

Intra-individual variations in acute and cumulative skin irritation responses

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It is well-known that humans show a wide range of variation in skin reactivity to irritant chemicals. This has been established through population studies, through the examination of inter-subject variability, and (to a limited extent) through studies of skin site variation in response within subjects. However, simple response variability within individual test subjects has not been examined as carefully, and this has implications for our ability to predict irritant reactivity. Some key questions are: (i) how consistently do human beings respond, even within a given study, to different equally irritating chemicals, or to the same chemical when comparing different concentrations or durations of exposure, and, (ii) Do individual test subjects' responses to one chemical (or exposure scenario) correlate with their responses to another? To examine these questions in some detail, we reexamined individual study subjects' responses from earlier published studies involving both acute and cumulative irritation patch test protocols. Acute irritation responses were compared across chemicals with similar irritation profiles. Cumulative irritation responses were compared across different concentrations of the anionic surfactant, sodium dodecyl sulfate (SDS). Acute (high concentration) and cumulative (low concentration) patch test responses to SDS were also compared. The analysis showed that, as might be expected, response correlations were greatest within test types, either when comparing chemicals of similar overall irritancy, or when comparing similar concentrations of a single chemical. However, individually divergent responses were also frequent, reinforcing the conclusion that a given individual's response to one chemical or exposure condition does not always predict their response to another. This has important ramifications for other questions related to population differences in skin reactivity.

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Various skin irritation test procedures have been developed over the years as a means to predict the skin irritation potential or skin compatibility of chemicals and products (1–3). Though it has been recognized that human test subjects have varying susceptibilities to chemical irritation (4), a recently developed acute human patch test procedure (2) has shown a high degree of intra-laboratory (across studies) and inter-laboratory reproducibility (5–7). This reproducibility has occurred in spite of certain discrepancies in individual subjects' response patterns and is likely due to the recruiting of sufficient numbers of test subjects in order to dilute the impact of such inter-individual variations in response (6, 7).

Variation in the pattern of skin reactivity within individual subjects is a facet of skin irritation that has not been evaluated to any great extent in previous investigative or predictive skin irritation studies. There have been occasional reports de-

scribing regional (i.e., body site) variations in skin irritation responses (8, 9). This has been noted for chemosensory irritation patterns as well (10). In fact, it has been recently reported that subjects' self-perceived sensory skin sensitivity does not necessarily correspond to their actual response to chemosensory chemical probes (11). We have also reported, to a limited extent, certain divergent patterns of individual responsiveness to different irritant chemicals (6, 7). However, there has been no extensive examination of intra-subject variations in acute and cumulative irritation response patterns within studies.

To examine this question in some detail, we reassessed individual subject data from previous clinical skin irritation tests conducted among large heterogeneous study populations (6, 12). Some studies included subjects of mixed sex and race (Caucasian and Asian) and also involved concurrent patch testing for both acute and cumulative

skin irritation responses. Since each subject's irritation response pattern was independently assessed within each study, for correlation analysis we pooled individual subject responses (for specific chemicals) across studies. We then examined intra-subject variability in skin irritation responses in 3 different ways: (i) comparison of the acute response pattern to pairs of similarly irritating chemicals, (ii) comparison of the cumulative response patterns to different concentrations of the anionic surfactant sodium dodecyl sulfate (SDS), and (iii) comparison of the acute/high dose v cumulative/low dose response susceptibility to SDS. All comparative response patterns were examined through regression analysis.

Methods

Chemicals

Test chemicals studied included sodium dodecyl sulfate (SDS), acetic acid (HAc), 1-decanol (Dec), octanoic acid (OAc), and distilled water (dH₂O). All test chemicals were obtained from a single supplier (Sigma Chemicals, St. Louis, MO) and were at least 99% pure. Lack of corrosivity of the test materials (under conditions of the test protocol) was assured prior to testing via reference to published data (7, 13, 14).

Human patch test procedures

Clinical testing was conducted at the Reed Hartman Research Center (Cincinnati, OH) or the California Skin Research Institute (San Diego, CA) under contract and according to protocols provided by Procter & Gamble. The procedures used were independently reviewed and approved prior to study initiation and all testing was conducted in accordance with the Helsinki Declaration of 1975. All procedures were described in detail to all study subjects, who individually read and signed informed consent forms. Specific details of the study populations have been reported previously (6, 12).

Acute irritation patch test

Study subjects were exposed on the upper outer arm to each test material for up to 4 h according to previously described procedures (2, 3, 5–7, 12). In each study, 20% SDS was tested as a positive control and dH₂O was tested as a negative control. Test materials were applied neat (OAc and Dec), or after dilution in dH₂O (10% HAc, 10% SDS), in a 0.2 ml volume to 25mm Hill Top Chambers (Hill Top Company, Cincinnati, OH). Test materials were applied to the skin sites progressively from 30 min through 1, 2, 3, and 4 h of exposure.

Thus, each subject had a maximum of 5 patches of each test material applied, with each successive patch being applied for an increased duration of time. This incremental exposure ensured that skin reactions stronger than mild (or at most moderate) were avoided.

