

The lactic acid stinging test predicts susceptibility to cumulative irritation caused by two lipophilic irritants*

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Summary

Background. Skin exposure to lipophilic irritants at workplaces is a risk factor for the development of occupational irritant hand dermatitis. Suitable lipophilic model irritants to be used in repeated irritation studies in volunteers are needed to gain a better understanding of the chemical-dependent mechanisms of skin irritation.

Objectives. To investigate acute and cumulative irritation elicited by two new lipophilic model irritants, octane (CAS No. 111-65-9) and cumene (CAS No. 98-82-8), in stingers as compared with non-stingers.

Methods. Short-time occlusive and repetitive occlusive irritation patch tests were performed over 5 days with octane and cumene in two groups of healthy volunteers ($n = 15$ each) who were classified as stingers and non-stingers according to a preceding lactic acid stinging test. Acute and cumulative irritation was quantified using clinical assessments and bioengineering methods (laser Doppler flowmetry, transepidermal water loss, capacitance measurements, and skin colour reflectance).

Results. Significantly stronger cumulative irritation was observed in stingers than in non-stingers for both irritants (visual scoring, stratum corneum hydration, and skin colour reflectance).

Conclusions. The preliminary results suggest the existence of a distinct sub-population with increased susceptibility to cumulative irritation induced by lipophilic irritants that can be identified by the lactic acid stinging test.

Key words: bioengineering; cumene; cumulative irritation; irritant contact dermatitis; lactic acid stinging test; lipophilic irritants; laser Doppler flowmetry; octane; solvents; skin capacitance; TEWL.

Disease mechanisms of irritant contact dermatitis are frequently studied in human experimental disease models *in vivo*. For this purpose, suitable model irritants are

needed that, at the same time, cause sufficient irritation and are still toxicologically acceptable.

In this article, two new lipophilic model irritants—octane and cumene (syn. isopropyl benzene)—and the ability of the lactic acid stinging test to predict sensitivity to them are presented.

Lipophilic substances comprise a chemically heterogeneous group, and include, among others, aliphatic and aromatic hydrocarbons, ketones, fatty acids, and glycol ethers. Some substances have been used on human skin as lipophilic irritants in the past, such as toluene (1–3), acetone (4), croton oil, kerosene (5), benzene (6), and other solvents and fuels (7). However, they are not as widely used as model irritants on human volunteers as is,

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Conflicts of interest: We declare that we do not have any conflicts of interest. The study was an investigator-driven clinical trial and did not receive any funding.

*This paper is dedicated to Jan E. Wahlberg, who performed pioneer research on the skin effects of industrial solvents.

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for example, sodium lauryl sulfate (SLS) when studying detergents, because of local toxicity, unknown or negative systemic safety profiles after cutaneous application, or simply the fact that many of them are volatile and not easy to handle in the experimental setting. Previous toxicological and experimental evaluation of the two chemicals introduced in this article ensured that both are safe and suitable for repetitive cutaneous testing on human subjects, as long as the exposure is restricted to small test areas (unpublished data).

Previous experimental studies with toluene, a solvent that is chemically related to cumene, showed considerable interpersonal variability in susceptibility to solvent-induced irritation (3). Up to now, the factors that define sub-populations with increased risk of skin irritation caused by solvents have been unknown. Tandem irritation tests with alternating exposure to toluene and SLS showed enhanced cumulative irritation as compared with irritation elicited with either irritant alone (8). Appropriate methods for the identification of people with increased susceptibility, who are more likely to develop irritant contact dermatitis, would be beneficial, but these have so far not been found. Two quick, non-invasive irritancy tests, with dimethyl sulfoxide (DMSO) and sodium hydroxide (NaOH) respectively, could not predict sensitivity to acute irritation with SLS (9). The lactic acid stinging test (LAST) (10) was reported to identify persons as 'stingers', who generally show an increased tendency to experience subjective irritant reactions to cosmetic products (11). It is frequently used in investigations on the so-called 'sensitive skin'-phenomenon (12–15). This test, however, could not predict higher reactivity to the detergent SLS (11, 16), and it could not predict higher sensitivity to three of five urticants (17) and stimuli causing sensorial irritation (15). With a few exceptions, it has been generally considered that higher sensitivity to a given irritant does not predict sensitivity to other irritants (15), perhaps because of high interpersonal variability in sensitivity to irritants (14, 18).

