same sex and weighing around 300 g each) with 0.1 ml each of 0.1, 0.25, 0.5 , 1 percent or more of the test substance in normal saline (0.9 percent sodium chloride) using a sterilized tuberculin or disposable syringe fitted with 26 gauge needle. Return the animals to their individual cages. After 24 h examine the intensity and extent of reactions by size in millimetres (length and breadth) for erythema (redness) and oedema (swelling). Select the concentration which produces a slightly irritant reaction, namely, 7 mm × 7 mm erythema and oedema (mean from 4 animals) for intradermal induction injection concentration.

6.2.4.2 *Test*

Saturate eight millimetre diameter chromatography paper (Whatman No. 3) discs with a range of concentrations, for example. 0.5, 1, 2, 5, 10 percent and so on of the test material in distilled water/suitable solvents and place them in 10 mm diameter aluminium discs/cup. Locate these cups/discs at least 1 cm apart on the clipped and shaved flank of four guinea pigs, each weighing about 400 g. Hold the patch test discs/ cups containing the paper in such a way that the paper is in contact with the skin. Fasten the discs/cups to the trunk with surgical adhesive plaster tapes and cloth bandage. Leave the animals in their individual cages. Remove the patches 24 h after application. Examine the treated sites 24 h and 48 h after the removal of patches. Score the resulting reactions for irritation on a 0-3 scale as described under **5.2.6.3** (*see* Table 2). For boosting the sensitization response through topical application, select the concentration giving a slightly irritant (score 1) reaction. Select the highest concentration which causes no visible reaction (score 0) for challenge treatment (final treatment).

6.2.5 *Main Sensitization Test*

Select 10 guinea pigs weighing about 300 g each from the stock as described under **6.2.1**.

6.2.5.1 *Sensitization treatment — Induction*

Carry out the sensitization treatment in two stages : Intradermal injection followed one week later by topical application.

a) *Intradermal injection* — Clip the hair from a 2 cm × 4 cm area of skin on the dorsal shoulder region and give 3 pairs of intradermal injections within the clipped area (*see* Fig. 1) using a sterilized tuberculin or disposable syringe fitted with a 23 gauge needle in the following manner:

   i) Two 0.1 ml injections of 50 percent FCA in normal saline on sites marked '1'.

   ii) Two 0.1 ml injections on sites marked '2' of the test material in normal saline at the concentration selected for sensitization induction from the intradermal irritancy test (*see* **4.2.4.1**).

   iii) Two 0.1 ml injections on sites marked '3' of test material in normal saline mixed with 1 : 1 FCA, such that the final concentration of test substance injected is the same as that in (ii) above.

b) *Topical treatment : boosting* — One week after the injection, clip and shave the same 2 cm × 4 cm treated area. Saturate a 2 cm × 3 cm chromatography paper (Whatman No. 3) with the test material at the selected concentration as determined under **6.2.4.2** and place over the shaved site. Cover this by 4 cm × 6 cm piece of thin polyethylene sheet. Hold the paper saturated with test substance covered by polyethylene sheet in place for 48 h by surgical adhesive plaster tape and cloth bandage. Remove the patches at the end of 48 h.

