

Review Article

Population differences in skin structure and physiology and the susceptibility to irritant and allergic contact dermatitis: implications for skin safety testing and risk assessment

MICHAEL K. ROBINSON

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH, USA

Skin safety testing and risk assessment utilize a comparative toxicological approach whereby the inherent toxicity (irritation or sensitization) is related to exposure to determine the potential risk to a consumer population. However, consumers cover a broad spectrum of individual characteristics in terms of skin types and functions, as well as their basic habits and practices in use of consumer products. Thus, we try to use very conservative estimates for both inherent susceptibility to the toxic effect as well as the potential worst-case exposures. While the inherent variation can be considerable even within a country, it is magnified even further when products are marketed globally. Questions have arisen, therefore, as to whether a skin safety testing approach conducted locally or regionally can adequately predict for adverse effects for the global consumer. A good deal of speculation exists that population differences are real and should mandate population-specific safety testing prior to marketing products in certain regions of the world. In an attempt to address this question, this review has summarized extensive literature covering basic skin biology and function and susceptibility to irritant and allergic skin responses. Throughout this literature, there are individual studies demonstrating population differences in skin properties or in the responsiveness to chemical insult. Some of the research on this topic has pointed fairly convincingly to a demonstrable population difference. The best example of this is the tendency for blacks to have a lesser skin reactivity than Caucasians, likely due to a more impenetrable barrier. In contrast, the comparative data for Caucasians and Asians, for males and females, and for different age clusters are far less compelling. In terms of tendencies, there are little data to support any real difference in skin barrier function or in skin irritation responsiveness between Caucasians and Asians or between males and females. Comparing different age profiles, there does appear to be a slight decline in reactivity to irritants among the elderly (>65 years). Susceptibility to skin sensitization tends to be more related to exposure than any inherent susceptibility, though some data exist to suggest a slightly increased sensitivity in females versus males. On the basis of these data, standard procedures for skin safety testing and risk assessment can be considered relatively conservative. Most clinical skin safety studies are conducted in test populations that are comprised primarily of female Caucasians between the ages of 18 and 65 years. If anything, the skin reactivity "character" of this population cohort tends to skew toward the more reactive. This is not to say that population-specific safety testing should not be done. There may be important reasons to pursue a population-specific testing strategy in certain situations, perhaps due to regional, sex-specific, or age-specific marketing or to satisfy a regulatory or external relations need. However, there is yet no scientific justification to mandate such a strategy, based on our current knowledge of skin responsiveness and how it compares across diverse populations. There are clearly gaps in our understanding of population differences, particularly in regard to skin irritation that should guide future clinical research efforts. Side-by-side population testing for both acute and chronic skin irritation, more comparative testing of Asian subpopulations, testing for neurosensory irritation, and testing objective skin responses among hyperreactive subpopulations of multiple races are all areas in which additional research is needed. While current testing strategies have provided an excellent track record of success in providing safe and effective products to the world's consumers, results

of such research will, in the future, help us to refine and further improve our skin testing and risk assessment capabilities.

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One of the fundamental questions in dermatotoxicology today is whether human subpopulations show quantifiable differences in skin reactivity based on race, age, or sex. The literature on this subject dates back many decades and, more recently, efforts have been made to apply new instrumental methods to address the question. In spite of this effort, definitive evidence of racial differences in skin reactivity is lacking. This is exemplified by a recent textbook on the subject of dermatotoxicology (1), which devotes less than 2 pages out of more than 600 to this topic. One reason for the lack of definitive data is the wide variation in human skin reactivity in general, even in relatively homogeneous subpopulations. Another is the enormous difficulty in controlling studies for the single variable of interest (e.g., race). Lastly, it is difficult to synthesize a point of view on populations differences in skin reactivity based upon a literature of diverse, and often conflicting, studies evaluating many different aspects of skin biology.

The question of racial differences in skin reactivity is taking on increasing importance in view of the global marketing of skin and beauty care, cosmetic and fragrance, paper, and laundry and cleaning products and the resulting increase in the racial diversity of the global consumer population. Pre-market skin safety testing and risk assessment involve a variety of clinical test methods to evaluate the potential of a new chemical or formulation to produce unacceptable adverse skin effects (sensory irritation, objective irritation, sensitization) in selected numbers of volunteer test subjects (2–16). While it has been recommended that one include volunteers of relevant age, sex, and race in clinical skin safety studies (17, 18), limitations in access to such diverse test subjects and the small number of contract clinical test facilities, often finds study populations that are generally disproportionately comprised of Caucasian, middle-aged female subjects. This leads to the question of whether or not testing in one population of test subjects is sufficient to predict for the potential reactivity of all consumers world wide.

