

Guidelines on sodium lauryl sulfate (SLS) exposure tests

A report from the Standardization Group* of the European Society of Contact Dermatitis

R. A. TUPKER, C. WILLIS, E. BERARDESCA, C. H. LEE, M. FARTASCH, T. AGNER AND J. SERUP
Department of Dermatological Research, Leo Pharmaceutical Products, 55 Industriparken,
DK-2750 Ballerup, Denmark

This report reviews the clinical and histopathological reactions caused by sodium lauryl sulfate (SLS), and the non-invasive methods that can characterize these reactions. Furthermore, SLS exposure techniques and factors that may influence the outcome of these exposures are discussed. Finally, guidelines are introduced for each exposure technique in order to have a uniform approach to SLS testing in man. Since different study aims warrant different testing conditions, we have proposed 2 categories, namely susceptibility testing and provocative testing, tailored to the aim with which the study is performed.

Key words: sodium lauryl sulfate; SLS; guidelines; irritancy testing methods; bioengineering methods; non-invasive methods; transepidermal water loss; TEWL; clinical scoring; exposure tests.
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Sodium lauryl sulfate (SLS) has been used extensively as a model irritant in the field of skin irritation testing. During its history, irritancy testing, aimed at answering different questions and evaluated by several clinical scoring systems, has been performed using various exposure methods. It is therefore not surprising that there is nowadays a multitude of SLS testing methods, which hampers the interpretation of results from different laboratories.

The present guidelines review the chemistry and biology of SLS, followed by a review of the various methods, with the ultimate goal of introducing a uniform approach to SLS testing in man. Since different study aims warrant different testing conditions, we propose several testing methods tailored to the purpose of study. With respect to aims, studies are divided into 2 categories. The 1st

category, *provocative testing*, concerns studies in which SLS is used to induce a definite skin reaction in 'all' individuals. Examples of aims of the first category are: (1) elucidate the mechanisms of skin irritation; (2) predict the irritant potency of different detergents; (3) study the time course after irritation; (4) compare the sensitivity of different non-invasive methods; (5) compare the efficacy of different moisturizers, barrier creams or corticosteroid creams in preventing or healing skin irritation. The 2nd category, *susceptibility evaluation*, concerns studies aimed to predict the irritant susceptibility of individuals, and investigate individual- and environmental factors determining this susceptibility.

Initially, evaluation of irritancy testing was based on visual scoring only. Examples are classic studies, such as those by Bettley (1) and Frosch & Kligman (2). Efforts were already being made to quantify the irritation reaction by means of transepidermal water loss (TEWL) measurement, using dry nitrogen as a carrier gas connected with an electrolytic water analyzer (3). The Evaporimeter (Fig. 1) was first used for this purpose by Van der Valk et. al. (4). It is by this relatively easy-to-handle instrument that TEWL measurement became a popular method of detergent irritation

* Members of the standardization group on non-invasive methods for quantification of patch test reactions: T. Agner (Copenhagen), E. Berardesca (Pavia), A. Bircher (Basel), P. J. Coenraads (Groningen), T. Fischer (Stockholm), P. J. Frosch (Dortmund), R. Golhausen (Munich), J.-M. Lachapelle (Brussels), A. Lathi (Oulu), H. I. Maibach (San Francisco), D. van Neste (Tournai), J. Pinnagoda (Singapore), J. Serup, chairman (Copenhagen), R. A. Tupker (Groningen), P. G. M. van der Valk (Nijmegen), J. E. Wahlberg (Stockholm).

evaluation. TEWL appears to be pre-eminently suited for this purpose. Today, many studies dealing with irritation by detergents are performed with TEWL measurement. TEWL measurement can be erroneous, and for details concerning correct TEWL measurement the reader is referred to the guidelines on this topic edited by the Standardization Group of the European Society of Contact Dermatitis (5). Quantification of the irritant reaction can also be carried out by other non-invasive techniques, such as laser Doppler flowmetry, colorimetry, ultrasound measurement and electrical capacitance measurement. The ultimate endpoint for doctors and patients however, is the clinical scoring of the irritant reaction.

Chemistry, purity and skin penetration properties of SLS

Chemistry

SLS (synonym: sodium dodecyl sulfate) is an anionic detergent with the molecular weight 288.38 g/mol. The molecule is a 12 carbon chain with the composition $\text{CH}_3-(\text{CH}_2)_{10}-\text{CH}_2-\text{O}-\text{SO}_2-\text{O}-\text{Na}$.

Pure SLS is white and the crystals, flakes or powder are soluble in water (1 g/10 ml) and somewhat soluble in ethanol. In aqueous solution SLS reduces the surface tension and forms oil-in-water emulsions. The critical micelle concentration for SLS in aqueous solution is 8.2×10^{-3} mol/l (0.23% w/v) at 25°C.

Purity and carbon chain length

Irritancy patch test studies with SLS generally have been performed using aqueous solutions, although petrolatum was also used as a vehicle (6, 7). Concentrations of SLS in the test material have varied from 0.1% to 10%. No study has directly

compared aqueous solution with petrolatum vehicle. SLS is available in different purities with a variable content of carbon chain lengths other than 12. C_{12} is known to elicit a maximum of irritant response clinically (8, 9). Analysis of different grades of SLS by high performance liquid chromatography revealed that in some grades of SLS part of the highly irritating C_{12} substance had been substituted by longer and less irritating carbon chains (10). The pharmacopoea quality of SLS (Ph. Eur., purity 96.5%) was shown to elicit a much weaker irritant reaction in human volunteers than a high purity (99%) solution, indicating a significant impurity in the pharmacopoea product not reflected in the purity claim. Thus, high purity SLS (99%) must be used in any study, preferably in water solution.

Release from patch test systems and skin penetration of SLS

Delivery from the patch test system of aqueous solutions of SLS was not found to be a major source of variation in an in vitro test system (11). Aqueous solutions of SLS and different types of gels were studied. Approximately 70% of the applied SLS in aqueous solution was released from the system while the release from 2 gels (Methocel and Pluronic gel) was significantly less (11). The penetration ability of radiolabelled SLS (^{35}S) into skin was studied in human cadaver skin (12). A Frantz diffusion cell was used. SLS was applied to the epidermal surface with Finn chambers for 24 h (0.5% and 1% SLS). SLS was quite capable of penetrating the skin barrier in the outer epidermis and could also be detected in small amounts in the dermis and receptor phase. The penetration of SLS into the skin was dose dependent and continued after removal of SLS from the skin surface and rinsing of the skin. Inter-individual variation was considerable (12). Thus, the release of SLS in aqueous solution from this occlusive test chamber system and the penetration of SLS into the skin is efficient. Skin penetration of SLS following open application and immersion was not studied. Skin penetration of SLS is expected to show a significant inter-individual and anatomical site variation.