The purpose of this study was to investigate acute and cumulative irritation elicited by the two new lipophilic model irritants, octane and cumene, in a single and repeated short-time occlusive irritation test, and to determine whether persons responding as 'stingers' in the LAST are more susceptible to irritation caused by octane and cumene.

Materials and Methods

Subjects

Thirty healthy Caucasian volunteers, aged 18–55 years, were selected according to the results of the stinging test

(see below), and allocated either to a group of stingers or to a group of non-stingers (each $n = 15$, 10 females and 5 males per group). The institutional ethics committee of the University Hospital Jena approved the protocol, and all subjects gave written informed consent. One week before and during the study, the subjects did not apply any cosmetic or topical preparations on the skin areas to be examined, namely the forearms and the back, and also abstained from washing, showering or exposing these areas to UV light. No pregnant or nursing females were included. Before any measurements, the volunteers were acclimatized in an air-conditioned room at constant temperature of $21 \pm 1^\circ\text{C}$ and a relative humidity of $45\% \pm 5\%$ for at least 20 min, and the measurements were performed under the same conditions. The study was conducted in March and April 2009.

Chemicals

Lactic acid, CAS No. 50-21-5, purity 90% (VwR International, Dresden, Germany), was used for the stinging test.

Neat n-octane, an aliphatic hydrocarbon, CAS No. 111-65-9, with highest purity of 99.2% (VwR International), and neat cumene (syn. isopropyl benzene), an aromatic hydrocarbon, CAS No. 98-82-8, purity 99% (Merck Schuchardt OHG, Hohenbrunn, Germany), were used as lipophilic irritants.

Test procedures

Pretest: LAST. The LAST was performed as described in (10). The subjects first washed their faces with tap water and a synthetic detergent. After drying of the face with paper napkins, a 5% aqueous solution of lactic acid and tap water were applied simultaneously on the two nasolabial folds with two cotton swabs. After 2, 4 and 5 min, the subjects ranked the presence and intensity of stinging, itching or burning during the test period on the both sides, using an arbitrary scale of 0 (none) to 3 (severe) for grading. Volunteers with a cumulative score of at least 3 for the lactic acid side and a more than 2-point difference from the control side were classified as stingers. The test was carried out double-blind, and the volunteers were unaware of the results until the end of the whole study.

Single application test with lipophilic irritants. The single occlusive application patch test was also conducted in a double-blind, randomized design, right versus left forearm, in order to investigate the resulting acute irritant reaction. On each forearm, a test area of 2.6×2.6 cm was marked.

Octane and cumene (240 µl each) were pipetted on filter discs in extra-large 18-mm Finn Chambers® (Epitest Ltd Oy, Tuusula, Finland) and simultaneously applied on the pre-marked test fields for 8 min. The test chambers were fixed with adhesive tape (Fixomull® stretch; Beiersdorf, Hamburg, Germany). Before and immediately after the patch test, the cutaneous blood flow was measured with a laser Doppler perfusion imager [Moor Laser Doppler Imager (MLDI); see below]. The distance between the MLDI scanner and the skin was 20 cm, and the area where the skin blood flow was measured was 1 × 1 cm, adjusted to be entirely inside the test area.

Repeated short-time occlusive irritation patch test with lipophilic irritants. The repeated occlusive irritation test was conducted after the single application test in the same subjects in order to investigate the cumulative irritation. Two test areas (2.6 × 2.6 cm) were marked with a surgical skin marker on the skin of the subject's para-vertebral mid-back. A control area was marked 5 cm below. It was left untreated and served as a negative control. Octane and cumene were applied occlusively under patches as in the single test, but for 10 min each, twice a day, with a time interval of 3 hr, for 4 days. This part of the procedure was conducted in a single-blind manner, with the subjects being unaware of the allocation of the substances to the test areas, which were randomized among the subjects. Bioengineering measurements (see below) and clinical assessments were conducted every day before the patch tests and on the fifth day. A TERFU® spot fume-extractor (Fumex, Skelleftea, Sweden) with activated carbon filter (L50; Asecos GmbH, Gruendau, Germany) was used during the application procedures in order to remove organic solvent vapours from the laboratory atmosphere.

