


Adapting epicutaneous patch testing protocols to assess immediate-type skin reactions

A. Mehling*, S. Benard[†], N. Braun[‡] , J. Degwert[§], M. Duttine[¶], C. Jassoy**, H. Merk^{††}, U. Rossow^{‡‡}, J. Welzel^{§§}, W. Wigger-Alberti^{¶¶}, K.P. Wilhelm^{***} and J. Ennen^{†††}

*BASF Personal Care and Nutrition GmbH, Duesseldorf, Germany, [†]Institute Dr. Schrader Hautphysiologie, Holzminden, Germany, [‡]DermaTronnier GmbH & Co. KG, Institute for Experimental Dermatology at Witten/Herdecke University, Witten, Germany, [§]SGS SIT GmbH, Skin Investigation and Technology, Hamburg, Germany, [¶]WIN COSMETIC GmbH & Co.KG, Floersheim-Dalsheim, Germany, **Henkel AG & Co. KGaA, Duesseldorf, Germany, ^{††}RWTH Aachen University, Aachen, Germany, ^{‡‡}CCR GmbH & Co. KG, Eschweiler, Germany, ^{§§}Universitätsklinikum Augsburg, Augsburg, Germany, ^{¶¶}BioSkin GmbH, Hamburg, Germany, ^{***}proDERM Institut für Angewandte Dermatologische Forschung GmbH, Hamburg, Germany and ^{†††}SkinNEXT Consulting, Hamburg, Germany

Received 25 October 2019, Accepted 26 March 2020

Keywords: skin compatibility testing, epicutaneous patch testing, in-use test, cosmetics, (non-immunological) contact urticaria, skin physiology, safety testing, emulsions

Abstract

OBJECTIVE: During the development of cosmetic formulations, in vitro and in vivo methods are essential tools used to reliably assess the skin irritation potential of a product or ingredient. Epicutaneous patch testing (single and/or multiple application protocols) has long been used as an initial in vivo method to screen for possible skin irritation properties of a substance or formulation. To confirm the mildness and dermatological and/or consumer acceptance of a product, use tests are often subsequently conducted. A study was therefore initiated to see how well patch test results correlate with use tests with respect to irritation elicited by skincare (leave-on) products.

METHODS/RESULTS: A number of different cosmetic formulations were assessed in both tests. Although the patch test results did not indicate substantial irritation potentials, immediate-type reactions (stinging and redness) were observed in some volunteers which disappeared within approx. 1 h. Although transient, these reactions suggested that consumer acceptance would probably be low and the studies were discontinued. Immediate-type reactions are rare but have been described for some substances used in cosmetics. These unexpected results were nevertheless intriguing and prompted the start of a journey to see if patch test protocols could be modified to assess these reactions. An occlusive short-term patch test protocol with an application period of 20 min was developed. Successful identification of the spontaneous reactions became possible. Furthermore, there was a correlation between the intensity of reactions observed in the short-term patch test and those observed in the controlled in-use studies. Short-term patch testing using the developed protocol can therefore reliably be used as a screening method, for example in the development and optimization of cosmetic formulations containing ingredients that could cause

spontaneous reactions, for instance of non-immunological contact urticaria type.

CONCLUSION: The lessons learned from this studies indicate that simple modifications of existing test protocols can lead to important insights into skin reactions. These modifications can then be used to create further building blocks in the development and optimization of test strategies for cosmetic formulations which offer reliable study designs for possible reactions product developers may encounter.

Résumé

OBJECTIF: Lors du développement de formulations cosmétiques, les méthodes in vitro et in vivo sont des outils essentiels utilisés pour évaluer de manière fiable le potentiel d'irritation cutanée d'un produit ou d'un ingrédient. Le test épicutané (protocoles d'application uniques et / ou multiples) est utilisé depuis longtemps comme méthode initiale in vivo pour dépister les éventuelles propriétés d'irritation cutanée d'une substance ou d'une formulation. Afin de confirmer la douceur et l'acceptation dermatologique et / ou consommateur d'un produit, des tests d'usage sont souvent effectués ultérieurement. Une étude a donc été initiée pour voir dans quelle mesure les résultats des tests épicutanés correspondent aux tests d'usage en ce qui concerne l'irritation provoquée par les produits de soin (sans rinçage).

MÉTHODES/RÉSULTATS: Un certain nombre de formulations cosmétiques différentes ont été évaluées dans les deux tests. Bien que les résultats du test épicutané n'indiquent pas de potentiels d'irritation substantiels, des réactions de type immédiat (picotements et rougeurs) ont été observées chez certains volontaires. Celles-ci ont disparu en à peu près 1 heure. Bien que transitoires, ces réactions de type 5 suggéraient que l'acceptation du consommateur serait probablement faible et les études ont été interrompues. Les réactions de type immédiat 6 sont rares mais ont été évoquées en relation avec certaines substances utilisées en cosmétique. Ces résultats inattendus étaient néanmoins intrigants et ont incité le lancement d'un processus pour voir si les protocoles de test épicutané pouvaient être modifiés pour évaluer ces réactions. Un protocole de test épicutané à court terme occlusif avec une période

