

Irritancy ranking of anionic detergents using one-time occlusive, repeated occlusive and repeated open tests

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Discrepancies between the one-time patch test and the wash test regarding the ranking of irritancy of detergents have been found in the literature. The aim of the present study was to investigate the concordance of irritancy rank order of 4 anionic detergents tested by 3 different exposure methods, namely one-time occlusive, repeated short-time occlusive and repeated short-time open tests. These detergents were sodium cocoyl isethionate (ISE), sodium lauryl sulfate (SLS), soap and disodium lauryl 3-ethoxysulfosuccinate (SUC). The reactions were evaluated by visual scoring and by transepidermal water loss (TEWL) measurement. When scored visually, the rank order in the one-time test was: SOAP $\geq$ SLS $\geq$ ISE $>$ SUC. The other test methods yielded a different order: SLS $>$ ISE $\geq$ SOAP $>$ SUC. A similar rank order was obtained with TEWL measurement for all exposure methods. Generally, the concordance among the different exposure methods was high when evaluated by TEWL. The concordance was lower when evaluation was performed by visual scoring. The present study demonstrates that the choice of exposure model and evaluation method may be important variables influencing the outcome of irritancy testing. It is proposed that the repeated open test is the best way to simulate most in-use situations where the uncovered skin is exposed to detergents. The repeated occlusive test or the one-time patch test may be better to simulate situations in which the skin is occluded after irritation by detergents.

Key words: detergents; irritancy; one-time patch test; prediction; rank order; repeated occlusive test; repeated open test; transepidermal water loss; sodium lauryl sulfate. © Munksgaard, 1999.

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Detergents are a major risk factor in the development of irritant contact dermatitis. This necessitates the search for less irritant detergents. It is possible to discriminate between detergents according to their irritancy by means of different types of exposure methods. These irritancy testing methods can be classified grossly as: one-time occlusive test, repeated occlusive test, repeated open test, immersion test and its modification, the wash test (1). These irritancy tests may yield different results regarding rank order of irritancy (1). Sodium laurate (soap) has been shown to be as irritant as sodium lauryl sulphate (SLS) only in the one-time occlusive (patch) test, but not in the immersion test, where sodium laurate was less irritant (2). To our knowledge, literature on the comparison of irritancy of detergents as tested by the patch test and the wash test, is sparse (3, 4). In 1 study (3), discrepancies were found in the rank or-

ders obtained by the 2 techniques. Another study found similar rank orders (4). In that study, the wash test appeared to have a greater power of discrimination.

Ideally, a test mirrors conditions encountered in daily practice. In that way, the repeated open test methods (repeated open test, immersion test and wash test) might be regarded as favourite for most situations. The disadvantage of repeated tests, however, is the fact that they are more time consuming than the 24-h patch test. A disadvantage of the immersion and wash tests is the fact that on the surface of 2 forearms only 2 different agents can be compared. The wash test has the further disadvantage of being less easy to standardize than repeated open exposure or immersion tests (5).

The aim of the present study was to investigate the concordance of irritancy ranking of 4 anionic detergents tested in 3 different exposure methods,

namely one-time occlusive, repeated short-time occlusive and repeated short-time open tests. These detergents were sodium cocoyl isethionate, sodium lauryl sulphate, soap (mixture of saponified fatty acids with different chain lengths) and disodium lauryl 3-ethoxysulfosuccinate.

Subjects and Methods

Subjects

25 healthy volunteers (15 female, 10 male, age from 18 to 33 years) participated in the study. None of them had past or current skin conditions. 6 subjects had a history of mucosal atopy. 1 person had skin type I, 9 persons had type II, 14 had type III, and 1 had skin type IV. The study was performed from May to June 1996. Informed consent was obtained from every subject, and the study was approved by the local ethical committee.

Agents tested

The following agents were tested: sodium cocoyl isethionate (ISE) (Elfan AT84, Akzo Nobel, Amersfoort, The Netherlands), sodium lauryl sulfate (SLS) (Merck 13760, USA), disodium lauryl 3-ethoxysulfosuccinate (SUC) (Elfanol 616, Akzo Nobel) and sodium soap of fatty acids derived from palm oil and coconut oil (SOAP) (Prisavon 9240, Uniqema International, Gouda, The Netherlands). This product also contained sodium chloride, glycerine, tetra sodium EDTA and tetra sodium etidronate. All agents were tested as an aqueous solution of 70 mM, except SLS, which was tested as an 8.75 mM solution. For ISE, SOAP and SUC a correction was made for the % active mass and chain length distribution. Analysis of alkyl chain length distribution was performed semi-quantitatively by gas chromatography of the methylated fatty acids. The fatty acids were isolated from the detergents by hydrolysis with 6 N HCl and extracted with ethyl acetate. The chain length distribution was calculated by normalization. As a result, the distribution percentage is a relative number instead of a mass %. Table 1 summarizes the concentration and chain-length distribution of each detergent.