Measurements. The laser Doppler blood flow assessments were conducted using a Moor LDI Mk1 (Moor Instruments, Millway Axminster, UK). The signal analysis was conducted with the Moor LDI V2.3 Research Software (Lawrenz Medizin-elektronik Vertriebs GmbH, Sulzbach/Taunus, Germany), and returned arbitrary flow units, which were used in the analysis. Erythema intensity was measured with a Chromameter® CR 300 (Minolta, Osaka, Japan), following the international guidelines and using the a^* -values (19). Stratum corneum hydration was assessed with a Corneometer® CM 825/MPA9 (Courage & Khazaka, Cologne, Germany), with output in relative units, in accordance with European Expert Group on Efficacy Measurement of Cosmetics and other Topical Products (EEMCO) recommendations (20). Transepidermal water loss (TEWL) was measured in g/hr m² with a Tewameter® TM 300/MPA9 (Courage & Khazaka),

according to the published guidelines (21, 22). Each value used in the final analysis represents the arithmetic mean of three consecutive measurements that were taken of the respective test area at each time point, except for the TEWL, where the means of two measurements were used.

The visual irritation score was calculated by a trained investigator, using a modification of the cumulative irritation score proposed by Frosch and Kligman (23) and the European Society of Contact Dermatitis (24). For each of the signs of irritation (erythema, oedema, dryness, scaling, and fissuring), a value of 1, 0.5, 1, 2 or 3 was given, and these were added to give the sum score, which served for the final analysis. For the purpose of this study, the score was further modified so that the scale for erythema was extended to 4 as the maximal value. This was done to facilitate the formulation of the break-off criteria for test areas developing irritation that was too intense. Irritation was to be prematurely discontinued for test areas that developed an excessive irritation, i.e. when erythema reached a score of 4 and/or oedema reached a score of 3. No such intensive irritation was observed in the study.

Statistics. SPSS for Windows, version 16.0, was used for the analysis. Microsoft Excel was used for plotting the figures and tables. Results are shown as medians, median differences from baseline, and conservative 95% confidence intervals. For the normally distributed values, the two-sample *t*-test was used to compare the groups of stingers and non-stingers. Alternatively, the Mann–Whitney *U*-test was used. For the within-group comparisons of the daily values versus baseline, the paired *t*-test was used in cases of normally distributed data; otherwise, the Wilcoxon signed rank test was used. The Mann–Whitney *U*-test was used for the tests with the visual score data. The level for statistical significance was $P < 0.05$ with Holm–Bonferroni corrections for multiple comparisons (25).

Results

Single application study on the forearms

Skin blood flow. Both lipophilic substances led to a significant increase in skin blood flow after a single 8-min patch test application on the lower arms (Fig. 1). The median increase caused by cumene was much higher than that caused by octane (586.15 and 20.05 relative units, respectively). No statistically significant difference was observed between the stingers and non-stingers (octane, $P = 0.976$; cumene, $P = 0.631$; Mann–Whitney-*U*-test, data not shown).

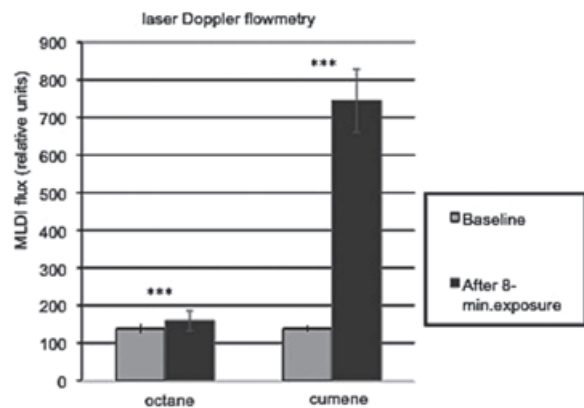


Fig. 1. Cutaneous blood flow, measured by Moor Laser Doppler Imager (MLDI), for both irritants before and after a 8 min patch test (median flux, conservative 95% confidence intervals). Stingers and non-stingers are grouped together, since no statistically significant difference was observed between them. Statistically significant differences between pre- and post-exposure values are marked as follows: *** $P \leq 0.001$.