Correspondence: Nicole Braun, DermaTronnier GmbH & Co. KG, Institute for Experimental Dermatology at Witten/Herdecke University, Witten, Germany. Tel +49 2302 2826300; fax: +49 2302 2826326; e-mail nicole.braun@uni-wh.de

Working group No 1: Safety and Skin Compatibility of the German Society for Scientific and Applied Cosmetic (DGK)

d'application de 20 min a été développé, permettant l'identification réussie des réactions spontanées. Il a été de plus constaté une corrélation entre l'intensité des réactions observées dans le test épicutané à court terme et celles observées dans les test d'usage contrôlés. Le test épicutané à court terme utilisant le protocole développé peut donc être utilisé de manière fiable comme méthode de dépistage, par exemple dans le développement et l'optimisation de formulations cosmétiques contenant des ingrédients qui pourraient provoquer des réactions spontanées, par exemple de type urticaire de contact non immunologique.

CONCLUSION: Les leçons tirées de ces études indiquent que de simples modifications des protocoles de test existants peuvent révéler des informations importantes sur les réactions cutanées. Ces modifications peuvent ensuite être utilisées pour créer d'autres blocs de construction dans le développement et l'optimisation de stratégies de test pour des formulations cosmétiques qui offrent des conceptions d'études fiables pour les réactions possibles que les développeurs de produits peuvent rencontrer.

Introduction

What is a cosmetic? According to EU Cosmetic Regulation (EC) N° 1223/2009 on cosmetic products, a 'cosmetic product' means any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours [1]. Similar definitions can be found in other regulatory frameworks, and these definitions govern products placed on the market in that region. Regulation (EC) N° 1223/2009 mandates that cosmetic products put on the market should not be harmful to human health with Annex 1 §8 specifying that a particular focus should lie on local toxicity which includes skin irritation.

During the development of cosmetic formulations, *in vivo* human test methods are indispensable tools used to assess the skin irritation potential of a product or ingredient. Epicutaneous patch testing for irritation (ECT; single and/or multiple application protocols) has long been used as an initial *in vivo* method to screen for possible skin irritation properties of a substance or formulation [2,3]. To confirm the mildness and dermatological and/or consumer acceptance of a product, use tests are often subsequently conducted. The types and protocols of tests that can be conducted are manifold, and it is necessary that test strategies are suitable for the products to be tested. A guideline for the assessment of skin compatibility of cosmetic products in humans is described by Cosmetics Europe [4] and a comprehensive overview of criteria and test possibilities was published by the DGK working group: Safety and Skin Compatibility of the German Society for Scientific and Applied Cosmetic [5].

Epicutaneous patch testing (ECT) remains the gold standard for skin compatibility testing and focuses on the acute irritation potential. To this accord, use of the exaggerated conditions of occlusion and relatively long single exposure times, depending on the protocol used generally ranging from 24 to 48 h, are involved. In contrast, 'use tests' are generally conducted under non-occlusive (open) conditions with repeated applications which represent the application procedures of skincare products more closely. Interestingly, literature is sparse in which direct comparisons between ECTs and 'use tests' were made, in particular for leave-on skincare products (e.g. topical skin creams and lotions [6]). The original

intention of this study was to investigate to what extent single application 24-h epicutaneous patch test results correlate with results obtained in use tests with regard to skin compatibility of skincare (leave-on) products. As a result of unexpected reactions in the use test, a detour was made to modify the single application 24-h patch test protocol to address these types of reactions.

Materials and methods

Materials

The following cosmetic leave-on formulations were developed following the current Guidelines of Good Manufacturing Practice. Different preservative systems were used (noted in italics) in accordance with Cosmetics Regulation (EC) N° 1223/2009 Annex V.

MD70001A-3 Cream Type W/O (*preservative-system: Ethylparaben/Methylparaben*):

Aqua, Caprylic/Capric Triglyceride, Helianthus Annuus Seed Oil, Glycerine, Polyglyceryl-3 Polyricinoleate, Butylene Glycol, Magnesium Stearate, Cera Alba, Glyceryl Oleate, Cetyl Palmitate, Methyl Caprylate, Magnesium Sulphate, Ethylparaben (0.4%), Glycerol, Ethylparaben (0.4%), Parfum, Sodium Pyrrolidone Carboxylic Acid.

MD70002-1 Cream Type O/W (*preservative-system: preservative-free*):

Aqua, Ceteary Isononaote, Alcohol, Caprylic/Capric Triglyceride, Dicaprylyl Ether, Cetearyl Alcohol, Glycerin, Glyceryl Stearate Citrate, Sucrose Stearate, Ethylhexylglycerin, Parfum, Xanthan Gum, Acrylates/C10-30 Alkyl Acrylate Crosspolymer.

MD70001A Cream Type W/O (*organic acid preservative-system: Potassium Sorbate/Sodium Benzoate*):

Aqua, Caprylic/Capric Triglyceride, Helianthus Annuus Seed Oil, Glycerin, Polyglyceryl-3 Polyricinoleate, Butylene Glycol, Magnesium Stearate, Cera Alba, Glyceryl Oleate, Cetyl Palmitate, Glyceryl Caprylate, Magnesium Sulphate, Potassium Sorbate (0.4%), Sodium Benzoate (0.3%), Parfum, Sodium Pyrrolidone Carboxylic Acid, Citric Acid.