Histopathology, ultrastructure and mediation of SLS reactions

The histopathological effects of SLS depend on concentration, mode of application, time of sampling, species, inter-individual factors etc. Nevertheless, some characteristic morphological and pathophysiological changes can be identified.

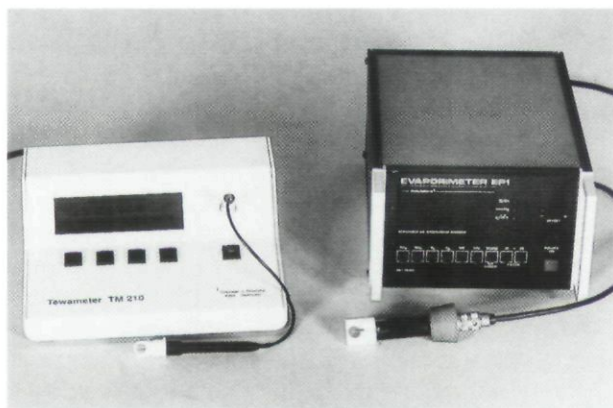


Fig. 1. Photographs of the Evaporimeter EP1C (ServoMed, Stockholm, Sweden) and the Tewameter TM210 (Courage & Khazaka, Cologne, Germany).

Table 1. Acute SLS reactions, histopathological changes of the epidermis

Conc.	Duration of exposure/ sampling time (h)	Species	Visual assessment of intensity of reaction		Ref.
1% aq.	24	g. pig	unspecified	spotted areas of necrosis, with nuclear pyknosis of epidermal cells in stratum Malpighii irregular hydropic degeneration of basal cell layer	(19)
5% aq.	24	g. pig	unspecified	total necrolysis – extensive hydropic degeneration of basal cells, often with total detachment of epidermis	
1% aq.	72	rat	mild to moderate	parakeratosis, spongiosis, intracellular lipid accumulation	(20)
5% in petroleum	24	g. pig	unspecified	necrosis of follicular ostia, some vesiculation/pustulation/bullae formation	(21)
5% aq.	24	g. pig	unspecified	complete necrosis with detachment of the epidermis	(21)
5% (aq. w/v)	47/48	man	mild to moderate	parakeratosis, spongiosis, mild or patchy intracytoplasmic and intranuclear vacuolation. Lipid accumulation	(22)
			severe	necrolysis of basal cells, with loss of dermo-epidermal junction	

Table 2. SLS acute reactions, effects on proliferation of keratinocytes

Conc.	Time	Sample size/ experimental design	Result	Ref.
0.5% 1% 2% aq.	1–4 days	unspecified no. of male volunteers/ Al tests, post biopsy staining with Feulgen reaction for DNA	1% SLS caused 30-fold increase in mitotic activity when compared with untreated control, peak increase at 48 h 0.5 and 2% SLS showed similar changes but to a smaller degree	(23)
(0.5, 1, 5, 10, 50) $\times 10^{-6}$ M in keratinocyte basal medium		in vitro, keratinocyte cultures	stimulation of proliferation of keratinocytes at 1 and 5×10^{-6} M SLS – rapid decline, i.e., toxicity at 50×10^{-6} M	(24)
7.5%	20 min/day for 3 weeks	18 male volunteers/open application, dansyl chloride staining	increased stratum corneum turnover	(25)
5% aq.	48 h	10 male volunteers/patch test followed by biopsy, immunocytochemical staining using Ki-67 Mab.	greatly increased numbers of dividing basal keratinocytes.	(26)

Effects on stratum corneum

Using routine fixation and staining for electron microscopy, detergents have been found to degrease the skin, with selective depletion of the lipids from the intercellular spaces (13). However, ruthenium tetroxide (RuO_4) fixation, a technique which permits the analysis of intimate structural changes (14), revealed that, after exposure to 0.5% SLS, the upper stratum corneum retained the regular lamellar arrangements of lipids (15,16). This corroborates biochemical studies which have shown that in experimentally-induced scaly skin the amount of ceramides did not differ from that in healthy control skin, with only a 4–7% removal of lipids (17). Lipid com-

position changes were observed which did not reflect the immediate consequences of detergent exposure (15,16,18). Ultrastructurally, SLS at low concentration does not alter the existing lipid structure, but rather influences the synthesis of new lipids. These changes might explain the protracted TEWL enhancement following SLS.

Effects on viable epidermis

Single application of SLS, with sampling between 24 and 72 h, induces spongiosis, intracellular vacuolation and lipid accumulation in those reactions which are mild to moderate, and necrosis in those that are more severe (Table 1), (19–22).

Parakeratosis is also a common feature, most probably arising from the stimulatory effects of SLS on keratinocyte stimulation which have been demonstrated in a variety of in vivo and in vitro systems (Table 2) (23–26). Immune-associated changes to keratinocytes also take place following exposure to SLS. The induction of intercellular adhesion molecule -1 (ICAM-1) expression has been described, as has the up-regulation of integrin receptors, $\alpha 3$, $\beta 1$, $\alpha 6$, $\beta 4$, $\alpha 5$, $\beta 5$ (27–29). Alterations in the pattern of epidermal CD36 staining also occur (27). Furthermore, there is evidence that SLS induces the production of cytokines, including interleukin-8 (IL-8) (30). In addition to the effects observed in keratinocytes, epidermal Langerhans cells also show a variety of changes following SLS exposure. A reduction in epidermal density frequently takes place, concomitant with ultrastructural indications of cellular degeneration and/or activation (31–34).

Effects on dermis

SLS reactions are characterized by the influx of leukocytes into the epidermis and dermis, accompanied in some reactions by oedema and collagen disruption/degeneration (19, 34–38). T lympho-

cytes are the predominant infiltrating cells, with CD4⁺ cells almost always outnumbering CD8⁺ cells (34–38). The majority of these are memory cells (CD45RO⁺) with naïve cells accounting for <5% (37). A significant proportion bear the receptor for interleukin-2 (IL-2), indicating an enhanced state of activation (36–38). Accompanying the T cell infiltration are CD11c⁺ and CD1a⁺ cells and neutrophils, whilst cell types such as B cells and NK cells are absent or rare (35–38).

Clinical appearance of SLS reactions and characterization by different non-invasive methods

Observations and findings again depend on choice of concentration and experimental design with a major difference between early, acute reactions and later, subacute reactions.

