Absence of human skin irritation and allergenic potential after repeated patch applications of a lamellar moisturizer

Stephanie J Nisbet BSc
GSK Consumer Healthcare, St George’s Avenue, Weybridge, Surrey, UK

Correspondence
Stephanie J. Nisbet, GSK Consumer Healthcare, St George’s Avenue, Weybridge, Surrey, UK. Email: Stephanie.J.Nisbet@gsk.com

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Summary
Background: New cosmetic products should undergo clinical evaluation for skin sensitization potential.

Objectives: To assess the irritation and sensitization potential of a moisturizer containing lamellar structured lipids after repeated patch application in humans, using human repeated insult patch test methodology.

Methods: This 6-week, single-center, open-label study compared a lamellar moisturizer with negative saline control in human subjects aged 18-70 years and skin phototype (Fitzpatrick) classification I–IV. During an initial induction phase, semi-occlusive multi-test patches were applied to the skin of participants’ backs three times per week for 3 consecutive weeks; clinical assessments were performed per International Contact Dermatitis Research Group criteria. Participants subsequently underwent a challenge phase, where a new patch was applied to a contact-naïve area of the skin to assess sensitization to the moisturizer.

Results: The study commenced with 233 voluntary participants, 214 of whom completed the study and underwent the final dermatological assessment. Most participants (232/233; 99.6%) demonstrated negative patch test results. One participant had a positive reaction at the lamellar moisturizer application site, with visible erythema and edema (classified as an adverse event [AE]); however, this reaction was observed 24 hours after a reaction to another product in the patch test panel (a prototype cleanser). Importantly, no skin reactions were detected during the challenge phase. Two participants had AEs of mild contact dermatitis in the area of patch adhesive application during the induction phase. No serious AEs occurred during the study.

Conclusions: These findings suggest that the lamellar moisturizer has low irritant and allergenic potential.

KEYWORDS
allergenic, human repeated insult patch test, irritation, moisturization, sensitization

1 | INTRODUCTION

Topical exposure to components of cosmetic products can result in a range of skin reactions, including irritation and allergic responses. Such dermatitis is characterized by redness, edema, oozing, crusting, scaling, and occasionally vesicles.1 Approximately 80% of cases of contact dermatitis are accounted for by irritant contact dermatitis, which occurs in response to contact between a substance and the skin.
skin. Allergic contact dermatitis is a type IV hypersensitivity reaction that has two phases: sensitization to an allergen and an allergic response to that allergen following re-exposure. During the development of a novel cosmetic product, it is important to identify any skin reactions indicative of potential sensitization, given that cosmetics are not expected to induce allergic skin reactions.

The human repeated insult patch test (HRIFT) methodology is commonly used to investigate the risk potential for a possible irritant and/or sensitizing agent that triggers a reaction when in contact with human skin, under exacerbated conditions of product exposure. HRIFT compatibility protocols are of particular benefit to validate a no-effect level of sensitization to a product or its components, in comparison with a control substance. The application of test products under occlusion favors a high level of skin contact, thus maximizing the detection of any irritant or allergenic potential of the product. The initial application of the product to the skin enables assessment of any primary irritancy and/or preexisting sensitization that may exist. Further detection of the irritant potential of the product is facilitated by repeated applications of the product to the same skin site; typically, the irritant action of the tested substance will induce test site reactions that subside within 24 h of patch removal. Sensitization to the product is further assessed by the subsequent application of the product to a different skin site.

HRIFT-induced skin sensitization reactions are typically characterized by erythema associated with other dermal manifestations, such as edema, papules, vesicles, blisters, and pruritus. Any response that occurs and persists at both the induction site (the site of primary and repeated product application) and a challenge site (subsequent testing at a different location) indicates skin sensitization and may be confirmed by repetition of the test. Products observed to be associated with extreme responses during such induction and challenge testing may be considered to present a risk for development of allergy in humans and therefore would not be recommended for cosmetic use.

The present study was conducted to assess the irritation and sensitization potential of a moisturizer containing lamellar structured lipids after repeated patch application in humans.

2 | METHODS

2.1 | Study design

This was a 6-week, open-label study comparing a lamellar moisturizer with a negative saline control in human subjects. The overall objective was to assess the irritation and sensitization potential of the moisturizer after repeated patch applications, following the HRIFT methodology. The study was conducted between April 28 and June 5, 2014, at a single center in Vila Martina, Valinhos, Brazil.

The study was approved by the Research Ethics Committee of Faculdade de Medicina de Jundiaí and conducted in accordance with Good Clinical Practice guidelines, the Declaration of Helsinki, and Conselho Nacional de Saúde Administrative Rule 466/12. All participants provided written informed consent prior to inclusion in the study.