MD70004-6 Lotion Type O/W (*organic acid preservative-type system: Potassium Sorbate/Sodium Benzoate*):

Aqua, Caprylic/Capric Triglyceride, Glyceryl Stearate Citrate, Cetearyl Alcohol, Glycerin, Glyceryl Caprylate, Sucrose Stearate, Potassium Sorbate (0.6%), Xanthan Gum, Sodium Benzoate (0.5%), Parfum, Carbomer.

Table 1 Irritation grading scale.

Visual Assessment ICDRG	Visual Assessment	Observed Skin Reaction
–	0	No visible skin reaction
+/-	0.5	Barely perceptible or spotty erythema
+	1/1.5*	Mild erythema covering most of the test site
++	2/2.5*	Moderate erythema, possible presence of mild oedema or a few papules
+++	3/3.5*	Marked erythema, possible extensive and oedema or papules
++++	4*	Severe erythema, possible oedema, vesiculation

*No further product application (Multiple EPI only).

Table 2 Sex, age and skin type distribution of test subjects

Study	Test subjects (n)	Age	Female/Male (ratio)	Skin sensitivity to SDS irritation (%)
Short-term EPI 20 min Single EPI 24 h Multiple EPI	30	22–70	~60%/40%	~30%
Short-term EPI 20 min Short-term USE (MD7001A and MD7004-6)	12	21–53	100%/0%	~41%
Short-term Use Long-term Use	22	24–76	100%/0%	~60%

Methods

Ethics and general design

Prior to use, safety assessments and microbiological tests were conducted to ensure the safety for human health under use conditions. The recommendations of the current version of the Declaration of Helsinki and the ICH Good Clinical and Research practices (GCP) guideline were observed as applicable to a non-drug study. All volunteers were informed concerning the objective, possible risks of the study and gave their informed consent prior to initiation of the study. The studies were performed at different single centres with double-blind randomized product applications and under dermatologically controlled conditions and the supervision of a dermatologist.

Test subjects

The subjects were selected according to the following inclusion and exclusion criteria: inclusion criteria: Caucasian female or male aged between 18 and 76 years, volunteers being in general good health and mental condition, and exhibiting healthy skin in the test areas. Exclusion criteria included pregnancy or breastfeeding, known allergies to cosmetics and cosmetic ingredients, previous adverse reactions to materials used in the study (e.g. plasters), skin diseases or dermatological disorders (e.g. scars, sunburn, tattoos, moles and/or irritation) which could interfere with the evaluation of possible reactions, medication such as antibiotics or anti-inflammatory drugs as well as the regular use of sun beds which could also affect the skin response.

Single application epicutaneous 24-h patch test (Single EPI 24 h) and multiple application epicutaneous patch test (Multiple EPI)

The single application epicutaneous 24-h patch test (Single EPI 24 h) and the multiple application epicutaneous patch test (Multiple EPI) were conducted as a combination study. 100 µL of the test product was applied using occlusive patches (1 cm diameter, Leukotest; Beiersdorf AG, Hamburg, Germany) and affixed [Fixomull stretch template (BSN medical, Hamburg, Germany, 15 cm × 20 cm)]. All products were applied to the upper back, where they remained for 24 h (day 1) and for 21 h (days 2, 3 and 4). Visual scoring was performed in accordance with a pre-defined irritation grading scale 3 h after removal of the patches (Table 1). Evaluations on day 1 were considered to be the Single EPI 24 h results, whereas the sum of reactions on days 1, 2, 3 and 4 represented the Multiple EPI results. If a level 1 (or higher) reaction in Multiple EPI had occurred, no further application of the test formulation for that specific study subject was applied. All of the

equipment and instruments needed to perform the study were prepared and operated by trained and experienced personnel. All assessments were performed under normal atmospheric conditions.

Short-term in-use study (Short-term USE) and long-term in-use study (Long-term USE)

Short-term in-use study (Short-term USE) and long-term in-use study (Long-term USE) were conducted as a combination study with both conducted as half-side tests. During the preconditioning phase and during the main study, subjects were allowed to use their personal daily cleansing products and their decorative products without any change in their normal regimens. Subjects were supplied with one unit of each test product and instructed to apply it to one side of the face twice daily as a substitute for their personal facial skincare product.

Baseline assessments were made prior to the test product application (t0). Approx. 30 min after first application, skin reactions were assessed (t1). These t1 reactions represent the Short-term USE test results. However, in case of unexpected skin reactions, the study was then terminated. After 2 weeks of specified use, possible reactions were assessed and this time point represents the Long-term USE results (t2). The following assessment methods were applied: dermatological assessment of visible or tactile skin conditions on each half of the face regarding erythema, oedema, dryness, scaling, fissures, papules, pustules and vesicles. Subjective assessment of skin conditions on each half of the face regarding itching, burning, tension, tickling and dryness by the volunteer via questionnaire.