Clinical appearance

Björnberg in his classical thesis on primary irritancy described the morphology of SLS reactions (39). Erythema, sometimes associated with infiltration and sometimes with superficial erosion of the epithelium, are the main features of acute reac-

Table 3A. One-time 24-h patch tests in normal skin of healthy volunteers and dermatitis patients using large Finn Chamber (12 mm)

Authors (Ref.)	No.	Quant.	Conc.	Anatomic site	Time	Pre-TEWL (SD)	Post-TEWL (SD)	Other remarks
Van der Valk et al. (4)	27	100 μ l	2%	volar forearm	1 $\frac{1}{4}$ h	7.7 (2.1)*	15.5 (4.0)	
Agner et al. (99)	17	ND	2.5%	volar upper arm	30 min.	8.0 (6.5–9.0) [#] 6.9 (6.2–9.2)	29.6 (14.9–55.0) 17.5 (14.8–26.2)	Winter Summer
Agner et al. (10)	12	ND	2.5%	volar upper arm	30 min	ND	30.2 (22.3–40.6) [#] 22.1 (15.7–28.4)	Quality A Quality B
Agner (117)	28	60 μ l	0.5%	volar upper arm	1 h	5.3 (4.3–6.1) [#] 9.5 (6.6–11.2)	14.0 (12.3–17.9) 18.7 (16.0–25.1)	controls atopic dermatitis
Agner (46)	16 14 9	60 μ l	0.5%	volar upper arm	1 h	5.4 (4.2–6.1) [#]	23.0 (17.2–29.7) 14.2 (11.9–17.7) 15.5 (11.5–21.2)	acute eczema chronic eczema healed eczema
Berardesca et al. (91)	9	ND	0.5% 2% 0.5% 2% 0.5% 2%	back	30 min	2.7 (0.7)	4.7 (3.9) ^o 14.7 (13.2) 7.4 (4.7) 8.5 (4.8)	pre-occluded white pre-delipidized white
	10	ND	0.5% 2% 0.5% 2%			3.3 (0.9)	12.9 (9.9) 20.3 (15.2) 8.4 (5.8) 24.1 (23)	pre-occluded black pre-delipidized black

No., number of subjects.

Quant., quantity of SLS solution.

Conc., concentration of SLS solution.

Time, time period between end of exposure and evaluation.

Pre-TEWL, pre-exposure TEWL value.

Post-TEWL, post-exposure TEWL value.

SD, standard deviation.

* Water control.

[#] Median value and 25/75 percentiles.

^o Differences between post- and pre-exposure TEWL values.

Table 3B. One-time 24-h patch tests in normal skin of healthy volunteers using Hilltop chamber

Authors (Ref.)	No. [‡]	Quant.	Conc.	Anatomic site	Time	Pre-TEWL (SD)	Post-TEWL (SD)	Other remarks
Van der Valk et al. (97)	10	200 μ l	2%	Elbow	15 min	2.8 (0.7) 2.3 (0.8) 2.8 (1.0) 4.0 (1.9) 5.4 (3.3)	24.6 (15.6) 16.5 (8.4) 16.2 (7.8) 14.9 (8.5) 14.0 (6.0)	
				Wrist				
Wilhelm et al. (56)	10	200 μ l	0.125% 0.25% 0.5% 1.0% 2.0% 3.0%	Volar forearm	24 h	4.7 (1.4)*	7.9 (2.9) 9.3 (2.6) 12.4 (5.3) 20.2 (11.3) 27.5 (13.6) 41.6 (21.1)	
Wilhelm et al. (118)	10	100 μ l	0.1%	Mid-volar forearm and upper back	24 h	4.2 (0.3)	11.1 (1.4)	
Cua et al. (89)	7	400 μ l	0.25%	Forehead Upper arm Volar forearm Dorsal forearm Postauricular Palm Abdomen Upper back Thigh Ankle	24 h	7.1 (1.1) 3.8 (0.7) 5.0 (1.4) 3.8 (0.7) 6.1 (0.8) 31.0 (7.7) 5.4 (0.9) 5.6 (1.2) 5.1 (1.6) 6.2 (1.7)	15.8 (2.1) 17.0 (5.2) 13.3 (4.5) 11.6 (2.8) 11.4 (2.3) 26.9 (4.0) 20.0 (4.7) 18.2 (3.5) 24.6 (7.6) 6.5 (1.8)	young adult
	8	400 μ l	0.25%	Forehead Upper arm Volar forearm Dorsal forearm Postauricular Palm Abdomen Upper back Thigh Ankle	24 h	4.8 (1.7) 1.8 (0.6) 2.3 (0.8) 2.1 (0.8) 6.8 (2.3) 26.2 (9.3) 1.9 (0.7) 3.3 (1.2) 2.2 (0.8) 2.5 (0.9)	14.1 (5.0) 4.6 (1.6) 5.3 (1.9) 4.2 (1.5) 11.6 (4.1) 22.5 (8.0) 5.1 (0.8) 6.6 (2.3) 7.1 (2.5) 3.7 (1.3)	old age
Lee et al. (125)	15	100 μ l	0.25% 0.50% 0.75% 1.0%	Volar forearm	30 min	5.9 (0.62) 5.9 (0.51) 5.7 (0.51) 5.9 (0.54)	22.3 (1.67) 22.3 (1.67) 22.7 (1.54) 25.4 (2.56)	

[‡] For explanation of abbreviations, see Table 3A.

* Water control.

tions. With higher concentrations vesicular and even pustular reactions may be seen. During healing of acute reactions, scaling and fissuring will take over. The same appearance with erythema, scaling and fissuring is seen during repeated application of SLS (40,41). Acute reactions read after 24 to 72 h mainly illustrate the immediate toxic insult to the skin while the appearance on later occasions or during repeated application is influenced and even dominated by healing and repair mechanisms. This may result in modification of the irritation threshold of the skin with either hardening or progressing irritancy (42). The so-called "soap effect" or "effet de savon" consists of a fine wrinkled surface contour associated or followed by chapping, the latter representing a characteristic roughening of the skin. This characteristic soap effect is however uncommon in SLS provocative experiments.

Characterization of irritant SLS responses by different noninvasive techniques

An extensive literature (see below) shows that TEWL measurement is a highly sensitive and precise measure of SLS irritant effects on skin with dose-effect relationship easily demonstrated in the concentration ranges normally used in provocative testing. Optimised measuring conditions and adherence to the ESCD guidelines is essential (5).

However, water barrier function and water evaporation from the skin is neither felt nor seen by patients or doctors. This is in contrast to parameters such as redness, skin thickening due to oedema, and, in later states, scaling due to increased epidermal turnover and changed corneocyte cohesion. Obviously, findings based on TEWL measurement are indirect and not automatically clinically relevant.