Superimposed upon the question of subpopulation differences in skin reactivity is the increasingly accepted notion of heterogeneity and variability in skin responsiveness among human subjects in general (19). There has been interest in the past, and

a recent resurgence of this interest, in understanding the phenomenon of “sensitive skin” (20, 21). Questions abound as to how to identify and characterize such individuals and then incorporate them into tests of product safety and efficacy. Key to the issue of population differences in skin reactivity is the question of whether any of these subpopulations (race, sex, and/or age) differ in the relative proportion of sensitive vs. normal-skin individuals and how to make that determination.

It is the intent of this review to summarize our state of knowledge on the subject of racial (and other population) differences in skin responsiveness. This will begin with a discussion of skin structure, function, and physiology followed by a review of the results of past studies on racial differences in skin irritation and the induction and elicitation of allergic contact dermatitis. Key data from published and heretofore unpublished studies will be presented and discussed. In addition to racial differences, a brief review of data on sex and age differences will also be presented. The data on reactivity to skin allergens and irritants will be discussed further in relation to the need (or lack thereof) for population specific safety testing and the implications for risk assessment.

Racial Differences in Skin Structure and Physiology

Skin structure

Studies on racial differences in structural features of skin have focused primarily on black versus Caucasian subjects. Most attention has been given to the thickness of the skin (number of cell layers and thickness of the stratum corneum), the density of the stratum corneum, the compactness of cells in the stratum corneum and viable epidermis, and melanosome size, distribution, and melanin content.

A study by Weigand et al. (22) looked at the cell layers and density of the stratum corneum in blacks and Caucasians. They first determined the number of tape strips required to completely remove the stratum corneum. Then they took 4 mm punch biopsies of lumbar skin and also examined abdominal skin samples from cadavers. Buoyant density of the stratum corneum was determined after removal of the viable cell layers.

In Caucasian subjects, it took 6–15 tape strips (mean = 10.3 ± 2.8) to remove completely the stratum corneum. In blacks, the range was 8–25 strips (mean = 16.6 ± 4.3). While these ranges overlapped, the mean difference was significant ($p < 0.01$). The variability was greater in blacks (greater range and standard deviation) but was not correlated with pigmentation. Consistent with the tape stripping result, the microscopically visualized difference in average stratum corneum layers was also significant ($p < 0.01$) between Caucasians (mean = 15.8, range = 13–20) and blacks (mean = 20.4, range = 14.8–26). Density in air of the stratum corneum was greater in blacks (1.68 gm/cm^3) than Caucasians (1.39 gm/cm^3); however, buoyant densities were similar.

These investigators surmised that a greater intercellular cohesiveness might explain both the increase in layers of stratum corneum and the relative resistance to removal by tape stripping. Because they found that the thickness of the stratum corneum was approximately equal in blacks and Caucasians, this would imply a tighter compacting of the layers in black skin (22). Intercellular lipid may be involved as the content tends to be higher on a percentage basis in black versus Caucasian skin (23). A more recent report (24) has confirmed the equivalent stratum corneum thickness, but greater compaction and lipid content, in black versus Caucasian skin. A study comparing corneocyte size and spontaneous desquamation between Asians, blacks, and Caucasians showed no significant size differences. Desquamation was greater in blacks than Asians or Caucasians, again with differences in lipid composition offered as a possible contributing factor (25). However, another study showed lower desquamation (on the forehead) in blacks vs. Caucasians (26).

In addition to the cellular/stratum corneum differences in skin structure between Caucasians and blacks, there is also the obvious pigmentation difference. There are reported to be 35 color gradations of black skin versus 7 for Caucasians (27). This is not due to any structural or quantitative differences in melanocytes. However, in blacks the melanosomes are larger, more oval, denser, and more evenly distributed than in Caucasians (27–29). This tendency for increased size and density of melanosomes is directly related to pigmentation (30). It is the variation in melanin content of the melanosomes that leads to the discrete variations in skin tone (28). Minimal erythema doses (seconds) ranged from 2.5 in Caucasians to 8.8, 15, and 83 in blacks of increasing pigmentation (30). Also, blacks were more prone to local changes in pigmentation (hyper or hypo) after chemical or physical insult (28).

