

Application of a 4-h human patch test method for comparative and investigative assessment of skin irritation

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A human 4-h patch test has recently been developed for testing the irritation hazard potential of chemicals. The original method was developed for comparative irritation assessments relative to benchmark irritants using simple statistical tests. In this context, the method has been shown to be robust in *intralaboratory* testing over time. Recent *interlaboratory* testing has also established the consistency of the method in assessment of the relative irritation potential of selected chemicals. These data help to position the method as a suitable replacement for animal test methods in assessment of skin irritation hazard. In addition, the method has great utility for investigating different parameters of clinical skin irritation. Using kinetic response patterns and curve fitting analysis, we have compared the relative irritation potential of chemicals in greater detail, using as a basis the time required for test subjects to respond as well as the incidence of positive responses. Also, using the response to 20% sodium dodecyl sulfate (SDS) as a benchmark, we've been able to examine the intersubject variation in clinical skin irritation responses. In general, subjects most reactive to 20% SDS, in terms of the exposure time required to produce a positive response, were relatively more sensitive to a 2nd irritant chemical as well. However, this was not an absolute correlation in that some test subjects showed divergent patterns of response. The method was also used to compare directly the relative skin reactivity of different populations, based on race (Caucasian versus Asian) or on neurosensory skin sensitivity. Our results using this acute exposure test method indicate little difference in visually assessed skin irritation among these diverse human subpopulations.

Key words: irritancy; human; chemicals; sodium dodecyl sulfate; patch testing; racial; neurosensitivity; irritant contact dermatitis; sodium hydroxide; acetic acid; 1-decanol. © Munksgaard, 1998.

Accepted for publication 3 December 1997

A human 4-h patch test has recently been developed and proposed as an alternative to the Draize rabbit test for assessing the irritation hazard potential of chemicals (1–4). It has been noted in multiple publications that the method is a superior alternative to rabbit skin testing, since it involves testing of the relevant species (man), in addition to the relevant target organ (skin), and the appropriate clinical endpoint (skin irritation) (3, 4). The method relies on a simple statistical comparison of results for the test chemical with those of 20% sodium dodecyl sulfate, included in each study as a positive control. SDS was selected as the control since it is the most widely used chemical for the study of skin irritation in man, has an acceptable safety profile, and can provide a suitable benchmark for determining the appropriate regulatory

classification of acute skin irritation potential (4). Most chemicals have some degree of irritation potential in man, depending on concentration and duration of exposure. This degree of irritation can also be influenced by intrinsic and extrinsic factors (5–7). Hence, skin irritant responses in man are almost always comparative. The use of 20% SDS provides an internal calibration check on any given irritation test, as well as a relevant control for irritation comparison. This concentration is the minimum level classified as irritant (R38) by EC criteria (8). Hence the relative irritancy of (generally) undiluted test materials compared to this benchmark enables one to make a reasonable determination of irritation hazard, based on a statistical comparison to the SDS response profile. Intralaboratory testing of several dozen chemicals of

varying classes has clearly demonstrated the ability of the method to detect irritation hazard relative to this SDS benchmark (3, 4). Moreover, interlaboratory testing has demonstrated the robustness of the method, by providing very similar classification of common test chemicals in spite of subtle variations in the test methodology from laboratory to laboratory (9).

An additional feature of this acute irritation patch test is the ability to assess different parameters of the skin irritation response. Recent studies using this method have shown that the population response to 20% SDS will show seasonal variation, with wintertime studies tending to give higher levels of irritation (6). Dose-response SDS studies showed small differences among ethnically diverse test populations when tests were performed in different countries and at different times of the year (6). Yet these population differences were minor compared to interindividual differences within the different test populations. The study of atopic vs. non atopic populations has also revealed minimal differences in reactivity (7).

The studies reported herein extend the investigative application of this methodology. In addition to the simple statistical testing of response differences to given test chemicals, we employed curve fitting analysis to calculate time-response patterns. This provides an additional means to assess, in greater detail, differences in skin irritation potential. The repeat testing of irritating chemicals in multiple studies has also enabled us to examine the clinical response correlation between irritants. Lastly, we recruited diverse test populations and conducted side-by-side testing to common irritants to assess more directly interracial differences in reactivity to skin irritants, and whether the acute irritation response to chemicals is different in subjects with differing neurosensory skin responsiveness.

Materials and Methods

Chemicals

Test chemicals studied included sodium dodecyl sulfate (SDS), sodium hydroxide (NaOH), acetic acid (HAc), and 1-decanol (Dec), all obtain from Sigma Chemicals (St. Louis, MO). Distilled water was the common diluent for all chemicals tested at <100% and was also tested as a control material. Lack of corrosivity of the test materials (under conditions of the test protocol) was assured prior to testing via reference to published data (10).

