e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2022; 12 (6); 174-184

Original Research Article

Assessment of Cumulative Irritation and Sensitization Potential of Himalaya's Baby Skin Care Products in Adult Healthy Volunteers by Human Repeat Insult Patch Test (HRIPT)

Rajesh Kumawat[1], Mukta Sachdev[2], Mukesh Ramnane[3], Swathi B[4], Babu U V[5]

[1]Head of Medical Services & Clinical Development,HWC, Bengaluru
[2]Head of Department & Consultant Dermatologist Dermatology, Manipal Hospital, Bengaluru, Medical Director of MS Clinical Research (MSCR), Bengaluru
[3]Senior Consultant Dermatologist, Manipal Hospital,Consultant, MSCR, Bengaluru
[4]Clinical Team Lead – Personal Care, Himalaya Wellness Company (HWC), Bengaluru
[5]Head-Research & Development (R &D), Himalaya Wellness Company (HWC), Bengaluru

Received: 15-04-2022 / Revised: 18-05-2022 / Accepted: 01-06-2022
Corresponding author: Dr. Rajesh Kumawat
Conflict of interest: Nil

Abstract

This study was to assess the mildness, gentleness and allergenic potency of 18 marketed baby skin care products. This open-label, controlled, single-center, HRIPT was conducted in 2 studies as per BIS 4011:2018 guidelines. Each study included 200 healthy participants in the age of 18-65years (of either sex). 10 products in study 1 and 8 products in study 2 were evaluated in different phases. Induction phase, products were repeatedly applied on the back of study subjects in the form of occlusive patch for 24 hours for 3 times a week, for 3 weeks. The reactions were assessed 48 hours after every patch application. Post 2-week rest period, subjects had 24-hour challenge contralateral patch application on treatment naïve site. The reactions were evaluated at 48, 72, and 96 hours post patch application. In study 1, 6 products were mild with cumulative irritation score of 0 and 4 products namely gentle baby soap, refreshing baby soap, nourishing baby soap, extra moisturizing baby soap elicited cumulative irritation score of 267.0, 289.0, 326.0, 137.0, respectively, they indicated probably mild in normal use. In study 2, 6 products were mild, with cumulative irritation score of 0 and 2 products namely gentle baby shampoo, extra moisturizing baby wash had cumulative irritation score of 375.12, 215.92 respectively, they indicated mild in normal use. All 18 baby skin care products concluded to be hypoallergenic and non-irritating, implies the products are safe, mild, and gentle in study population.

Keywords: HRIPT, induction phase, contralateral patch application, cumulative irritation score

Introduction

Skin care products must be tested appropriately to evaluate the skin toxicity and tolerance to avoid adverse effects on consumers prior to marketing [1]. Results obtained from animal experiments may not be valid for humans, particularly when dealing

with irritants, due to its complex mechanisms and high variability of human skin [2]. The irritation potential of any substance can be evaluated through primary irritation patch test (PIPT) or human repeat insult patch test (HRIPT) in healthy adults, these are the initial safety studies which are conducted for skin care products before testing in target population . Some products may cause irritation on single application and a few others may cause irritation on repetitive application (cumulative irritation). HRIPT helps assess the cumulative irritation and skin sensitization potential of any skin care product [3].

Based on the nature, concentration and duration of exposure, skin irritants may cause damage to the skin, which manifest as erythema, oedema, vesiculation, or dryness commonly called as irritant contact dermatitis (ICD), the most common inflammatory skin disease [4].

HRIPT includes standardized procedure/protocol for prediction of potential allergy to particular product (Allergic contact dermatitis (ACD)) which is a major parameter in quantitative risk assessment for chemicals/drugs [5]. HRIPT is considered as indirect confirmatory test to evaluate the safety of skin sensitizers and ensures the safety of the products on human skin [6].

In order to conduct the patch test, Bureau of Indian Standards (BIS) guidelines and other global guidelines (USFDA elaborates the study methodology, and interpretation of data in such type of studies [7,8].

If any potentially allergen product is applied on the allergenic skin for a long time, the skin may react and result in dermatitis. HRIPT should not be performed on individuals already allergic or has acute dermatitis as it may aggravate the reaction, leading to false positive results [3].