The enzyme tyrosinase is rate limiting in the production of melanin (31). Given the greater content of melanin in black skin, Talwar and coworkers studied basal and inducible levels of tyrosinase activity in black and Caucasian subjects (32). At baseline, the melanin content of black skin was approximately $3\times$ that of Caucasians. Correspondingly, tyrosinase activity was $2.3\times$ greater in blacks. Upon stimulation with retinoic acid, the tyrosinase activity of Caucasian skin increased $1-3\times$. This was not simply the result of the irritant effect of retinoic acid, since SLS treatment produced a similar degree of irritation, but no induction of tyrosinase. Also the induction of tyrosinase activity did not result in augmented melanin production. Black skin did not show a similar induction in tyrosinase activity with retinoic acid treatment.

Transmission of ultraviolet light through excised skin is approximately 5 fold greater in Caucasians versus blacks. In Caucasians, UV filtration is mainly in the stratum corneum. In blacks, most filtration is in the Malpighian layers. Increased melanin and the manner in which melanosomes are packaged and distributed all contribute to increased photoprotection in blacks (33).

Innervation is another facet of skin structure that has recently been studied in the context of racial comparisons. Until a few years ago, skin innervation was described only in the context of the dermal components (34). However, epidermal innervation has now been well established through ultrastructural and immunohistochemical methods (35, 36). A recent study characterized epidermal nerve fibers through the use of immunohistochemistry (staining for neuropeptides) and laser-scanning confocal microscopy (37). These investigators detected thin unmyelinated nerve fibers through all viable layers of the epidermis. They were capsaicin sensitive (i.e., C-afferent fibers). The findings were similar across a study population that included 20 Caucasian subjects, 8 native Japanese subjects, and 8 native Chinese subjects. Hence, no racial differences were detected at the neurostructural or neuromolecular levels.

Skin biomechanical characteristics

Moving from the cellular/biochemical level to the physical or biomechanical characteristics of skin, other differences have been noted between the races. Berardesca and coworkers compared the skin of blacks, Caucasians, and Hispanics for skin extensibility, elastic modulus, elastic recovery, viscoelasticity, and elasticity (38). All are measures of the degree of stretch, deformation, and recovery of skin after application of a turning or twisting force

(torque). The evaluation included both the sun exposed and unexposed surfaces of the arm.

In general, Caucasians showed greater differences, between exposed and unexposed skin sites, in these parameters than blacks. Blacks showed little mean difference in skin extensibility readings (arbitrary units) between the exposed and unexposed sites while Caucasians had much lower readings on the exposed site vs. the unexposed site. On the sun-exposed sites, blacks had higher values (more elastic), but on the unexposed sites their values were slightly lower than Caucasians. Racial differences at both sites were significant. Caucasians and blacks also differed significantly in elastic modulus and elastic recovery (black skin values were lower). However, these differences were site (exposed vs. unexposed) specific. Also, in another study, blacks showed significantly higher elastic recovery values than Caucasians (26). Hispanics tended to show intermediate values between blacks and Caucasians, with no statistically significant differences seen in comparison with either of the other races.

The authors concluded that the differences between sun-exposed and unexposed sites, in Caucasians especially, reflected the damaging influence of solar radiation on skin properties, with the narrow differences in blacks being due to the protective effects of melanin (see above). What is also clear is that significant differences in values obtained for different biomechanical properties occur as a result of skin site-to-site variability as well as possible investigator variability. This needs to be accounted for in addition to any differences based on race.

Skin physiology

Though only a few studies have examined basic structural differences in skin between races, a larger number have focused attention on evaluation of physiological parameters of skin and racial differences in such skin properties as barrier function, percutaneous absorption, electrical resistance, water content, and vasodilatation. In regard to percutaneous absorption of chemicals into the skin, black skin is generally considered to be somewhat more impervious than Caucasian skin (39–41). Wedig & Maibach (39) observed >30% less absorption of ^{14}C -dipyrithione in black versus Caucasian subjects when combining all of several skin sites, vehicle systems, and pretreatments (i.e., tape stripping). Within site comparisons showed no significant differences. Williams et al. (40) examined the transdermal penetration of nitroglycerine from two different formulations by monitoring plasma concentrations of parent material

and metabolites. 4 of 16 test subjects were black and 2 of these subjects had such low plasma concentrations that the mean population values of the black subjects were statistically significantly reduced relative to the Caucasian or Asian subjects. Lotte et al. (41) also included Asians in their study. They saw no statistically significant differences among the races in the penetration and excretion of 3 chemicals. Directionally, they saw slightly increased percutaneous absorption in Asians and slightly reduced percutaneous absorption in blacks, relative to Caucasians. Overall, these findings suggest a slightly lesser degree of chemical penetrability in blacks, which may help explain reduced skin irritation responses reported in black subjects (see below).