Human patch test procedures

Experiments were performed under contract at the Reed Hartman Research Center (Cincinnati, OH),

Hill Top Research (Cincinnati, OH), and the California Skin Research Institute (San Diego, CA). All studies were conducted under protocols supplied by Procter & Gamble. Approximately 30 subjects per study were exposed to each test material for up to 4 h. In each study, 20% SDS was tested as a positive control and dH₂O was tested as a negative control. SDS (20%) is a model irritant that gives reasonable, but not excessive, levels of irritation. Test materials were applied in 0.2 ml volumes, either neat or after suitable dilution in dH₂O, to 25mm Hill Top Chambers. The patches were affixed to the skin with BlenDerm[®] tape. Test materials were applied progressively from 15 or 30 min through 1, 2, 3, and 4 h of exposure. The 15 and 30 min exposures were omitted if investigators were satisfied that excessive reactions would not occur after 1 h of exposure. After patch removal, the skin sites were gently wiped with a damp towel to remove excess residual test material. The sites were graded at 24, 48, and 72 h after patch removal using a 4-point grading scale of increasing irritation (0, +, ++, and +++). Erythema was the primary response detected. Subjects not responding to a test material after a given exposure interval, were reexposed for the next longer interval at the same or naive skin sites. Once a positive response (+ or greater) occurred in a given subject, there were no further exposures to that test material.

In 1 clinical study, we recruited test subjects of Caucasian or Asian descent but imposed an additional selection criterion based on subjective reactivity to the neurosensory stimulant lactic acid. Subjects were tested with 5% or 10% lactic acid or distilled water in blinded fashion following a method similar to that recently described (11). Subjects were included in the "sensitive skin" subpopulations (Caucasian or Asian) if they (i) reproducibly described a stinging or burning response to 10% lactic acid, and (ii) did not show similar reactivity to distilled water. Subjects not responding to lactic acid were placed in the "normal skin" subpopulations.

The principles of the patch testing procedure and the testing approach are intended to avoid the production of strong irritation responses. The procedures used in these studies were subjected to review and approval by local ethical review committees and all experiments were performed in accordance with the Helsinki Declaration of 1975. All procedures were described in detail to all study subjects, who read and signed informed consent forms.

Data analysis

The effective number of panelists completing the test included all panelists who received a 4-h ex-