Short-term epicutaneous patch test (Short-term EPI 20 min)

For the short-term epicutaneous patch test (Short-term EPI 20 min), products were applied to the backs of approx. 30 volunteers with occlusive patches [Hill Top Chamber[®], 1 cm diameter, exposure area: 0.79 cm² (Hill Top Research, St. Petersburg, FL, USA)] for 20 min. 100 µL of each test product was applied to the patch with a pipette. Positioning of treatment locations was permuted from subject to subject by means of a randomization scheme. Visible skin reactions (e.g. erythema) were assessed by clinical scoring 15 min after the removal of the patches under day light conditions and applying an irritation grading scale (Table 1).

Data analysis

Data analysis was primarily based on the frequency of skin reactions. A skin reaction graded as 1 or greater was considered to be a positive reaction. Information on the skin reactions are presented

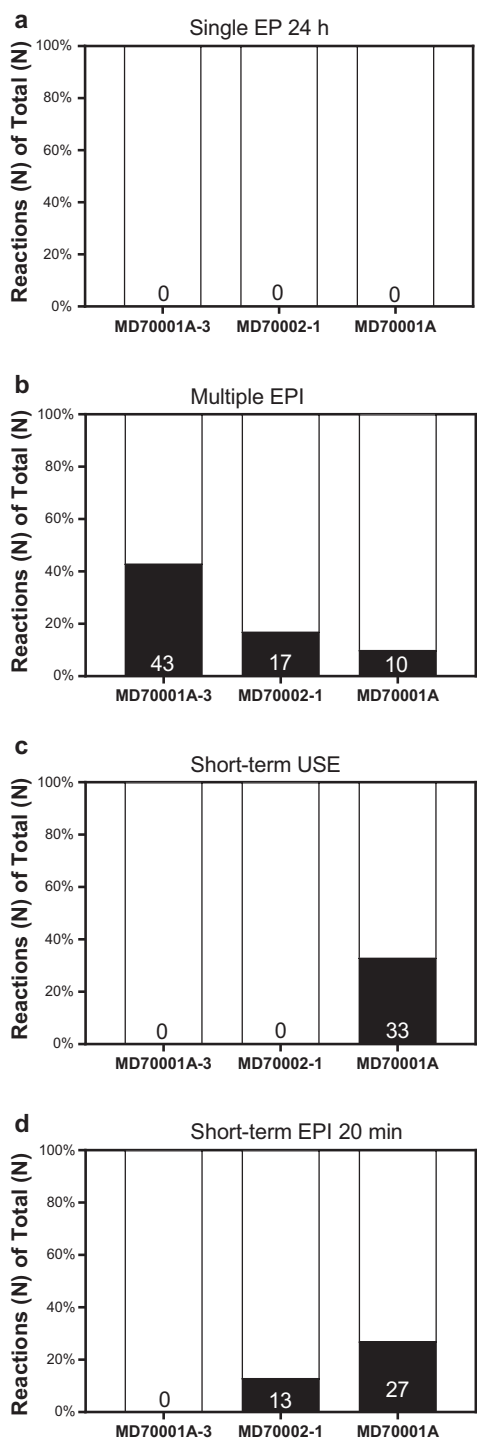


Figure 1 Reactions to formulations MD70001A-3, MD70002-1 and MD70001A and are depicted [as % of volunteers reacting to the formulations with objective reactions in ECTs or USE studies with an irritation level 1 (or higher) with or without subjective discomfort reactions (USE test only)]: (a) Single EPI 24 h, (b) Multiple EPI, (c) Short-term USE and (d) Short-term EPI 20 min.

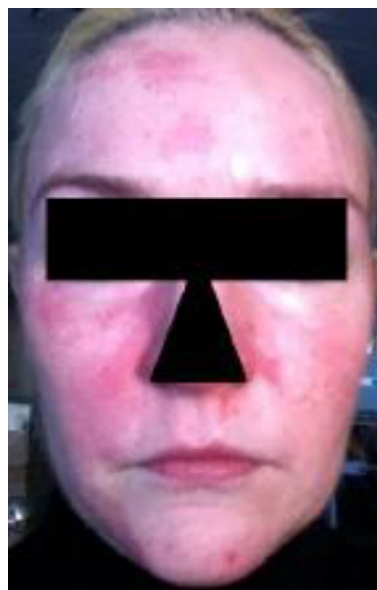


Figure 2 Representative photograph of immediate-type skin reaction after application of formulation MD70001A in the Short-term USE test.

as the number and percentage of volunteers having skin reactions. To calculate the intensity of test reactions, the scores for all reactions were summed and divided by the number of test subjects.

For the pairwise comparison of reaction rates between groups, the Fisher's exact test was applied on an alpha-level of 5% two-sided to identify significant differences in rates.

Results

In an attempt to verify predictivity of results derived from closed epicutaneous patch tests for possible irritant reactions that may occur in open application in-use studies, three different formulations MD70001A-3, MD70002-1 and MD70001A were developed. These formulations were of different composition regarding their ingredients and emulsion types, and in the preservative systems used. They were developed with the initial aim of possibly having different degrees of irritation take place. Table 2 gives an overview of the test subjects characteristics for the different tests and formulations.

