SAFETY STUDIES

1.1 Skin Irritation through Open Patch Test
1.2 Skin Irritation through Patch Test.
1.3 Human Skin Irritation Test
1.4 Repeat Open Application Test
1.5 Test For Hypoallergenicity
1.6 Modified 21 Days Cumulative Irritation Test
1.7 'Modified Draize-95' Test
1.8 Repeat Insult Patch Test (Ript).
1.9 Patch Test On Sensitized Individuals
1.10 Human Maximization Test
1.11 Extended Prospective Product Use Testing
1.12 Comedogenicity Test
1.13 Human Ocular Irritation
1.14 Assessment Of Skin Following Repeated Wash
1.15 Safety Assessment Of Baby Bath Using Use Test

EFFICACY STUDIES

1.16 Assessment Of Whitening Effect Following Repeated Use Of Test Materials.
1.17 Assessment Of Antiwrinkle Effect Following Repeated Use Of Test Materials
1.18 Barrier Efficacy Studies
1.19 Assessment Of Skin Barrier Recovery
1.20 Efficacy Assessment Of Topical Skin Products On Cracked Heel
1.21 Assessment Of Effect On Nail Cuticles Following Repeated Use Of Test Materials On The Fingernails

1.1 SKIN IRRITATION THROUGH OPEN PATCH TEST

Objective
To determine the immediate irritation effects and the irritation power of a particular materials.

Principle of method
The method consist of a non-occlusive application of the materials by means of plastic chamber on 20 selected subject's back or forearm, for 48 hours. The irritation activity is clinically

evaluated at he following time interval:
- 30 minutes (T1) after application (immediate irritation power)
- 48 hours after application (T2 irritation power)

Number of subject: 20 subjects
Duration: 1 month

1.2 SKIN IRRITATION THROUGH PATCH TEST

Objective
To determine the immediate irritation effects and the irritation power of a particular materials using a closed patch test

Principle of method
The method consist of a non-occlusive application of the materials by means of plastic chamber on 20 selected subject's back or forearm, for 48 hours. The irritation activity is clinically evaluated:
- 30 minutes (T1) after application (immediate irritation power)
- 48 hours after application (T2 irritation power)

Number of subject: 20 subjects
Duration: 1 month

1.3 HUMAN SKIN IRRITATION TEST

Objectives
To determine the irritation effects of a material on the skin using patch test technique.

Principle of method
Application of test material
The test sample is applied and secured onto the skin of the back, by means of an occlusive tape. The patches are held in contact with the skin by means of suitable non-irritating dressing, including non-irritating tape, for the duration of the exposure period.

Pilot Group – Limited exposure (3 subjects)
A cautious approach to testing is adopted by using a sequential patch procedure. The patches are applied progressively for 1hour, 2 hours, 3 hours and 4 hours. The test surfaces are then evaluated upon removal of patch and 1hour to 2 hours, 24 hours and 48 hours later.
Test group- A total of 30 subjects
If the tested sample, does not show any reaction in the pilot group after completing the 48 hours observation post 24 hours and 48 hours exposure period, the test is then be completed in a group of 30 subjects with a 24 hours and 48 hours.
Number of subject: 30 subjects
Duration: 2 months

1.4 REPEAT OPEN APPLICATION TEST

Objective
To determine the effect of a material following repeated application.

Principle of method
The method involves application of the material according to instructions on the forearms of 20 selected subjects, once a day for a total of five days. The irritation effects of test materials is visually assessed based on the time they take to appear and the duration of the visible reaction.
Number of subject: 20 subjects
Duration: 1 month

1.5 TEST FOR HYPOALLERGENICITY

Objective
To determine the allergenic response of the test materials.

Principle of method
The method consist of an occlusive application of the materials by means of plastic chamber on 20 selected subject's back or forearm, for 48 hours. After removal of the occlusion, the reactions induced by the test materials are evaluated. These evaluations are performed 24 and 48 hours later.

1.6 MODIFIED 21 DAYS CUMULATIVE IRRITATION TEST

Objective
To determine the incidence and severity of cumulative irritation.

Principle of method
Using the predictive patch technique, the materials are applied daily for 21 days. This is followed by a rest period of 14 day and then a challenge with test material.
Number of subject: 25 subjects
Duration: 2 months

1.7 ‘MODIFIED DRAIZE-95’ TEST

Objectives
To evaluate whether and ingredient is present at levels that may induce Type IV allergy in the unsensitized general user population are present in a finished product.

Principle of method
The study is conducted in two stages. In the first stage, a population of 50 human subjects are tested to evaluate the product for the potential to cause irritation or sensitization. The second stage is initiated on a further 150 individuals after the first stage has shown that the test product does not indicate a potential to induce dermal irritation and does not show sensitization capability.