Table 3C. One-time 24-h patch tests in normal skin of healthy volunteers using round flat plastic chamber (22 mm)

Authors (Ref.)	No. [#]	Quant.	Conc.	anatomical site	Time	pre-TEWL (SD)	post-TEWL (SD)
Pinnagoda et al. (126)	44	300 μ l	0.5%	Volar forearm	2 h	5.24 (1.03)	14.41 (3.47)
Tupker et al. (68)	35	300 μ l	0.5%	Volar forearm	2 h	6.0 (1.3)	17.8 (4.1)

[#] For explanation of abbreviations, see Table 3A.

Table 3D. One-time 48-h patch tests in normal skin of healthy volunteers using large Finn Chamber

Authors (Ref.)	No. [#]	Quant.	Conc.	Anatomical site	Time	pre-TEWL (SD)	post-TEWL (SD)	Other remarks
Van der Valk et al. (127)	9 14	100 μ l	5%	Volar forearm	15 min	3.6 (1.5)* 4.0 (1.3)*	13.7 (9.2) 33.9 (21.7)	controls allergic contact dermatitis
Goh et al. (94)	23 15 15 12 11	50 μ l	2%	Right scapula	20 min	5.5 (2.61) 2.9 (1.62) 4.0 (1.9) 4.9 (2.9) 4.7 (3.2)	26.7 (12.76) 29.8 (17.8) 35.3 (16.2) 21.7 (9.3) 24.7 (13.8)	male female Chinese Malay Indian

[#] For explanation of abbreviations, see Table 3A.

* Water control.

Table 3E. Repeated short contact occlusive exposure tests

Authors (Ref.)	No. [#]	Quant.	Conc.	Anatomical site	Time	Pre-TEWL (SD)	Post-TEWL (SD)	Other remarks
Tupker et al. (73)*	35	300 μ l	0.5%	Volar forearm	24 h	4.9 (1.9)	15.2	total exposure period: 3 weeks
Tupker et al. (71)*	21	300 μ l	0.5%	Volar forearm	24 h	5.9 (0.42) 6.7 (0.4)	13.2 (2.33) 17.9 (2.29)	controls atopic dermatitis
Zhou et al. (128)*	18	300 μ l	2%	Volar forearm	24 h	3.0 (0.4) 3.6 (0.4)	32.5 (9.8) 10.3 (1.8) 49.7 (5.7)	day 5 day 8 day 5, after 3 months

[#] For explanation of abbreviations, see Table 3A.

* 2 $\times$ application a day for 1 week; the interval between 2 applications 4 h.

Table 3F. Repeated short contact open exposure tests

Authors (Ref.)	No. ^a	Quant.	Conc.	Anatomical site	Time	Pre-TEWL (SD)	Post-TEWL (SD)	Other remarks
Van der Valk et al. (80)*	10	100 μ l	7.5%	Volar forearm	24 h	3.6 (1.4)	6.4 (3.4) 5.2 (2.7)**	plexiglass ring (11 mm)
Van der Valk et al. (97) [#]	10	100 μ l	7.5%	Elbow fold Midvolar Wrist	24 h	2.8 (0.7) 2.8 (1.0) 5.4 (3.3)	14.0 (8.1) 6.2 (3.4) 6.5 (3.0)	Plexiglass ring (11 mm)
Wilhelm et al. (25) ^{##}	18	200 μ l	7.5%	Midvolar forearm	24 h	3.5 (0.3)	13.0 (1.6)	Glass ring (18 mm)
Lee et al. (78) ^o	11	80 μ l	2.0% 5.0% 7.5%	Volar forearm	24 h	6.0 (3.2) 5.8 (0.30) 6.0 (0.28)	10.2 (0.81) 11.2 (1.13) 11.5 (1.19)	Absorbent disc (12 mm)

^a For explanation of abbreviations, see Table 3A.

* 1 $\times$ daily 45 min for 5 days.

** Alternate day application.

[#] 1 $\times$ daily 45 min for 4 days; normal skin was stripped 15 times before irritation test.

^{##} 1 $\times$ daily 20 min for 3 weeks.

^o 1 $\times$ daily 20 min for 4 days on subclinically irritated skin with short term (30 min) patch application of 1% SLS solution.

Acute SLS reactions measured 24 to 72 h after exposure show increased a^* and decreased L^* by CIE colorimetry, and increased blood flow by laser Doppler flowmetry (7, 42–48). Furthermore, if reactions are stronger, increased skin thickness by ul-

trasound, increased number of low echogenic pixels in the dermis, and increased number of high echogenic pixels in the epidermis by ultrasound image analysis were demonstrated (6, 43–46, 49). Electrical capacitance can indicate increased or de-

creased values, depending on the state of the epidermis (40, 50). Reduced skin surface temperature, due to the formation of a thermo-insulating crust, was measured by thermography (51). Stereo microscopic evaluation of silicone rubber replica revealed a non-papular change of the skin surface contour with alterations of the primary skin relief with non-parallel deep grooves or parallel lines (52). In comparison with clinical reading and the various noninvasive methods, TEWL measurement, being highly sensitive with linear dose-irritation curves, tends to "overestimate" irritant SLS reactions in the skin (42). The correlation between TEWL and other methods is generally best in reactions of weak to moderate strength. The clinical dose-response curve is typically not linear.

Subacute and cumulative irritant responses to SLS show similar principal changes. However, dryness and decrease of electrical capacitance, scaling and fissuring become more prominent as a consequence of ongoing repair and epidermal hyperproliferation. Details were described by Wilhelm et al. (40).

SLS exposure methods

Irritancy testing methods can be classified grossly as: one-time occlusive tests, repeated occlusive tests, repeated open tests and immersion tests. In Tables 3 A-F, the literature is summarized, organized according to testing methodology.

One-time occlusive tests (closed patch tests)

For many years, closed patch testing with SLS has been the favoured test method, both for practical and traditional reasons. It is undoubtedly easy and rapid to perform. The disadvantage of this one-time occlusive test is the fact that it mimics only an acute irritant reaction. The more common situation in real life is the development of chronic irritant contact dermatitis resulting from multiple repeated exposures (53). Regrettably, numerous studies have used a multitude of exposure techniques. Variables include type of test chamber, quantity of test solution, concentration of detergent solution, evaporation of the solution, temperature of the solution, duration of exposure, and time of evaluation.