Determination of the skin irritation potential in the Single EPI 24 h and Multiple EPI

In a first phase, Single EPI 24 h and Multiple EPI tests (both with occlusive patches) were initiated in order to evaluate possible differences in irritation potentials of the formulations MD70001A-3, MD70002-1 and MD70001A depending on the study protocol. As depicted in Figure 1a, these three formulations did not cause any reactions in the Single EPI 24 h, whereas differences in the irritation potential in the Multiple EPI were observed (Fig. 1b). Furthermore, 43% of the subjects developed erythema after application of formulation MD70001A-3; the percentages of reactions for formulations MD70002-1 and MD70001A were significantly lower with 17% and 10% reactions occurring, respectively (Fig. 1b).

Table 3 Mean intensity scores after patch test removal or after short-term exposure under use conditions

	Single EPI 24 h	Short-term EPI 20 min	Short-term USE (Erythema)
MD70001A-3	0.15	0.02	0.00
MD70002-1	0.08	0.17	0.00
MD70001A	0.00	0.41	0.60
MD70004-6	0.40	2.46	1.54

Determination of the skin compatibility in the Short-term USE test

The formulations were then tested using the Short-term USE and Long-term USE test protocols. In these studies, products were applied in open application under use conditions thereby more closely resembling the use of cosmetics by the consumer. The objective of study was to answer the question, whether differences in irritation potentials of formulations in the epicutaneous patch test can be substantiated in the open application in-use study types (USE). The results of Short-term USE are given in Figure 1c: Formulation MD70001A unexpectedly induced immediate-type effects in 33% of study subjects, which were characterized by the subjects spontaneously developing erythema and subjective sensations of discomfort within 30 min after application of the test product (Fig. 2). These reactions disappeared in the following 1–2 h. The reactivity to formulation MD70001A was neither predicted by the Single EPI 24 h nor by the Multiple EPI study results, most likely because of the much longer time period of at least 24 h until reactions are assessed. As a consequence of the reactions, further application of the products for the long-term use with formulation MD70001A to the subjects exhibiting reactions was subsequently discontinued.

Short-term EPI: Alternative to Single EPI 24 h and Multiple EPI

To further analyze the spontaneous reaction types observed when applying formulation MD70001A and to answer the question, whether a modified epicutaneous patch could predict these type of reactions, the following study type was initiated: a short-term epicutaneous patch test with an application period of 20 min and with evaluations made after 15 min (Short-term EPI 20 min). An application period of 20 min was specifically selected, as in the application test, reactions occurred within 30 min after leave-on product application. The formulations MD70001A-3, MD700002-1 and MD70001-A were investigated. A seemingly inverse reaction profile in comparison to the Multiple EPI was obtained: Formulations MD70001A-3 and MD70002-1 demonstrated no or only a low number of short-term reactions, whereas formulation MD70001-A triggered erythematous reactions in 27% of study subjects (Fig. 1d). This result is in good correlation with the spontaneous reactions observed in the earlier open application study with this formulation, and in which further applications were then discontinued. Moreover, the mean intensity scores were similar in the Short-term EPI 20 min and Short-term USE test. The mean intensity scores were 0.02 (MD70001A-3), 0.17 (MD70002-1) and 0.41 (MD70001A) for the Short-term EPI 20 min and are 0.00 (MD70001A-3), 0.00 (MD70002-1) and 0.60 (MD70001A) for the Short-term USE test. However, in the Single EPI 24 h, the mean intensity score ranges were below 0.15 (Table 3).