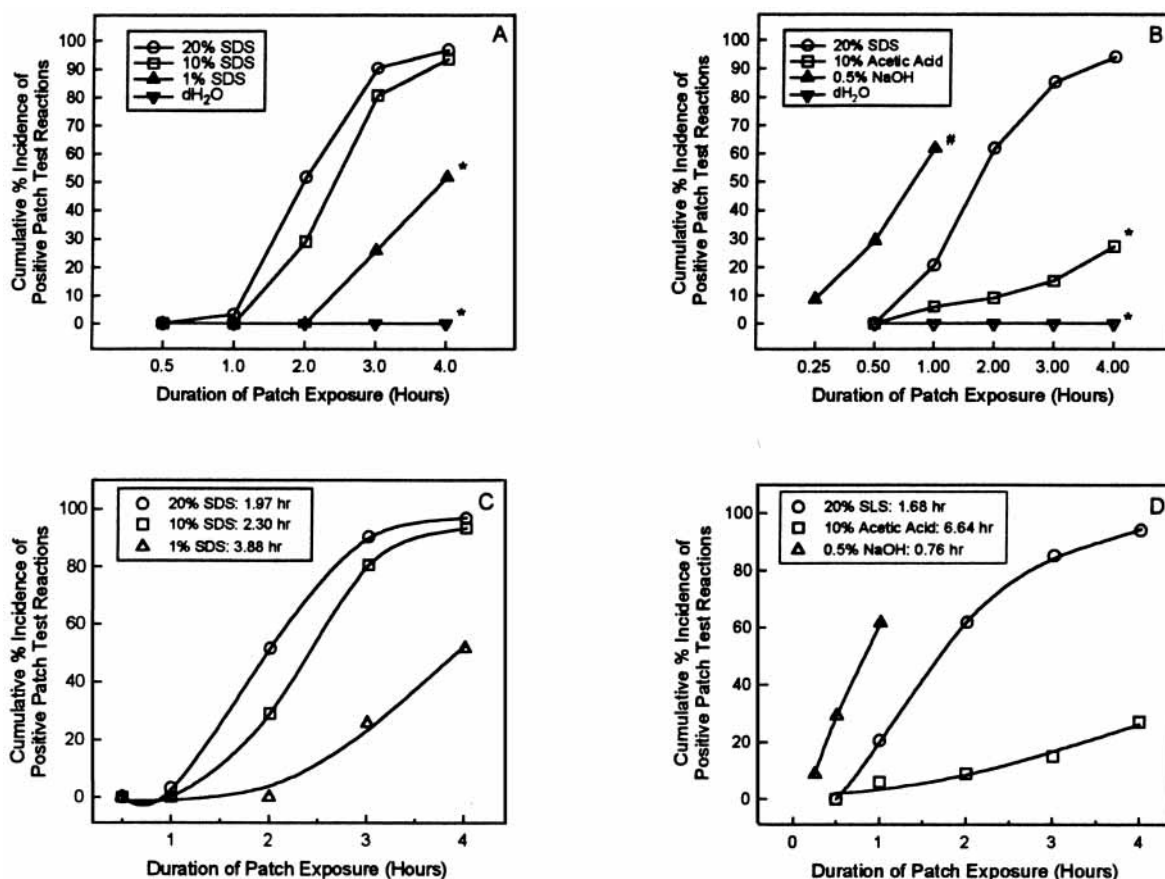


Fig. 1. (A) Patches containing the indicated concentration of SDS were applied for 0.5 to 4-h, increasing the duration of exposure until a positive response was seen. The asterisk (*) designates a statistically significant difference (the Fisher exact test) at $p < 0.05$ relative to the response to 20% SDS. (B) Patches containing the indicated test materials were applied for 0.25 to 1 h (0.05% NaOH) or 0.5 to 4 h (other materials), increasing the duration of exposure until a positive response was seen. The asterisk (*) or pound (#) designate a statistically significant difference (the Fisher exact test) at $p < 0.05$ relative to the response to 20% SDS at the same time point. (C, D) The data from Figs. 1A and 1B were regraphed using logistic curve fitting procedures and a TR_{50} value (time for 50% of test subjects to respond) was determined for each test material.

posure to that test material (or 2-h exposure to 0.5% NaOH) plus any panelist who showed a positive reaction at a shorter exposure time. Dropouts for any reason (none were due to skin reactions) were not included in the data analysis. The number of subjects per study ranged from 27 to 34, except for the study in which subjects were recruited on the basis of race and neurosensory skin response. Because of a more limited number of neurosensory reactive or "sensitive skin" subjects available, the number of subjects per subgroup in that study ranged from 13 to 20.

The data were compiled as the cumulative % incidence of positive skin reactions to a given test material. Responses to the various test materials in a given study were compared to one another using Fisher's exact test. The temporal responses to the more irritating materials were also determined by curve fitting (SigmaPlot for Windows, version 2.0) and TR_{50} values (the time of exposure necessary

for 50% of the exposed population to show a positive skin response) were determined. Linear regression analysis was also used to assess the correlation, within a study, between the population response to one irritant versus another. Criteria for selection of subjects for this analysis are listed in the figure legend.

Results

Chemical irritation testing

2 studies were conducted to verify the clinical irritation response to SDS and other irritants relative to previously published data (1). The 1st study compared the cumulative incidence of the response of test subjects to different concentrations of SDS. The 2nd compared the responses to 20% SDS, 0.5% NaOH, and 10% HAC. The protocol used in these studies was slightly different than that previously published, in that repeated weekly ex-

posures to test chemicals were made over the same skin sites instead of naive skin sites (9).

The results of the SDS dose-response study are shown in Fig. 1A and the results of the SDS/NaOH/HAc study are shown in Fig. 1B. The response to 20% SDS in these studies was slightly greater than that previously reported (3, 4), probably due to our practice of repatching the same skin sites (9). However, the relative responses to 3% SDS, 10% SDS, 0.5% NaOH, and 10% HAc were the same as those reported previously. Based on simple statistical analysis using Fisher's exact test, 10% SDS would be considered equal in potency to 20% SDS, 0.5% NaOH would be considered more potent (based on the 1-h exposure time point), and 3% SDS and 10% HAc would be considered significantly less potent. There was no response to water in either study. In addition, we compared the relative potencies of these materials by fitting a logistics curve to the time-response data for each chemical. For each curve, a TR_{50} value was calculated. This represents the time of exposure necessary for 50% of the test population to show a posi-

tive irritation response. These data are shown for the SDS dose response study in Fig. 1C and for the SDS/NaOH/HAc study in Fig. 1D. As would be expected, as the cumulative incidence of positive patch test reactions tended to increase, the TR_{50} values tended to decrease in a directly proportional pattern.