Type of test chamber. An important variable is the internal diameter of the chamber. The most frequently used chamber is the Finn device, constructed by Pirilä (54). Originally, this chamber had an inner diameter of 8 mm, with a small capacity (20 μ l), which was found too small to yield positive reactions to certain irritants (55). Nowadays, Finn Chambers with 11 mm and 18 mm



Fig. 2. Standard (left), large (middle) and extra large (right) Finn Chambers[®]. Small chamber (height 0.5 mm, diameter 8 mm, maximal capacity 20 μ l); large chamber (height 0.8 mm, diameter 12 mm, maximal capacity 70 μ l); extra large chamber (height 1.2 mm, diameter 18 mm, maximal capacity 300 μ l). Filter paper disks are optional. The small chamber (diameter 8 mm) is only recommended for allergy patch testing while the large and extra large chambers or similar test systems are recommended for irritancy testing.

inner diameters, having capacities of 70 and 300 μ l, respectively, are available (Fig. 2). Frosch & Kligman (55) introduced the Dühring chamber, and found that this chamber had better test properties compared with the small Finn Chamber. 2 other types of chambers used in this field are the Hilltop chambers, with various inner diameters, and the plastic Agfa-Bayer chamber, the latter with an inner diameter of 22 mm.

Quantity and concentration of test solution. In closed patch testing with SLS, as with other irritants, the quantity of test solution per mm² skin is of importance for the skin response (55). Even when applied at the same concentration larger quantities of SLS solution tend to induce more intense skin reactions. Furthermore, small test areas may be too limited to elicit an irritant skin reaction (55). Several studies demonstrated a dose-dependence for SLS reactions in one-time occlusive tests (45, 56–58).

Evaporation and temperature of the solution. Evaporation from the patch test before application on the skin may diminish the inflammatory response, even though the relative concentration of test substance per mm² is the same (59). Also, the temperature of the test substance may be of significant importance. Berardesca et al. (60) recently reported significantly different skin responses to 4°, 20° and 40°C SLS test solution (60).

Duration of exposure. For closed testing with SLS, application times of 24 h and 48 h are the commonly used. In a study comparing these two time periods, Staberg & Serup found that for SLS concentrations of 1, 2, 5 and 10% in petrolatum

higher mean blood flow values were present after 48 than after 24 h application, as evaluated by laser Doppler flowmetry (7). In recent literature, short-time single occlusive tests have been described. A 4 h exposure to 5% SLS induced mild to moderate erythema and a rise in TEWL (61). Others have used a 7 h occlusive test in order to study the beneficial effect of various lipids (62) or moisturizers (63). 50 μ l of 14% SLS, applied in large Finn Chambers, induced a very weak or slight erythema in most volunteers, accompanied with a moderate increase in TEWL (62).

Time of evaluation. For closed patch testing, the time between removal and reading of the patch should be carefully considered. The time course for TEWL after occlusive patch testing with SLS, demonstrated a significant decrease in TEWL from 0–30 min and from 30 to 60 min after removal of the patch, while the values from 60 to 180 min were stable (64). In the same study the time course for TEWL after patch testing with an empty chamber was studied. A statistically significantly increased TEWL, as compared to normal skin, was reported 30 min after removal of the empty patch, but not after 60 min. In contrast, other investigators found that longer times between removal and reading were necessary (55,65). The TEWL value of a 24 h water patch had returned to a level slightly elevated above its own baseline value only after 2 h in most individuals (65). A delay of 2–3 h after removal yielded somewhat more intense irritant reactions and more consistent clinical readings (55).

Differences in the time course of the reaction following patch test removal have been noted. In some studies an early peak (measuring at 0.5–1 h after patch removal, on day 2) of mean TEWL values was observed (40, 66, 67), whereas in another study a late peak (on day 3) of mean TEWL was found (68). In the latter study, TEWL was measured 2 h after removal of the patch, on day 2. In fact, different patterns in individual time courses could be recognized, dividing the group into subjects with early peak values, late peak values or very late peak values (45, 66, 68, 69). The time necessary to restore barrier function exceeds 10 days (40, 68).

Repeated occlusive tests

The concept of repeated occlusive testing is not new. Kligman and Wooding developed a test method in which substances are brought into contact with the skin under occlusion 24 h a day for 20 consecutive days (8). This method was designed for substances with a very low expected irritant potency. Later, Frosch & Kligman (2) used an exposure model in which a substance is applied re-

peatedly during 5 consecutive days: the first day for 24 h and the following days for 6 h a day (2).

In order to reflect better the conditions in daily practice, models were developed in which detergents were applied in a multiple repeated short-time way, namely 2 times daily 45 min during the working days of 1 week (68,70–72) or 3 consecutive weeks (73–75). Using this test method, it was possible to rank detergents according to irritant potency, SLS being by far the most irritant detergent (73).

The advantages of this multiple repeated short-time occlusive test method are that it offers a reasonable simulation of the daily practice, and has a relatively high reproducibility. However, being occlusive rather than open, it does not entirely mimic the situation in real life and, furthermore, it is time consuming to perform.

Repeated open tests

Solutions of 2%, 5% and 7.5% SLS were pipetted onto areas on the back and allowed to air-dry, in studies by Lammintausta et al. (76,77). Applications were done once daily for 6 days in total. Concentration-dependent TEWL increases were observed, accompanied with no (76) or only minor (77) visible reactions. SLS in similar concentrations was exposed on the forearm using filter paper discs once daily for 4 days in another study (78). Increased TEWL values were noticed with minor visible signs, independent of concentration (78). Other repeated open test models were based on once daily applications on the forearm skin using a glass ring during 20–45 min for 1–2 weeks (25,79,80). Increasing dose-dependent values for TEWL and erythema could be noticed (25,79,80).

The repeated open test can offer a good simulation of conditions in daily practice, as it mimics repetitive and cumulative toxic insults, whilst avoiding occlusive influences. The disadvantage is the fact that it is time-consuming for the subjects.

Immersion tests

Even a single 1 h occlusive test with 10% SLS has been shown to produce a clinical reaction, accompanied by a TEWL increase (3). Twice daily 30 min immersions of various detergents of 0.1% concentration were shown to induce different degrees of erythema and scaling, with SLS proving to be the most irritant (81). SLS at 0.5% appeared to elicit erythema in most subjects who had undergone 3 immersions of 10 min duration (82).

The skin response markedly increased in arms immersed in solutions of higher temperature (40°C), as compared with low temperature (20°C)

(83). The wash test represents another open test model, in which the act of washing is mimicked in a repetitive way (84,85).

The advantage of the immersion model is that it reproduces the cumulative insults by detergents in a more realistic way, but it is cumbersome compared to patch testing. Another disadvantage is the fact that only 2 agents can be compared in the same volunteer.

Correlation between occlusive and open tests

Studying the irritant potency of a limited number of detergents, Smeenk showed that there was a relatively good correlation between the results from the one-time occlusive patch test and those from the immersion test. The exception was soap, which was irritating in the patch test, but not irritating in the immersion test (81). This discrepancy may be explained by the earlier observation that the pH of a soap solution decreased after contact with human skin (86), and that soap at low pH (7.5) induced erythema and pruritus, which did not occur with soap solution at higher pH (9.5) (86).