After each patch removal, the skin sites were gently wiped with a damp towel (to remove excess residual test material) and allowed to dry, taking care not to cross-contaminate the sites. The sites were graded at 1, 2, and 3 days (D) after patch removal using a 4-point grading scale of increasing irritation:

- 0 no significant reaction,
- + weak but unequivocal erythema over most or all of test site; the response may include some edema or other surface effects,
- ++ moderate erythema that may spread beyond the edge of the treatment site. The response is usually accompanied by edema and/or surface effects (e.g., glazing, scaling) and possibly other focal injuries (hemorrhage, erosion, scabbing);
- +++ strong, usually spreading erythema commonly accompanied by edema and possibly with injuries in depth (vesiculation, scabbing, hemorrhage).

Erythema was the primary response detected and recorded. Purely surface effects (e.g., dryness, glazing) were recorded, but were not used as sole determinants of positive responses. Subjects not responding to a test material after a given exposure interval were re-exposed for the next longer interval at naive skin sites (adjacent to the prior site or on the alternate arm). Once a positive response (+ or greater) occurred in a given subject, there were no further exposures of that subject to that test material. The principles of the patch testing procedure were intended to avoid the production of strong irritation responses. Greater than 90% of all skin reactions were mild in severity and resolved quickly.

Cumulative irritation patch test

Some study subjects included in acute irritation testing (above) were concurrently exposed to each of 4 concentrations of SDS (0.025%, 0.05%, 0.1%, and 0.3%) on the upper back for 14 consecutive days. Each SDS concentration was applied in 0.2 ml volumes to 25 mm Hill Top chambers. Each patch was worn for 1 D, then was removed and the skin sites graded 15 min after patch removal. The sites were graded using a 7-point scale of increasing irritation.

- 0 no reaction
- 1 minimal (barely perceptible) erythema

- 2 mild but well defined erythema only
- 3 moderate erythema only OR Mild erythema plus edema and/or papules
- 4 severe erythema only OR Moderate erythema plus edema and/or papules
- 5 severe erythema plus edema and/or papules OR any vesicular reaction
- 6 bullous reaction or any grade 3–5 skin reactions that spread beyond the patch site

New patches were then applied at the same sites. Once a skin grade of ≥ 3 (moderate erythema or mild erythema plus edema and/or papules) was observed at a patch site, no further patches were applied and a grade 3 was carried out for the remainder of the study for data evaluation purposes.

Data analysis

For the acute irritation patch test, the effective number of panelists completing the test included all subjects who received a 4-h exposure to that test material, plus any subjects for which treatment with a given test material was stopped at an earlier exposure time due to a positive reaction. Dropouts for any reason (none were due to skin reactions) were not included in the data analysis.

Individual subjects' response patterns were compared across chemicals to see if rapid or slow responses to 1 chemical would correspond to rapid or slow responses to the 2nd chemical. The 3 chemical comparisons studied were 20% SDS versus 10% SDS, 20% SDS versus 100% OAc and 100% Dec versus 10% HAc because, at the study population level, these chemical pairs had similar overall irritation time-response profiles. For each subject, the time of response to 1 of the pairs of chemicals was plotted against the time of response to the 2nd chemical. The scatter plots were then processed by linear regression analysis, the correlation coefficient (r) determined, and assessed for statistical significance.

For the cumulative irritation test, an irritation index was calculated for each subject for each concentration of SDS by summing each subject's irritation grade for all time points and dividing by 42 (theoretical maximum cumulative grade if a cutoff grade 3 were assigned for all 14 grading time points). To examine intra-subject variability in response patterns, the irritation indices for all test subjects to each of the SDS concentrations were cross-compared giving 6 comparisons for analysis (0.025% versus 0.05%, 0.025% versus 0.1%, 0.025% versus 0.3%, 0.05% versus 0.1%, 0.05% versus 0.3%, and 0.1% versus 0.3%). The scatter plots were evaluated by linear regression analysis as described above.

Lastly, because acute and cumulative irritation

testing of SDS were done concurrently in some test subjects, a comparison could be made between these responses. To do this, the duration of patch exposure giving a positive reaction in the acute test was "converted" to an arbitrary severity grade as described previously (6). For example, a response after 0.5 h of patch exposure was assigned a maximum grade of 6 and no response after 4 h of patch exposure was assigned a minimum grade of 1. The severity grade for each subject's response to 20% SDS was cross-compared to the irritation index recorded, for that subject, for each of the SDS concentrations tested for cumulative irritation. Each of the 4 resulting scatter plots was then evaluated for correlations by linear regression analysis as described above.

In addition to the analyses conducted on the combined test population, a separate analysis was made for the subjects comprising 2 racial subpopulations. There were sufficient numbers of Caucasian ($n=46$) and Japanese ($n=37$) subjects to enable a cross-comparison of acute responses (20% SDS versus 100% OAc), cumulative responses (0.025% SDS versus 0.05% SDS), and acute/cumulative responses (20% SDS/acute versus 0.3%/cumulative). The low SDS concentrations were used for the cumulative irritation response comparison because the irritation indices covered almost the entire range of responses (0–1). The analysis procedures used were the same as those described above.

