

## INVESTIGATIVE REPORT

# The Tandem Repeated Irritation Test: A New Method to Assess Prevention of Irritant Combination Damage to the Skin

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The effect of a protective cream was tested in a new tandem repeated irritation test with tandem application of 0.5% sodium lauryl sulphate (SLS) and undiluted toluene. The irritants were applied twice daily for 30 min to the ventral forearms of 20 volunteers. Irritant cutaneous reactions were quantified by a visual score, transepidermal water loss, chromametry and skin capacitance. Concurrent application of SLS/toluene induced stronger reactions than those caused by twice daily application of each irritant on its own. A protective effect of the protective cream was obtained against all treatment combinations and was significant for SLS/SLS ( $p \leq 0.01$ ) and SLS/toluene ( $p \leq 0.05$ ). Our results indicate that the tandem repetitive irritation test has great potential in the evaluation of skin care products to prevent irritant contact dermatitis. *Key words: tandem repeated irritation test (TRIT); irritant contact dermatitis; protective cream; sodium lauryl sulphate; toluene; bioengineering methods.*

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Much effort has been undertaken to develop valid methods for evaluation of the benefit of protective creams to prevent irritant contact dermatitis (ICD). Since Suskind introduced the “slide test” to evaluate protective creams in the 1950s (1) various *in vitro* and *in vivo* studies have been performed to investigate both the effects of irritants on skin barrier function and the benefit of protective creams under experimental conditions (1–8). However, none of these studies is considered to be close enough to real workplace situations.

In 1994, Frosch & Kurte introduced the repetitive irritation test (RIT) with cumulative irritation over a 2-week period by standard irritants such as sodium lauryl sulphate (SLS), sodium hydroxide, lactic acid and toluene (9). This model has been used in many laboratories as a routine procedure, as it was considered to be suitable for comparing protective creams simultaneously with non-pretreated control sites on the backs of

volunteers (10). However, manufacturers of skin-care products prefer easy study protocols that provide valid data in a short time with few restrictions for the volunteers. Therefore, short duration and easy application given in a one-week test using the forearms of healthy volunteers was highly desirable.

As a first step, a test model on the basis of the RIT was developed to optimize the concentration of irritants against which protective creams are tested and to evaluate the necessary cumulative application time (11, 12). It could be demonstrated that a one-week period was enough to evaluate the efficacy of protective creams against most irritants even if lower concentrations of irritants were used.

Based on the RIT, a multicentre study subsequently was designed to standardize a test procedure for the evaluation of skin-protective products. In this irritation study, a repeated short-time occlusive irritation test (ROIT) was evaluated (13). Using 2 irritants (SLS and toluene, each applied twice daily for 30 min), the evaluation showed that significant results could already be achieved with the 5-day protocol.

It could be criticized that in all models presented, the investigation of protective cream efficacy has been limited to the exposure of a single irritant only. The anionic surfactant SLS and the organic solvent toluene have mainly been used although repetitive contact to both hydrophilic and hydrophobic substances together or, more commonly, one after the other, occurs regularly in the workplace setting.

We recently investigated concurrent application of SLS and toluene, which showed that a mixed application of these irritants induced significantly stronger reactions than those caused by twice daily application of each irritant on its own (14). It is obvious that the additive effect is important for the use of protective creams in practice and the way they should be tested.

Therefore, the sequential application of 2 irritants in the so-called tandem repeated irritation test (TRIT) was investigated in the present study to evaluate the benefit of a commercially available protective cream compared with non-pretreated control sites.

## MATERIAL AND METHODS

### Subjects

Twenty healthy non-preselected Caucasian volunteers (17 females and 3 males; aged 18–49 years, median 29 years,)

without any skin diseases were included. Informed consent was obtained from all participants, and the study was approved by the local ethics committee. Subjects were allowed to bathe as usual, but were instructed to avoid direct application of detergents, moisturizers or emollients on their forearms during the 5 days of investigation.