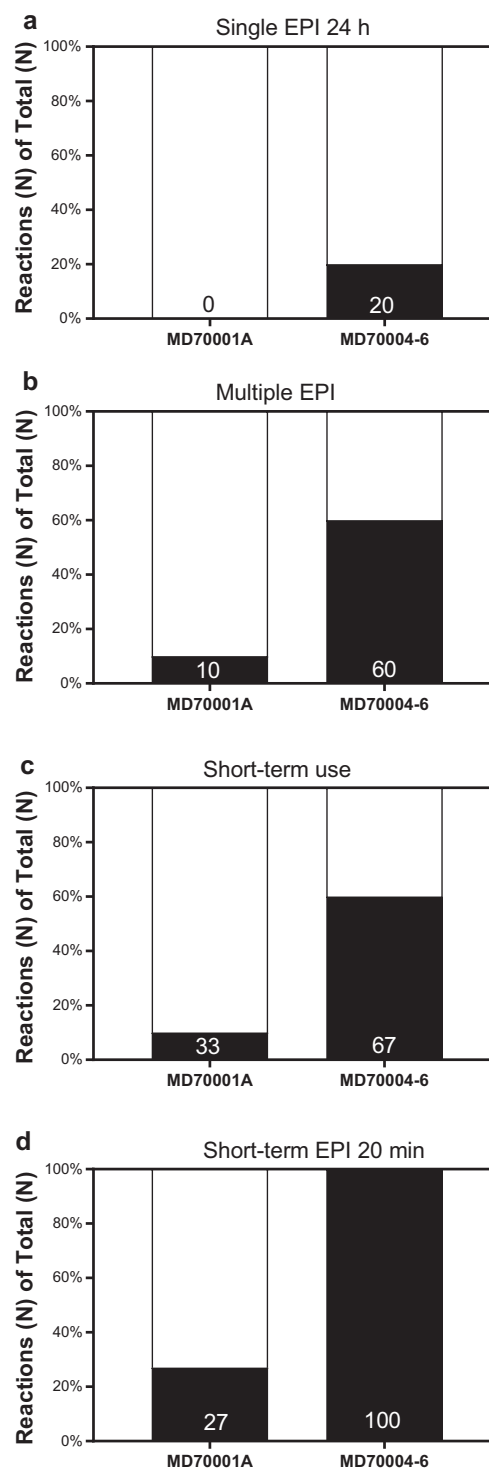


Figure 3 Reactions to formulations MD70001A and MD70004-6 are depicted as % of volunteers reacting to the formulations with objective reactions in ECTs or USE studies with an irritation level 1 (or higher) with or without subjective discomfort reactions (USE test only): (a) Single EPI 24 h, (b) Multiple EPI, (c) Short-term USE and (d) Short-term EPI 20 min.

Table 4 Statistical analysis of reaction rates between the different skin compatibility tests

Test		P-value
Single EPI 24 h	Short-term USE	0.1219
Single EPI 24 h	Short-term EPI 20 min	0.0699
Short-term EPI 20 min	Short-term USE	0.8339
Multiple EPI	Single EPI 24h	0.0005
Multiple EPI	Short-term USE	0.1060
Multiple EPI	Short-term EPI 20 min	0.1134

Skin irritation results with a formulation containing a higher concentration of the suspected urticant

To experimentally challenge the study protocols EPI 24 h and Multiple EPI and to validate the first results of the Short-term EPI 20 min as being indicative of these types of reactions, a further formulation (MD70004-6) containing an even higher concentration of the ingredients of formulation MD70001A suspected of causing the previously described immediate reactions was developed. To this accord, the Potassium Sorbate/Sodium Benzoate concentrations were increased from 0.4%/0.3% (MD70001A) to 0.6%/0.5% (MD70004-6).

As hypothesized, a higher number (100%) of study subjects demonstrated immediate-type erythema in the Short-term EPI 20 min and 67% of the subjects developed reactions in Short-term USE studies with formulation MD70004-6 (Fig. 3c/d). More interestingly, even in the EPI 24 h, 20% of subjects and in the Multiple EPI 60% of subjects reacted with erythema (Fig. 3a/b) indicating that the higher concentrations may also lead to typical irritative reactions. In addition, the mean intensity of irritant reactions was similar in the Short-term EPI (2.46) and Short-term USE test (1.54) compared to the Single EPI 24 h (0.40). Thus, a clear difference between the Single EPI 24 h and Short-term USE test values was observed (Table 3). The results show also that the intensity scores are concentration dependent.

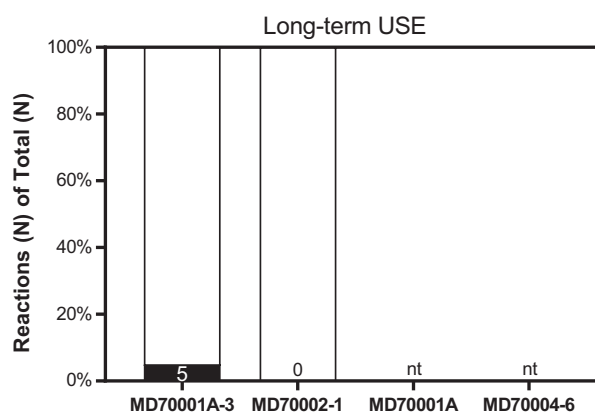
The reaction results for all formulations obtained in the skin compatibility tests were also statistically analysed for differences using Fisher's exact test. Table 4 presents the results of the statistical analysis. Since the reaction rates between Short-term EPI and Short-term Use, were almost identical, the statistical analysis showed a *P*-value of 0.8339, indicating a lack of statistically relevant differences in reactions. In contrast, the generally used value of *P* = 0.05 indicating statistical significance was almost achieved

Table 5 Summary of study results based on percentage of reactions

Formula	Characteristics	Short-term EPI 20 min*	Single EPI 24 h	Multiple EPI	Short-term USE*	Long-term USE
MD70001A-3	Cream Type W/O	0%	0%	43%	0%	5%
MD70002-1	Cream Type O/W	13%	0%	17%	0%	0%
MD70001A	Cream Type W/O	27%	0%	10%	33%	nt
MD70004-6	Lotion Type W/O	100%	20%	60%	67%	nt

Possible concerns for further formulation development of the formulation is depicted by colour: Criteria for green (GO), orange (ATTENTION), and red (STOP) are study type-specific considering intensity and kinetics of the reaction, data are offered as reactors in percentage, nt not tested.