Results

Intra-subject variations in acute skin irritation responses to equally irritating chemicals

To examine acute irritation response patterns, the time-response profile of each test subject was compared across chemicals. 3 paired chemical comparisons were analyzed; 20% SDS versus 10% SDS, 20% SDS versus 100% OAc and 100% Dec versus 10% HAc. These comparisons were made because the population level time responses to each pair of chemicals were similar (6, 12). The time of exposure resulting in a positive patch test response was plotted for each test subject for each chemical and linear regression analysis conducted. The results are shown in Fig. 1.

All of the paired chemical comparisons showed positive correlations that were statistically significant. This was much more apparent for the comparisons of the responses to the more irritating chemicals (20% SDS versus 10% SDS and 20% SDS versus 100% OAc). The population responses to 20% and 10% SDS were virtually identical (6). In comparing the individual subject response pattern, 29/31 subjects had positive response exposure time differences of 1 h or less (Fig. 1A). Among subjects

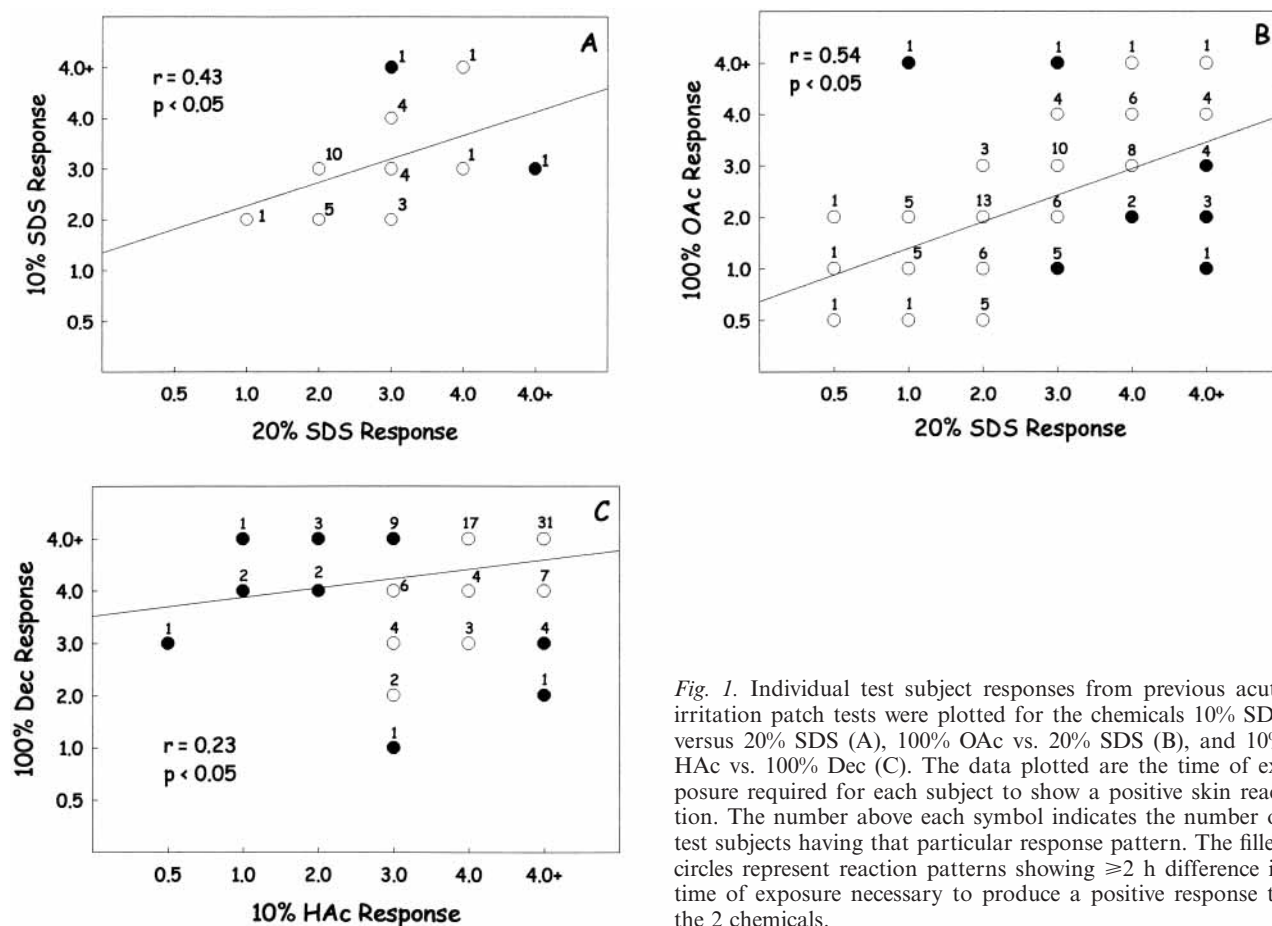


Fig. 1. Individual test subject responses from previous acute irritation patch tests were plotted for the chemicals 10% SDS versus 20% SDS (A), 100% OAc vs. 20% SDS (B), and 10% HAc vs. 100% Dec (C). The data plotted are the time of exposure required for each subject to show a positive skin reaction. The number above each symbol indicates the number of test subjects having that particular response pattern. The filled circles represent reaction patterns showing ≥ 2 h difference in time of exposure necessary to produce a positive response to the 2 chemicals.

