

Long-term repetitive sodium lauryl sulfate-induced irritation of the skin: an *in vivo* study

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Skin may adapt to topical irritants through accommodation. This study focuses on long-term exposure to irritants and attempts to demonstrate accommodation. Sodium lauryl sulfate (SLS) induced irritant contact dermatitis at 3 concentrations (0.025% to 0.075%). Distilled water, acetone and an empty chamber served as controls. Experimental compounds were applied to forearms of 7 healthy volunteers for 24 hr before replacing by a fresh chamber for 6 non-consecutive weeks over 103 days. Possible accommodation was quantified by visual scoring (erythema and dryness) and by bioengineering parameters: transepidermal water loss (TEWL), capacitance, chromametry and laser Doppler flowmetry (LDF). Significant erythema, dryness, elevated TEWL, skin colour reflectance and LDF values occurred during the exposure periods. Upon repeat exposure, an immediate and augmented response in erythema, TEWL, skin colour reflectance and LDF developed. However, irritant skin changes were not sustained. Irritation parameters return to baseline after cessation of exposure. There was no evidence of sustained irritation or accommodation after the last exposure. Study findings do not document sustained accommodation or adaptive hyposensitivity after long-term repetitive irritant exposure under these test conditions. Alternative models should be developed to prove or disprove the accommodation hypothesis.

Key words: accommodation; bioengineering; contact dermatitis; hardening; hypoirritation; sodium lauryl sulfate (SLS); transepidermal water loss (TEWL). © Blackwell Munksgaard, 2005.

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Irritant contact dermatitis (ICD), a common cause of occupational disease, results from cumulative, repetitive exposure of skin to weak irritants (1–3). In the 1910s, Jadassohn (4) studied ICD and the development of tolerance to sensitizing chemicals. He introduced the term ‘hardening’ to characterize the phenomenon of hyporeactivity that was observed in the skin of industrial workers. This phenomenon has since been named accommodation. In more recent studies, the defining characteristic of hardening has been a progressively decreasing inflammatory response with prolonged exposure to an irritant (5–8). Skin hardening has been associated with allergic contact dermatitis and ICD. While there have been studies demonstrating accommodation in ICD (9–14), the induction of hypoirritation with prolonged exposure to known irritants such as sodium lauryl sulfate (SLS) has been minimally studied (15, 16).

A single exposure to an irritant can induce skin changes that render it more resistant to subsequent exposures (16, 17). Resistance develops in

the form of an adaptive downregulation of the inflammatory response to topical irritants (15, 16, 18–22). Stone and Willis (12) attempted to explain accommodation by analysing the inflammatory reaction in intraepidermal pustule formation. They hypothesized that the reactive inflammatory process may be downregulated and even blocked by multiple non-specific reactions (23–26). This concept of hyporeactivity appears paradoxical to mechanisms involved in chronic ICD (CICD) where irritation is amplified and the skin is progressively hyperreactive with repeated irritant exposure (27).

This multidose study was performed over 103 days to evaluate irritation and the concept of hypoirritation as a response to long-term irritant exposure.

Materials and Methods

Subjects

7 healthy Caucasian volunteers (1 woman and 6 men, median age of 46 years, range 24–65 years)

were randomly selected from a pre-existing volunteer database. Informed consent was obtained, and the study was approved by the University of California Committee on Human Research. Subjects did not have a history of atopic dermatitis nor any other chronic skin disease. During the study, subjects were allowed to bathe as usual but were instructed to avoid direct application of detergents, moisturizers, emollients or topical medications to their forearms.

Materials

SLS (99% purity; Sigma Chemical, St Louis, MO, USA) was dissolved in deionized water to form 0.025%, 0.050% and 0.075% solutions. Deionized water served as the vehicle control. Acetone at 100% (Sigma Chemical) is known to deplete skin's lipid content and functioned as a positive control. Polypropylene chambers (12-mm diameter; Hill Top Companies, Cincinnati, OH, USA) were used as occlusive compartments. An empty chamber served as the negative control.

Study design

The study was executed in 4 phases. Phases 1 and 2 involved continuous irritant exposure and ICD induction, whereas Phases 3 and 4 served as post-irritation evaluation periods. There was no irritant exposure during Phases 3 and 4. Only bioengineering and clinical evaluation of skin changes occurred in this period. Phases 1 and 2 spanned 3 weeks each and were separated by a 3-week break period. A 2-week break period preceded Phases 3 and 4, which took place over 1 week each and were separated by a 2-week break.

The study was performed between December and March 2003, inclusive.