*Primarily immediate-type reactions.

**Figure 4** Study results of Long-term USE test. Reactions to formulations MD70001A and MD70004-6 are depicted as % of volunteers reacting to the formulations with objective reactions with an irritation level 1 (or higher) with or without subjective discomfort reactions.

(*P*-value of 0.0699) when comparing the Single EPI 24 h and the Short-term EPI 20 min. The difference in the reaction rates between Single EPI 24 h and Short-term USE is also not significant (*P* = 0.1219) but a general trend in differences in reactions is observable. Thus, the *P*-values support differences in reactions between the Single EPI 24 h and the Short-term Use or Short-term EPI 20 min. On the other hand, similar reaction rates are observed between Short-term EPI 20 min and Short-term Use. The Multiple EPI shows significant differences in results obtained when compared to the Single EPI 24 h (*P* = 0.0005); when compared to the Short-term EPI 20 min (*P* = 0.1134) and Short-term use test (*P* = 0.1060) differences are only just not significant. The Multiple EPI does not therefore show similar reaction rates to any of the other three tests.

Results of epicutaneous patch test versus long-term USE

Differentiation of the irritation potentials of formulations MD70001A-3 and MD70002-1 using the Multiple EPI was possible, but not when using the Single EPI 24 h (Fig. 3a/b). What about their potential to cause reactions in Long-term USE studies (Long-term USE)? The results are shown in Fig. 4. The reactions elicited by these formulations were no cause for concern in these types of studies, and only formulation MD70001A-3 demonstrated reactions in 5% of study subjects. In other words, there was no

clear correlation of Single EPI 24 h and Long-term USE results, and the differences in Multiple EPI between both formulations being on a moderate level are only minor and are mirrored by Long-term USE study protocols.

Importance for interpretation

Table 5 offers a summary of study results with an indication the level of concern of a study specific result that would typically influence approvals of formulations for market release. Three categories are depicted: category green represents results with little concern, category orange indicates the results be further verified by further studies or alternative study protocols and, finally, category red specifies results of high concern typically meaning a stop of the further formulation development. Clearly, only formulations MD70001A-3 and MD70002-1 are market-compatible, whereas both formulations MD70001A and MD70004-6 failed but primarily because of transient immediate-type effects.

Discussion

Epicutaneous patch testing (single and/or multiple application protocols) is an initial in vivo method to screen for possible skin irritation properties of a substance or formulation. Although the ECT results in this study did not indicate substantial irritation potentials, immediate-type reactions (stinging and redness) were observed in some volunteers during the use tests which disappeared within approximately 1 h. Although transient, these reactions suggested that consumer acceptance would probably be low and these studies were discontinued. These unexpected results were nevertheless intriguing and prompted the start of a journey to see if patch test protocols could be modified to assess these reactions. An occlusive 20 min short-term patch test protocol (application time based on the time observed when reactions were observed in the short-term USE test) with evaluations made 15 min after patch test removal was developed. It should be noted that there are parallels with the open patch test in terms of duration of application, but the open patch test is designed to exclude irritant reactions due to substances or formulations with a potentially higher irritation potential for example for rinse-off products which, such as household products, remain on skin just for a short time only [5]. Successful identification of the spontaneous reactions was possible and there was a correlation between the intensity of reactions observed in the short-term patch test and those observed in the controlled in-use studies.

Immediate-type reactions to cosmetic products and ingredients are rare but have been described for some substances. Immediate-type urticarial reactions can be categorized into two major types – immunologic (allergic; ICoU) and non-immunologic (irritative) contact urticaria (NICoU). NICoU is more prevalent than ICoU but rarely associated with serious adverse effects and/or systemic reactions. Although possibly not full-fledged NICoU, the induction of transient skin redness and tingling or stinging sensations has been reported for a number of cosmetic ingredients, for example cinnamic acid/aldehyde, methyl salicylate, dimethyl sulfoxide, nicotinic acid esters, benzoic acid/aldehyde, sodium benzoate and potassium sorbate (non-exhaustive) [7-9]. Vehicle effects have also been described, with propylene glycol and isopropanol enhancing the effects of benzoic acid reactions [7]. Coverly et al. (1998) conducted comparative studies to assess the relationship between stinging, NICoU and skin irritation [10]. A short-term type patch test was used to assess NICoU in which the products were applied to the volar forearm for 20 min

and reactions assessed 10 min after patch removal. The substances were applied in petrolatum. During this time, lactic acid was applied to the nasolabial fold to assess if the volunteer is prone to develop stinging sensations ('stinger'). This group reported that for SDS-induced irritancy and most of the tested urticants there was no difference between 'stingers' and 'non-stingers' and their propensity to develop irritation and/or NICoU or differences in the intensity thereof. Reactions to trans-cinnamic acid and benzoic acid were higher in stingers. Adverse reactions to one urticant was not predictive for that of other urticants. Zhai et al. (2012) initiated a study to gain more insights into effects the anatomic region may have and studied the elicitation of NICoU of benzoic acid and hexyl nicotinate (HN) on the scalp, back and face of human volunteers. The back was more sensitive to HN at low concentrations than the other two regions, but no differences were observed at higher concentrations. Clear cut differences in sensitivity were not observed for reactions with benzoic acid [11,12].