whose response times were different, most (17/22) responded more quickly to 20% SDS. Overall, there was a significant correlation. Comparing 100% OAc and 20% SDS responses across 2 studies (12), 81/98 subjects had positive response exposure time differences of 1 h or less (Fig. 1B). For this comparison, there were 17 subjects with divergent responses (≥ 2 h), all but 2 of whom were more rapidly reactive to the more irritating chemical, OAc. Again, the correlation was significant. Occasional divergent responses to stronger irritants have been reported before (6, 7). For the weaker irritant chemicals (100% Dec and 10% HAc) the correlation was much less obvious visually, though still statistically significant due to the large number of test subjects included in the analysis (Fig. 1C). In this comparison 24/98 subjects had divergent responses (exposure times ≥ 2 h), most of whom (18/24) were more rapidly reactive to 10% HAc.

Intra-subject variations in cumulative skin irritation responses to different concentrations of SDS

By testing 4 concentrations of SDS concurrently in the cumulative irritation phase of these studies, it

was possible to also examine each subject's consistency in response to each of the 4 concentrations. With 4 concentrations tested, it was possible to make 6 dual concentration comparisons for each study. Table 1 lists all of the correlation coefficients for each comparison of SDS exposure concentrations. Fig. 2 shows the actual scatter plots. Overall, the correlation coefficients ranged from 0.43 to 0.68. All were statistically significant. If there was any trend apparent, it was that the comparisons between more disparate test concentrations (e.g., $\geq 4\times$ differences) showed poorer correlations. In spite of the general tendency for indi-

Table 1. Correlation between individual subject cumulative irritation indices from different SDS exposure concentrations

Test concentrations	Correlation coefficient (<i>r</i>)	<i>p</i>
0.025% versus 0.05%	0.53	<0.05
0.025% versus 0.1%	0.43	<0.05
0.025% versus 0.3%	0.43	<0.05
0.05% versus 0.1%	0.50	<0.05
0.05% versus 0.3%	0.50	<0.05
0.1% versus 0.3%	0.68	<0.05