#### Protective cream

The composition (Stokodem®, Stockhausen, Krefeld, Germany) was in accordance with the INCI declaration: aqua, octyl stearate, glyceryl stearate SE, glycerin, cetearyl alcohol, sodium bischlorophenyl sulphamine, isopropyl palmitate, glycol distearate, xanthan gum, cetareth-6, phenoxy-ethanol, methylparaben, ethylparaben, propylparaben, butylparaben, cetareth-25, fragrance.

#### Procedure

The application area was the clinically normal skin of the medial volar forearms. The placement of test fields and arms (6 chambers on the right and left forearms) was randomized. Three test fields were treated with 0.05 ml protective cream rubbed onto a skin area 2 cm in diameter with a gloved finger. The other test fields served as untreated controls. After 10 min pretreatment, the irritants were applied on all 6 premarked test sites on the forearms for 30 min under occlusion (Finn Chambers, 12 mm diameter, filling volume 0.05 ml; Epitest Ltd., Hyrlä, Finland). The volunteers were tested with 0.5% aqueous SLS (Sigma, St. Louis, MO) or undiluted toluene (E. Merck, Darmstadt, Germany). After removal of the patches, the skin was cleaned with a dry paper tissue. A second exposure with 0.5% aqueous SLS or undiluted toluene was performed the same day, after 3 h. Thus, 3 treatment combinations were investigated, resulting in a repeated irritation caused by SLS/SLS; toluene/toluene; and SLS/toluene and the pre-irritation application of Stokodem on the respective test areas. Since in a previous study the exact chronological order of the irritants was shown not to have any effect on the degree of irritation (14), the combination toluene/SLS was not included in the present study. Using this scheme of application, the volunteers were treated from day 1 to day 4 (in each case at the same time of day).

#### Evaluation methods

The study was carried out from October to November 2000. All visual scorings and bioengineering measurements to compare the intensity of reactions were performed daily before starting treatments (days 1–4) and on day 5, by the same observer under controlled environmental conditions. All measurements were carried out in an air-conditioned room (room temperature 20–22°C, relative humidity between 34% and 46%) after 30 min for equilibration.

*Clinical score* graded for erythema, scaling and fissuring was recorded according to Frosch & Kligman (15).

*Transepidermal water loss* (TEWL) (expressed in g/m<sup>2</sup>h) was measured using an evaporation meter (Tewameter TM 210, Courage & Khazaka, Cologne, Germany). Measurements were taken according to the Guidelines of the Standardization Group of the European Society of Contact Dermatitis (16).

*Instrumental colour measurements* were taken with a Minolta Chromameter (CR-200, Minolta, Osaka, Japan) according to published recommendations (17). The colour coordinates were expressed in the L\*a\*b\* 3-dimensional colorimetric system. The a\* value is the component of separation between red (positive value) and green (negative value) as a sensitive measure for quantifying erythema.

*Electrical capacitance*, indicating the hydration level of the skin, was measured by a Corneometer CM 825 (Courage & Khazaka, Cologne, Germany) (18).

If the clinical score progressed to a severe degree ( $\geq 5$ ), exposure was discontinued. For these test areas, the maximal scores and the measured values for TEWL, chromametry, and capacitance obtained on the day of discontinuance were used for the final calculation.

#### Statistics

Statistical analysis was conducted using SPSS/PC+ (Version 10.0, SPSS, Chicago, ILL, USA). Data of visual scoring are presented as means  $\pm$  SEM. TEWL, skin colour a\*, and skin hydration (differences between baseline values and after irritation) were determined. As the data were not normally distributed, the differences between means were checked for significance using the Wilcoxon test for paired data for the erythema score, the comparison of TEWL, skin capacitance and skin colour. The chosen level of significance was  $p \leq 0.05$ .

## RESULTS

The results of the TEWL are presented in Figs. 1–3 as means  $\pm$  SEM. All data of day 5 are presented in Table I.

Repeated application of SLS 0.5% twice daily induced an irritant reaction indicated by a moderate increase in the visual scoring, a more pronounced increase in the TEWL values, a decrease in skin hydration, and an increase in the a\*-values that confirmed the visual scoring. There was a highly significant difference ( $p \leq 0.01$ ) between SLS-treated sites and those that were pretreated with Stokodem on day 5 for the visual score, TEWL (Fig. 1) and chromametry.