As the typical symptoms of facial redness and/or the unpleasant sensory effects are transient in that they usually appear after 10 min and disappear after 1 h, they can considerably diminish consumer acceptance of products eliciting reactions. They may also be involved in why consumers consider themselves to have 'sensitive skin'. These can occur although rigorous toxicological evaluations and safety assessments have been conducted. As benzoates and sorbates were used as preservatives in this study (at concentrations allowed by the EU Cosmetics Regulation) and immediate-type reactions typical of these products occurred, this was indicative of these substances being the culprits eliciting these reactions. If ingredients associated with immediate-type effects are used, it may be beneficial to assess the proclivity to induce reactions early on in product development and in particular prior to large scale consumer studies. One possibility is to use the short-term patch test protocol described in this study for formulation or substances containing potential urticants.

Conclusion

Although the epicutaneous patch testing is routinely used as a screening method in the development and optimization of cosmetic formulations, it does not allow all reactions to be assessed and surprises can be encountered. A number of ingredients can cause spontaneous although transient reactions, such as redness possibly indicative of reaction such as non-immunological contact urticaria. As these reactions are transient and usually resolve within 1–2 h, typical ECT protocols do not allow detection of immediate-type reactions. The lessons learned from this study indicate that simple modifications of existing test protocols can lead to important insights into skin reactions. Subsequently, these modifications can be used to create further building blocks leading to an optimization of test strategies for newly developed cosmetic formulations and finally provide reliable study designs for possible reactions. The preliminary goal of this study was to investigate to what extent the patch test results and the use test show similar results regarding skin compatibility of skincare products. Interestingly, it resulted in improved insights for other reactions – an unexpected journey but this is one path science can take.

Acknowledgements

We thank all the volunteers who made this investigation possible. This study was funded by the Deutsche Gesellschaft für

Wissenschaftliche und Angewandte Kosmetik (DGK: German Society for Scientific and Applied Cosmetics) and conducted by the DGK Safety and Compatibility Task Force.

Disclosure

Other than employment, the authors report no conflicts of interest in this work.

References

1. EU Cosmetic Regulation (EC) No. 1223 \2009; <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF>.
2. Mehling, A., Chkarnat, C., Degwert, J. *et al.* Interlaboratory studies with a proposed patch test design to evaluate the irritation potential of surfactants. *Contact Dermatitis*. **62**, 157–64 (2010).
3. Walker, A.P., Basketter, D.A., Baverel, M. *et al.* Test guidelines for the assessment of skin tolerance of potentially irritant cosmetic ingredients in man. European Cosmetic, Toiletry and Perfumery Association. *Food Chem. Toxicol.* **35**, 1099–106 (1997).
4. Cosmetics Europe. (1997) "Product test guidelines for the assessment of human skin compatibility"; https://www.cosmeticseurope.eu/files/6014/6407/8875/Product_Test_Guidelines_for_the_Assessment_of_Human_Skin_Compatibility_-_1997.pdf.
5. Ennen, J., Degwert, J., Duttine, M. *et al.* Studies on the skin compatibility of cosmetics involving human subjects - Study portfolio and test strategy. *IFSCC*. **17**, 19–23 (2014).
6. Horita, K., Tanoue, C., Yasoshima, M., Ohtani, T. and Matsunaga, K. Study of the usefulness of patch testing and use test to predict the safety of commercial topical drugs. *J. Dermatol.* **41**, 505–13 (2014).
7. Lahti, A. and Nojonen, S.L. Propylene glycol in an isopropanol vehicle enhances immediate irritant reactions to benzoic acid. *Contact Dermatitis*. **39**, 150–1 (1998).
8. Lahti, A. Non-immunologic Contact Urticaria. In: *Handbook of Occupational Dermatology*. (Karneval, L., Wahlberg, J.E., Elsner, P. and Maibach, H.I., ed.), pp. 221–224. Springer, Heidelberg (2000).
9. Merk, H.F. Contact Urticaria and Contact Urticaria Syndrome. In: *Environment and Skin*. (Krutmann, J. and Merk, H. ed.), pp. 51–55. Springer Nature Switzerland AG, Basel (2018).
10. Coverly, J., Peters, L., Whittle, E. and Basketter, D.A. Susceptibility to skin stinging, non-immunologic contact urticaria and acute skin irritation; is there a relationship? *Contact Dermatitis*. **38**, 90–5 (1998).
11. Zhai, H., Zheng, Y., Fautz, R., Fuchs, A. and Maibach, H.I. Reactions of non-immunologic contact urticaria on scalp, face, and back. *Skin Res. Technol.* **18**, 436–41 (2012).
12. Gimenez-Arnau, A., Maurer, M., De La Cueva, J. and Maibach, H. Immediate contact skin reactions, an update of Contact Urticaria, Contact Urticaria Syndrome and Protein Contact Dermatitis – "A Never Ending Story". *Eur. J. Dermatol.* **20**, 552–62 (2010).