Irritant exposure and induction of ICD (Phases 1 and 2)

Both medial volar forearms of each subject were used for test sites. 3 sites on each forearm were designated. Specific sample placements (0.025% SLS, 0.050% SLS, 0.075% SLS, deionized water, acetone or empty chamber) were randomized to the 6 test sites on each subject. Cumulative ICD was induced at the 3 SLS sites. 180 µl of each sample was placed in each occlusive chamber immediately prior to application to minimize drying of the filter. The occlusive sample chambers were then placed on the 6 experimental sites for a 24-hr period. A distance of 2 cm separated 1 chamber from the next. 24 hr after the sample application, the chambers were removed and the

skin gently wiped (Kimwipe, Kimberly Clark Corporation, Roswell, GA, USA) to remove residual moisture. Test sites were not rinsed. 30 min after chamber removal, clinical scoring and bioengineering measurements of the sites were taken. Fresh samples and occlusive chambers were then re-applied to the designated sites in the aforementioned fashion. Consistency in test sites was ensured with stencils of the first application. The process of continuous sample application followed by evaluation occurred 4 times weekly (Monday through Thursday). Only chamber removal and evaluative measurements were taken on Fridays. There were no interventions during the weekends.

The induction phase and the respective irritant exposure spanned 6 non-consecutive weeks and totalled 24 applications per subject.

Post-irritation evaluation (Phases 1 through 4)

Measurements were taken to evaluate and compare the quality and intensity of irritation. All measurements were taken by the same investigator and conducted under controlled ambient conditions (temperature $20 \pm 3^\circ\text{C}$, relative humidity 46–58%).

Visual scoring (VS)

Clinical changes in the skin were evaluated according to a 5-point erythema scale and dryness scale. The erythema scale is as follows: 0 (no visible reaction); 0.5 (slight patchy erythema); 1 (moderate, uniform erythema, possibly slight infiltration); 2 (intense erythema, possibly infiltration and/or vesicles); 3 (fiery erythema with oedema, infiltration, vesicles, possibly bullae). The scaling/dryness is graded as follows: 0 (none); 1 (weak); 2 (moderate); 3 (large flakes); 4 (intense peeling).

Baseline assessment was performed prior to the first sample application in both induction phases. Clinical assessment of the test sites were done daily during the weekdays in Phases 1 and 2 and on Mondays and Fridays during Phases 3 and 4. VS measurements were taken before the bioengineering methods.

Bioengineering evaluation

Measurements were taken every Monday and Friday in each phase for a total of 16 data points per subject.

Transepidermal water loss (TEWL). TEWL is a measure of the functional integrity of the stratum corneum water barrier and was measured with an evaporimeter (Tewameter TM 210,

Courage & Khazaka, Cologne, Germany and Acaderm, Menlo Park, CA, USA) (28–30).

Capacitance. Capacitance reflects the hydration status of the stratum corneum. Measurements were taken with a corneometer (CM 820 PC, Courage & Khazaka and Acaderm) (31).

Skin colour reflectance. To quantify erythema, skin colour reflectance was measured with a tristimulus colourimeter (Chroma Meter CR 200, Minolta, Osaka, Japan) (32).

Laser Doppler flowmetry (LDF). Superficial cutaneous blood flow was measured (Laser Doppler Flowmeter, Moor Instrument, Axminster, England) to evaluate the intensity of inflammation (33).

Statistical analysis

Statistics were performed using SIGMASTAT computer program (SPSS Science, Chicago, IL, USA). 1-way repeated-measures ANOVA evaluated the differences among the test sites for values of TEWL, capacitance, skin colour reflectance and LDF. Friedman repeated measures were performed for the non-parametric VS data points. All comparison tests were 2-sided with a significance level of 0.05.

Results

All data are compared to negative control (empty chamber).

Visual score

After 4 consecutive days (a work week) of exposure, significant erythema and dryness developed at the SLS and acetone sites. The intensity of erythema and dryness increased with increasing SLS concentration and with continued SLS

exposure during Phase 1. Erythema and dryness then returned to baseline. After the re-introduction of SLS exposure, significant erythema developed over a shorter time period (3 days versus 4 days), while development of significant dryness occurred after a longer period of exposure (10 days versus 4 days). In Phase 2, the intensity of erythema correlated with SLS concentration and with continued exposure as in Phase 1. After the discontinuation of the irritant exposure, both irritation parameters gradually returned to and remained at baseline for the remainder of the study (Figs 1 and 2).