Induction Phase
The induction phase of the test includes application of ten patches on the skin.

Rest Period
Two to three weeks.
Challenge Phase
Test materials are applied consecutively to a virgin site for 48 hours. The test site is evaluated for reaction at the time of patch removal and again two and four days later.
Number of subject: 200 subjects
Duration: 6 months.

1.8 REPEAT INSULT PATCH TEST (RIPT).

Objective
To provide an exaggeration of anticipated product use/misuse exposures through an extended duration of exposure, testing higher than use concentrations, minor skin irritation of the test material, and, for many product types, through the occluded patch.
-not done to confirm sensitization potential but to confirm ingredient and/or product safety under exaggerated conditions relative to anticipated consumer exposure.

Principle of method

80-120 test subjects are employed for human repeat insult patch testing

Induction phase
Nine 24 hour patches at a single site with a 24-hour rest between patches
Rest phase: 14-17 day rest
Challenge phase - 24-hour challenge patch.
Skin reactions are scored during induction (just prior to patch reapplication), and 24 and 72 hours after challenge patch removal, although scores from 48 hours, 96 hours.

1.9 PATCH TEST ON SENSITIZED INDIVIDUALS

Objectives
To determine whether a test product contains residual chemicals which might cause skin reaction in individuals who are already allergic to one of its ingredients.

Principle of method
The test material is applied to each of the 25 human subjects who are previously diagnosed to be allergic to one or more of its ingredients.
In this test procedure the patch is applied (day 1) with all edges continuously secured with a non-reactive adhesive tape (to ensure complete occlusion) for 48 hours. The test sites are evaluated at the time of patch removal (day 3) and repeated two days later (day 5).
Number of subject: 25 subjects
Duration: 2 months

1.10 HUMAN MAXIMIZATION TEST

Objective
Assessment of the skin sensitization potential of chemicals in humans

Principle of method
25 subjects are subjected to repeated 48 hour occlusive patch treatment with as high a concentration of test chemical as possible on five occasions over a two week period.
Five sets of patches are worn on the same site for 48 hours each; with a 24 hour rest period between removal and reapplication.
Following a 2 week rest period after the last induction patch, an SLS provocative patch

procedure is performed to prepare the skin for challenge.
The extent of sensitization in the panel is assessed by 48 hour treatments on a slightly irritated skin site using the maximum non-irritant concentration of the test substance. The challenge sites are scored at 48 hours and 96 hours after application.

1.11 EXTENDED PROSPECTIVE PRODUCT USE TESTING

Objective
To determine the potential for a product to induce sensitization or both induce sensitization and elicit allergic skin reactions under typical conditions of product use.

Principle of method
Up to 100 to 500 subjects and extended up to three to six months
Diagnostic patch testing at the conclusion of the study is done to verify lack of patch test reactivity or to identify sub clinical sensitization
responses Any evidence of induced sensitisation would necessitate
consideration of withholding a developmental product (or ingredient) from the market

1.12 COMEDOGENICITY TEST

Objectives
To evaluate the potential of test material to induce the onset or the increase of comedones on the face after repeated application.

The acne visual count is taken by the dermatologist at the beginning of the test and then weekly until the end of the study.

The product has no comedogenic power if no statistically significant variations in acne lesion count are shown during the study

Principles of method
Dermatological assessment
Products potential comedogenicity after repeated application on facial skin is visually performed by acne lesion count. The dermatologist counts open and closed comedones, papules, pustules and nodules separately and records the number of each lesion. Total lesion count is also calculated for each subject.

1.13 HUMAN OCULAR IRRITATION

Objectives

To determine the possible irritation and the safety of the product on the human eyes.

Principle of method
Prior to this study Primary skin irritation test is performed to ensure that the test material is not irritating.
One drop of test material is gently instilled into the culder sac of the assigned eye. Within 10 second,eyes are examined and scored according to the mentioned grading scale. Subjects are interrogated for the sensorial component. In sufficient discomfort is initially experienced by the volunteers upon material instillation.

1.14 ASSESSMENT OF SKIN FOLLOWING REPEATED WASH

Objective
To determine any possible irritation or reaction induced by cosmetic or skin care products on the skin following repeated wash.

Principle of method
The effects of a product could be assessed through the application of a reasonable quantity of test product with an exaggerated frequency. The exaggerated dosing of the test subject is therefore comprised of varying the quantity and frequency of use with the product configuration. The effects of the particular products on the skin could be assessed visually.
The product will be used on specified test area of the skin, from the elbow to wrist of the right forearm, 4 times daily, for 4 consecutive days of use.

The skin was assessed visually, and using chromameter that measures the changes in pigmentation, and degree of erythema of the skin.