In recent years, demand for baby care products has been increasing due to increase awareness of the requirements for these products. In India, there are around 50 million babies in the age group of 0 to 2 years and 304.8 million children in the age group of 0 to 12 years [9].

The products, specially formulated to be applied on baby's delicate/immature skin, need thorough and careful evaluation for toxicity and tolerance [10]. There is a sudden change from the intrauterine environment to the external environment at birth. Baby's skin is most delicate of all skin types as the skin is very sensitive, thin, and fragile [11]. The low defence mechanism of the baby's skin makes the skin more vulnerable to trauma and toxicity. Hence, skin care products intended for use in babies requires high caution and intense testing [12].

Hence HRIPT study was conducted to revalidate the safety of baby skin care products.

The study was conducted to evaluate the hypo-allergenicity and skin sensitization potential of these baby skin care products through HRIPT in healthy adult volunteers.

Materials and Methods

Two separate open label, controlled clinical HRIPT safety studies were conducted at MS Clinical Research (Bengaluru, Karnataka, India), between February 2020 and December 2020. In these studies, 18 baby skin care products of Himalaya Wellness Company were evaluated for cumulative irritation and sensitization potential on human skin in two different sets with 200 healthy adult volunteers in each study (10 products in study 1 and 8 products in study 2). The studies were conducted according to the BIS guideline 4011:2018, and as per the ethical standards and principles laid down in the Helsinki Declaration after obtaining approval from the Independent Ethics Committee on 4 May 2020 and 26 May 2020 and due registration at the Clinical Trials Registry of India (CTRI/2020/03/023725 and CTRI/2020/06/026085, respectively). A

written consent was obtained from all the study participants.

Inclusion criteria

The study included healthy men and women aged

18 to 65 years willing to avoid UV exposure (ie, exposure to sunlight or artificial UV), excessive water contact (eg, swimming), or activities that cause excessive sweating (eg, exercise and sauna) during the course of study.

Exclusion criteria

Subjects with significant systematic or cutaneous diseases, which may interfere with study treatment or procedures; with scars; with excessive hair or tattoo or dermatological infection/pathology; with known hypersensitivity; with allergy antecedent (to any cosmetic product, raw material or hair dye) were excluded in the study. Pregnant women, nursing mothers and those who participated in any other food, cosmetic, or therapeutic clinical studies within past 1 month of screening were also excluded from the study.

Study procedure

In study 1, 215 subjects were enrolled, of which 200 completed the study and in study 2, 205 subjects were enrolled, of which 201 completed the study as per the protocol.

Test products

In this HRIPT study, 18 Himalaya's baby skin care products were tested. In study 1, 10 products were included (1. gentle baby bath, 2. refreshing baby wash, 3. baby diapers/baby pants, 4. baby massage oil, 5. soothing baby wipes, 6. prickly heat baby powder 7. extra moisturizing baby soap, 8. gentle baby soap, 9. refreshing baby soap, and 10. nourishing baby soap). Whereas study 2, included 8 products (1. gentle baby shampoo, 2. extra moisturizing baby wash, 3. baby cream, 4. baby lotion, 5. diaper rash cream, 6. soothing calamine baby lotion, 7. baby powder, and 8. gentle baby wipes/gentle baby wipes [extra-large]) for the assessment . In both the studies, 0.9% saline was used as a negative control.

The study included 4 phases: induction phase, rest phase, challenge phase, and re-challenge phase. All the activities and procedures carried out in each phase of the study are mentioned in Table 1 (schedule of events).

Induction phase

All test products were patched using Finn Chamber with 40 μL of the test products (diluted or pure). The patches were applied on the back of the subjects for 3 applications per week, for 3 weeks. A negative control of 0.9% normal saline was also applied along with the test products on every patch application visit. The patches were retained for 24 hours.

The skin evaluation was performed of by dermatologist 24 hours after patch removal. Reactions on the skin were scored as per Draize scale. If any product showed a reaction of > 2 on the erythema scale, then the patch was to be relocated to an adjacent site. Two relocations were allowed per product for each volunteer in induction phase. All assessments, once a score of 3 on Draize scale was observed, then the same was to be maintained from that point onward.

Rest phase

During the rest phase of 2-3weeks (14 to 22 days), no patches were applied to develop any sensitization of the skin to the test product. The previous reactions observed in the test sites were allowed to completely subside in this phase.