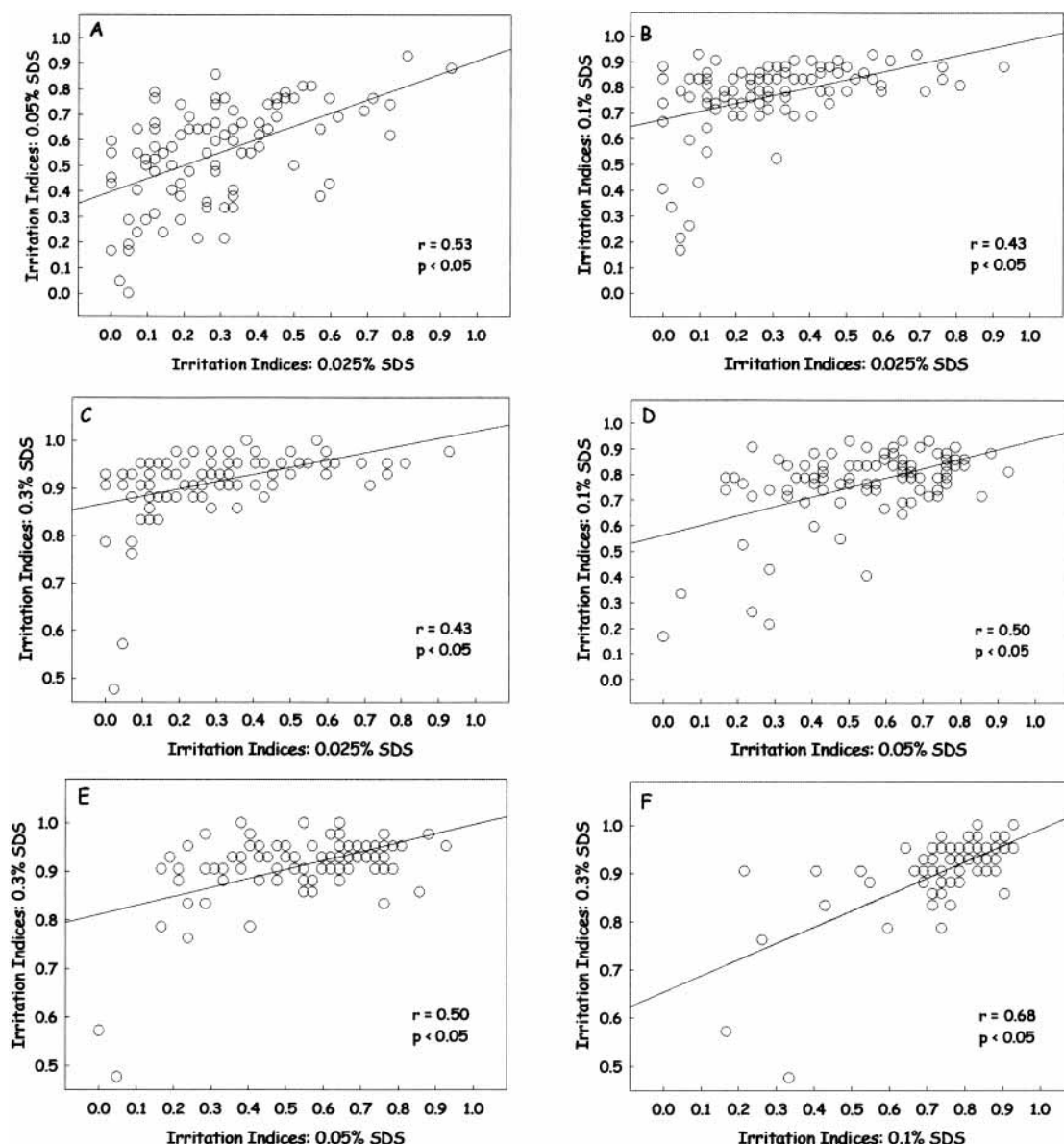


Fig. 2. Irritation indices were calculated for each test subject's response to each concentration of SDS in the 14-D cumulative irritation test (as described in *Materials and Methods*). (A)–(F) show the scatter plots obtained when each subject's irritation index value for one SDS concentration was plotted against their irritation indices for each of the other SDS concentrations. Linear regression analyses were run for each scatter plot.

vidual responses to different SDS concentrations to correlate, several test subjects did show highly divergent responses.

Correlation between susceptibility to skin irritation responses from acute/high dose versus cumulative low dose SDS exposures

The correlation analyses described above were each focused on comparative responses within 1 of 2 distinct test types (acute or cumulative irritation patch tests). In addition, the unique concurrent

test design of certain studies (12) enabled a comparison of individual subject's irritation responsiveness to acute/high dose vs. cumulative/low dose exposures to the same chemical (SDS).

To facilitate this comparison, each subject was assigned a score for the acute irritation response (to 20% SDS) depending on the time of exposure required to produce a positive skin reaction. A reaction following the shortest exposure period (0.5 h) was assigned a maximum score of 6 and a failure to respond to the longest exposure period (4 h) was assigned a minimum score of 1. Positive re-

sponses after 1, 2, 3, and 4 h were assigned scores of 5, 4, 3 and 2, respectively.

For each study subject, the assigned acute irritation score was plotted against their cumulative irritation index value for each of the 4 SDS concentrations examined in the cumulative irritation test. The scatter plots are shown in Fig. 3. In this comparison across test types (acute vs. cumulative), the correlation values derived from the analysis were much lower than those obtained from the other comparisons within test types. None of the correlation coefficients reached a value of 0.3 and only 2 of the 4 were statistically significant.

Racial differences in the intra-subject skin irritation response correlations

Because racially distinct test subjects were enrolled in some of these studies, we were also able to ask whether the response pattern comparisons would differ among racial subpopulations. In this comparison, Caucasian and Japanese subjects were included and 3 comparison analyses were made: acute response to 20% SDS versus 100% OAc, cumulative response to 0.025% SDS versus 0.05% SDS, and acute response to 20% SDS versus cumu-

lative response to 0.3% SDS. The results are shown in Fig. 4.

Comparisons between the Caucasian and Japanese test subjects were quite similar to the results obtained with the combined larger study population. Positive and significant correlations were observed for the relative acute responses to 20% SDS and 100% OAc and for the relative cumulative responses to 0.025% and 0.05% SDS. The correlation coefficients were somewhat lower for the smaller Japanese test population. In contrast, there was no positive correlation between the individual subject acute high dose and cumulative low dose responses to SDS.

Discussion

Unraveling the complexity of the human skin irritation response has been hampered to a great extent by response variability. It has been well-recognized that there is significant variability in the human skin irritation response, both in terms of objective (e.g., visual) and symptomatic endpoints (4, 8, 9, 15). This has been documented in studies of regional (skin site) variation within individual study subjects (8–10, 16, 17) as well as inter-indi-

