

Sensory irritation caused by two organic solvents—short-time single application and repeated occlusive test in stingers and non-stingers

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Summary

Background. Neurosensory irritation is a subjective phenomenon induced by a number of chemicals.

Objectives. To investigate the sensory irritation induced by two organic solvents – n-octane and cumene – between two groups of volunteers, stingers and non-stingers, identified as such according to the results of a lactic acid stinging test (LAST).

Methods. The immediate effects of the solvents were directly compared in a single simultaneous application test. The reaction intensities over time were studied in a repetitive irritation test over 4 days. The volunteers graded the reaction intensities by the use of a labelled magnitude scale.

Results. Cumene induced significantly stronger sensory irritation than octane in both the single and the repeated applications. Both substances induced less subjective irritation the more times the volunteers were exposed. The decline with time for cumene was statistically significant for the non-stingers but not for the stingers. However, no significant differences regarding the reaction intensities were detected in the direct comparisons of stingers and non-stingers.

Conclusions. Further studies with larger sample sizes are needed to investigate a potential connection between the responsiveness to the sensory irritation caused by lipophilic irritants and lactic acid.

Key words: cumene; cumulative irritation; irritant contact dermatitis; labelled magnitude scale; lactic acid stinging test; lipophilic irritants; octane; sensory irritation; solvents.

Neurosensory perceptions of stinging, burning, smarting or even pain can be induced when the skin comes into contact with a wide range of compounds, such as chloroform/methanol, organic acids, and plain lipophilic substances such as benzene, phenol, and other organic

solvents. With some compounds, for example strong caustic chemicals, these sensations can be viewed as an early warning signal and an initial phase of irritation, because the continued exposure would invariably produce damaging effects (1). In contrast, other substances may cause only subjective irritation without any visible signs of irritation at certain concentrations and depending on the vehicle (1), and the paramount feature of this phenomenon is that the perceptions of irritation (stinging, burning, etc.) are experienced only by some of the exposed individuals.

In this study, the effects of two lipophilic model irritants – octane and cumene (syn. isopropyl benzene) – on sensory irritation in human skin were compared with the well-established lactic acid stinging test (LAST) for prediction of skin sensitivity. Octane and cumene are examples

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of organic solvents that are in widespread use in many industries. Whereas octane is a typical aliphatic hydrocarbon, cumene is structurally related to the aromatic toluene. Both solvents are volatile at room temperature, develop a typical pungent odour, and may cause central nervous symptoms, necessitating precautions to evacuate vapours effectively in the experimental setting. Previous toxicological and experimental evaluation of the two solvents described in this article have identified them as safe and suitable for repetitive cutaneous testing on human subjects, as long as exposure is restricted to small test areas and inhalation of vapours is prevented (unpublished data).

Occupational skin contact with solvents typically extracts lipids from the stratum corneum, and clinically causes dryness and a so-called whitening of the skin after a single exposure (2), whereas repeated skin contact is a risk factor for the development of chronic irritant contact dermatitis (3). The LAST (4) was developed to identify persons as 'stingers'. It is frequently used in investigations on the so-called 'sensitive skin' phenomenon, a condition that is especially associated with only subjective discomfort caused by certain cosmetics (5–8). We recently investigated use of the LAST to predict susceptibility to objective cumulative irritation caused by octane and cumene in a repetitive irritation test in stingers and non-stingers. Significantly stronger cumulative irritation was observed in stingers than in non-stingers for both irritants (visual scoring, stratum corneum hydration, and skin colour reflectance) (9). As organic solvents are also capable of eliciting sensory irritation after prolonged skin contact, we were additionally interested in their sensory irritant potential in relation to the subjects' stinging status in the LAST. Our aim was to investigate the neurosensory irritation induced by the two solvents and to compare the sensory effects between the stingers and non-stingers. Therefore, we used a single simultaneous application test with the lipophilic irritants to directly compare the immediate effects of both chemicals. In addition, we performed a repetitive irritation test to study the effect of repeated exposure on the intensity of the subjective irritation over time.

Material 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 a group of non-stingers (each $n = 15$, with 10 women/5 men per group). The institutional ethics committee of the

University Hospital Jena approved the protocol, and all subjects provided 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 they also abstained from washing, showering, or exposing these areas to ultraviolet light. No pregnant or nursing women were included. The study was conducted in March and April 2009, together with the cumulative irritation study (9).