One-time occlusive test

The one-time occlusive test was performed as follows: 0.2 ml of the solutions were applied on paper discs fitting in the extra large Finn Chamber, with an inner diameter of 18 mm (Epitest Ltd Oy, Tuusula, Finland). These chambers were fixed on the right side of the upper back by means of Fixomul (Beiersdorf, Hamburg, Germany). The exposure time was 24 h, after which the skin was washed thoroughly with a gauze wetted with tap water. The 24-h occlusive test was performed in the 3rd week of the experiment.

Repeated occlusive test

In the repeated occlusive test, a plastic strip on which 5 extra large Finn Chambers were mounted, was fixed on the volar side of the left forearm by means of non-adhesive bandages. The chambers were filled with 0.2 ml of the solutions. The distance between the most distal exposure site and the wrist joint was kept at 6 cm. In each subject, this most distal site was exposed to distilled water. Exposures had a duration of 2 h, 1× daily for 3 consecutive weeks, 5 days per week, according to the guidelines on SLS exposure tests from the European Society of Contact Dermatitis (1). After their removal the skin was cleaned with running water and gently dried with a cotton towel.

Repeated open test

For the repeated open test, use was made of a plastic strip with 5 holes (diameter, 20 mm) in which 0.8 ml of the solutions was applied. The strip was fixed to the volar side of the right forearm with non-adhesive bandages. The distance between the most distal exposure site and the wrist joint was kept at 6 cm. In each subject, this most distal site was exposed to distilled water. The solutions were exposed with a duration of 20 min, 1× daily for 3 consecutive weeks, 5 days per week. After their removal the skin was cleaned with running water and gently dried with a cotton towel. In all exposure methods the detergent solutions were exposed in a sequence which changed cyclically from

Table 1. Characteristics of the detergent solutions used: concentration and alkyl chain-length distribution

Detergent	Conc. ^{a)}	Chain-length distribution ^{b)}							
		C8	C10	C12	C14	C16	C18	C18:1 ^{c)}	C18:2
ISE	2.9	5	6	52	19	9	9		
SLS	0.25			100					
SOAP	2.3	1	1	9	4	38	5	34	8
SUC	11.6			58	42				

^{a)} Figures given are % (g/100 ml). ^{b)} Chain-length distribution in %. ^{c)} Figure behind colon refers to number of unsaturated bonds.

Table 2. Median values and range of total visual scores after last exposure

Exposure method	Exposed sites				
	Water	ISE	SLS	SOAP	SUC
one-time occlusive	0	0.5 (0–4.0)	1.0 (0–2.0)	1.0 (0–3.0)	0 (0–0.5)
repeated occlusive	0	1.0 (0–2.0)	3.0 (0.5–5.0)	1.0 (0.5–2.5)	0 (0–1.5)
repeated open	0	0.5 (0–1.5)	1.0 (0–3.0)	0.5 (0–1.5)	0 (0–0.5)

one subject to the next. The use of emollients was not allowed.

Evaluation

On each day the sites were graded visually for erythema, scaling and fissures, according to the “elaborate” scoring system for acute and subacute/cumulative irritant reactions, described in the guidelines on SLS exposure tests (1). The irritation qualities were recorded separately, and sum scores (total visual scores) were also recorded.

In all tests TEWL measurements were performed on day (D) 1, i.e., before exposure. In the one-time occlusive test TEWL was also measured on D 3, i.e., 24 h after removal of the one-time patch. In the repeated exposure tests TEWL was measured on the 5th day of each week. For TEWL measurement use was made of the Tewameter TM210 (Courage & Khazaka, Cologne, Germany). The measurements were performed in an air-conditioned room at a temperature of 19–21°C and a relative humidity of 40–50%, in which the volunteers acclimatized for 15 min, following the guidelines of the Standardization Group of the European Society of Contact Dermatitis on this topic (6).