The use of the TR_{50} calculation has also enabled us to assess the consistency of the irritation response to SDS across repeated studies. Fig. 2 shows the compiled results for 20% SDS from multiple (21) studies conducted by Procter & Gamble and Unilever. Fig. 2A shows the mean responses ($\pm$ standard error) for SDS exposures of 0.5 to 4 h for all of the studies. The fitted curve gives a TR_{50} value of 2.64 h. Fig. 2B shows the TR_{50} values for the individual studies. In spite of minor differences in the study procedure across studies (e.g., repatching of sites in some P&G studies vs. movement of new patches to naive skin sites) and use of multiple laboratories, there has been reasonably good consistency across the studies. All 6 of the P&G studies and 11/15 of the Unilever studies resulted in TR_{50} values between 1.5 and 3 h.

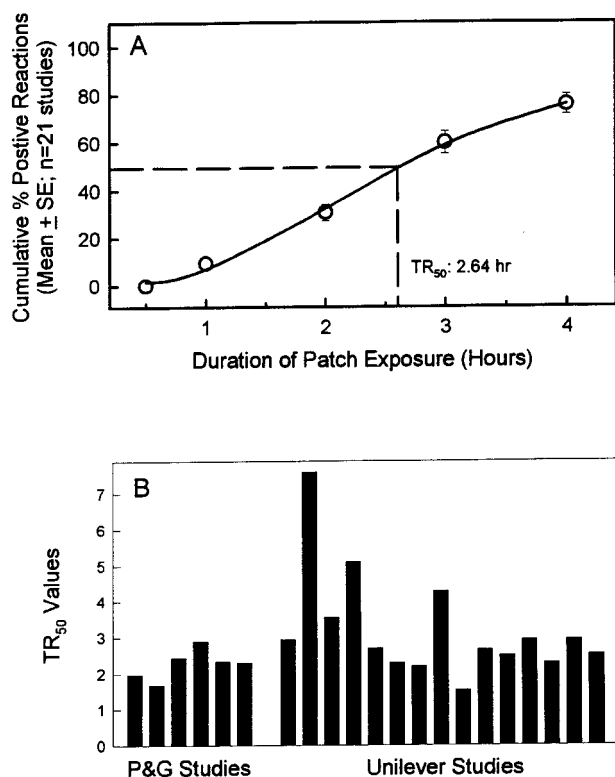


Fig. 2. (A) The mean cumulative % incidence of positive responses to 20% SDS across 21 studies conducted by P&G or Unilever was assessed using time to respond calculations from 0.5 to 4 h and applying curve fitting analysis. (B) The TR_{50} values were determined for each of the 21 individual studies. These were plotted individually to show the degree of consistency across studies. Seventeen of the 21 studies (81%) resulted in TR_{50} values between 1.5 and 3 h.

Correlation within study subjects between responses to different chemical irritants

Skin irritation responses tend to show interindividual variability even within generally homogeneous study populations (5, 6, 12). The human 4-h patch test protocol enables one to assess the relative irritation responses to multiple chemicals of varying irritation potentials within and between study subjects. This allows examination of very basic questions, such as whether individuals that are very sensitive (i.e., react after short term exposure) to one material tend to be the most reactive to all materials or at least those materials with similar irritation potencies. An example of this type of correlation analysis is shown in Fig. 3.

Test subjects were assigned an arbitrary response grade, based on how quickly they responded with a positive erythema skin reaction to 20% SDS and 0.5% NaOH. Subjects not reacting after the longest patch exposure received a grade of "1" and subjects reacting positively to the shortest patch exposure (15 min for NaOH and 30 min for SDS) received a grade of "6". Intermediate grades were assigned as determined by the patch exposure needed to produce a positive reaction. Fig. 3A shows the values for 19 of 34 test subjects that participated in a single study comparing these 2 test materials. These 19 subjects had arbitrary skin grades for the 2 materials that were either equal or within 1 numerical unit, and therefore showed a