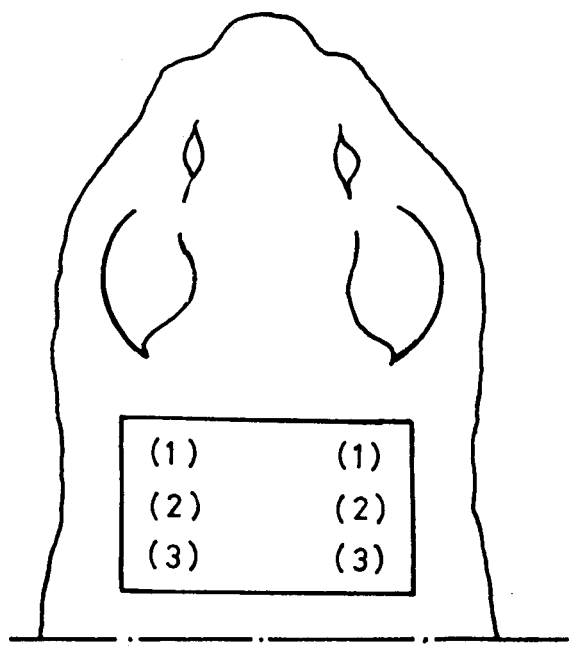

FIG. 1 GUINEA PIG VIEWED FROM ABOVE SHOWING THE SITES OF INTRADERMAL INDUCTION INJECTIONS

6.2.5.2 *Topical challenge*

Fourteen days after the boosting treatment, challenge (final treatment to determine sensitization) the guinea pigs using an occluded patch. For each animal, saturate an 8 mm diameter chromatography paper (Whatman No. 3) disc with the test material at the selected challenge concentration (highest topical non-irritant concentration (*see* **6.2.4.2**) and place it in a 10 mm diameter aluminium patch test disc/cup. Apply this on to the clipped and shaved flank (*see* Fig. 2) and hold in position using surgical adhesive plaster tape and cloth bandage for 24 h. Examine the challenged site for inflammatory response, redness (erythema), swelling (oedema) 24 h and 48 h after removal of the patches. One week after the first challenge, a further challenge on the opposite flank may be given exactly in the same manner as the first one. Provide a third challenge one week later on the opposite flank if the earlier challenge results are inconclusive.

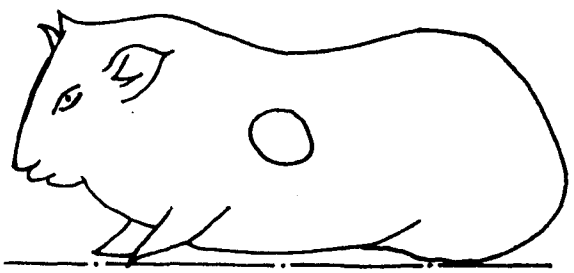

FIG. 2 SITE OF TOPICAL CHALLENGE ON GUINEA PIG

6.2.6 *Treated and Untreated Controls*

6.2.6.1 *Treated control*

At the same time as the main test, select 4 guinea pigs of the same weight range as treated controls. Give them mock sensitization treatment at the same time and in the same way as for the main test animals except that the test substance is omitted from the intradermal injection induction and topical application boosting. But treat them exactly in the same way as the test animals at every challenge with the test material.

6.2.6.2 *Untreated controls*

Challenge 4 animals which did not receive any treatment previously but are of the same weight range as the main test animals, exactly in the same manner as the test and treated control animals.

6.2.7 *Scale for Evaluation*

Score the skin reactions resulting from treatment using the scale given in **5.2.6.3** (*see* Table 2).

6.2.8 *Conclusion*

Consider a reaction in a test animal as positive response if it is significantly greater than the response on treated and untreated control animals. When there is no reaction on treated and untreated control animals, a reaction score of 1 or more in any of the test animals is considered to be a positive sensitization response. Fabric washing synthetic detergent product producing a positive sensitization response in 30 percent of the animals in this test poses a risk to the consumer.

6.2.9 *Reporting*

The test report must include the information as required under **5.2.8**.

ANNEX A

(Foreword)