Bioengineering measurements

TEWL. It linearly increased with continuous exposure and linearly decreased to near baseline with the interruption in exposure during the weekend. At the end of Phase 1, only the 0.05% and 0.075% SLS sites demonstrated significantly elevated TEWL. TEWL measurements for all test sites returned to baseline with cessation of exposure. In Phase 2, TEWL for all samples again linearly increased with continued exposure and linearly decreased with weekend discontinuation. All SLS concentrations developed significant TEWL elevation with re-exposure in Phase 2. TEWL from all sample sites linearly decreased to baseline by Phase 3 and remained insignificant throughout the evaluation phases (Fig. 3).

The water test sites temporarily achieved significant TEWL elevation during Phase 2.

Acetone exposure did not demonstrate any significant effect on TEWL.

Capacitance. Only water test sites achieved significant capacitance during the entire study (Fig. 4).

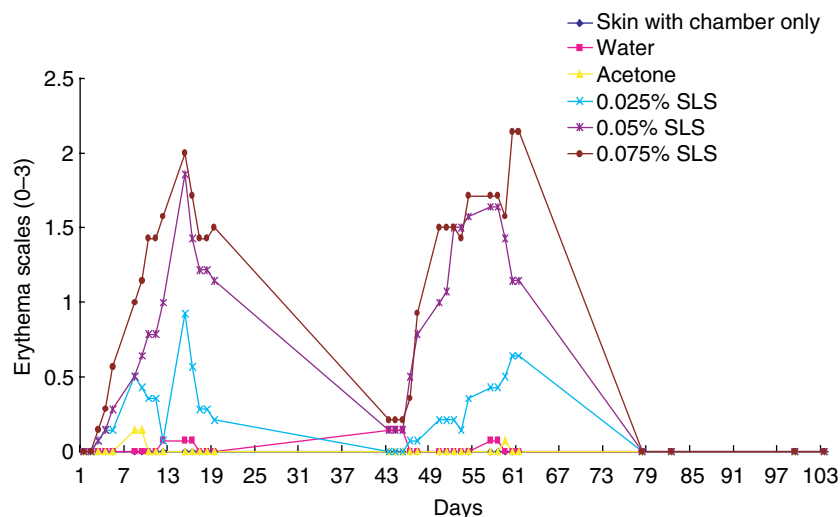


Fig. 1. Irritation as evaluated by visual scoring of erythema.

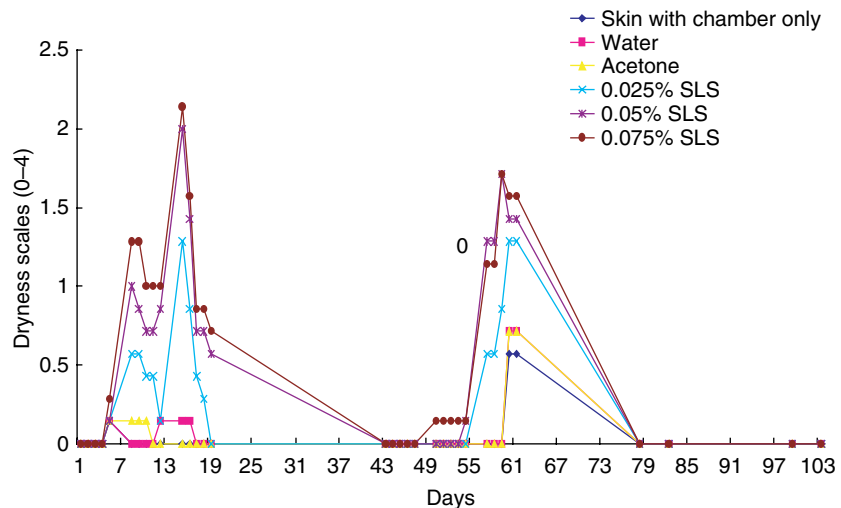


Fig. 2. Irritation as evaluated by visual scoring of dryness.

Skin colour reflectance. All SLS concentration sites demonstrated significant a^* values. The time to onset and a^* values correlated with concentrations. Higher SLS concentrations achieved significance earlier and reached higher peak values. The a^* values for all SLS samples progressively increased throughout Phase 1. All a^* values gradually returned to baseline over the first break period. With re-exposure, significant skin colour reflectance developed over a shorter period of time and reached a higher peak value. At the end of the induction period, a^* values for all SLS concentrations gradually decreased. Only the highest SLS concentration maintained significance by the start of the evaluation phases (Fig. 5).

Both acetone and water did not yield significant a^* values.