In contrast to SLS/SLS, the application of toluene/toluene caused only a moderate increase in the TEWL over the study period, which was slightly suppressed by Stokodem (Fig. 2). A moderate benefit of the test product against toluene was also confirmed by the measurement of skin capacitance and the chromametry, whereas the visual score showed contrary results.

Monitoring of the instrumental measurements and the visual score following sequential application of SLS/toluene showed that the induced reactions were significantly stronger than those caused by twice daily application of the single irritants SLS or toluene. Additionally, pretreatment with Stokodem suppressed the irritant reaction presented by all measurements. The TEWL and the visual scoring indicated a significant benefit of the product tested (Fig. 3).

## DISCUSSION

Though protective creams are one of the common measures to prevent ICD, their actual benefit in the workplace is still regarded with scepticism (19) and has been debated in recent reviews (20–22). Reasons explaining a lack of protection in practice are inefficient products (23), products that are effective against a special irritant while aggravating reactions caused by other irritants (11) or insufficient application of products on exposed skin areas (24). However, so far, an investigation of relevant

Table I. Effects of various treatment combinations (SLS, toluene) at day 5 for visual scoring, transepidermal water loss (TEWL,  $\text{g}/\text{m}^2\text{h}$ ), skin redness (chromametry,  $a^*$ ) and skin hydration measured by capacitance (arbitrary units). Mean change and SEM

Treatments		Visual Score	TEWL	Chromametry	Capacitance	
SLS/SLS	Control	Mean	1.95	20.92	3.14	-9.07
		SEM	0.25	3.34	0.75	2.15
	Stokoderm	Mean	0.95	12.15	1.12	-8.21
		SEM	0.18	1.74	0.41	2.07
Toluene/Toluene	Control	Mean	1.55	3.57	1.78	-14.33
		SEM	0.32	0.82	0.69	2.74
	Stokoderm	Mean	1.80	2.38	1.62	-11.43
		SEM	0.22	0.33	0.53	2.97
SLS/toluene	Control	Mean	3.75	32.65	5.76	-14.27
		SEM	0.27	5.92	0.79	3.50
	Stokoderm	Mean	2.65	14.54	4.60	-13.97
		SEM	0.34	2.44	0.84	2.10

TEWL: transepidermal water loss; SLS: sodium lauryl sulphate.

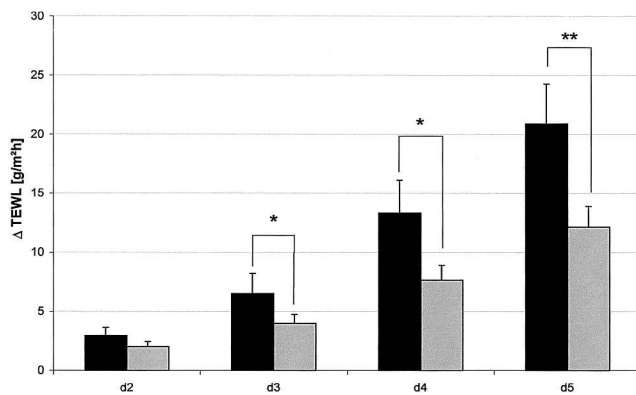


Fig. 1. Change in transepidermal water loss ( $\Delta$  TEWL) (mean  $\pm$  SEM,  $n=20$ ) after sequential application of SLS/SLS (sodium lauryl sulphate) (■) and Stokoderm/SLS/SLS ( $\eta$ ). On days 3, 4 and 5 the protective effect of Stokoderm on the irritation was statistically significant ( $*p \leq 0.05$ ,  $**p \leq 0.01$ ).