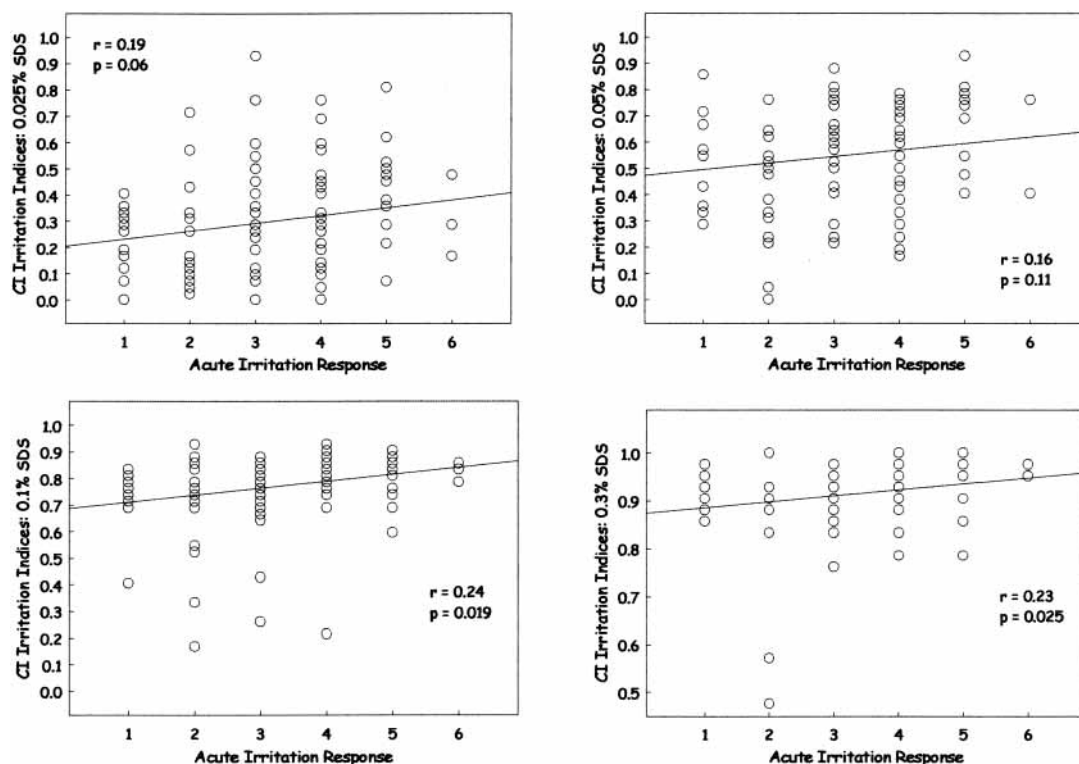


Fig. 3. Individual subject's acute irritation responses to 20% SDS were assigned a response value of 1 to 6 depending on the time of exposure required to produce a positive skin reaction (1=no response after 4 h of exposure; 6=positive response after 0.5 h of exposure). These values were plotted against the same individuals' responses (irritation indices) to each of the 4 SDS concentrations tested in the cumulative irritation patch tests. Linear regression analyses were run for each scatter plot.