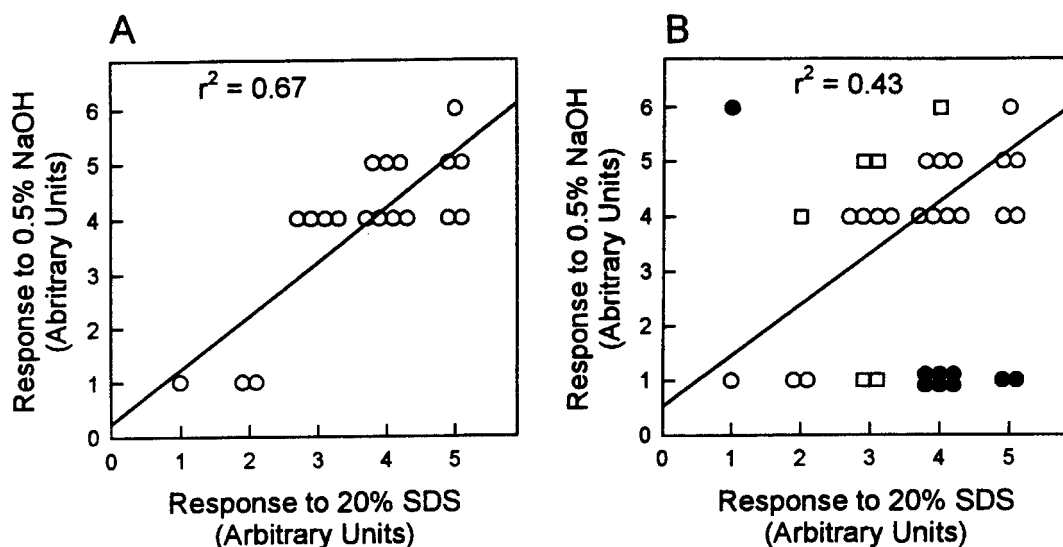


Fig. 3. (A) Within a given study, comparisons were made among test subjects as to their relative response to different irritants. Grades were assigned based on how rapidly a subject responded (higher grade=faster response). Comparing the response to 2 strong irritants (0.5% NaOH and 20% SDS), most subjects (open circles: 19/34) showed a positive correlation (arbitrary grades within 1 unit). (B) A larger number of subjects (open circles plus open squares: 25/34) fell at least within 2 arbitrary grade units resulting in a weaker, but still positive, correlation. There were also several subjects (9/34) that showed very divergent responses (filled circles). The latter were not factored into the correlation analysis.

good correlation between the response to the 2 materials. If subjects who responded within 2 numerical units were included in the analysis (now a total of 25/34 subjects), the correlation was weaker, but still positive (Fig. 3B). Thus, 74% of the subjects in this study tended to show similar degrees of reactivity (in terms of time to respond) to the 2 chemicals. It is important to note, however, that 9 study subjects showed very divergent responses to the 2

test materials. 8 of these responded very quickly to SDS, but did not respond at all or responded relatively slowly to NaOH. In contrast, 1 person responded quickly to NaOH but not to SDS. These results underscore the interindividual variability in skin reactivity to chemicals that occurs, but also reinforce the concept that information on the relative acute skin irritation potential of chemicals can be derived from panels of this size.

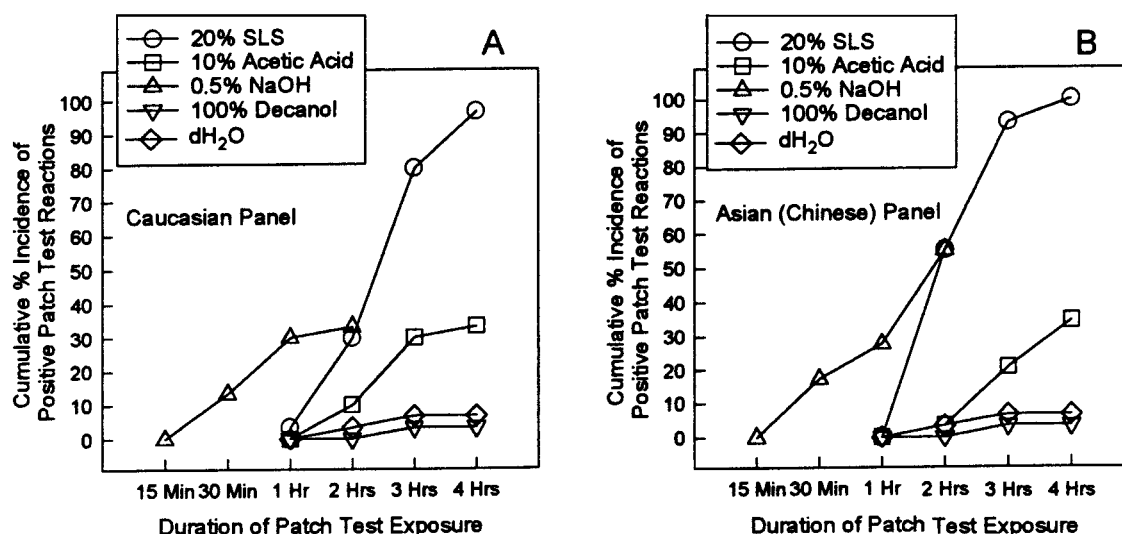


Fig. 4. Separate panels of Caucasian (A) and Asian (B) test subjects were exposed to the indicated irritant materials in a single side-by-side study (29–30 subjects per panel). Based on the cumulative response incidences (at 2 h of exposure for 0.5% NaOH and 4 h of exposure for all other test materials), there were no significant differences between the panels in their response to any of the materials tested.