COMMITTEE COMPOSITION

Soaps and Other Surface Active Agents Sectional Committee, CHD 25

Organization	*Representative(s)*
Drugs Controller General of India, New Delhi	SHRI ASHWINI KUMAR (***Chairman***) SHRI B. R. WADHAWAN (*Alternate*)
Central Board of Excise and Customs, Ministry of Finance, New Delhi	CHIEF CHEMIST DEPUTY CHIEF CHEMIST (*Alternate*)
Central Pollution Control Board, Delhi	DR AJAY AGARWAL DR M. Q. ANSARI (*Alternate*)
Consumer Guidance Society of India (Regd.), Mumbai	SHRI N. G. WAGLE SHRIMATI R. TALWANI (*Alternate*)
Consumer Education and Research Centre, Ahmedabad	DR C. J. SHISHOO SHRI SANTOSH YELLORE (*Alternate*)
Department of Industrial Development, Ministry of Industry, New Delhi	SHRI SHAISH KUMAR SHRI B. B. SHARMA (*Alternate*)
Development Commissioner, Small Scale Industries, New Delhi	SHRI J. S. REKHI
Directorate General of Supplies and Disposals (Inspection Wing), New Delhi	SHRI P. JAYAKUMARAN SHRI M. A. KHAN (*Alternate*)
Federation of Associations of Small Scale Soap and Detergent Manufacturers of India, Delhi	SHRI SANTOSH KUMAR SHRI R. C. DOSHI (*Alternate*)
Godrej Soaps Limited, Mumbai	SHRI A. RANGARAJAN DR (SHRIMATI) ROHINI THAKKAR (*Alternate*)
Gujarat Detergent Manufacturers Association, Ahmedabad	SHRI S. A. PATEL SHRI MAHENDRA VYAS (*Alternate*)
Hindustan Lever Limited, Mumbai	DR V. R. DHANUKA
Indian Soaps and Toiletries Manufacturers Association, Mumbai	SHRI V. P. MENON
Karnataka Soaps and Detergents Limited, Bangalore	DR K. B. PATIL SHRI S. G. KULKARNI (*Alternate*)
Khadi and Village Industries Commission, Mumbai	SHRI G. K. GHOSH
K. S. Krishnan Associates (P) Limited, New Delhi	SHRI K. S. KRISHNAN SHRI S. KRISHNAN (*Alternate*)
Ministry of Defence (DGQA), Kanpur	SHRI M. S. SULTANIA SHRI SURJIT GHOSE (*Alternate*)
Nand Kishore Khanna and Sons, Mumbai	SHRI P. P. KHANNA SHRI A. C. KHANNA (*Alternate*)
National Test House, Kolkata	SHRI A. K. CHAKRAVORTY SHRI P. K. CHAKRABORTY (*Alternate*)
Nirma Limited, Ahmedabad	SHRI K. K. PATEL SHRI ASHISH R. PATEL (*Alternate*)
Oil Technologists Association of India, Kanpur	DR B. R. GAIKWAD SHRI P. K. TIWARI (*Alternate*)
Procter and Gamble India Hygiene and Healthcare Limited, Mumbai	DR S. MAZUMDAR SHRIMATI SHWETA PURANDRE (*Alternate*)
Resarch,Designs and Standards Organization (Ministry of Railways), Lucknow	DEPUTY DIRECTOR (Chemicals) ASSISTANT RESEARCH OFFICER (CM II) (*Alternate*)

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Organization	*Representative(s)*
The Non-Power Soap Manufacturers Association, Mumbai	SHRI R. C. DOSHI SHRI Y. R. DOSHI (*Alternate*)
Colgate Palmolive (I) Ltd, Mumbai	SHRI SUNIL AGARWAL SHRI VILAS TULLE (*Alternate*)
Kopson Surfactants, Chennai	SHRI S. S. KOPPIKAR
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Raw Materials and Methods of Tests Subcommittee, CHD 25 : 6

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Consumer Education and Research Centre, Ahmedabad	DR (SHRIMATI) FALGUNI MAZUMDAR SHRI SANTOSH YELLORE (*Alternate*)
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Hindustan Lever Limited, Mumbai	DR V. KRISHNAN DR V. R. DHANUKA (*Alternate*)
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(*Continued from second cover*)

guidelines of IFRA (International Fragrance Association). Products formulated on the above lines are likely to be safe and such products may not warrant any safety testing. However, if the manufacturer feels it necessary to generate data on such formulations, he/she has the freedom to do so. Whereas, products which contain novel ingredients will require safety assessment using the methods as given in this standard.

All testing whether involving animals or human subjects are to comply with the legislative/regulatory requirements with reference to animal experimentation/testing on volunteers. All animal tests should comply with the '*Prevention of Cruelty to Animals Act*, 1960 and *Breeding of and Experiments on Animals (Control and Supervision) Rules*, 1998' updated from time to time. Any study involving human subjects should be as per the 'Ethical Guidelines for Biomedical Research on Human Subjects' formulated by ICMR and revised from time to time.

The composition of the Committee responsible for formulation of this standard is given in Annex A

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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