Chemicals

Lactic acid (CAS no. 50-21-5), supplied as 90% by VwR International (Dresden, Germany), was used for the stinging test.

Neat n-octane, an aliphatic hydrocarbon (CAS 111-65-9), purity 99.2%, supplied by VwR International, and neat cumene (syn. isopropyl benzene), an aromatic hydrocarbon (CAS 98-82-8), 99% purity, supplied by Merck Schuchardt OHG (Hohenbrunn, Germany), were used as lipophilic irritants. A TERFU® spot fume-extractor (Fumex, Skelleftea, Sweden) with an activated carbon filter (L50; Asecos GmbH, Gruendau, Germany) was used during all application procedures, in order to remove organic solvent vapours from the laboratory atmosphere.

Test Procedures

Pretest: LAST

The LAST was based on the previously described procedure (4). The subjects first washed their faces with tap water and a synthetic detergent. After drying the face with normal 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 past period on 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-points 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. However, the stinging status of the participants was disclosed to the investigator for the subsequent procedures in the single and repetitive application studies.

Single application test with lipophilic irritants

The single occlusive application patch test was conducted in a double-blind, randomized design, right vs.

left forearm, in order to simultaneously compare the sensory irritation intensities of both substances immediately after patch removal. 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). Immediately after patch removal, the subjects were requested to grade the intensity of the experienced sensory irritation, separately for the two sites, by means of a labelled magnitude scale (LMS) (see below).

Repeated short-time occlusive test with lipophilic irritants

The repeated occlusive irritation test was started after the single application test in the same subjects, in order to investigate the course of the sensory irritation induced by each chemical over time. Two test areas (2.6×2.6 cm) were marked with a surgical skin marker on the skin of the subject's paravertebral mid-back, with a horizontal distance of approximately 20 cm between the areas. The volunteers were unaware of the allocation of the substances to the test areas. The areas were randomized among the subjects, but remained constant for each subject for the repeated applications. Octane and cumene were applied under patches as in the single test, but for 10 min each, twice a day, with an interval of $3 (\pm 1)$ hr in between, for 4 days. The irritants were applied one after another, and the second solvent was applied only after the sensory irritation from the first one had worn off completely, in order to help the volunteers to focus more on the perceived irritation from the tested compound and less on the comparison between the two. Immediately after removal of each chamber, the volunteers ranked the experienced sensory irritation with the LMS.

Bioengineering measurements and visual scoring were conducted every day before the first application of the patch tests, and on the fifth day by a trained investigator (data presented elsewhere). Irritation would have been prematurely discontinued in respective test areas that developed too intense irritation, that is, when the erythema score exceeded a certain level. No such intensive irritation was observed in the study.

Grading – the LMS

The volunteers graded the intensity of the sensory irritation with the help of an LMS that was introduced by Green et al. (10) and used in previous studies (11, 12). Its advantage is that it yields ratio-level data, in contrast

to the arbitrary category scales (10). The numerical scale is continuous from 0 (no sensation) to 94.53 (strongest imaginable sensation). Orientating verbal descriptors are distributed in between with a pseudo-logarithmic spacing, whereas the numerical values are linear. The descriptors are 'barely detectable' (1.37 numerical value), 'weak' (5.76), 'moderate' (16.27), 'strong' (33.48), and 'very strong' (50.41) (11, 12). The volunteers underwent a training session with the LMS before it was used in the single and repeated application studies, as proposed by Robinson and Perkins (11), in order to make them familiar with the method and to investigate eventual principle differences in the imagined perception of stingers and non-stingers. The subjects were requested to recall 14 different sensory stimuli and to rank the imagined sensations on the LMS (Table 1). In all measurements with the LMS in our studies, the subjects had to give a single value in order to represent the intensity of all the possible irritating sensations altogether, including stinging, burning, itching, tingling, and pain.

Statistics

The data were analysed with SPSS™ 16.0 for Windows. Microsoft Excel was used for plotting of the figures and tables. Results are shown as medians, median differences from baselines and conservative 95% confidence intervals for the medians, as most of the measured values were not distributed normally. For comparisons between

Table 1. The following descriptions of various stimuli were read to the volunteers during the training session with the labelled magnitude scale (LMS)

Recalled or imagined stimuli	
1	The cold of tap water as you wash your hands.
2	The warmth from dipping your hand in lukewarm water.
3	The burn from putting your hand into scalding hot water.
4	The sting from cutting your finger with a knife or razor blade.
5	The cold from briefly resting your fingertip on an ice cube.
6	The cold from holding an ice cube in the palm of your hand.
7	The heat as you briefly touch a hot light bulb.
8	The sensation of getting into a hot bath.
9	The tingling in your hand after it 'falls asleep' and the numbness begins to go away.
10	The prick from a needle when getting a vaccination or allergy shot or having your finger pricked for a small blood sample.
11	The cold from putting your hand into ice water.
12	The burn from touching a hot stove or the flame from a match.
13	The itch from a mosquito bite.
14	The sting from applying alcohol on an open sore or cut.