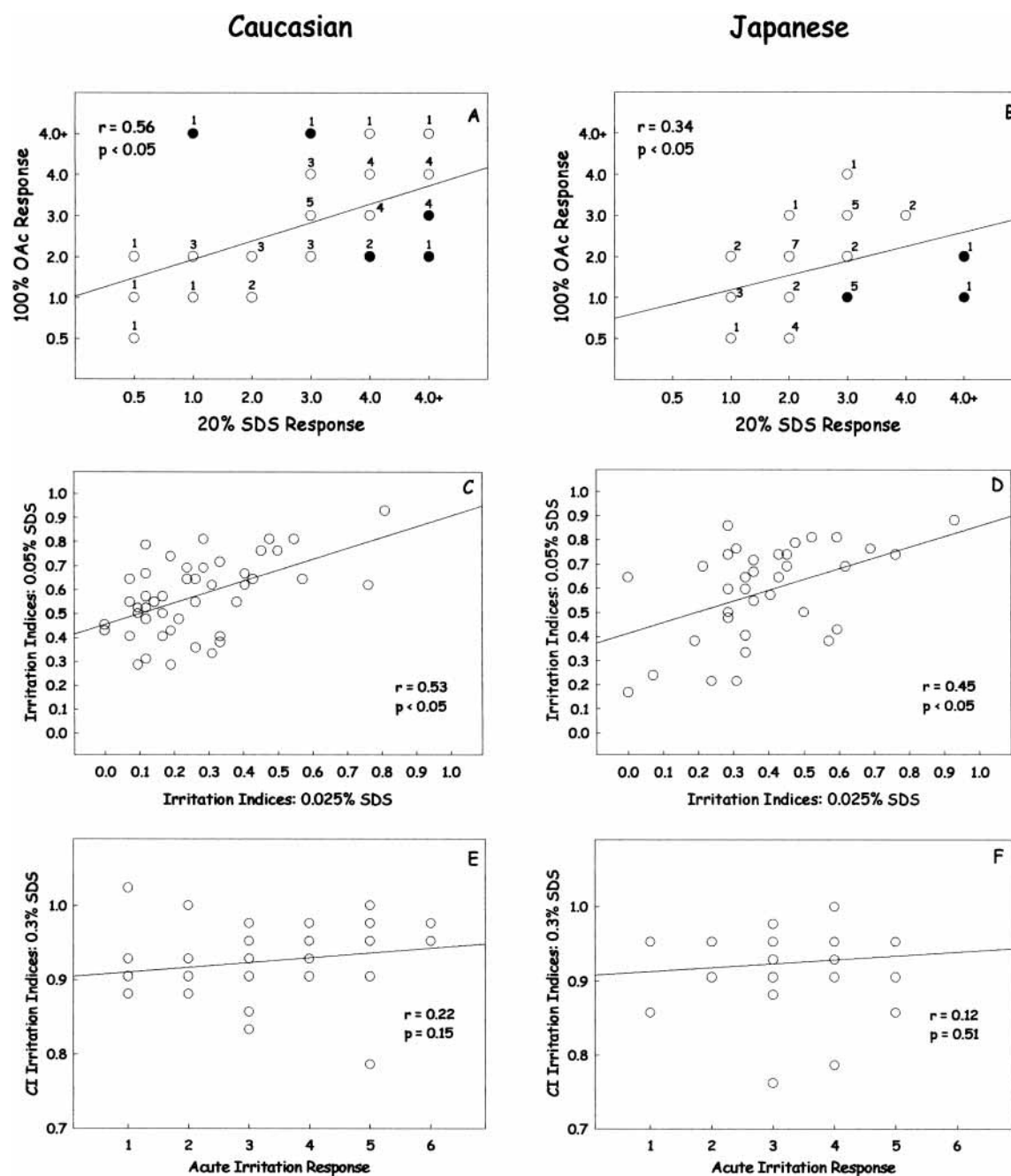


Fig. 4. Comparison of acute and cumulative irritation responses among Caucasian and Japanese subjects. (A) and (B) show the acute response comparison to 20% SDS and 100% OAc (see legend to Fig. 1). (C) and (D) show the cumulative irritation response comparison to 0.05% and 0.025% SDS (see legend to Fig. 2). (E) and (F) show the acute vs. cumulative response comparison to SDS (see legend to Fig. 3).

vidual responses (4, 10, 17). Attempts to understand the influence of other factors on skin irritation responsiveness; factors such as age, race, sex, or environment, can be undermined by an inadequate appreciation of intra-subject variability in skin irritation responsiveness. This may account for at least some of the inconsistencies in the clin-

ical dermatology literature on the subject of population differences in skin reactivity (18).

While there have been studies addressing inter-subject variability and intra-subject variability between skin sites, less effort has been devoted to simply measuring the consistency of responses within individual test subjects. It was the intent of

this current investigation to re-examine past studies (6, 12) for intra-subject response patterns, by measuring responses to multiple chemicals or chemical concentrations across different irritation test methods.

The results presented here were obtained in 3 separate studies, 2 of which combined both the 4 h patch test and a cumulative irritation patch test. The latter 2 studies (12) were conducted under the same exact protocol at a 4-month interval. Because these 2 studies used the same exact protocol and test materials, the individual subjects' data were combined for the correlation analyses. A variety of relative response profiles were compared. These included: (i) the acute 4-h patch response to different chemical pairs with differing irritancy potentials, (ii) the cumulative irritation responses to different concentrations of SDS, and (iii) a comparison of the acute response to 20% SDS versus the cumulative response to various lower concentrations of SDS. Lastly, a subset of responses was examined among Caucasian and Japanese test subjects to see if any racially distinct response patterns would emerge.

The acute response comparison between 20% and 10% SDS and between 20% SDS and 100% OAc showed clear positive (and statistically significant) correlations, with the majority of subjects responding to each chemical within a 1 h or less time of exposure. This similarity in intra-individual response patterns was comparable to that reported previously (7). The correlation was much weaker (though still significant) when less irritating chemicals (10% HAc and 100% Dec) were compared.

Moderate but positive (and statistically significant) correlations were seen between all SDS concentration comparisons in the cumulative irritation test. As one might expect, the correlations were poorer for concentrations that were most different (i.e., 0.025% versus 0.3%). The best correlation was seen for the comparison between the two highest concentrations tested (0.1% versus 0.3%).

Unlike the comparisons within test types, correlations between acute and cumulative skin irritation responses were relatively poor, even though the test chemical was the same (SDS). Only the comparisons between the acute response to 20% SDS and the 2 highest cumulative irritation test concentrations (0.1% and 0.3%) showed statistically significant correlations, although the coefficients were low. Separating out the responses among the Caucasian and Japanese test subjects, there were no substantive differences between these populations in the correlations examined.

Prior studies have reflected upon the variability in human skin irritation responses and the limi-

tations this imposes upon predictive skin irritation testing and the ability to discern reactivity differences between populations (4, 7, 12, 18). We observed some intra-individual variation in the acute irritation response to SDS and various organic acids (7). The variation was greater for unrelated chemicals and less for structurally related chemicals with similar overall irritation potential. Those findings were consistent with the observation here of a positive correlation between the 20% SDS and 10% SDS and between the 20% SDS and 100% OAc responses, albeit with some individual subjects showing divergent responses. Andersen reported similar variation within individuals and suggested that less variation was seen with more severe irritants because background irritancy from the test method itself would be overwhelmed (19).