Repeated irritation test. No significant changes over time were observed in the control areas for any of the parameters. Table 1 (octane) and Table 2 (cumene) show

the changes in the bioengineering parameters over time. With octane, statistically significant differences from baseline were evident from day 2 on for all of the parameters measured and for both stingers and non-stingers, except for the stratum corneum hydration in the non-stinger group, which reached statistical significance 1 day later (Table 1). The latter parameter, but with the other hydrocarbon, cumene, exhibited a similar pattern; that is, it became statistically significant for the stingers on day 2 and for the non-stingers 1 day later (Table 2). The changes in the cumene-induced erythema and the visual irritation score achieved significance only in the stinger group (Table 2). In contrast to octane, cumene did not induce a statistically significant increase in the TEWL in either group (Table 2). The comparisons between the stingers and the non-stingers are shown in Table 3 (octane) and Table 4 (cumene). For the corneometry (Figs. 2 and 3) and the visual irritation score data, the two groups exhibited statistically significant differences on all days and with both irritants (Tables 3 and 4). Stingers reacted significantly differently from non-stingers, with stingers losing skin humidity more rapidly and to a greater extent than non-stingers. The octane-induced erythema showed

Table 1. Skin bioengineering parameters over time and within-group comparisons for octane

Days	Erythema (chromametry a* values)		SC hydration (corneometry)		TEWL (g/hr m ²)		Visual irritation score	
	Stingers	Non-stingers	Stingers	Non-stingers	Stingers	Non-stingers	Stingers	Non-stingers
	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)
	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)
1	3 (2.55; 3.45) n.a.	2.9 (2.17; 3.63) n.a.	38.2 (36.53; 39.87) n.a.	36.8 (33.66; 39.94) n.a.	5.8 (4.86; 6.74) n.a.	5.6 (4.87; 6.33) n.a.	0 (0; 0) n.a.	0 (0; 0) n.a.
2	7.9 (6.55; 9.25) <0.001	6.6 (4.03; 9.17) <0.001	33.8 (31.47; 36.13) <0.001	36.4 (33.01; 39.79) 0.141	6.7 (5.68; 7.72) 0.002	6.0 (5.14; 6.86) 0.025	3.5 (2.07; 4.93) <0.001	0.5 (-0.52; 1.52) 0.002
3	8.1 (5.29; 10.91) <0.001	7 (3.7; 10.3) <0.001	31.1 (28.53; 33.67) <0.001	35.7 (32.23; 39.17) 0.002	7.6 (6.38; 8.82) <0.001	6.3 (5.77; 6.83) 0.001	4 (2.57; 5.43) <0.001	1 (-0.22; 2.22) <0.001
4	7.5 (4.40; 10.60) <0.001	5.8 (4.13; 7.47) <0.001	29.0 (25.78; 32.22) <0.001	33.1 (29.96; 36.24) 0.001	6.5 (5.52; 7.48) <0.001	6.5 (5.72; 7.28) <0.010	3 (1.57; 4.43) <0.001	2 (1.18; 2.82) <0.001
5	7.8 (5.23; 10.37) <0.001	4.7 (3.39; 6.01) <0.001	23.5 (20.36; 26.64) <0.001	31.8 (28.05; 35.55) 0.002	7.7 (6.64; 8.76) <0.001	6.6 (5.95; 7.25) <0.005	3.5 (1.46; 5.54) <0.001	1.5 (0.68; 2.32) <0.001

CI, confidence interval; n.a., not applicable; SC, stratum corneum; TEWL, transepidermal water loss.

Comparisons of each day's values versus day 1 (baseline) and within each group only. For each day, the median value, the conservative 95% CIs and the *P*-value after Holm–Bonferroni adjustment for multiple comparisons are provided.

Bold values indicate significance.

Table 2. Skin bioengineering parameters over time and within-group comparisons for cumene