Statistical analysis

Mean TEWL values on the exposed sites after the last exposure day were compared by paired *t*-tests. Also 95% confidence intervals for mean differences were calculated. Presented *p*-values are those of individual 2-sided *t*-tests. The results were considered significant if the *p*-value was below 0.05/6=0.008 (The Bonferroni correction for multiple comparisons). The visual scores were compared in a similar fashion using Wilcoxon’s signed rank test.

The irritancy rankings of the 4 detergents under each of the 3 exposure methods were compared as follows. It was assumed that the true order was SLS > ISE > SOAP > SUC. This assumption was based on the rankings of the mean values derived from the repeated occlusive and open tests. For each of the 6 pairwise rankings (e.g., SLS > SOAP) it was determined for each individual and under each exposure method whether the observed re-

sponses were concordant with this ranking or not. For each exposure method the percentage of concordant rankings was calculated. These percentages were compared by means of the McNemar test. Presented are individual 2-sided *p*-values, not corrected for multiple comparisons.

Results

Table 2 shows the median values of the total visual scores on the day after the last exposure. It is striking that, unlike the other 2 tests, in the one-time occlusive test the median value of SOAP was as high as SLS. Differences regarding visual scores in the one-time test were significant only for comparisons with SUC (Table 3A). The ranking was: SOAP ≥ SLS ≥ ISE > SUC. In contrast, the ranking in the repeated occlusive and open tests was different: SLS > ISE ≥ SOAP > SUC. In both repeated test methods visual scoring led to significant differences, with the exception of the comparison ISE – SOAP (Tables 3B,C). In the one-time test, erythema was a more prominent feature than scaling and fissuring, whereas in the repeated exposure tests, scaling was more prominent than

Table 3. *p*-values for pairwise differences between detergents regarding their total visual scores after exposure, for the different exposure models

	ISE	SOAP	SUC
(A)			
One-time occlusive test			
SLS	0.79	0.018	0.001
ISE		0.019	<0.001
SOAP			<0.001
(B)			
Repeated occlusive test			
SLS	0.001	<0.001	<0.001
ISE		0.297	<0.001
SOAP			<0.001
(C)			
Repeated open test			
SLS	<0.001	<0.001	<0.001
ISE		0.079	<0.001
SOAP			<0.001