The stimuli are the same as described by Robinson and Perkins (10), except for one question relating to poison ivy, which does not grow in Europe.

the groups (stingers vs. non-stingers), the two-sample *t*-test was used for the normally distributed data, and the Mann–Whitney *U*-test otherwise. For the within-groups comparisons (changes compared with baseline), we used either the paired *t*-test or the Wilcoxon signed rank test, depending on the presence or absence of a normal distribution. The level for statistical significance was $p \leq 0.05$, with Holm–Bonferroni corrections for multiple comparisons (13).

Results

Training with the LMS

The quantified intensities of all of the individual subjects' imagined and recalled perceptions are plotted in Fig. 1. As in the referenced study (11), there was great variability among the volunteers, especially for the answers to questions recalling stronger stimuli, such as Q3 (burning from scalding water), Q4 (cutting the finger with a knife), Q7 (the heat from a hot light bulb), Q12 (the burn from a hot plate or the flame of a torch), and Q14 (applying alcohol on an open sore or cut). Much lower variability was observed for milder stimuli, such as Q1 (the cold from tap water) and Q2 (the sensation from lukewarm water). Some subjects tended to give higher ratings in general, whereas others gave predominantly lower ratings for most of the imagined stimuli. However, no statistically significant differences were detected between stingers and non-stingers for any of the 14 imagined stimuli.

Sensory irritation with octane and cumene

Single application study on the forearms. Direct comparison of the sensations induced by both irritants applied simultaneously revealed that cumene induced much stronger sensory irritation than octane ($p < 0.001$,

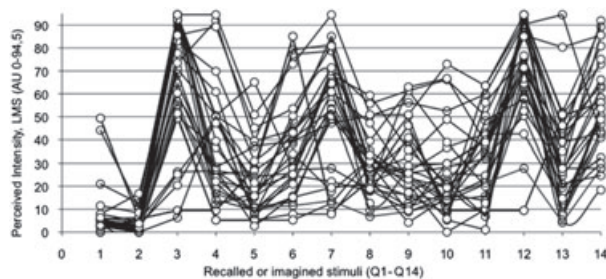


Fig. 1. Results of all the individual ratings of the imagined or recalled stimuli during the training session with the labelled magnitude scale (LMS) plotted together ($n = 30$). The lines connect the ratings of the individual volunteers.

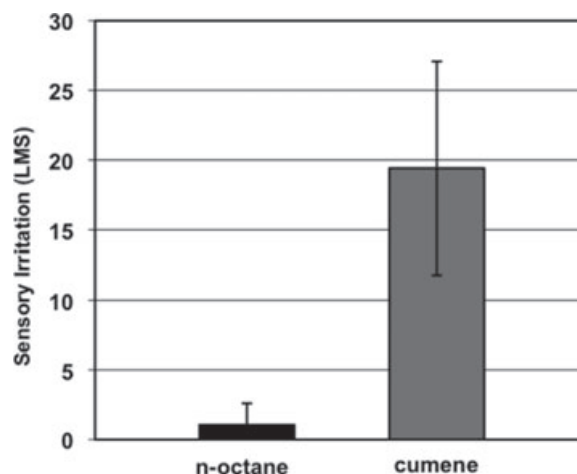


Fig. 2. Median values ($\pm$ conservative 95% confidence intervals) of the sensory irritation rated immediately after a single simultaneous exposure to both solvents on both volar forearms. The results for stingers and non-stingers were pooled, as they did not differ significantly between the groups. LMS, labelled magnitude scale.

Wilcoxon test; Fig. 2). Stingers and non-stingers did not react significantly differently with respect to the intensity of the subjective irritation caused by both octane ($p = 0.892$) and cumene ($p = 0.713$).