Days	Erythema (chromametry a* values)		SC hydration (corneometry)		TEWL (g/hr m ²)		Visual irritation score	
	Stingers	Non-stingers	Stingers	Non-stingers	Stingers	Non-stingers	Stingers	Non-stingers
	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)
	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)	<i>P</i> (versus baseline)
1	3.0 (2.35; 3.65) n.a.	3.4 (2.34; 4.46) n.a.	37.8 (35.15; 40.45) n.a.	36.4 (32.24; 40.56) n.a.	5.3 (4.36; 6.24) n.a.	4.9 (3.8; 6.0) n.a.	0 (0; 0) n.a.	0 (0; 0) n.a.
2	3.6 (1.97; 5.23) 0.02	3.6 (2.74; 4.46) 0.531	34.7 (29.97; 39.43) <0.001	35.00 (31.17; 38.83) 0.328	4.9 (3.76; 6.04) 0.091	4.9 (4.04; 5.76) 0.283	0.5 (0.3; 0.7) <0.001	0.0 (0.0; 0.0) 0.500
3	4.7 (3.15; 6.25) 0.001	3.5 (2.68; 4.32) 0.057	32.7 (27.40; 38.0) <0.001	35.2 (31.12; 39.28) 0.004	6.3 (5.08; 7.52) 0.031*	5.6 (4.74; 6.46) 0.213	0.5 (-0.11; 1.11) 0.001	0.0 (0.0; 0.0) 0.500
4	4.3 (3.12; 5.48) 0.019	3.7 (2.76; 4.64) 0.487	29.4 (26.71; 32.09) 0.001	34.5 (29.73; 39.27) 0.001	6.4 (5.22; 7.58) 0.029*	5.3 (3.91; 6.69) 0.358	0.5 (0.3; 0.7) <0.001	0.0 (-0.2; 0.2) 0.125
5	4.0 (3.06; 4.94) 0.005	3.1 (2.04; 4.16) 0.599	28.9 (24.7; 33.1) 0.001	35.0 (30.84; 39.16) 0.024	6.3 (5.77; 6.83) 0.055	5.4 (4.79; 6.01) 0.512	0.5 (0.3; 0.7) 0.001	0.0 (0.0; 0.0) 0.250

CI, confidence interval; n.a., not applicable; SC, stratum corneum; TEWL, transepidermal water loss.

Comparisons of each day's values versus day 1 (baseline) and within each group only. For each day, the median value, the conservative 95% CI and the *P*-value after Holm–Bonferroni adjustment for multiple comparisons are provided.

**P*-values that did not remain significant according to the adjusted thresholds.

Bold values indicate significance.

a statistically significant difference between the stingers and non-stingers on day 5 only (Table 3). No significant difference was observed between the groups regarding cumene-induced erythema or TEWL resulting from both irritants (Tables 3 and 4).

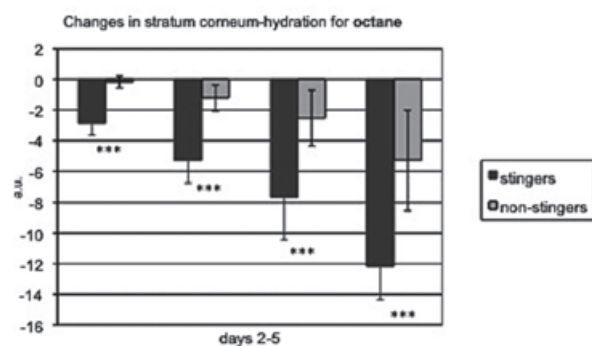


Fig. 2. Median changes ($\pm$ 95% confidence intervals) of the corneometry-data compared to baseline over time induced by octane. a.u.: arbitrary units. Statistically significant differences between stingers and non-stingers are marked as follows: ****P* $\leq$ 0.001.

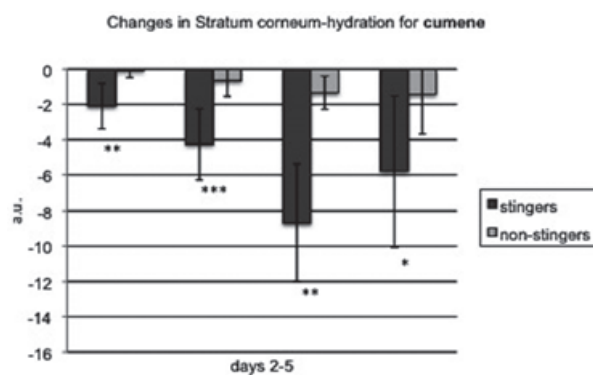


Fig. 3. Median changes ($\pm$ 95% confidence intervals) of the corneometry-data compared to baseline over time induced by octane. a.u.: arbitrary units. Statistically significant differences between stingers and non-stingers are marked as follows: ****P* $\leq$ 0.001; ***P* $\leq$ 0.01; **P* $\leq$ 0.05.