LDF. Only 0.05% and 0.075% SLS solutions yielded significant superficial blood flow. Significance was not sustained in Phase 1, since LDF values decreased to baseline with interruption of irritant exposure during the weekends and breaks. In Phase 2, the 0.075% SLS sample

developed significant values that were sustained throughout that phase (Fig. 6).

Water, acetone, and 0.025% samples never achieved significant levels.

Discussion

This study included the longest controlled irritant exposure duration in the literature. The daily continuous irritant exposures during the weekdays and exposure-free weekends simulated occupational exposures. Daily continuous irritant exposure induced significant changes in several irritation parameters (VS and bioengineering methods) during the initial irritant exposure period and perhaps reflected the direct cytotoxicity of the irritant and the skin's immunological naïveté. Upon repeat irritant exposure to irritated skin, irritation parameters varied in the intensity and speed of response. Most irritation parameters showed a quick amplified response consistent with the previously proven concept of skin memory and possibly enhanced by increased

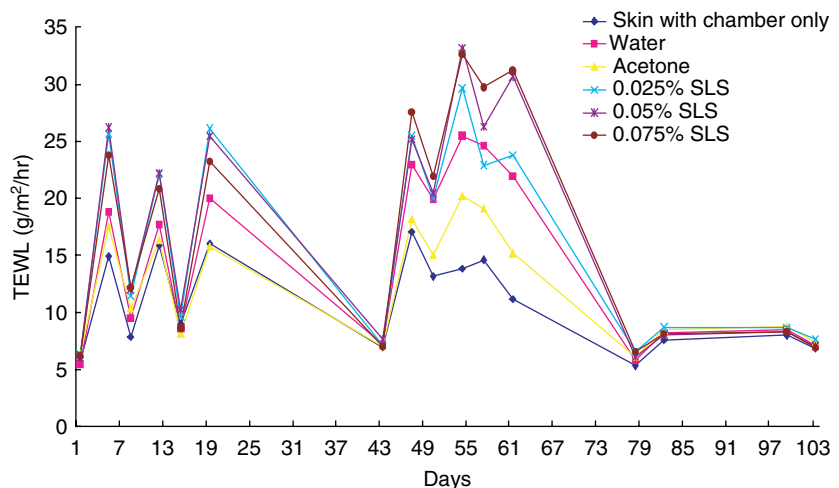


Fig. 3. Irritation as evaluated by trans-epidermal water loss.

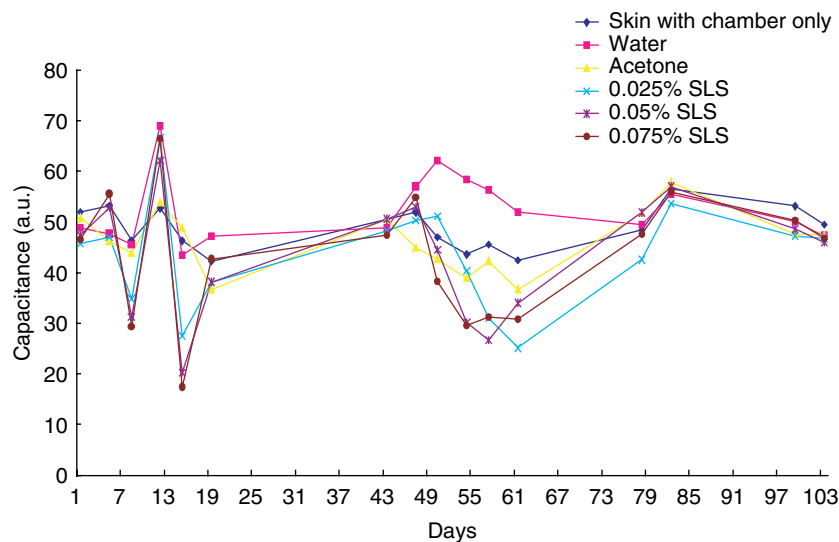


Fig. 4. Irritation as evaluated by capacitance.

irritant penetration, given the previously disrupted skin barrier. All irritation parameters returned to baseline, upon the discontinuation of irritant exposure at the end of the induction phases.

Of the parameters studied, TEWL, capacitance and visible dryness are considered the most important in evaluating chronic irritation in skin. These parameters correspond with barrier changes that often accompany long-term exposures. LDF, skin colour reflectance and visible erythema are mainly correlated with acute inflammation that is more common with initial or short-term exposure.

In this study, there was no evidence of accommodation or adaptive hyposensitivity after chronic irritant exposure. This study also showed no evidence of long-term or sustained skin changes after removal of the irritant, SLS.