A few studies have compared the patch test with the wash test with respect to the irritancy potential ranking order of a series of detergents (84,85). In one study (84), discrepancies were found in the ranking order obtained by the two techniques. Another study found similar ranking orders (85). However, the wash test appeared to have a greater power of discrimination (85).

Constitutional factors and SLS reactivity

Age

The peak of TEWL during life occurs in childhood, decreasing and remaining steady during adult life (87). A further decrease appears after the age of 60 (88). The decrease of TEWL with age is uniform all over the body and occurs at all sites in a constant manner (88). More pronounced irritative responses were noted in young subjects than in elderly individuals (89). However, once increased, the raised TEWL levels are more persistent in elderly subjects implying that healing capacity decreases with age (90). No significant differences in SLS reactivity were reported between the ages of 18–50 years (46).

Race

In one study, TEWL responses after SLS exposure tended to be higher in blacks as compared with caucasians (91). There is no racial difference in the thickness of the stratum corneum,

but the number of cell layers is higher in blacks (92). The finding may also be related to a different qualitative and quantitative composition of intercellular lipids between races (93). Among Asians, Chinese showed significantly higher values than Malays (94). Fair skin within the caucasian population has been shown to correlate with increased SLS sensitivity (46, 77). Subjects with skin type I, i.e., higher susceptibility to UV-light, had a higher susceptibility to SLS (77). Also increased light reflection from the skin ('fair' skin) was associated with increased susceptibility to SLS (46).

Sex

Although epidemiological studies show increased prevalence of contact dermatitis in females as compared with males (95), experimental studies do not confirm a sex related difference in skin susceptibility to irritants (4,46,70,76). Variation in skin susceptibility during the menstrual cycle has been demonstrated (96). In this study of 29 females with regular menstrual cycles, an increased skin reactivity at day 1 as compared to day 10 was confirmed by measurement of TEWL and oedema formation (skin thickness).

Anatomical site

Regional variations in baseline TEWL exist, with higher levels on the palms and forehead as compared with arm and back (3,5,88). However, there are also significant differences within the same area of the body. On the forearm, for instance, significantly higher TEWL values were found next to the wrist as compared with the other sites of the forearm (72, 97, 98). It has been suggested therefore that this area should be excluded when testing (98). There is also a site variation in susceptibility to SLS on the forearm, as evaluated by TEWL and visual scoring (97). Post-exposure TEWL next to the elbow was significantly higher than the other sites on the forearms, the area next to the wrist having the lowest value (97). Surprisingly, the baseline TEWL values had an inverse pattern (97). This points to the fact that susceptibility to SLS is not solely dependent on barrier function of the stratum corneum. Other factors, such as reactivity of the layers underneath the stratum corneum, are also important.

Environment-related variables

Season and climate

Seasonal factors have a major influence on skin reactivity (2). A significantly higher skin suscepti-

bility to SLS was found in winter as compared with summer, in healthy volunteers (72, 99). Differences between winter and summer were even more pronounced in patients with atopic dermatitis (72). A significantly lower pre-exposure barrier function and hydration state of stratum corneum was reported in winter in healthy volunteers and atopic dermatitis patients (72, 99). The pathophysiological background for this may be that the low ambient water vapour pressure in the winter impairs the ability of stratum corneum to retain water. Water vapour pressure is a better estimate of quantity of water vapour in the air than % relative humidity, since the latter is dependent on the ambient temperature. Air of low temperature can contain a lower quantity of water vapour than air of high temperature. Water vapour pressure is proportional to dew point, which is defined as the temperature of the air at which the gaseous moisture begins to condense in the air (100).

It is likely that differences in atmospheric conditions influence skin susceptibility in various geographical regions, determined by water vapour pressure, temperature, wind and number of sunshine hours.

SLS testing in abnormal skin

Dryness/ichthyosis

Skin dryness is a symptom with a heterogeneous background. In atopic dermatitis patients, the barrier function (baseline TEWL) was found to correlate well with dryness (101), whereas in individuals with ordinary winter xerosis dryness was poorly correlated with barrier function (102). In subjects with dry skin (of which the majority had atopic dermatitis) a higher susceptibility to SLS was observed than in subjects having a non-dry skin (75). In contrast, Lammintausta et al. did not observe a difference in susceptibility between normal skin and skin that looked and felt rough (77).

Several investigators found TEWL to be significantly increased in ichthyosis compared with healthy control subjects (103–105). However, others found normal TEWL values in ichthyosis vulgaris patients (106, 107).

Dermatitis

A higher TEWL was noted in the involved skin in various types of dermatitis as compared with uninvolved sites (106, 108, 109). Shahidullah et al. have observed an increased TEWL on the involved and uninvolved skin in several types of dermatitis (108), TEWL of uninvolved skin being related to the severity of the dermatitis (108). In a more recent study, it was noted that susceptibility to SLS was related to

the severity of manifest atopic dermatitis (71). In other studies on several types of dermatitis, it was observed that existing dermatitis anywhere on the body may enhance susceptibility to irritants on another body location (39, 110, 111). This has been termed the "excited skin syndrome" (112). Since the type of dermatitis present did not appear to influence the reactivity, the enhanced susceptibility noted was regarded as secondary to dermatitis activity per se (111). However, van der Valk found a significantly elevated TEWL before and after SLS exposure only in the subgroup of patients with manifest atopic dermatitis (113). In patients with manifest irritant contact dermatitis there was no difference in the post-exposure TEWL values compared with control subjects (113). In a study comparing patients with a history of atopic dermatitis (but without manifest dermatitis) and patients with a history of contact dermatitis, higher pre-exposure and post-exposure TEWL values could be noted only in patients with a history of atopic dermatitis (75). In another study, patients with active and those with inactive atopic dermatitis reacted more strongly to SLS as compared with nonatopic controls (58). Also in the open test model, subjects with a history of atopic dermatitis were proven to be more susceptible (77). Therefore, atopic dermatitis skin may be considered as unique with respect to irritant susceptibility.

Hyperreactive and hyporeactive skin

In summary, hyperreactive skin was demonstrated in healthy subjects and atopic dermatitis patients in the winter season (72, 99). Personal characteristics that are probably characterized by hyperreactive skin are dryness (75), manifest dermatitis (several types) on another body location (39, 71, 110, 111), history of atopic dermatitis (58, 75), impaired barrier function (see below), and recent short-time irritant contact (66, 78). It is a clinical impression that the uninvolved skin of patients with psoriasis is not characterized by elevated susceptibility to irritants, in contrast to dermatitis. An exception to this statement may be generalized pustular forms.