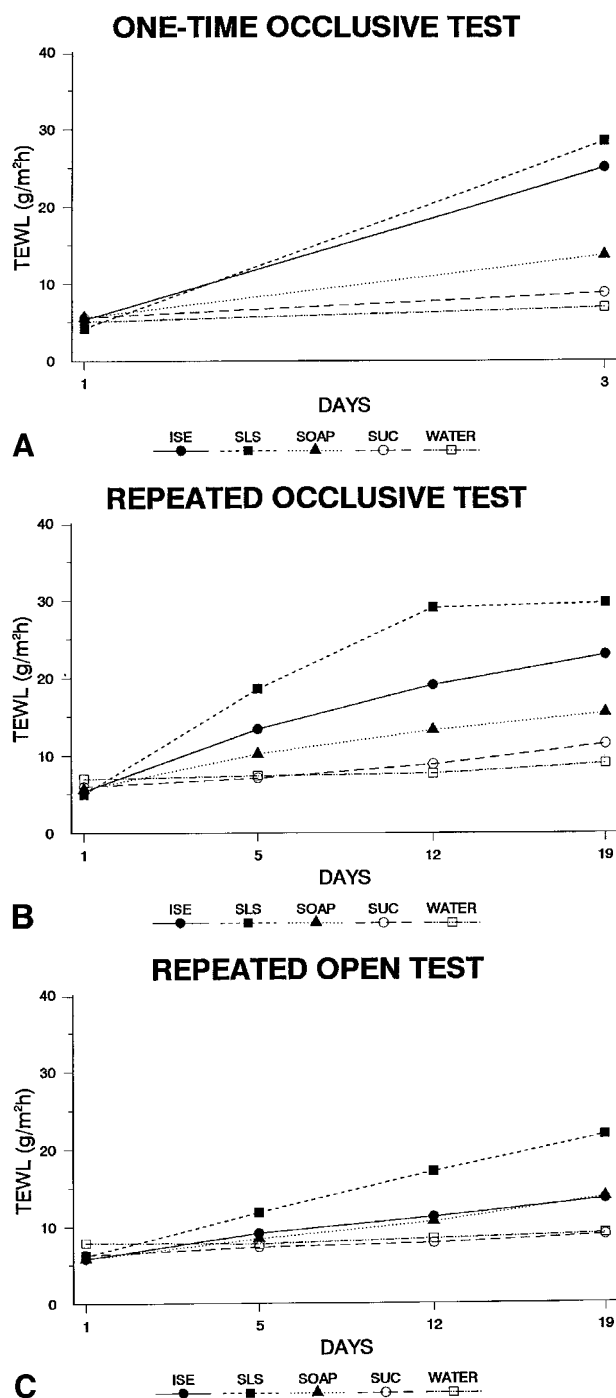


Fig. 1. Time course of transepidermal water loss (TEWL) before and after exposure to sodium cocoyl isethionate (ISE), sodium lauryl sulfate (SLS), soap, disodium lauryl 3-ethoxysulfosuccinate (SUC) and water. (A) one-time occlusive test; (B) repeated occlusive test; (C) repeated open test.

erythema (data not shown). The scores in the repeated occlusive test were approximately two-fold higher than in the repeated open test.

Figs. 1A–C shows the mean values for the time course in TEWL. In the one-time and repeated oc-

Table 4. Mean values, 95% confidence intervals and *p*-values for pairwise differences between detergents regarding their transepidermal water loss (TEWL) values after exposure, for the different exposure models

	ISE	SOAP	SUC
(A) One-time occlusive test			
SLS	3.2 ^{a)} −0.9–7.3 ^{b)} 0.122 ^{c)}	14.5 9.8–19.2 <0.001	20.4 16.3–24.5 <0.001
ISE		11.4 8.0–14.8 <0.001	16.7 13.0–20.3 <0.001
SOAP			5.1 3.6–6.7 <0.001
(B) Repeated occlusive test			
SLS	6.7 2.0–11.4 0.007	14.2 9.8–18.7 <0.001	18.1 13.9–22.4 <0.001
ISE		7.5 4.9–10.1 <0.001	11.4 8.9–14.0 <0.001
SOAP			3.9 2.5–5.3 <0.001
(C) Repeated open test			
SLS	8.2 6.0–10.3 <0.001	8.0 5.8–10.1 <0.001	12.8 10.6–15.1 <0.001
ISE		−0.2 ^{d)} −1.2–0.7 0.644	4.7 3.6–5.8 <0.001
SOAP			4.9 3.7–6.1 <0.001

a) Mean value for difference between SLS and ISE.

b) 95% confidence interval belonging to this difference.

c) *p*-value belonging to this difference.

d) The negative value is the result of the higher value of SOAP, in the subtraction: ISE – SOAP, assuming that the 'real' ranking order was SLS > ISE > SOAP > SUC.

clusive tests the rank order was: SLS > ISE > SOAP > SUC. In the repeated open test ISE and SOAP had almost the same value. By means of TEWL measurement it was possible to discriminate between the detergents regarding their irritancy in the one-time test, with the exception of the comparison SLS – ISE (Table 4A). In the repeated occlusive test all comparisons yielded significant results (Table 4B), whereas in the repeated open test the only exception to this rule was the comparison ISE – SOAP (Table 4C).

In Fig. 2, the concordance between the exposure methods regarding the total visual scores after exposure is depicted. The repeated occlusive test had very high prediction rates for most comparisons, except for the comparison ISE – SOAP. The repeated open test yielded lower prediction rates than the re-