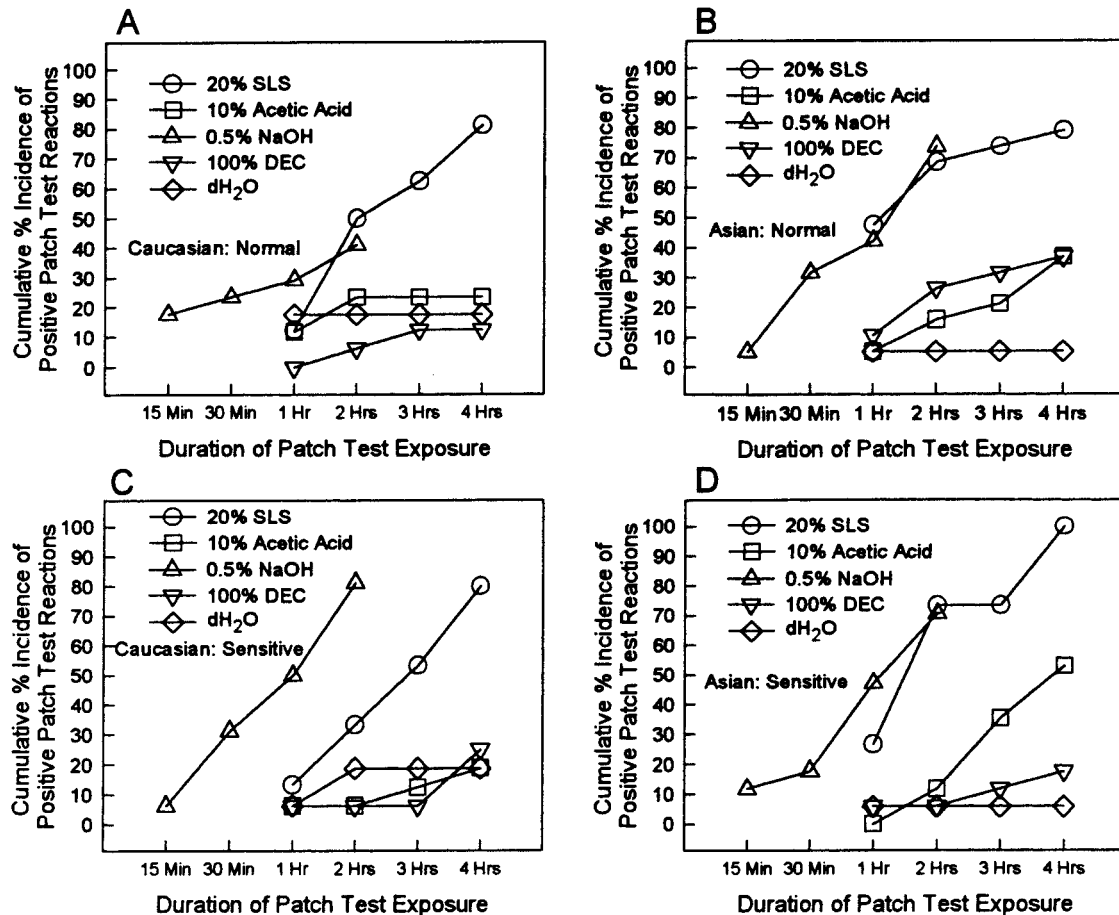


Fig. 5. Caucasian and Asian test subjects were screened for neurosensitivity to lactic acid, as described in Materials and Methods, and subdivided into "sensitive" and "normal" subpopulations. The 4 test populations were then exposed to the indicated irritant materials in a single side-by-side study (13–20 subjects per panel). See Table 1 for statistical comparison of responses across race and skin sensitivity categories.