Repeated irritation test. When the substances were applied one after another, the volunteers again rated the sensations induced by cumene more strongly than those induced by octane, as in the single simultaneous application test. The course of the sensory irritation in the repeated application study showed two trends (Fig. 3). The main trend was an overall decrease in the sensory irritation intensity for both irritants from days 1 to 4. Second, within the single days, the second application induced more intense sensations than the first on the same day in the stingers reacting to cumene, in contrast to the non-stingers. However, with respect to octane, this tendency was observed both in the stingers and in the non-stingers, but only on days 1 and 2. The trend for enhanced sensitivity within the day achieved statistical significance only for octane on day 1 in both groups (stingers, $p = 0.028$; non-stingers, $p = 0.023$; Wilcoxon test). All other comparisons between the first and the second applications on any given day, for both octane and cumene, in both stingers and non-stingers gave insignificant results, although for cumene in the stingers group, the values were close to the significance threshold (day 1, $p = 0.13$; day 2, $p = 0.078$; day 3, $p = 0.054$; day 4, $p = 0.075$). In general, the sensory irritation seemed to follow a divergent course in the two

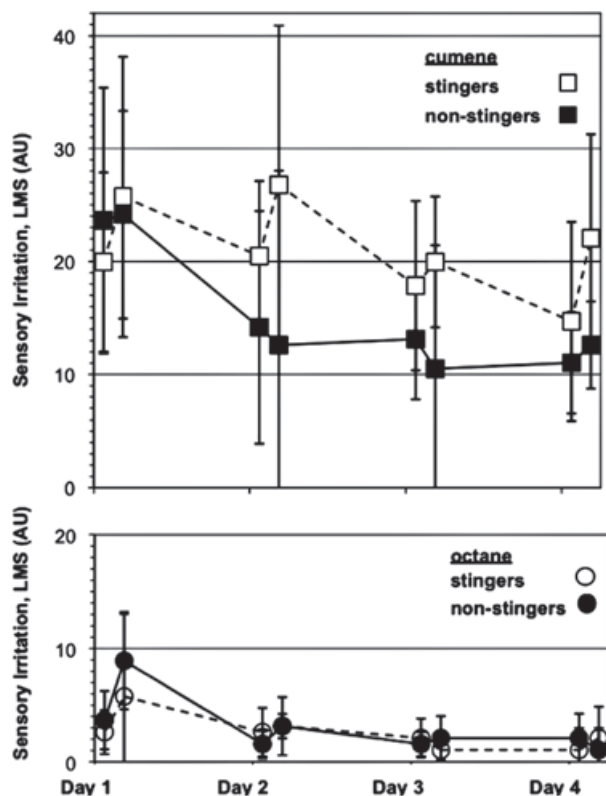


Fig. 3. Median values ($\pm$ conservative 95% confidence intervals) of the sensory irritation induced by cumene (above) and octane (below) in the course of the repeated application study for stingers and non-stingers from days 1 to 4. LMS, labelled magnitude scale.

groups only for cumene (Fig. 3); that is, the stingers seemed to have experienced higher levels of sensory irritation, but the direct comparison with the non-stingers was not statistically significant.

In order to investigate the overall trend of the sensory irritation in the course of the study, daily values were calculated as the arithmetic means of each day's two measurements. These daily values were then compared with the day 1 results (within both groups). Additional 'change from day 1' values were also estimated, and used for a comparison between stingers and non-stingers.

Decline in the sensory irritation caused by octane

The sensory irritation in both the stinger and non-stinger groups became significantly weaker from day 2 onwards, when compared to day 1. The non-stingers appeared to have a more distinctive decrease than the stingers; that is, on day 4, the non-stingers perceived the sensory irritation as only 24% of the value from day 1, whereas the stingers still perceived it as 60% of the day 1 value. The difference

between the stingers and non-stingers, however, was not statistically significant on any of the days (day 2, $p = 0.48$; day 3, $p = 0.835$; day 4, $p = 0.493$; data not shown).

Decline in the sensory irritation caused by cumene

The decline in the sensory irritation caused by cumene became significant only in the non-stinger group, and only on days 3 and 4, when compared with baseline. The values of the stingers also had a tendency to decrease, but no statistical significance was achieved. Nevertheless, this dissimilarity in the course of the sensory irritation could not be confirmed by the direct comparisons of stingers and non-stingers, although, on the last day, the test results were close to the significance threshold (day 2, $p = 0.110$; day 3, $p = 0.418$; day 4, $p = 0.056$). In order to exclude the possibility that this finding was caused by the applied calculation method (i.e. using the arithmetic means of the two daily assessments to form the daily values), additional statistical comparisons only within the first and the second applications, respectively, were performed and compared. The trend towards a decrease in the stinger group did not reach significance, and nor could the divergent course of the sensory irritation in the two groups be corroborated by eventual significant differences (with the single exception of day 4, second application, $p = 0.033$).