CONCORDANCE OF VISUAL SCORE RANKING ORDER % PREDICTED BY EXPOSURE METHODS

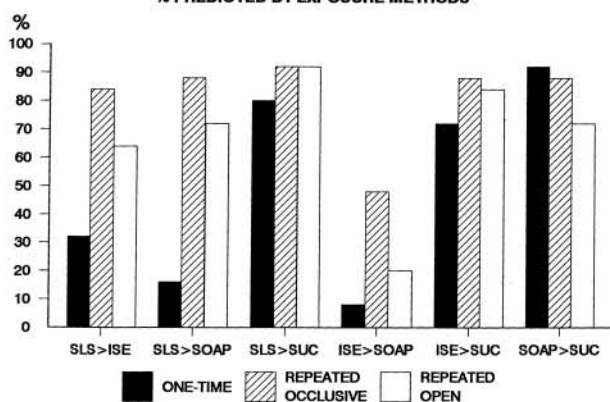


Fig. 2. Concordance among one-time occlusive, repeated occlusive, and repeated open tests with respect to the rank order of total visual score after exposure to the detergents. For explanation of abbreviations, see legend to Fig. 1. It was assumed that the "real" rank order was as follows: SLS > ISE > SOAP > SUC. For each exposure method, it was determined for each individual in a pairwise manner whether the observed responses were concordant with this rank order or not. In that way, the number of cases that were classified "right" (i.e., according to the above-mentioned ranking) and the number of cases that were classified "wrong" (not according to this ranking) was calculated for each pair of agents. The Y-axis represents the % of cases that were classified right, i.e., the prediction rate.

peated occlusive test, and in the one-time test prediction was low in 3 comparisons. The sign-test on the concordance rates of pairwise comparisons yielded low *p*-values in the following cases. When SLS and ISE were compared, the repeated occlusive and repeated open test predicted better than the one-time test ($p=0.04$ and $p=0.012$, respectively). The same held true for the comparison SLS – SOAP (both *p*-values, <0.001). The repeated occlusive test had a better prediction than the open test in the comparison ISE – SOAP ($p=0.012$).

The concordance between the exposure methods regarding rank order of TEWL values is shown in Fig. 3. Generally, the concordance was very high, except in the comparisons SLS – ISE, and ISE – SOAP. In the comparison of SLS versus ISE, the prediction rates of the one-time test and the repeated occlusive test were 62% and 76%, respectively; these prediction rates were lower than that of the repeated open test (sign-test, $p=0.004$ and $p=0.032$, respectively). When ISE was compared with SOAP, the repeated open test had a low predictability (56%), which was lower than the predictability of the one-time test ($p=0.022$) and repeated occlusive test ($p=0.038$).

Discussion

When evaluated visually, there was a discrepancy in irritancy rank order between the one-time oc-

CONCORDANCE OF TEWL RANKING ORDER % PREDICTED BY EXPOSURE METHODS

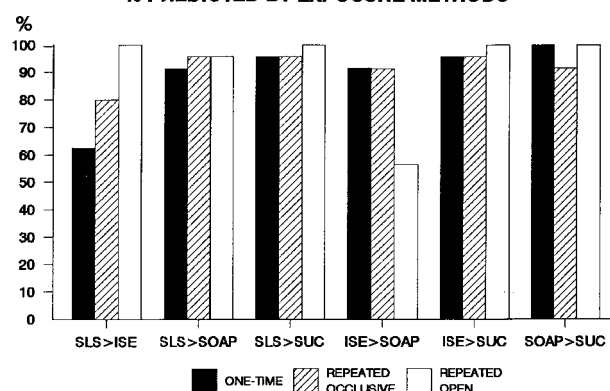


Fig. 3. Concordance among one-time occlusive, repeated occlusive, and repeated open tests with respect to the rank order of transepidermal water loss (TEWL) values after exposure to the detergents. For further explanation, see legend to Fig. 2.

clusive test on the one hand, and the repeated occlusive and open tests on the other hand. An important finding in this respect was the fact that soap had a high irritancy score in the one-time patch test. The higher irritancy of soap in the patch test in this study can partially be explained by the fact that the molarity of SLS was much lower than that of soap, namely 8.75 mM (SLS, concentration, 0.25%) versus 70 mM (soap, concentration, 2.3%). Based on pilot studies, SLS was tested in such a low molarity because otherwise SLS would have induced irritant reactions out of the range of the other detergents. Because of this low molarity, which was lower than recommended in the guidelines on this topic (1), and the low irritancy of the other detergents, the exposure duration in the repeated open test was doubled. The other detergents were tested on an equimolar basis, which is considered the best way to compare their irritancy (7).

The same discrepancy regarding soap as tested in the one-time test versus the immersion test has been noticed by Smeenk (2). Imokawa and Mishima have found that the roughness-inducing effect (tested by a washing assay) of a series of 4 anionic detergents correlated poorly with their irritating effect, tested in a one-time patch test (3). Thus, the type of exposure method determines to a certain extent the way in which a detergent exerts its irritant action on the skin. Occlusion in the one-time test promotes a clinical reaction mainly consisting of erythema (i.e., acute reaction) (1). In contrast, the repeated tests promote the occurrence of scaling and roughness (subacute/cumulative reaction) (1).