Hyporeactive skin may be induced by preceding long-time irritant exposure, as was demonstrated by Lammintausta (114). Hyporeactivity was also noted by Widmer et al., 6 and 9 weeks after short-time repeated 3-week occlusive exposures, but not after 3 weeks (115).

Baseline TEWL and predictive testing

In 1986 Murahata et al. (116) found that subjects with increased basal TEWL reacted more severely

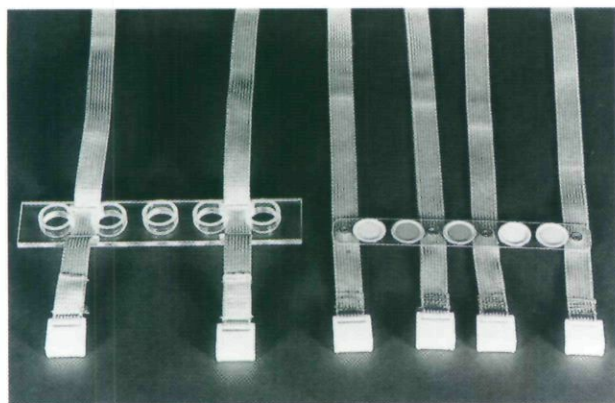


Fig. 3. Examples of a strip with 20 mm diameter holes (left) for repeated open testing on the forearm, and a strip with extra large Finn Chambers (right) for repeated occlusive testing on the forearm.

to detergent irritation, indicating an association between high basal TEWL and sensitive skin. Subsequently, Tupker and Pinnagoda demonstrated that pre-exposure baseline TEWL value was strongly correlated with the TEWL value after 1 week of repeated exposures (65, 70, 75), and only moderately correlated with TEWL after 3 weeks of exposures (75). A positive correlation between basal TEWL and TEWL after skin exposure to SLS was also found by Agner (46, 110, 117). In a multiple regression analysis, including basal TEWL, laser Doppler flow, skin colour (a^* , b^* and L) and skin thickness, only basal TEWL was highly significantly positively correlated with TEWL after SLS-exposure (46). In other studies using one-time occlusive tests, these results could not be confirmed; no correlation (66, 118) or low correlation (91, 119) was found. Using repeated open exposures to SLS, a correlation between baseline TEWL and postexposure TEWL was found by Wilhelm et. al. (25), in contrast with the study by Lammintausta (77), in which no correlation was found.

The relationship between basal TEWL and risk of occupational dermatitis was first studied by Coenraads and Pinnagoda (120). In a group of 54 metal workers, 4 workers developed irritant contact dermatitis. In these 4 cases, the pre-exposure TEWL was significantly higher than in those 50 workers who did not develop dermatitis (120). However, in a prospective study of 74 apprentice hairdressers and 111 apprentice nurses, no correlation between baseline TEWL and the occurrence of hand dermatitis was found (121). In the group of hairdressers with a basal TEWL below $15 \text{ g/m}^2\text{h}$, there was no clear relationship with dermatitis incidence rate. However, for basal TEWL above $15 \text{ g/m}^2\text{h}$ the incidence rate was elevated

(121). In another study basal TEWL was not predictive for occupational dermatitis risk, as there was no significant difference between those workers who developed cutting fluid dermatitis and those who did not (122). However, workers who developed dermatitis exhibited higher mean increases in TEWL during the first 3 weeks of work (122).

Guidelines on SLS exposure tests with evaluation by non-invasive methods

Laboratory facility, measuring device and operating procedure

The previous guideline of the ESCD Standardization Group on TEWL measurement with recommendations about the device and control of ambient humidity and temperature conditions, draught etc. as well as general principles for design and performance of a good bioengineering and skin study should be followed (5,123,124).

Test individuals, reactivity and location

The possible influences of age, race, sex, menstrual cycle, anatomical site and of season and climate on SLS reactivity should be considered. Also previous conditions, diseases and exposures including topical and oral medications need consideration. Flexor side forearm skin with cubital fossa and the wrist excluded is a preferred study site. When different detergents or treatments are to be compared, it is recommended to change the position of these treatments cyclically from one subject to the other.

Pre- and postexposure values should be given. The use of differences between pre- and postexposure values is optional. For repeated testing, measurements should be performed at least twice weekly, namely on the first day of exposure and on the last day, before the applications.

Test substance and applications

High purity (99%) SLS must be used in any study, dissolved in distilled water (w/v%) in occlusive and open testing, while tap water may be acceptable in immersion testing.

Standard sized occlusion chambers with filter paper discs corresponding to large (12 mm Ø, maximal capacity $70 \mu\text{l}$, recommended volume $60 \mu\text{l}$) and extra large (18 mm Ø, maximal capacity $300 \mu\text{l}$, recommended volume $200 \mu\text{l}$) (Fig. 2) Finn[®] chambers are recommended, the extra large chambers for repeated applications. Test sites on the skin must be properly marked and remarked during repeated exposure studies. For repeated exposure the use of chambers on a strip is to be pre-

Table 4. ESCD guidelines on SLS exposure tests with TEWL measurement

	Susceptibility evaluation		Provocative testing	
	acute reaction	cumulative reaction	acute reaction	cumulative reaction
One-time occlusion test				
application time	24 h	not	24 h	not
mode of application	chamber 12 mm	applicable	chamber 12 mm	applicable
SLS w/v%	0.5% ^a		2%	
Repeated occlusion test				
application time	not	2 h 1× daily	not	2 h 1× daily
application period	applicable	3 weeks*	applicable	3 weeks*
mode of application		chamber 18 mm		chamber 18 mm
SLS w/v%		0.25%		1%
Open test				
application time	60 min 2× daily	10 min 1× daily	not possible ^a	10 min 1× daily
application period	1 day	3 weeks*		3 weeks*
mode of application	20 mm guard ring	20 mm guard ring		20 mm guard ring
SLS w/v%	10%	1%		2%
Immersion test ^b				
immersion time	30 min 2× daily	10 min 1× daily	30 min 2× daily	10 min 1× daily
application period	1 day	3 weeks	1 day	3 weeks*
mode of application	forearm immersion	forearm immersion	forearm immersion	forearm immersion
SLS w/v%	0.5%	0.5%	2%	2%

^a May also performed as one-time application of a dilution series (SLS 4, 2, 1, 0.5, 0.25, 0.125, 0.0625%, 0% w/v).

* 1 week is 5 application days.

^b Water temperature 35°C.

^a In temperate zones, it is not possible to elicit an irritation response in all subjects using 10% SLS for 60 min twice daily, and longer exposure times are not feasible.