2.2 | Participants

Male or female subjects aged 18-70 years and with skin phototype (Fitzpatrick) classification I-IV were eligible for inclusion. Participants were required to agree to comply with study procedures and requirements, including prespecified attendance for assessments, and to provide informed consent. Exclusion criteria included: pregnant and nursing women, or their partners; active dermatoses or skin marks in the experimental area that could interfere with study results; a history of severe allergic reactions to topical products, cosmetics, or drugs; immunodeficiency; intensive sun exposure or tanning session exposure up to 15 days before initial assessment or during the study; esthetic or dermatological body treatment up to 3 weeks before screening or during the study; topical or systemic use of immunosuppressants, antihistamines, nonsteroidal anti-inflammatory drugs, and corticosteroids up to 2 weeks prior to screening; vaccination up to 3 weeks before or during the study; participation in another clinical study; any history of allergy to materials used in the study; dermatographism; participation during the study in any activity leading to intensive sweating; or a history of noncompliance with a study protocol. Individuals who did not meet the study eligibility criteria or who decided not to participate in the study at screening were considered to be screening failures.

During the study, participants were asked not to apply any product at the experimental region that could interfere with study assessments, or to change other cosmetic habits, including hygiene products. In addition, participants were requested not to: have facials, body exfoliation, or other esthetic treatments performed at the product application area; expose themselves to excessive sunlight or use artificial tanning beds; change dietary habits; change hormonal treatment; wet the patches during baths, or by means of pool or sea bathing, or through use of saunas or excessive sweating; remove the patches, or wear tight clothes that may remove the patch by friction or cause skin redness; miss one of the scheduled clinic visits; use any of the following restricted medications, including nonsteroidal anti-inflammatory drugs continuously for more than 3 days, corticoids, antihistamines, immunosuppressants, vitamin A and its derivatives, or any esthetic, cosmetic, or dermatological treatment at the product application site.

2.3 | Study procedures and assessments

The lamellar moisturizer evaluated in this study contained the following ingredients: aqua, butyrospermum parkii butter, caprylic/capric triglyceride, carboxer, ceramide NP, cocos nucifera oil, glycerin, hydrogenated lecithin, hydroxyethylcellulose, pentylen glycol, sodium carboxer, squalane, and xanthan gum.

Semi-occlusive patches made of a hypoallergenic material (transparent adhesive plaster [CREMER S.A], paper filter disks [FILLTRUS Ind. e Com. Ltda], Silicone Paper BR M2 [Adere Prod. Auto Adesivos...
Participants could be withdrawn from the study under the following circumstances: at their own request; if they or their partner became pregnant; or at the discretion of the principal investigator if there was a failure to comply with the research protocol, in the event of any reaction considered to pose a risk to the participant’s integrity, in the event of any complication that could interfere with data analysis, or if AEs made it impossible to continue to use the study product. All participants meeting these criteria were reassessed at all follow-up visits.

### 2.4 Statistical considerations

A total of 233 participants were planned for inclusion to ensure that at least 200 evaluable participants would complete the study. All results obtained until study completion, discontinuation of study product use, or participant withdrawal from the study were included in the final analysis, with descriptive statistics used to summarize the data.

### 3 RESULTS AND DISCUSSION

#### 3.1 Participants

Participant flow through the study is presented as a CONSORT diagram in Figure 1. Overall, 234 participants were screened, with one participant considered a failure of selection. Therefore, the study commenced with 233 voluntary participants, 214 of whom completed the study and had the final dermatological assessment performed.

Participant characteristics are shown in Table 1. The study population was predominantly female (83.7%), with a mean age of 44.8 years (range: 18-70 years). The majority of participants had skin phototype classification II or III.

#### 3.2 Patch tests

Skin reactions detected during the study are summarized in Table 2. The majority of study participants (232/233; 99.6%) demonstrated negative patch test results. Only one skin reaction was detected...
during the study. During the induction phase, one participant had a positive reaction at the site of the lamellar moisturizer application, with visible erythema and edema; however, this reaction occurred 24 hours after a reaction was observed to another product in cell 01 of the patch test panel (a prototype cleanser). Importantly, no skin reactions were detected during the challenge phase of the study.

3.3 | Safety

AEs and participant reports of discomfort are presented in Table 3. No serious AEs occurred during the study. Two participants developed mild contact dermatitis in the area of patch adhesive application during the induction phase of the study; both events were considered to be unrelated to the lamellar moisturizer. A third participant, who had reported no prior cases of allergy at screening, experienced a skin reaction during the induction phase; this participant’s reaction has also been described above in the patch test section. An equivocal reaction (classification +?, as per Table 2) to a prototype cleanser in cell 01 of the patch test panel (the lamellar moisturizer was in cell 02 and the saline control in cell 16) was recorded at clinic visit 6. At clinic visit 7, a strong positive skin reaction (classification ++, as per Table 2) was recorded, comprising erythema with strong infiltrate and microvesicles (no papules), which was visible at the lamellar moisturizer test site; the participant also reported itching at the patch site and removed the patch at home to alleviate the itching. The investigator discontinued study product application, and subsequent assessments 72 hours and 96 hours after patch removal indicated that the condition had regressed; total regression was recorded 192 hours after patch removal. Given that this participant experienced clinical signs of mild-to-moderate intensity in response to more than one product, the investigator related the causality of the reaction to the test product and defined the event as a strong allergic reaction to an ingredient in the test products in the patch test panel. However, this patch test did not include the individual ingredients in the test product, so the investigator was unable to determine which ingredient had caused the reaction.