Impact of racial and neurosensory response differences on the objective irritation response to chemicals

Most objective skin irritation tests use severity of response as the primary visual indicator of reactivity. The use of a "time to respond" evaluative endpoint in the 4-h patch test provides a unique means by which to assess epidemiological factors impacting on the skin irritation response. We used this approach to examine the question whether racially diverse study populations (Caucasians versus Asians) would differ in skin reactivity to different chemical irritants when tested in parallel at the same study site.

In this study, Caucasian and Asian populations were concurrently patched with 20% SDS, 10% HAc, 0.5% NaOH, 100% decanol, and water. The results are shown in Fig. 4 (A&B). The level of response seen with the Caucasian panel was very similar to that seen in prior studies (9). Also, based

upon the statistical comparison of the cumulative responses to all 5 test and control materials, there was no difference in the level of response between the Caucasian and Asian subject panels. Thus, we were unable to identify a racially based difference in reactivity to multiple chemical irritants of differing potencies.

In a follow-up study, similar racially distinct test panels were again tested with the same study materials. In this case, the subject panels were further subdivided on the basis of whether the individual test subjects showed heightened neurosensory responses to lactic acid. The reproducible ability to manifest a stinging response to a facial exposure to 10% lactic acid has been used as a criterion to identify individuals as "stingers" or individuals with increased neurosensory reactivity (11). The purpose here was to determine if individuals with heightened neurosensory responses also tend to show the greatest objective skin responses to a range of chemical irritants, and again to examine

Table 1. Statistical comparison between populations (racial and neurosensory reactivity) in terms of cumulative irritation response

Race comparison ^{a)}	Test material				
	20% SDS	10% HAc	0.5% NaOH	100% DEC	dH ₂ O
CS versus CN	= ^{b)}	=	CS > CN ^{c)}	=	=
AS versus AN	=	=	=	=	=
CS versus AS	=	=	=	=	=
CN versus AN	=	=	=	=	=
CS versus CN versus AS versus AN	=	=	=	=	=

^{a)} Statistical (the Fisher exact test) comparison between racial and neurosensory sensitivity distinct subpopulations in terms of cumulative incidence of skin response over a 2-h (NaOH) or 4-h (other materials) exposure time. Populations include: Caucasian sensitive (CS), Caucasian normal (CN), Asian sensitive (AS), and Asian normal (AN).

^{b)} Designates no statistically different response.

^{c)} Population to left of symbol showed statistically greater ($p < 0.05$) cumulative response than population to right of symbol.

whether this relative pattern of response is subject to racial influences.

Prospective test subjects were given sequential exposures to facial patches containing 10% or 5% lactic acid or distilled water. Subjects of either race (Caucasian or Asian/Chinese) were classified as neurosensory reactive only if they showed a reproducible stinging or burning response to 10% lactic acid with no (or much reduced) responses to water. In most cases the “stinger/burners” were also able to distinguish 10% and 5% lactic acid. The nonresponders were classified as “normal skin subjects”. The neurosensory reactive and normal subjects from each population were then tested for their objective irritation responses to 20% SDS, 10% HAc, 0.5% NaOH, 100% decanol, and water in the 4-h patch test. The results of this study are shown in Fig. 5 and the statistical results are summarized in Table 1.

Comparing within racial subpopulations, there was very little evidence of enhanced skin irritation responses in subjects with “sensitive” versus normal skin. For Caucasian subjects (Fig. 5A/5C), the responses were equivalent for all test materials with the exception of 0.5% NaOH. Here, the “normal skin” subjects responded a bit more quickly, but the “sensitive skin” subjects showed a statistically higher cumulative incidence of response. The 2 Asian populations also showed equivalent responses across all the test materials (Fig. 5B/5D). We also compared the responses for the most neuroreactive subpopulation of Asian or Caucasian test subjects with less neurosensitive or normal subpopulations. Even here, there was no evident heightened susceptibility of these most neurosensitive subjects to objectively assessed chemical irritation (data not shown).

Comparing within skin type categories but across races, there were, again, no significant differences in objective response. Asian “sensitive skin” subjects had a directionally higher cumulative incidence of positive responses to acetic acid

than the Caucasian “sensitive skin” subjects. All other cross racial comparisons were about the same. For each test material, we also compared reactivity in parallel across all 4 subpopulations and, again, were able to discern no significant differences in response (Table 1).

Discussion

Our results with the human 4-h patch test confirm and supplement a series of previous studies showing a reproducible acute irritation response to 20% SDS and a consistent differential response to other irritant chemicals (1, 3, 9, 13). In repeat testing at 3 different laboratory facilities, we obtained a consistent relative ranking for a set of chemicals in comparison to 20% SDS as a benchmark. Not surprisingly, when the data were analyzed as the time to respond (as compared to the total cumulative response), the time course of response followed the same relative ranking. Moreover, the time-response curves for SDS from multiple studies was consistent, in spite of the use of multiple testing laboratories and slight modifications of the test procedure. These results support the prior contention (4) that this method is robust and a valid alternative to the rabbit skin irritation (Draize) test for the assessment of skin irritation hazard to humans.