Table 5A. ESCD guideline on clinical scoring of acute SLS irritant reactions, simple scoring system*

Score	Qualification	description
0	negative	no reaction
$\frac{1}{2}$	doubtful	very weak erythema or minute scaling
1	weak	weak erythema, slight oedema, slight scaling and/or slight roughness
2	moderate	moderate degree of: erythema, oedema, scaling and/or roughness, or minor degree of erosions, vesicles, crusting and/or fissuring
3	strong	marked degree of: erythema, oedema, scaling, roughness, erosions, vesicles, bullae, crusting and/or fissuring
4	very strong/caustic	as 3, with necrotic areas

Reading 25 to 96 h after one-time exposure.

* The ESCD simple scoring system is based on the scoring system according to Frosch and Kligman (2), and may be used when no subdivision into the different qualities of irritation (erythema, scaling, roughness, oedema, fissures) is necessary.

Table 5B. ESCD guideline on clinical scoring of acute SLS irritant reactions, elaborate scoring system*

Each of the irritation qualities is graded according to the following system:

quality	$\frac{1}{2}$	1	2	3
erythema	very weak	weak, diffuse or spotty	moderate	marked
roughness/ contour	shiny surface	slight roughness or wrinkled surface	moderate roughness	marked roughness
scaling	—	minute flakes	moderate flakes	large flakes
oedema	—	slight	moderate	marked
fissures	—	fine fissures	broad fissures	wide fissures with hemorrhage or ex- udation

* The elaborate scoring system is based on the scoring system according to Frosch and Kligman (2), and may be used when a subdivision into the different qualities of irritation (erythema, scaling, roughness, oedema, fissures) is necessary, or when a better balanced estimation of reaction intensity is warranted.

From these separate scorings an estimate of the overall intensity of the reaction can be made by means of the following sum scores:

Sum score Qualification: 0, negative; $\frac{1}{2}$, doubtful; 1–2, weak; 3–4, moderate; 5–7, strong; >7, very strong or caustic.

Table 6A. ESCD guideline on clinical scoring of *subacute/cumulative* SLS irritant reactions, simple scoring system*

Score	Qualification	Description
0	negative	no reaction
$\frac{1}{2}$	doubtful	very weak erythema and/or shiny surface [#]
1	weak	weak erythema, diffuse or spotty, slight scaling and/or slight roughness ^a
2	moderate	moderate degree of: erythema, scaling, roughness and/or weak oedema and/or fine fissures
3	strong	marked degree of: erythema, scaling, roughness, oedema, fissures and/or presence of papules and/or erosions and/or vesicles
4	very strong/caustic	as 3, with necrotic areas

* The ESCD simple scoring system may be used when no subdivision into the different qualities of irritation (erythema, scaling, roughness, oedema, fissures) is necessary.

[#] The term 'shiny surface' is used for those minimal reactions that can only be discerned when evaluated in skimming light as a 'shiny' area.

^a The term 'roughness' is used for reactions that can be felt as rough or dry, sometimes preceded or followed by visible changes of the surfaces contour, in contrast to 'scaling', which is accompanied by visible small flakes.

Table 6B. ESCD guideline on clinical scoring of *subacute/cumulative* SLS irritant reactions, elaborate scoring system*

Each of the irritation qualities is graded according to the scoring system described in Table 5B.

From these separate scorings an estimate of the overall intensity of the reaction can be made by means of the following sum scores:

Sum score	Qualification
0	negative
$\frac{1}{2}$	doubtful
1-2	weak
3-5	moderate
6-9	strong
>9	very strong or caustic

* The elaborate scoring system may be used when a subdivision into the different qualities of irritation (erythema, scaling, roughness, oedema, fissures) is necessary, or when a better balanced estimation of reaction intensity is warranted.

ferred (Fig. 3). Manufacturer's standard filter discs should be used.

Chambers should be applied to the skin immediately, i.e. within one minute after preparation with the test solution. There must be good contact between the application system and the skin.

For open exposures, a 20-mm diameter plastic ring is advised. This ring must be fixed to the volar forearm skin preferably by means of a strip and nonadhesive bandages (Fig. 3). The volume of the solutions must be such that the total exposure area is covered (about 800 μ l). TEWL measurement should be performed a minimum of 1 hour after removal of test chambers, with the skin uncovered under laboratory room conditions in the meantime.

Specific test procedures

The ESCD standards for one-time occlusion, repeated occlusion, open and immersion tests are presented in Table 4.

Procedures fall into the following categories:

Provocative testing aims to induce an irritant reaction in "all" test individuals, without creating a caustic burn, in order to *study irritant reactions*, their modification by therapeutic or prophylactic

measures, or for comparison with other irritants, particularly detergents.

Susceptibility testing aims to *study the individual* by determining the susceptibility to SLS either by a simple procedure with one standard application (concentration) or more detailed using a dilution series and assessment of the irritation dose-response curve. Dilution series application may also be used in provocative testing in order to provoke reactions of a defined strength.

Acute reaction testing aims at experimental *study of the initial irritative damage* to the skin.

Cumulative reaction testing aims at *clinically realistic study of chronic irritant contact dermatitis* taking ongoing repair, with increase or lowering of the threshold, into consideration. The characteristic time course is erythema in the 1st week changing in scaling and roughness in the 3rd week, al-

Table 7. ESCD guideline on subjective scoring of the SLS irritant reactions during or after exposure (optional)

Qualification	score	description
negative	0	no burning/stinging sensation
weak	1	weak burning/stinging
moderate	2	moderate burning/stinging
strong	3	strong burning/stinging

though in some subjects erythema (together with the other signs) may be observed in the 3rd week only.

The clinical appearance of acute and cumulative reactions differ. The guideline therefore includes separate *standard clinical scoring schemes* for the two types of reactions, see Tables 5A, B and 6A, B). The use of a scoring system for subjective irritation is optional (see Table 7).

Individual pilot study

In recognition of the range of influencing variables and their complex interaction it is recommended that a pilot study is performed in order to ensure that any deviation from the recommended standard concentrations given in the guidelines is justifiable.

Interpretation of SLS exposure/results from non-invasive evaluation methods

SLS is a model irritant, suited for precise testing and the highly sensitive measurement of response by means of TEWL. However, irritant substances in general differ greatly in skin penetration capabilities and mode of responses. Results of testing with SLS cannot therefore be uncritically projected to other types of irritation. TEWL is not a clinical sign or symptom per se, but is an indirect measure of the skin response. The same holds true for some of the other non-invasive evaluation methods, all of which measure only one particular aspect of skin irritation. Results must be compared with clinical observations, epidemiological data and comparative data obtained using other noninvasive methods.

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Address:

Jorgen Serup
Department of Dermatological Research
Leo Pharmaceutical Products
55 Industriparken
DK-2750 Ballerup
Denmark

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