Intra-individual variability in skin reactivity is a factor that needs to be considered in conducting predictive skin irritation tests or in attempting to document population differences in skin reactivity. Predictive test methods are feasible if enough test subjects are recruited to minimize the impact of the intra-individual variability. Inclusion of positive and negative control test materials as a quality control check is also important (3, 20). Although a significant response correlation was commonly observed in these studies, the correlation coefficients were generally low and divergent responses were frequent. Therefore, it is clearly inappropriate to define an individual's reactivity to one chemical purely on the basis of their response to another. This is even truer when making predictions across different test methods. This variation in reactivity is also important to consider before ascribing to theories of population differences in skin reactivity based on divergent responses obtained in studies on limited numbers of racially or otherwise distinct test subject populations.

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References

1. Berger R S, Bowman J P. A reappraisal of the 21-day cumulative irritation test in man. *J Toxicol-Cutan Ocul Toxicol* 1982; 1: 109-115.
2. Basketter D A, Whittle E, Griffiths H A, York M. The identification and classification of skin irritation hazard by a human patch test. *Food Chem Toxicol* 1994; 32: 769-775.
3. York M, Griffiths H A, Whittle E, Basketter D A. Evaluation of a human patch test for the identification and classification of skin irritation potential. *Contact Dermatitis* 1996; 34: 204-212.
4. Judge M R, Griffiths H A, Basketter D A, White I R,

- Rycroft R J G, McFadden J P. Variation in response of human skin to irritant challenge. *Contact Dermatitis* 1996; 34: 115–117.
5. Griffiths H A, Wilhelm K P, Robinson M K, Wang X M, McFadden J, York M, Basketter D A. Interlaboratory evaluation of a human patch test for the identification of skin irritation potential/hazard. *Food Chem Toxicol* 1997; 35: 255–260.
 6. Robinson M K, Perkins M A, Basketter D A. Application of a 4-h human patch test method for comparative and investigative assessment of skin irritation. *Contact Dermatitis* 1998; 38: 194–202.
 7. Robinson M K, Whittle E, Basketter D A. A two-center study of the development of acute irritation responses to fatty acids. *Am J Contact Dermat* 1999; 10: 136–145.
 8. van der Valk P G, Maibach H I. Potential for irritation increases from the wrist to the cubital fossa. *Br J Dermatol* 1989; 121: 709–712.
 9. Cua A B, Wilhelm K P, Maibach H I. Cutaneous sodium lauryl sulphate irritation potential: age and regional variability. *Br J Dermatol* 1990; 123: 607–613.
 10. Green B G. Regional and individual differences in cutaneous sensitivity to chemical irritants: Capsaicin and menthol. *Journal of Toxicology Cutaneous and Ocular Toxicology* 1996; 15: 277–295.
 11. Bowman J P, Floyd A K, Znaniecki A, Kligman A M, Stoudemayer T, Mills O H. The use of chemical probes to assess the facial reactivity of women, comparing their self-perception of sensitive skin. *Journal Of Cosmetic Science* 2000; 51: 267–273.
 12. Robinson M K. Racial differences in acute and cumulative skin irritation responses between Caucasian and Asian populations. *Contact Dermatitis* 2000; 42: 134–143.
 13. Barratt M D. Quantitative structure activity relationships for skin corrosivity of organic acids, bases and phenols. *Toxicol Lett* 1995; 75: 169–176.
 14. Lewis R W, Basketter D A. Transcutaneous electrical resistance: Application in predicting skin corrosives. In: Elsner P, Maibach H I (eds): *Irritant dermatitis: new clinical and experimental aspects*. S. Karger AF: Basel, 1995: 243–255.
 15. Green B G. Measurement of sensory irritation of the skin. *Am J Contact Dermatitis* 2000; 11: 170–180.
 16. van Neste D, de Brouwer B. Monitoring of skin response to sodium lauryl sulphate: clinical scores versus bioengineering methods. *Contact Dermatitis* 1992; 27: 151–156.
 17. Erntestam L, Ollmar S. Electrical impedance index in human skin: measurements after occlusion, in 5 anatomical regions and in mild irritant contact dermatitis. *Contact Dermatitis* 1993; 28: 104–108.
 18. Robinson M K. Population differences in skin structure and physiology and the susceptibility to irritant and allergic contact dermatitis: implications for skin safety testing and risk assessment. *Contact Dermatitis* 1999; 41: 65–79.
 19. Andersen K E. Reproducibility of the chamber scarification test. *Contact Dermatitis* 1996; 34: 181–184.
 20. Basketter D A, Chamberlain M, Griffiths H A, Rowson M, Whittle E, York M. The classification of skin irritants by human patch test. *Food Chem Toxicol* 1997; 35: 845–852.

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