Discussion

Sensory irritation caused by organic solvents is well known, but has not been a matter of systematic investigation in the general context of occupational contact dermatitis (14, 15). It is therefore of interest to study the relationship between the subjective and objective cumulative irritation caused by lipophilic irritants, and to compare the findings with reactions induced by other sensory irritants, such as lactic acid. The results of the bioengineering measurements of the objective cumulative irritation induced by octane and cumene were presented recently (9).

As the pattern of the objective cumulative irritation caused by octane and cumene was significantly different between the stingers and the non-stingers (9), we expected the sensory irritation caused by the solvents to differ as well. However, such differences could not be confirmed in general. The first main finding in our study was that both substances induced less subjective irritation the more times the volunteers were exposed, thus implying some probable mechanism of adaptation to the stimulus. Second, although we could not identify an association

between the LAST status of the volunteers and the overall intensity of the solvent-induced subjective irritation in the single application experiments, we observed that the stingers failed to show the same reduction in stinging after repeated exposure as the non-stingers, at least with the stronger sensory irritant cumene. The lack of statistically significant differences in the direct comparisons of stingers and non-stingers might be attributed to the fact that, in view of the known wide interpersonal variability in the perception of irritation, our sample size (15 per group) might have been insufficient. Larger samples would therefore be more appropriate for examination of the potential differences between stingers and non-stingers in the sensory irritation induced by lipophilic irritants. Most studies on stinging apply the substances on the face, and especially the nasolabial fold. The type of stinging that the LAST is intended to model, termed by Frosch 'delayed-type stinging', shows considerable regional variations, with the back being 'virtually unreactive' (1). Although we performed the LAST on the nasolabial folds, as usual, the choice of the forearms and especially the back as test body sites may have lowered the perceptive abilities of the volunteers, and might thus have influenced the results, preventing us from detecting differences between the stingers and the non-stingers.

After a single application, cumene elicits not only stronger sensory irritation than octane, but a greater increase in the cutaneous blood flow (9). Both effects can be explained by the known quicker and better penetration of the aromatic hydrocarbons (such as cumene) than of the aliphatic ones (such as octane) (16, 17). In general, poorly absorbed solvents and solvents that extract lipids are known to cause more severe skin damage and fewer systemic symptoms (15). After repeated exposures, cumene still produced more potent subjective sensory irritation than octane (Fig. 3), although cumene has a weaker potential to induce objective cumulative irritant contact dermatitis. This was shown by the standard bioengineering measurements obtained after 4 days of twice-daily exposures (9). Thus, our results show the divergence of subjective sensory and objective cumulative irritation potential in two ways: first, the stronger irritant according to the objective cumulative irritation (octane) was a weaker sensory irritant; second, the interrelations between the courses of the objective and of the sensory irritation were not the same with octane and cumene. At this point, it cannot be determined whether these differences can be ascribed to the class dissimilarities in structure and mode of action between the aliphatic (such as octane) and the aromatic (such as cumene) hydrocarbons, or whether the reaction patterns are specific to the individual compounds. The inconsistency of the objective and the

sensory irritation with octane and cumene confirms, for the lipophilic organic irritants, what is well known across a wide range of structurally varied substances, including cosmetic preservatives (18), esters, and others (1).

Our results add to the growing data on the functional differences (or the lack of such) between the stingers and non-stingers. Recently, the reactivity of the sensory nerves was shown to differ between these groups (19). Electric current, adjusted to selectively activate the A-delta and C nerve fibres, which are responsible for the stinging response, had a lower detection threshold for the stingers than for the non-stingers (19). No such difference was observed when the large A-beta fibres were selectively stimulated. Another study showed that people with the tumour necrosis factor- α polymorphism at position 308 have more intense stinging responses to lactic acid (20). Previous investigations have reported a connection between the responses in the LAST and the skin bioengineering parameters at baseline or after provocations with test substances (21–24) [reviewed in (25)]. However, as the sensory and the objective irritation most likely follow divergent courses, it is not surprising that, in many instances, sensitivity to the sensory irritant lactic acid did not predict sensitivity to objective irritants such as the detergent sodium lauryl sulfate (23, 26) and other detergents (8) and urticants (8, 27).