Discussion

There is a lack of suitable lipophilic irritants that can be conveniently used in repetitive irritation studies on human volunteers. Octane and cumene are candidates

Table 3. Median changes (difference from baseline values) of the skin bioengineering parameters with comparisons between stingers and non-stingers for octane

	Erythema (chromametry a* values)		SC hydration (corneometry)		TEWL (g/hr m ²)		Visual irritation score	
	Stingers	Non-stingers	Stingers	Non-stingers	Stingers	Non-stingers	Stingers	Non-stingers
	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)
Days	<i>P</i> (stingers versus non-stingers)		<i>P</i> (stingers versus non-stingers)		<i>P</i> (stingers versus non-stingers)		<i>P</i> (stingers versus non-stingers)	
2	4.86 (2.92; 6.80)	2.69 (0.72; 4.66)	-2.87 (-3.63; -2.11)	-0.17 (-0.56; 0.22)	0.95 (0.46; 1.44)	0.42 (-0.03; 0.87)	3.5 (2.07; 4.93)	0.5 (-0.52; 1.32)
	0.057		<0.001		0.113		0.001	
3	5.94 (3.48; 8.4)	2.91 (1.46; 4.36)	-5.3 (-6.76; -3.84)	-1.2 (-2.04; -0.36)	1.49 (0.42; 2.56)	0.78 (0.38; 1.18)	4 (2.57; 5.43)	1 (-0.22; 2.22)
	0.040*		<0.001		0.151		0.016	
4	4.56 (2.14; 6.98)	2.28 (1.01; 3.55)	-7.64 (-10.43; -4.85)	-2.53 (-4.35; -0.71)	1.48 (0.42; 2.54)	1.48 (0.93; 2.03)	3 (1.57; 4.43)	2 (1.18; 2.82)
	0.032*		<0.001		1.0		0.008	
5	4.66 (2.67; 6.65)	1.5 (0.61; 2.39)	-12.17 (-14.34; -10.00)	-5.27 (-8.53; -2.01)	1.31 (0.05; 2.57)	1.07 (0.45; 1.69)	3.5 (1.46; 5.54)	1.5 (0.68; 2.32)
	0.002		<0.001		0.418		0.012	

CI, confidence interval; SC, stratum corneum; TEWL, transepidermal water loss.

The difference from baseline values of each day were compared between stingers and non-stingers. For each day, the median value, the conservative 95% CIs and the *P*-value after Holm–Bonferroni adjustment for multiple comparisons are provided.**P*-values that did not remain significant according to the adjusted thresholds.

Bold values indicate significance.

to fill this gap, and may help in the investigation of new aspects in the pathogenesis of irritant contact dermatitis. Exposure to octane and cumene may occur in a variety of industries where they are ingredients of fuels and organic solvents or where they are used to produce other chemicals (cumene: phenol, acetone, and methyl styrene). Examples of such industries include the fabrication of rubber, iron and steel, paints, lacquers, and enamels; and activities involving cleansing, metal degreasing, painting, dyeing, and working with fuels. Octane and cumene may prove to be suitable model irritants for industries involving skin contact with organic solvents, with the caveat that many lipophilic substances used in the working environment are diverse mixtures. These are not easy to model in experimental studies, because their compositions are not standardized and they may contain many components. Some of these compounds are toxic and unsuitable for epicutaneous application, others are volatile, and some do not have sufficient irritant potential.

In our study, single and repetitive occlusive applications were performed in order to investigate both acute and cumulative irritation in stingers and non-stingers. In the single application experiment, we were interested in the penetration of the lipophilic irritants as indicated indirectly by their vascular effects. Therefore, we used

laser Doppler imaging immediately after exposure. The repetitive application study was designed on the basis of the repeated short-time occlusive irritation test (1) and its modifications (3). Methodologically, it has to be taken into account that open skin contact is the predominant means of exposure in the real occupational environment. However, occlusive contact may nevertheless occur because of trapping under gloves, watches, or clothes. For the experimental model, it was necessary to choose occlusive applications, because of the volatility of the substances and the impossibility of standardizing multiple open repeated exposures. Typical sensations after single and quick contact with solvents and fuels include the sensation of cooling on the skin, because of their rapid evaporation, followed by whitening of the skin, considered to be caused by lipid extraction from the stratum corneum and not vasoconstriction (26, 27). More intense contact with some substances is followed by sensory irritation such as stinging, burning, or even discomfort, which is also the case for octane and cumene. The analysis of this aspect will be presented separately. Other effects may be induced only by individual lipophilic irritants, such as immunological and non-immunological contact urticaria (28).