Table 1: Schedule of events for both the studies

Visit	V1 Screenin	V2 D1	V3 D3	V4 D5	V5 D8	V6 D10	V7 D12	V8 D15	V9 D17	V10 D19	V11	V12		V13	V14	V15	V16	V17	V18	V19	V20
	Week1				Week 2			Week 3			Week 4 D1 and D2			W6/7	W6/7D3	W6/7D4	W6/7D5	W7/8D1	W7/8D3	W7/8D4	W7/8D5
Phase	Induction phase												Rest phase: 14 to 22 days	Challenge phase				Re-challenge phase			
Informed consent	X																				
Medical History	X																				
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Dermatologi -cal examination	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Eligibility	X																				
Pregnancy test	X																				
Patch application	-	X	X	X	X	X	X	X	X	X	X			X				X			
AE	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Exit form																	X				X

*Shaded boxes indicate that assessments are not applicable for that visit

X = assessments to be performed on that visit

AE= Adverse events

Challenge phase

After completion of rest phase, the test products were reapplied, following the same procedure at the same test site and at a new contralateral naive test site. A series of scoring was done at 24, 48, and 72 h post patch removal. The reactions were scored as per International Contact Dermatitis Research Group (ICDRG) scale.

Re-challenge phase

In this phase, only those participants who showed allergic dermatitis were to be analysed. In both the studies, none of the participants developed any indicative allergic reaction in the challenge phase; hence none of the participants required to undergo re-challenge phase.

Statistical analysis

Cumulative irritation potential: The cumulative irritation potential is represented by means of cumulative irritation score (CIS). Categories of test products were based on the maximum possible score of 3 (severity score) for 9 induction visits. 200 participants data from study 1 and 201 participant data from study 2 were considered for analysis.

The irritation score obtained in the induction

phase was considered to identify the cumulative irritation potential and categorize the participants based on the below-mentioned 5 classes of irritants. The test products were classified based on CIS score.

Class	Mean Cumulative Irritant Score	Indication From Test	Description of Observed Responses
I	0 to 205	Mild material: no experimental irritation	Essentially no evidence of cumulative irritation under conditions of test (i.e. continuous) at concentration specified).
II	>205 to 675	Probably mild in normal use	Evidence of slight potential for very mild cumulative irritation under conditions of test.
III	>675 to 1890	Possibly mild in normal use	Evidence of moderate potential for mild cumulative irritation under conditions of test.
IV	>1890 to 2430	Experimental cumulative irritant	Evidence of strong potential from mild to moderate cumulative irritation under conditions of test.
V	>2430 to 2700	Experimental primary irritant	Evidence of potential for primary irritant irritation under conditions of test.

Allergenicity response

To be regarded as a hypoallergenic product, the frequency of affirmative allergenic reaction should be less than 5% per test product in the study population.

Results

Demographic details such as age, gender, and disposition of the study participants in study 1 and study 2 is as below.

Study 1						
	N	Mean	SD	Min	Median	Max
Women	160	34.13	10.62	18	33.5	62
Men	55	26.60	10.15	18	23	58
Total	215	32.20	10.98	18	31	62
Study 2						
	N	Mean	SD	Min	Median	Max
Women	155	32.80	9.32	18	33	55
Men	50	28.68	11.56	18	23.5	63
Total	205	31.80	10.04	18	32	63

From study 1 results, it was observed that gentle baby bath, refreshing baby wash, baby diapers/baby diaper pants, baby massage oil, soothing baby wipes, and prickly heat baby powder had cumulative irritation score of 0, which indicates that these are mild materials, with no irritation. However, gentle baby soap, refreshing baby soap, nourishing baby soap, and extra moisturizing baby soap elicited cumulative irritation score of 267.0, 289.0, 326.0, and 137.0, respectively, thus emerged as probably mild materials as illustrated in Table 2 (summary of CIS score). All the test products in study 1 were considered as hypoallergenic as demonstrated in Table 3.