The central finding of our study, that is, the decrease in the sensory irritation with time, may be attributed to the neurotransmitter depletion of the free nerve endings. Such emptying of neuropeptides such as substance P and calcitonin gene-related peptide was observed in experiments with rodents after epicutaneous application of some lipophilic irritants (28–30). Similarities exist between the course of the sensory irritation reported here and the better-studied effects of capsaicin on sensory irritation. When capsaicin was applied repeatedly over short intervals (such as 15 min), sensitization was observed, whereas after longer intervals (such as 90 min), desensitization occurred (31). A trend for increased sensory irritation within the 3-hr applications was also visible in our study. The discussed mechanisms for the desensitization with capsaicin included tachyphylaxis and degeneration of the nerve fibres, besides depletion of neuropeptides (31, 32). All of these could be relevant to our irritants. The degeneration of nerve fibres after a single intradermal application of capsaicin has been shown in human volunteers (33). The speed of capsaicin penetration, which depends on body sites and vehicles, can also influence the dynamics of the perceived sensations (31). Additional potential mechanisms influencing the course of the sensory irritation after repeated exposure may be related to the induction of

epidermal, keratinocyte-related, cytokine-derived inflammation. Inflammatory mediators are known to influence the sensitization and desensitization of afferent nerve fibres from studies in pain research (34). Accumulating evidence from experimental animal and human studies (35, 36) links so-called neurogenic inflammation and cutaneous inflammatory responses, such as contact dermatitis. Neurogenic inflammation and hyperreactivity of the neural response of the skin is also considered to contribute to the 'sensitive skin' phenomenon, in which certain individuals report subjective sensory effects induced by cosmetic (personal-care) products. Possibly related factors include variations in epidermal nerve density, sensitivity thresholds, and receptor densities, as well as functional differences (37) [reviewed in (38)].

Neuropeptides have been shown to enhance both allergic and irritant contact dermatitis, when topically applied on the skin (39). Neurogenic inflammation might therefore be a mechanism of particular importance in solvent-induced chronic irritant contact dermatitis, owing to the ability of the lipophilic chemicals to penetrate the epidermis and to primarily affect the free nerve endings. It can be speculated that there is a relationship between the fact that the sensory responses of the stingers decreased to a lesser extent over time than those of the non-stingers, and the fact that they developed stronger objective cumulative irritation caused by cumene. If the release of neuropeptides parallels the sensory response (which is speculative), this would mean that the stingers obtain higher levels of proinflammatory neuromediators, contributing to stronger objective irritation. Such a hypothesis would require substantiation in further investigations.

The responses to the recalled and imagined stimuli from the training with the LMS in our study did not reveal a statistically significant difference between the stingers and the non-stingers. Robinson and Perkins also could not correlate the stinging caused by lactic acid and the

recalled or imagined perceptions (11), although such a correlation was shown for capsaicin. The sensitivity to any one given sensory irritant, including lactic acid, is considered to be unable to reliably predict susceptibility to other sensory irritants (8). Thus, a link between the stinging status in the LAST and the sensitivity to the sensory irritation caused by the lipophilic irritants tested here would have been an exception to the rule, just like our finding that the LAST predicts sensitivity to the cumulative irritation caused by octane and cumene (9).

Whenever organic solvents are to be used in human *in vivo* irritation studies, particular consideration has to be given to the safety of the volunteers. Both octane and cumene qualified in previous clinical studies as suitable model irritants to be used in bioengineering studies with human volunteers. Previous toxicological expert opinions (unpublished data) concluded that cumene was safe, as long as the exposure is restricted to small skin areas and inhalation of vapours is prevented, in contrast to toluene, the related solvent that has been used in experimental studies in the past (40–42). Meanwhile, critical new data on cumene safety have shown evidence of potential toxicity with respect to high inhalation doses (43), and the foregoing recommendations suggesting the choice of cumene as a model irritant have to be considered in relation to this.

In conclusion, the course of the sensory irritation induced by subsequent repeated applications of two organic solvents was characterized by a decline in the intensity. Differences between stingers and non-stingers regarding the reduction of the sensory irritation over time were noticed for the more potent sensory irritant cumene. The LMS proved to be applicable for quantification of the subjective irritation. Further studies with larger sample sizes are needed to verify a potential connection between the responsiveness to the sensory irritation caused by lipophilic irritants and that caused by lactic acid.

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