In the single application study, both of the irritants were capable of inducing an immediate increase in

Table 4. Median changes (difference from baseline values) of the skin bioengineering parameters with comparisons between stingers and non-stingers for cumene

Days	Erythema (chromametry a* values)		SC hydration (corneometry)		TEWL (g/hr m ²)		Visual irritation score	
	Stingers	Non-stingers	Stingers	Non-stingers	Stingers	Non-stingers	Stingers	Non-stingers
	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)	Median (95% CI)
	<i>P</i> (stingers versus non-stingers)		<i>P</i> (stingers versus non-stingers)		<i>P</i> (stingers versus non-stingers)		<i>P</i> (stingers versus non-stingers)	
2	0.35 (−0.86; 1.56) 0.128	−0.04 (−0.74; 0.66)	−2.1 (−0.81; −3.39) 0.001	−0.03 (−0.48; 0.42)	0.47 (−0.18; 1.12) 1.00	0.16 (−0.72; 1.04)	0.5 (0.3; 0.7) <0.001	0.0 (0.0; 0.0)
3	1.20 (0.48; 1.92) 0.013*	0.27 (0.14; 0.40)	−4.27 (−2.25; −6.29) 0.001	−0.66 (−1.55; 0.23)	0.78 (−0.13; 1.69) 0.454	0.7 (−0.26; 1.66)	0.5 (−0.11; 1.11) 0.001	0.0 (0.0; 0.0)
4	0.54 (−0.23; 1.31) 0.087	0.16 (−0.18; 0.5)	−8.7 (−12.02; −5.38) 0.005	−1.34 (−2.28; −0.4)	1.29 (0.2; 2.38) 0.480	0.19 (−0.89; 1.27)	0.5 (0.3; 0.7) <0.001	0.0 (−0.2; 0.2)
5	0.83 (0.10; 1.56) 0.036*	0.11 (−0.26; 0.48)	−5.8 (−10.08; −1.52) 0.021	−1.43 (−3.68; 0.82)	0.33 (−0.28; 0.94) 0.675	0.74 (−0.52; 2.0)	0.5 (0.3; 0.7) <0.001	0.0 (0.0; 0.0)

CI, confidence interval; SC, stratum corneum; TEWL, transepidermal water loss.

The difference from baseline values of each day were compared between stingers and non-stingers. For each day, the median value, the conservative 95% CIs and the *P*-value after Holm-Bonferroni adjustment for multiple comparisons are provided.

**P*-values that did not remain significant according to the adjusted thresholds.

Bold values indicate significance.

the cutaneous blood flow, as measured with MLDI. This increase is a manifestation of quick and transient vasodilatation, probably mediated by a direct impact on the free nerve endings located in the lower epidermis and the upper dermis. It has been shown that lipophilic irritants trigger the release of vasodilatory neuropeptides, such as substance P and calcitonin gene-related peptide, from the nerve endings in rat experiments (29, 30). Wide variability among the lipophilic irritants in increasing the cutaneous blood flow has been reported previously (27). In our study, the increase in cutaneous blood flow was significantly higher with cumene than with octane, thus confirming indirectly the well-known extraordinarily quick penetration of cumene through the epidermis, as is the case for all aromatic hydrocarbons. Our results suggest that octane penetrates and reaches the deeper epidermis/upper dermis as well, albeit to a much lesser extent. The different abilities of the aliphatic and the aromatic hydrocarbons to penetrate the skin have been established in studies examining the dynamics of the stratum corneum and the blood levels after epicutaneous application (31, 32). Aromatic compounds penetrate the stratum corneum and the epidermis faster than the aliphatic hydrocarbons, such as octane, which are retained for longer in the epidermis (31). This may serve

as an explanation for the difference in irritant potential in the cumulative irritation model. In previous experimental studies with the aromatic hydrocarbon toluene (1, 3, 8), to which cumene is structurally related, the results were explained by toluene quickly penetrating the stratum corneum barrier, damaging it to a lesser extent. This may explain why aromatic solvents, in general, do not increase the TEWL significantly after repeated application. Visual scoring and the measurements of the stratum corneum hydration better reflected the degree of cumulative irritation induced in our study. Both parameters showed higher irritation resulting from octane than from cumene.

Further reasons for the different irritant characteristics of octane and cumene include the mechanisms of the cytokines released from keratinocytes after interaction with the lipophilic irritants (33). In studies with cultured human keratinocytes, aromatic hydrocarbons displayed higher cytotoxicity, but the aliphatic ones led to a higher per cell release of interleukin (IL)-8, IL-1, and tumour necrosis factor- α (34). Additional potential differences between octane and cumene could arise at the level of the interaction with the free nerve endings and the facilitation of neurogenic inflammation (29). Release of neuropeptides is a major mechanism in the induction of inflammation caused by lipophilic irritants (30), and there

is increasing evidence that neurogenic inflammation plays an important role in the development of both irritant and allergic contact dermatitis (35, 36).