Evaluated by TEWL measurement, SLS had the

highest value in the one-time occlusive test, followed by ISE. The TEWL values of soap and SUC were much lower. Thus, there is a discrepancy between visual scores and TEWL values regarding soap. The same discrepancy between TEWL measurement and clinical findings was observed for soap in the repeated occlusive test of an earlier study by our group (8). This discrepancy of the irritancy of soap evaluated by TEWL versus visual scoring might be explained by the finding that soap had the highest score regarding permeability-enhancing effect of the stratum corneum in in-vitro studies (2, 9). In this way, one may postulate that soap can penetrate better through the stratum corneum, promoting its own permeability, thereby still causing less damage to the stratum corneum, resulting in a less pronounced TEWL increase. Another explanation might be that all detergents penetrate through the stratum corneum but differ in the way and magnitude they induce synthesis of lipids of the stratum corneum (directing the TEWL), and release mediators of inflammation (directing the erythema). Induction of stratum corneum lipid synthesis has been found for SLS, accompanied by TEWL increase (10). SLS also induces intercellular adhesion molecule-1 expression and the production of cytokines (1, 11).

Based on the 3 exposure methods in this study, it may be concluded that soap and ISE may be regarded as detergents with intermediate irritant properties, positioned between the extremes of SLS on the one hand, and SUC on the other. This finding is different from the result of our previous study, in which soap had values for visual scoring and TEWL that were slightly lower than those of SLS, which was tested in that study in the same molarity as soap (12). In the previous study, soap had a highly irritant nature since it was tested as pure sodium laurate (containing 99% alkyl chains with 12 carbon atoms), in contrast to the current study, in which soap had a 'broad' chain length distribution (Table 1). In all detergents a fully saturated chain length of 12 carbon atoms induces the most irritant skin changes (12).

In the current study, soap and ISE were tested in concentrations that are similar to those used in domestic products; the concentration of SUC was tested in a higher concentration (13). Therefore, the irritancy rank order found in this study is realistic.

The concordance among the methods as evaluated by TEWL was generally high. Exceptions to this statement were the low predictability of the one-time test in the comparison SLS – ISE, and an even lower predictability of the repeated open test in the comparison ISE – soap. These lower concordance figures are caused by the fact that the

values belonging to the detergent pairs in these tests were equal or almost equal. In the case of the repeated open test the mean TEWL value of soap was even slightly higher than that of ISE. This may be due to the fact that TEWL values after repeated open exposures were low, which might turn into a rank order comparable with the other tests in the case that exposure conditions would be more severe. However, one cannot rule out the possibility that in the open test the irritant action of detergents is different compared to in the occlusive tests.

The moderate and low concordance rates among the methods as scored visually may be explained by the fact that the values of the total visual scores exhibited less spread than the TEWL values, with many equal values (data not shown).

The central question is the following: which exposure method gives the best prediction of the results of real-life exposure to these kinds of detergents? That supposes another question: what are the real-life situations encountered? In most in-use situations the uncovered skin is exposed to detergents several times daily. In some situations, already-exposed skin is covered by 'protective' gloves or other materials. In the first situation mentioned the repeated open test seems the best way to simulate this, whereas the repeated occlusive test is the second best. In the second situation however, the repeated occlusive test or the one-time patch test might be chosen. In order to prove this point, comparison with field studies on in-use situations are necessary. To our knowledge, there is only 1 study that complies with this criterion (14). In this study, 3 metalworking fluids showed the same irritancy ranking when tested in a modified one-time patch test and a repeated occlusive test model. However, this ranking did not correspond to epidemiological data from the field study on 3 trainee workshops, in which the hands were apparently not protected by gloves.

The present study demonstrates that the choice of exposure model and evaluation method may be important variables influencing the outcome of irritancy testing. In our opinion, visual scoring remains the reference ('gold standard'). Other non-invasive methods may give additional information on specific mechanisms of action of the irritants studied.

In conclusion, there was a good concordance between the exposure methods regarding rank order as measured by TEWL. However, a lower concordance was found when judged clinically. The real rank order of the detergents tested was probably SLS > ISE > SOAP > SUC. An exception to this rule was the aberrant ranking in the one-time patch test, scored visually, namely, SOAP ≥ SLS ≥ ISE > SUC. The only in-use situations that may

possibly correspond to this aberrant ranking may be those in which the skin is occluded after irritation by detergents.

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