The main focus of the current studies was to use this acute human irritation test method to investigate certain parameters of the human skin irritation response. If one thing is clear about clinical skin irritation, it is the extreme variability in response across the human population. Many factors are known to influence the susceptibility to skin irritants (14). Some are extrinsic, such as the chemical nature of the irritant, the dose, the body site, mechanical factors, and environmental conditions. Others are endogenous and include race, age, sex, preexisting disease, and state of hyperreactivity. It has been established that irritation re-

sponse thresholds will vary considerably across even reasonably homogeneous subject populations (5, 6). The pattern of both the time- and dose-response to SDS in the current study support this finding. However, we also wanted to ask the simple question of whether a positive irritation response to 1 chemical would predict the response to a 2nd chemical. We selected the response to 20% SDS and 0.5% NaOH, since the cumulative levels of response to the 2 chemicals were similar albeit over a different time course. We used the time to respond across the population as the index for comparison. The intriguing finding was that, while there was a good correlation in response across most of the subjects in the study population, there were also a large number of subjects (~25%) who responded selectively to only 1 chemical. Thus, one cannot assume, for a specific individual, that reactivity to 1 irritating chemical predicts their reactivity to other irritating chemicals, especially across chemicals of different classes. Nevertheless, the group correlation supports previous recommendations for panel sizes of approximately 30 subjects for the human irritation patch test (3, 4).

A 2nd application of the human irritation test investigated in this study was the relative reactivity of human subpopulations as distinguished by race or neurosensory hyperreactivity. There is speculation in the literature that different races react differently to chemical irritants and allergens. Some classic studies in dermatology suggest, for example, that Blacks are more resistant than Caucasians to developing visual skin irritation or allergic patch test reactions (15–18). The situation with Caucasian and Asian populations is more complex. A single study in the literature has reported an increased cumulative irritation response in Japanese subjects versus Caucasians to various chemicals and product formulations (19). More recently, the human acute skin irritation test method was applied to different racial subpopulations and failed to show any enhanced reactivity between Asian (Chinese) and Caucasian (German or English) populations (6, 9). In the present study, we directly compared Caucasian and Asian subpopulations tested in parallel with the same chemicals in a single study. Again, we failed to detect significant differences in skin reactivity to a series of chemicals of widely varying inherent irritation potentials. These data would tend to support the contention that interindividual variability within a given racial subpopulation is sufficiently large so as to obscure any potential interracial differences.

Lastly, we extended the question of differences in population reactivity to include the issue of sensitive skin. Differences in levels of skin hyperreactivity is thought to impact on the skin irritation

response. However, it is not clear if differences in neurosensory reactivity to chemical stimuli such as lactic acid translates to differences in the objective (visual) irritant response to the same or other chemicals. The literature on this point is somewhat conflicting. Simion and coworkers (20) reported a direct correlation between subject perceived sensory responses and objectively graded irritation responses to soap or synthetic detergent. This finding differs from those in which “stingers” and “non-stingers” (to lactic acid) were initially recruited and then compared for their objective irritation responses to surfactant. In an early study (21), no difference in visual skin reactivity to SDS was seen in stingers and non-stingers. This negative finding was later confirmed (22).

In our current study, we expanded upon the question by combining both the racial population difference and the difference in neurosensory reactivity into a single assessment and evaluated multiple chemical irritants. In this way we asked 2 questions: 1) do stingers and non-stingers differ in objective irritation responses to chemical irritants of different classes, and 2) is this pattern of reactivity different between Caucasian and Asian subpopulations. Our results confirm the findings of Lammintausta et al. (21) and Basketter and Griffiths (22) in that minimal differences in objective acute skin irritation responses were seen between stinger and non-stinger populations, either within or across racial populations. This was also the case when the most severely “neurosensitive” subjects were compared with less sensitive or normal test subjects; no difference in objective irritation response to the chemicals was evident. Thus, with regard to objective skin irritation responses to chemicals, interindividual variation in response obscures any recognizable differences in response across these different human subpopulations.

Acknowledgements

The authors thank Dr. Greg J. Carr for his statistical support for these studies. They also thank Drs. G. Frank Gerberick, Elizabeth E. Sikorski, and Karen L. Blackburn for their critical review of the manuscript.

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