Table 2: Summary of cumulative irritation scores (Study 1)

Control and Investigational Product	Cumulative Irritation Score for 200 Subjects	Indications From Test
Gentle Baby Bath	0.00	Mild material: no experimental irritation
Refreshing Baby Wash	0.00	Mild material: no experimental irritation
Baby Diapers 100 subjects Baby Pants: 100 subjects	0.00	Mild material: no experimental irritation
Soothing Baby Wipes	0.00	Mild material: no experimental irritation
Baby Massage Oil	0.00	Mild material: no experimental irritation
Prickly Heat Baby Powder	137.00	Mild material: no experimental irritation
Extra Moisturizing Baby Soap	267.00	Probably mild in normal use
Gentle Baby Soap	289.00	Probably mild in normal use
Refreshing Baby Soap	326.00	Probably mild in normal use
Nourishing Baby Soap	0.00	Mild material: no experimental irritation
Negative Control	0.00	Mild material: no experimental irritation

Table 3: Summary of allergenic responses (Study 1)

Product	No. of Reactions	Indicative allergenicity
0.9% Normal Saline (Negative Control)	0	Hypoallergenic
Gentle Baby Bath	0	Hypoallergenic
Refreshing Baby Wash	0	Hypoallergenic
Baby Diapers: 100 subjects Baby Pants: 100 subjects	0	Hypoallergenic
Soothing Baby Wipes	0	Hypoallergenic
Baby Massage Oil	0	Hypoallergenic
Prickly Heat Baby Powder	0	Hypoallergenic
Extra Moisturizing Baby Soap	0	Hypoallergenic
Gentle Baby Soap	0	Hypoallergenic
Refreshing Baby Soap	0	Hypoallergenic
Nourishing Baby Soap	0	Hypoallergenic

In study 2, baby cream, baby lotion, diaper rash cream, soothing calamine lotion, baby powder, and gentle baby wipes/gentle baby wipes (extra-large) elicited cumulative score of 0, which indicates that these products are mild materials, with no irritation. Gentle baby shampoo and extra moisturizing baby wash had the cumulative irritation score 375.12 and 215.92, respectively, indicating that they are probably mild in normal use as illustrated in Table 4 (summary of CIT score). All the test products were considered as hypoallergenic as demonstrated in Table 5.

Table 4: Summary of cumulative irritation scores (Study 2)

Control and Test Products	Cumulative Irritation Score for 201 Subjects	Indications From Test
Gentle Baby Shampoo	375.12	Probably mild in normal use
Extra Moisturising Baby Wash	215.92	Probably mild in normal use
Baby Cream	0.00	Mild material: no experimental irritation
Baby Lotion	0.00	Mild material: no experimental irritation
Diaper Rash Cream	0.00	Mild material: no experimental irritation
Soothing Calamine Lotion	0.00	Mild material: no experimental irritation
Baby Powder	0.00	Mild material: no experimental irritation
Gentle Baby Wipes/Extra Large Wipes	0.00	Mild material: no experimental irritation
Negative Control	0.00	Mild material: no experimental irritation

Table 5: Summary of allergenic responses (Study 2)

Product	No. of Reactions	Indicative Allergenicity
0.9% Normal Saline (Negative Control)	0	Hypoallergenic
Gentle Baby Shampoo	0	Hypoallergenic
Extra Moisturising baby Wash	0	Hypoallergenic
Baby Cream	0	Hypoallergenic
Baby Lotion	0	Hypoallergenic
Diaper Rash Cream	0	Hypoallergenic
Soothing Calamine Lotion	0	Hypoallergenic
Baby Powder	0	Hypoallergenic
Gentle Baby Wipes/Extra Large Wipes	0	Hypoallergenic

Discussion

The cosmetic products for children should be specifically formulated as the paediatric skin is very sensitive and more susceptible to percutaneous toxicity [10]. Due to immature sensitive skin barriers the risk of developing ACD when using cosmetics is very high in infants and young babies. That necessitates for careful assessment of tolerance during the development of dermo-cosmetics [13,14].

Although clinical studies are not mandatory for the development of cosmetics, considering the ethical aspect and safety perspective, manufacturers may decide on conducting clinical studies under ethical and scientific guidance.

Patch tests in healthy adult subjects is an ethical approach for the effective prediction

of skin irritation potential of skin care categories including baby care products. These present HRIPT studies are conducted as per the BIS guidelines 4011:2018 for safety testing of cosmetic products in India (the land of study conduct).