1.15 SAFETY ASSESSMENT OF BABY BATH USING USE TEST

Objective
To evaluate the effects of bubble bath under exaggerated use condition on babies

Principle of method
The effects of a product could be assessed through the application of a reasonable quantity of test product with an exaggerated frequency. The exaggerated dosing of the test subject is therefore comprised of varying the quantity and frequency of use with the product configuration. The effects of the particular products on the skin could be assessed visually.

Thorough clinical examination is done on each subject twice, within one day prior to commencement of exposure period and within one day of completion of the test procedure. Any sign of irritation is noted and recorded on the subject report form. The effects of the particular

products on the skin was assessed visually.

EFFICACY STUDIES

1.16 ASSESSMENT OF WHITENING EFFECT FOLLOWING REPEATED USE OF TEST MATERIALS ON THE HAND

Objective
To evaluate the whitening effects following repeated use of test materials.

Principle of method
The exaggerated dosing of the test subject comprise of varying the quantity and frequency of use with the test material. The whitening effects of the particular test materials are assessed using skin colorimeter. Any sign of irritation by usage of the test material are recorded on the subject report form.
The assessment and measurement are done periodically until the completion of study. The data collected are then analysed and compared statistically.
Number of subject: 20 subjects
Duration: 2 months

1.17 ASSESSMENT OF ANTIWRINKLE EFFECT FOLLOWING REPEATED USE OF TEST MATERIALS ON THE HAND

Objective
To evaluate the antiwrinkle effect following repeated use of test materials.

Principle of method
The exaggerated dosing of the test subject is comprised of varying the quantity and frequency of use with the test material.
The effects of usage of test materials on the skin surface of the hand are assessed by evaluating the topography of the skin surface by light transmission of a very thin, and a silicone replica. Any sign of irritation induced by usage of the test material are recorded on the subject report form.
Number of subject: 20 subjects
Duration: 2 months

1.18 BARRIER EFFICACY STUDIES

Objective
To determine the barrier efficacy of a particular product against four standard irritant, sodium

hydroxide, sodium laurel soleplate, 1% lactic acid toluene.

Principles of method
Evaluation of fluorosilicone, aqueous and zinc oxide as a barrier cream is assessed against 2 standard irritants (10 % sodium lauryl sulphate and 1% sodium hydroxide).

Induction of irritation
Cumulative irritant contact dermatitis will be induced using the repetitive irritant test (RIT) with a set of 2 standard irritants, a model quantification of skin protective creams published by Forsch & Kurte (5).
Number of subject: 20 subjects
Duration: 2 months

1.19 ASSESSMENT OF SKIN BARRIER RECOVERY

Objective
To assess the therapeutic effects of topical application in recovering skin reactions to irritants will be looked into.

Principles of method
Skin barrier recovery
The skin protects the body from excessive water loss and at the same time hinders the entrance of chemical substances and microorganisms. These exposures, including solvents, water and detergent eventually damages the stratum corneum. Exposure of normal skin to organic solvents or detergents will remove the lipids and affect the barrier integrity (Grubauer et al., 1989).

A specific concentration of Sodium Lauryl Sulfate in water will be applied in occlusion for 24 hours on the subject’s forearm. Visual assessment and measurements with Transepidermal water loss meter (TEWL), Corneometer, Chromameter and Mexameter are taken on both the treated and untreated forearm, 30 minutes after the removal of the occlusion.

The skin is then treated with test materials and the other is used as control. Serial measurements of TEWL, corneometer and chromameter, are carried out 24 and 48 hours after the test product application.
Number of subject: 20 subjects
Duration: 2 months

1.20 EFFICACY ASSESSMENT OF TOPICAL SKIN PRODUCTS ON CRACKED HEEL

Objective

1. To evaluate the efficacy of a topical skin product on cracked heel.
2. To evaluate the effect of skin product on transepidermal water loss of crack heel.
3. To evaluate the effect of skin product on the degree of moisturizing effects on the skin

Principle of method
Efficacy assessment of topical skin product on crack heel can be assessed clinically, by visual assessment and by transepidermal water loss measurement. Corneometer measurement can be used to measure the moisturizing effect of the product on the skin.

1.21 ASSESSMENT OF EFFECT ON NAIL CUTICLES FOLLOWING REPEATED USE OF TEST MATERIALS ON THE FINGERNAILS

Objective
To evaluate the effects on the nail cuticles following repeated use of test materials.

Principle of method
The exaggerated dosing of the test subject comprise of varying the quantity and frequency of use of the test material. The effects of usage of test materials on the nail cuticles is clinically evaluated.
The assessment and measurement of the nail cuticular surface are done 3 times, i.e. at baseline, day 14 and after completion of test (28 days).
Number of subject: 20 subjects
Duration: 2 months