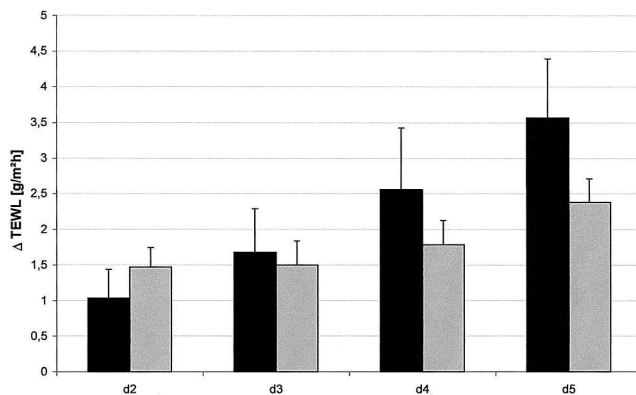


Fig. 2. Change in transepidermal water loss ( $\Delta$  TEWL) (mean  $\pm$  SEM,  $n=20$ ) after sequential application of toluene/toluene (■) and Stokoderm/toluene/toluene ( $\eta$ ). The protective effect of Stokoderm on the irritation was not statistically significant.

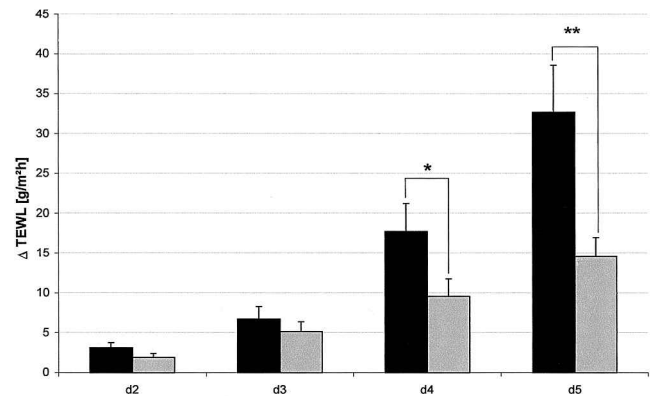


Fig. 3. Change in transepidermal water loss ( $\Delta$  TEWL) (mean  $\pm$  SEM,  $n=20$ ) after sequential application of SLS/toluene (sodium lauryl sulphate) (■) and Stokoderm/SLS/toluene ( $\eta$ ). On days 4 and 5 the protective effect of Stokoderm on the irritation was statistically significant ( $*p \leq 0.05$ ).

combinations of irritants against which protective creams can be tested has not been taken into account.

The most important risk factor for occupational contact dermatitis is the exposure to irritants. Well-known irritants are water (wet work), detergents and cleansing agents, hand cleansers, chemicals, cutting fluids and abrasives (25). Additionally, organic solvents are extensively used in many industrial applications. Moreover, in some professions contact with hazardous substances can be complex and manifold. For instance, workers in the metal industry are repeatedly exposed to water-based metal working fluids, neat oils, detergents and organic solvents. Therefore, the interaction between irritant chemicals has significant practical consequences.

Stokoderm<sup>®</sup> has previously been shown significantly to suppress the irritation caused by SLS and toluene as single irritants in an animal model. The irritants were applied daily for 2 weeks to the shaved back skin of young guinea pigs and the cream was applied 2 h prior

to and immediately after exposure to the irritants. Control animals were treated with the irritants only (26). It is gratifying that our results confirm the protective effect of Stokoderm against the single irritants, although the benefit against toluene was not significant in our test, which confirms the results for other creams. Additionally, the animal study did not assess combined irritation in a tandem model.

To establish a new method to evaluate the benefit of protective creams, we performed the study with SLS and toluene, which have been used as standard irritants in various types of patch tests (2, 3, 9–14, 20, 23, 26). These authors are aware of the necessity to evaluate further irritants. However, our results show that the TRIT seems to have considerable potential in differentiating the efficacy of protective creams in a relevant experimental setting that is quite close to a workplace situation where detergents and organic solvents are the major irritants used not exclusively, but concurrently. Nevertheless, this model must be validated by field studies under actual conditions of use. Interaction of further irritants should be investigated, with attention to professions where a multitude of hazardous substances may cause ICD. We hope that this model also proves useful in other hands to investigate both the effect of combinations of irritants to the skin and the way skin-care products may prevent contact dermatitis.

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