Ribet et al suggested that standardized ethical stepwise development approach is required to ensure the commercialization of safe and well-tolerated dermo-cosmetics for paediatrics population, and our study adhered to these basic principles [10]. It was inferred that using this methodology to test a dermo-cosmetic product intended for paediatric population reduces potential risks of irritation and contact dermatitis in this population. Draize scale is used to interpret the irritation potential of the test product and ICDRG was used for the scoring in challenge phase. Both the scores are globally acknowledged and thus provide the authenticity/ credibility of such type of assessments.

In HRIPT, any formulation's irritation and allergic potential can be evaluated. In order to study the sensitization potential, a larger group of individuals, that is, up to 100, are to be considered15. However, the present study included 200 individuals (in each study) as per the requirements of the guideline for conducting such type of study.

The probability of detecting a response for a range of conditions demonstrates the statistical limitations of the test. It is unlikely to observe 0.1% of skin sensitization even with 200 participants. The factors that further increase the sensitivity and reliability of the test are exaggeration through possible minor skin irritation by a test material and use of occluded patches. Based on industry experience and following guidelines, a study population of 100 is also considered to be reasonable to test sensitivity and reliability [16].

As suggested by FDA-recognized consensus standards (2018), Henderson and Riley (1945) demonstrated that a test sample size of 30,000 should be included to ensure statistically that there would be no more than 0.1% sensitization. If there are no allergic responses in a sample size of 200 participants with exposures comparable to those of the population, then there could be as many as 1.5 allergic reactions per 100 users [17,19].

The European Commission Scientific Committee on Consumer Safety stated that when a confirmatory HRIPT in 100 subjects yields the (expected) result of no sensitised individual (i.e. 0%), based on statistical considerations, a confidence interval has to be considered. This implies that for a sample of 100, a confidence interval of 95% would include up to 3 individuals (i.e. 3%) who still could be sensitive.

To ensure an adequate safety evaluation, approximately 100 subjects must complete the entire HRIPT protocol. The statistical calculations of patch tests adapted for the detection and evaluation of chemical agents were investigated by Henderson and Riley (1945). It was observed that if no reactions were observed in a group of 100 test subjects, then the rate of positive reactions in a larger population is not likely to exceed 2.9%, based on a confidence level of 95%, under identical conditions18,19. Hence based on HRIPT study in 100 subjects, we can make inference about the population that positive response probably would not exceed 2.9%.

The resulting predictions of allergy and irritation response are the scientific goals of HRIPT. Multiple international agencies, that is Eurofins, BioScreen Testing Services Inc, and Evalulab Inc [20-22], suggest a minimum of 50 human volunteers in HRIPT studies. However, data from up to 200 subjects are better for statistical prediction, which was adopted in the present study.

It is estimated that 1 to 5.4% of the general population is sensitive to a cosmetic or cosmetic ingredient. Allergens like fragrances and preservatives which are majorly synthetic in nature increases incidence of cosmetic

dermatitis which may result in sensitization of the skin [23].

Irritant dermatitis and ACD are the major common adverse effects caused using cosmetics [24]. It is hypothesized that 15 to 20% of the general population are sensitive to one of the common allergens; and the incidence of contact dermatitis in the general population in 1 year is around 7%. The patch test helps prove sensitization to substances that cause ACD [25]. ACD is considered as a disorder of immature immune system in adults but children are expected to be safe due to a lack of exposure to potential allergens. However, recently contact sensitization is observed in the paediatric population also, including just-born babies [26].

The guidelines are quite stringent with regard to clinical trials to be conducted on drugs, whereas there are no mandatory registration and stringent guidelines for conducting studies on cosmetic products. We believe that the development of dermo-cosmetic products for the pediatric population should involve a multidisciplinary team and should be based on robust clinical studies performed according to Good Clinical Practice (GCP), and data thus obtained must be assessed ethically.

As the products tested in our studies are designed for babies, the HRIPT study was conducted on healthy adult volunteers as a first step to evaluate skin irritation and sensitization potential of the products. Once the safety is established, these products must be studied for efficacy in the actual target population. The study results indicated most of the products having mild materials and a few other products as having probably mild materials. These facts validated that these products can be used in paediatric population. The methodology or procedures followed, and interpretation of data are consistent with the multiple other studies carried out earlier and the literature available worldwide [27,28]. To conclude, 18 baby skin care products were tested on Indian adult population through HRIPT study.