4 | DISCUSSION

Our findings indicate that the lamellar moisturizer has low irritant and allergenic potential, as only one positive skin reaction was

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**TABLE 1** Participant characteristics

| Category                              | Participants enrolled n = 233 |
|---------------------------------------|-------------------------------|
| Female, n (%)                         | 195 (83.69)                  |
| Mean age, years (SD)                  | 44.8 (13.37)                 |
| Range                                 | 18-70                         |
| Phototype classification, n (%)       |                               |
| I (always burns, never tans)          | 12 (5.15)                    |
| II (always burns, tans minimally)     | 106 (45.49)                  |
| III (burns moderately, tans gradually)| 79 (33.91)                   |
| IV (burns minimally, tans well)       | 36 (15.45)                   |

MED, minimal erythemal dose; PS, pigmentation scale; SD, standard deviation.

aTypical MED, according to the Fitzpatrick classification.8

bMeasured using the Dermatone Skin Analyzer™ device.

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**TABLE 2** Irritant and allergic reactions to the test product during the study (study population, n = 233)

| Reaction grade* (interpretation) | Reaction description                                                                 | Participants with reactions, n (%) |
|----------------------------------|--------------------------------------------------------------------------------------|-----------------------------------|
| – or absent (negative test)      | No skin changes at the test area                                                     | 232 (99.6)                        |
| +? (equivocal reaction)b         | Weak macular erythema, not palpable                                                | 0                                 |
| +/++ (weak/strong reaction)      | Erythema and edema (± vesicles):                                                    | 1 (0.4)c                          |
|                                 | • Palpable erythema                                                                 |                                    |
|                                 | • Presence of edema/infiltrate                                                      |                                    |
|                                 | • Absence of papules or vesicles-many papules, vesicles, and/or microvesicles       |                                    |
| +++ (extreme reaction)           | Coalescent vesicles and/or blisters ulceration                                       | 0                                 |
| IR (irritant reaction)           | Irritant reaction of different types:                                               | 0                                 |
|                                 | • No infiltration                                                                   |                                    |
|                                 | • Small petechiae                                                                  |                                    |
|                                 | • Pustules                                                                         |                                    |
|                                 | • Efflorescences different from papules or vesicles                                 |                                    |
|                                 | • Inflammation limited to the exposed area                                          |                                    |

ICDRG, International Contact Dermatitis Research Group.

*ICDRG criteria for irritant and allergic reactions classification.9

bConsidered as a negative test; participants would not be withdrawn from the study, patch application continued.

cParticipant withdrawn from the study, patch application discontinued.
recorded during the study, and this participant experienced a reaction to more than one of the test products in the patch test panel. Given that this subject presented a positive reaction to multiple products, it is possible that they were presensitised to a common ingredient within these formulations. This one strong positive skin reaction was substantially below the cutoff of ≤3% of weak reactions or ≤2% of strong reactions above which the product would be considered an irritant, and was not classified as an extreme reaction, for which the product would be considered as presenting a risk for development of allergy in humans. Strengths of this study include that these data from human subjects are directly applicable to risk assessments. Moreover, due to the number of study product applications in the induction phase, this study allows assessment of primary irritability and irritant potential after repeated applications (accumulated irritability), enabling two cases of irritation caused by the study patch adhesive to be detected.

The 6-week total duration of this study is sufficient time for development of a humoral immune response, meaning that this study was also able to evaluate the sensitising potential of this formula, in addition to any preexisting sensitization for the test product. Given that no sensitization to the lamellar moisturizer was detected when applied to the skin under occlusion in all but one participant in this study, this suggests that this product has low allergenic potential. A limitation of the study is that while there was considerable variability between the individuals tested, including a wide age range, no participants had skin pigmentation levels (Fitzpatrick phototype classification) above IV, limiting generalizability of the results to populations with deep skin pigmentation. Considerable differences in skin properties and the ability to develop responses against chemical agents exist in individuals with skin pigmentation of I–IV compared with those above IV, which may be due to a more impenetrable barrier in the latter group. The irritant and sensitization potential of the lamellar moisturizer remains to be evaluated in populations with skin pigmentation levels of V and VI.

5 | CONCLUSION

Our findings suggest that the investigated lamellar moisturizer has low irritant or allergenic potential.

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