Limitations in this study include exposure chronicity, use of specific irritants, and methodical

flaws in data collection. While daily 8-hr irritation for a period of months to years would be more realistic in reflecting occupational exposures, in this study, a 24-hr irritation period was used to compensate for the short duration of the study and to increase subject compliance with the study protocol. This study's findings are also limited to SLS, water and acetone as the irritant and cannot be generalized to other chemical irritants' effects on skin. Bioengineering measurements were taken only 30 min after the removal of the chambers. Measurements of skin hydration such as TEWL and capacitance taken shortly after occlusion may be artificially elevated. This concept may explain both the high TEWL and capacitance values of all samples after week-long occlusion (days 5, 12 and 19) and the large variation in TEWL values for all samples, including the negative control. The artificially elevated hydration measures may be minimized in future studies with greater elapsed time after removal of

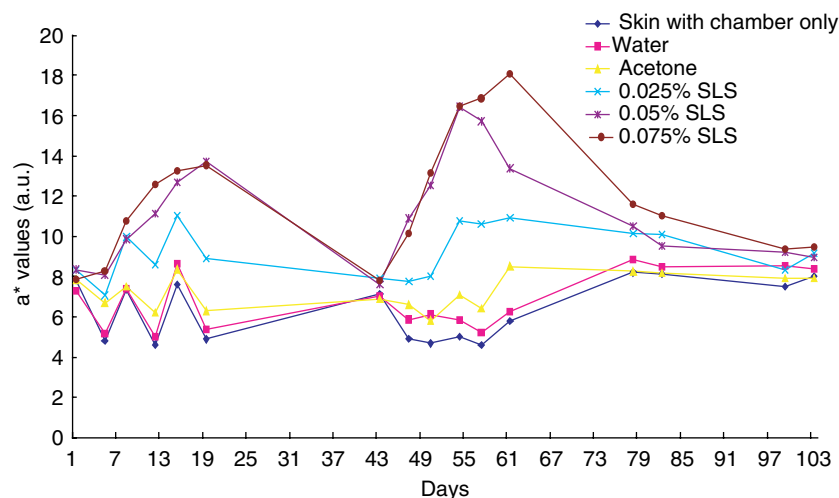


Fig. 5. Irritation as evaluated by skin colour reflectance.

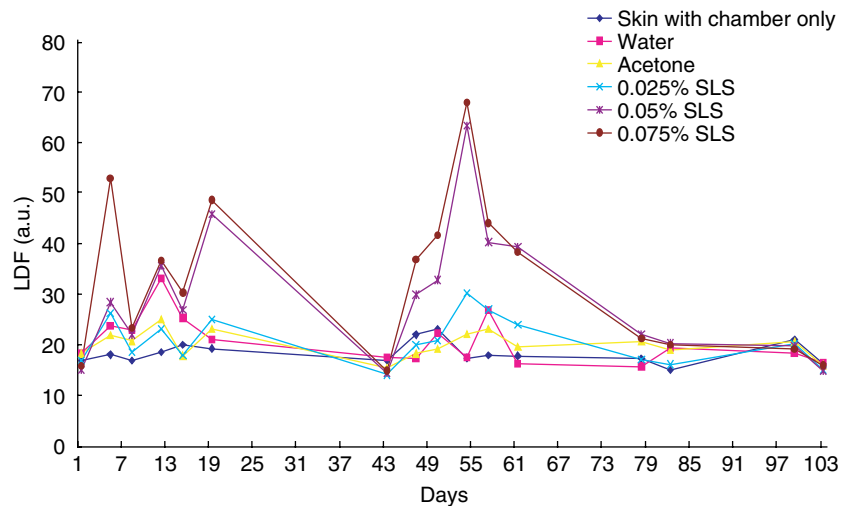


Fig. 6. Irritation as evaluated by laser Doppler flowmetry.

Table 1. Possible experimental variables in accommodation to irritants.

- molecular weight, charge, and solubility (partition coefficient) of irritants
- open application vs. occlusion
- number applications/days
- continuous vs. intermittent dosing
- dose (concentrations) response effects
- effect of multiple irritants ("tandem exposure")
- anatomic site specificity
- skin temperature (cold & hot)

the occlusive chambers. This would allow skin-hydration levels to reach a steady-state equilibrium that more accurately reflects post-irritation changes.

As many occupational dermatologists' clinical impressions support the accommodation hypothesis, we note that failure to document the phenomena here can be taken as only one step in the process of defining the role of many experimental variables. Table 1 presents a partial listing of such variables.

Addendum

Since review of this manuscript, Heinemann et al. (*Acta Derm Venereol* 2005; 85: 290–295) provide additional experimental data on hardening.

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