These products have assessed and shown to confirm to the highest testing standard for sensitization as per Indian and International acceptable guidelines. All these products displayed to be “hypo-allergenic”, “non-allergenic”, “mild & gentle” and “dermatologically tested” on study population.

These studies were conducted as per prevailing guidelines after due approvals from ethics committee and acknowledgment from national registry. Apart from these strong credentials, study included sufficient number of subjects as discussed above.

However, further studies under supervision of dermatologist/ paediatrician are warranted in actual population in sufficient number of babies to validate the safety of the products and these studies in healthy volunteers. The application of these products on baby skin may further validate the sensitization/allergic potential for safer use in this vulnerable population. Nevertheless, even after conducting the clinical studies, there is always a potential risk of intolerance when the product is used in a larger population. Cosmetovigilance is a major asset for detecting early safety signals that are eventually not observed during the development program. The European Union Cosmetics Regulation (EC) No. 1223/200910 requires companies to collect and assess reports of undesirable effects caused by the cosmetic products they market, and serious undesirable effects should be reported as per defined timelines. Mandatory Cosmetovigilance should be in place for actual safety claims of cosmetic products.

Acknowledgements: The authors would like to thank all the experts, especially Ms. Ritambhara from MS Clinical Research, who were involved in conducting this clinical study.

References

1. M K, Robinson, R., Osborne, M A., Perkins. In vitro and human testing strategies for skin irritation. Ann N Y Acad Sci. 2000:919, 192-204.
2. W, Wigger-Alberti., A, Rougier., A, Richard., P, Elsner., P. Efficacy of protective creams in a modified repeated irritation test. Methodological aspects. *Acta dermato-venereologica, 1998:78 4*, 270-3.
3. Bureau of Indian Standards. Methods of Test for Safety Evaluation of Cosmetics. BIS guideline 4011:2018
4. M, Soltanipoor., T, Stilla., C, Riethmüller., J P, Thyssen., J K, Sluiter., T, Rustemeyer., T W, Fischer., S, Kezic., I, Angelova-Fischer. Specific barrier response profiles after experimentally induced skin irritation in vivo. Contact dermatitis, 2018:79(2), 59–66.
5. M K, Robinson., A P, Mary. A strategy for skin irritation testing. American journal of contact dermatitis, 2002:13 (1), 21-9.
6. R M, Walters., P, Khanna., M, Hamilton., D A, Mays., L, Telofski. Human Cumulative Irritation Tests of Common Preservatives Used in Personal Care Products: A Retrospective Analysis of Over 45 000 Subjects. Toxicol Sci. 2015:148(1),101-107.
7. Combined Cumulative Irritation Potential and Repeat Insult Patch Test of LEO 90100 Aerosol Foam [Internet]. Leo Pharma. Eudra CT. 2014 Jun 18. Available from: https://fdocuments.in/document/clinical-study-report-combined-cumulative-irritation-clinical-study-report.html
8. C, Galzote., M, Thomas., M, Sachdev. Assessment of Irritation and Sensitization Potential of Eight Baby Skin Care Products. Journal of drugs in dermatology: JDD, 2016:15(10), 1244–1248.
9. Business Standard. Baby and child care market set at boom with foreign brands. [Internet]. updated 2014 Mar 30; cited 2021 May 19]Available from: https://www.business-standard.com/article/pti-stories/baby-and-child-care-market-set-to-boom-with-foreign-brands-114033000476_1.html
10. V, Ribet., M, Gurdak., P J, Ferret., E, Brinio., F, Giordano Labadie., A B, Rossi. Stepwise approach of development of dermo-cosmetic products in healthy and atopic dermatitis paediatric population: safety evaluation, clinical development and postmarket surveillance. Journal of the European Academy of Dermatology and Venereology: JEADV, 2019:33(12), 2319–2326.
11. R, Sarkar., S, Basu., R K, Agrawal., P, Gupta. Skin care for the newborn. Indian pediatrics, 2010:47(7), 593–598.
12. J D,Fernandes., M C R, Machado., Z N P, de Oliveira. Prevention and skin care for children and newborns**.** An Bras Dermatol, 2011:102–110.
13. U, Blume-Peytavi., J,Tan., D, Tennstedt., F, Boralevi., G, Fabbrocini., A, Torrelo., R, Soares-Oliveira., M, Haftek., A B, Rossi., M D, Thouvenin., J, Mangold., M F, Galliano., H, Hernandez-Pigeon., M F, Aries., C, Rouvrais., S, Bessou-Touya., H, Duplan., N, Castex-Rizzi., V, Mengeaud., P J, Ferret., R, Dauskardt. Fragility of epidermis in newborns, children and adolescents. Journal of the European Academy of Dermatology and Venereology: JEADV, 2016:30 Suppl 4, 3–56.
14. G N, Stamatas., J, Nikolovski., M C, Mack., N, Kollias. Infant skin physiology and development during the first years of life: a review of recent findings based on in vivo studies. Int J Cosmet Sci, 2011:33, 17–24.
15. V S, Bhuvaneswari., R S, Chandan., R.S. Review on Safety Assessment of a