Regarding the differences between stingers and non-stingers, the stingers were clearly more susceptible to the cumulative irritant effects of octane and cumene in the repetitive irritation study. This was shown best by the corneometry data and visual irritation scoring. Thus, our study shows a distinct difference between the stingers and the non-stingers in the cumulative reactivity to both irritants, even though the two lipophilic irritants are quite dissimilar from one another in structure and in the responses that they produce.

Our findings are in contrast with the general notion that sensitivity to one irritant cannot reliably predict sensitivity to other irritants (15, 18). This notion is based on studies where the correlations among the reactivities to various chemicals and irritants had low coefficients, albeit significant, and divergent responses were frequently observed (18). In another example, a prediction of the reactivity to SLS by means of DMSO and NaOH tests was investigated, but again, the correlations were, at best, weak and insufficient (9). Particularly in the case of the LAST, prediction of the sensitivity to other irritants could not be consistently established, but previous studies predominantly used acute irritation or single application models and three types of irritants – urticants, detergents, and sensorial irritants. Moreover, the previous studies had diverse aims, such as possible applications in occupational dermatology, cosmetics, and toxicology. In a comparison of 8 stingers with 15 non-stingers (11) in an open repetitive application of SLS once daily for 6 days, no statistically significant difference in the TEWL increase could be established. Only the sensitive laser Doppler velocimetry could detect a significant difference. There was, however, a difference in the response to the urticants benzoic and sorbic acid. The stingers reacted slightly, but significantly more strongly. In a later examination with five urticant substances and more volunteers, 42 stingers versus 44 non-stingers (17), a slightly stronger, but nevertheless statistically significant, response to benzoic acid in the stingers could be reproduced. The stingers also reacted more strongly to *trans*-cinnamic acid, but the response to three other urticants, which probably act through other mechanisms, was not statistically different. The authors concluded that the LAST and the response to urticants were not closely correlated (17). In a study on acute irritation with SLS and visual scoring of the induced irritation, (16), the stingers did not react more strongly than the non-stingers. In another investigation (15), the sensory responses to lactic acid and three other sensorial irritants and the reactivities

to three surfactants and two urticants were investigated in a panel of 58 volunteers, who were known from previous studies to have a higher sensitivity to lactic acid or SLS. The tests with the various irritants were shown to be independent from one another, with a *P*-value of 0.0937. The reactivity to lipophilic irritants, however, has not been studied before in the context of the LAST.

Our findings may have implications for identifying people at risk in professions where lipophilic irritants are possible factors in the pathogenesis of occupational contact dermatitis. However, in many occupations, the workers are exposed to chemically diverse irritants, some of which may be more relevant. Such additional irritants are frequently detergents, for which the LAST has not shown good predictive capabilities (15, 16). Tandem studies would be required to address this problem (37). Other issues with the practical use of the LAST concern its reliability and reproducibility in the same subjects at different time points (38). This is related to the question of whether the LAST does indeed identify a sub-population with distinct characteristics. Although many would disagree (15, 17), a study on the pH changes after the application of lactic acid (39) showed that stingers had higher pH values on the nasolabial folds. This was interpreted as indicating quicker penetration, probably because of deficient barrier function. As lactic acid is a fairly hydrophilic substance, penetration through the intact lipid barrier is, in any case, theoretically unexpected. Another study found higher baseline TEWL values in 44 stingers than in 209 non-stingers (40). Other explanations for the higher susceptibility to cumulative irritation induced by lipophilic irritants in stingers that should be further studied include potentially higher levels of neurogenic inflammation with higher cytokine release and/or higher susceptibility to such mechanisms on a constitutional or acquired basis.

Our study also lends support to the existence of a distinct sub-population identifiable by the LAST. Even if such a sub-population can be identified with a simple test, it is doubtful whether this is suitable in the occupational setting and relevant in the development of hand dermatitis with multiple influencing factors. In conclusion, our study proposes two new lipophilic model irritants for the study of contact dermatitis with non-invasive bioengineering methods. It is also the first report of a correlation between the responses in the LAST and the susceptibility to cumulative irritation caused by lipophilic irritants. However, these preliminary results will need to be confirmed in further investigations with these irritants.

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