Cosmetic product. International Journal of Current Pharmaceutical Research, 2018:1-6.
16. P M, McNamee., A M, Api., D A, Basketter., G, Frank Gerberick., D A, Gilpin., B M, Hall., I, Jowsey., M K, Robinson. A review of critical factors in the conduct and interpretation of the human repeat insult patch test. Regul Toxicol Pharmacol, 2008:52(1), 24-34.
17. US Food and Drug administration. Recognized Consensus Standards [Internet]. USA:US Food and Drug administration;2018 [updated 2020 Dec 21; cited 2021 May 19]Available from:https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/detail.cfm?standard__identification_no=36973
18. T P, Valerie., M A, Marie.The Research Institute for Fragrance Materials' human repeated insult patch test protocol. Regulatory Toxicology and Pharmacology, 2008:(52)1, 35-38.
19. Henderson, C.R., Riley, E.C., 1945. Certain statistical considerations in patch testing. J. Invest. https://www.eurofins.com.au/6, 227–232.
20. Eurofins. Human repeat insult patch test. [Internet]. cited 2021 Jun 15. Available from: https://cdnmedia.eurofins.com/apac/media/601381/hript.pdf
21. BioScreen Testing. Human repeat insult patch test. [Internet]. Cited 2021 Jun 24 Available from: https://www.bioscreen.com/index.php/human-repeat-insult-patch-test
22. Evalulab. HRIPT Test: Allergy patch, Hypoallergenic claim. [Internet]. Cited 2021 Jun 24 Available from: https://www.evalulab.com/en/clinical-testing/services/product-safety-testing/hript-test.
23. P,Kumar., P,Rekha. Patch Testing in Suspected Allergic Contact Dermatitis to Cosmetics. Dermatology research and practice. (2014).
24. M F S, Hafner., A C, Rodrigues., R, Lazzarini., Allergic contact dermatitis to cosmetics: retrospective analysis of a population subjected to patch tests between 2004 and 2017. An Bras Dermatol. 2020:95(6),696-701.
25. A, Schnuch., W,Aberer., M,Agathos., D,Becker., J,Brasch., P,Elsner., P, Frosch., T,Fuchs., J, Geier., U, Hillen., H, Löffler., V, Mahler., G,Richter., C, Szliska. Patch testing with contact allergens Guideline of the German Dermatologic Society (Deutsche Dermatologische Gesellschaft, DDG) and the German Society for Allergy and Clinical Immunology (Deutsche Gesellschaft für Allergie und klinische Immunologie, DGAKI) (2008).
26. V K, Sharma., D P, Asati. Pediatric contact dermatitis. Indian journal of dermatology, venereology and leprology, 2010:76(5), 514–520.
27. Y S, Mulyanti., Y., B, Kasemchainan., P, Mitra., P, Evans., H, Hartono. Evaluation of the Skin Irritation and Sensitization Potential of the Cussons Baby Sensicare Skin Range of Products in Healthy Volunteers. Journal of Cosmetics, Dermatological Sciences and Applications, 2019:9, 207-215.
28. V S, Bhuvaneswari., R S, Chandan. Review on Safety Assessment of Cosmetic Product. International Journal of Current Pharmaceutical Research. 2018:10(3). 1-6.