Transepidermal water loss (TEWL) is considered to be a measure of barrier function of skin at least in terms of the evaporation potential for water (42). It has been used in numerous studies to examine changes in barrier function due to disease states or to chemical or physical treatment of skin. Several studies have looked at racial differences between Caucasian, black, and Asian populations, with somewhat variable results.

Kompaore et al. (43) compared TEWL values in black, Caucasian, and Asian subjects *in vivo*. Prior to any treatment, they observed significantly increased values among Asian and black subjects vs. Caucasians and no difference between blacks and Asians. After 8 or 12 tape strips, the TEWL values increased for all 3 racial groups. The % increase was about the same for Caucasians and blacks after 8 (24–27%) or 12 (49%) tape strips, but was greater in Asians (74% and 95%) after these treatments. In contrast to the above findings, other studies found either lower baseline TEWL values for blacks versus Caucasians (26) or no differences (38). Reed et al. (44) used TEWL after tape stripping Caucasian and Asian subjects to determine both barrier perturbation and recovery. Neither parameter was different. They did find, however, that subjects (regardless of race) with lighter pigmented skin had more easily perturbed barrier function and a longer recovery time.

Part of the difficulty in evaluating TEWL is the need to acclimate subjects carefully prior to recording TEWL values in order to avoid undesired environmental influences (45). This difficulty likely accounts for some differences among study results. In an *in vitro* study, TEWL measurements were taken in the absence of vital functions such as sweating, that can often confound results from *in vivo* testing (46). When TEWL was determined as a function of skin temperature, there was a direct relationship. Samples of black skin also had a significantly greater mean TEWL reading across all

temperatures, than samples of Caucasian skin. Asian skin appeared to be similar to black skin. Measurements of water content in the stratum corneum, via electrical capacitance or conductance, tend to show equal or somewhat greater values (more water content) in black versus Caucasian skin depending on the site of measurement (26, 38). Other studies comparing racial differences in TEWL readings after exposure to skin irritants are discussed below.

Another way to assess skin penetration and barrier properties of skin is to measure a pharmacodynamic response to chemical exposure, e.g., vasodilatation. Berardesca & Maibach (47) and Kompaore et al. (43) used laser Doppler flowmetry (LDF) to examine blood flow changes after application of nicotines in blacks, Caucasians and Asians. Kompaore et al. used lag time from application of methyl nicotinate (MN) to the beginning of an increase in vasodilatation as a way to examine barrier function. Basal blood flow values were not significantly different between races. However, the lag time was significantly shorter in Asians and significantly longer in blacks versus Caucasians. After 8 or 12 tape strips, the lag times decreased considerably for all 3 racial subpopulations, but the ordering remained the same.

Berardesca & Maibach (47) used peak response and area under the curve to assess the pharmacodynamic vasodilatory response to hexyl and methyl nicotinate in blacks and Caucasians. They observed weaker responses in blacks, again indicative of decreased penetration of the chemicals. It should be noted, however, that an earlier study from the same group showed no significant differences between blacks and Caucasians in that study population (48), although the magnitude of the peak response was greater in Caucasians.

It is clear from the above discussion that some measurable differences do exist between races in the biophysical properties of skin. Making definitive conclusions as to how these properties differ among racial subpopulations is complicated by the intrastudy and interstudy variability within and among these relatively small study populations, as well as the inherent variability in the test methods per se. Also important, is how these differences, if real, impact the susceptibility of individuals to chemical or physical skin irritation (irritant contact dermatitis) or allergic contact dermatitis.

Racial Differences in Susceptibility to Skin Irritation

As in the case of biophysical properties of skin, studies of skin irritation have included numerous endpoints of evaluation and single or multiple in-

terracial comparisons. Published studies of skin irritation in racial subpopulations go back to the early 1900s with the work of Marshall et al. (49) who examined black and Caucasian subjects for their irritant responses to dichlorethylsulphide (DCES) or mustard gas. Black and Caucasian male test subjects were exposed (uncovered forearm) to 0.1% or 1% DCES in paraffin oil solution for 10 min. A positive skin reaction was denoted by the appearance of erythema 24 h after exposure. Far more Caucasians than blacks showed positive erythema responses to the chemical vapor especially at the high test concentration (59% versus 15%), suggesting that Caucasian subjects were the more susceptible.

The same result was obtained over 50 years later in testing for the primary irritation response to dinitrochlorobenzene (DNCB). Weigand & Gaylor (50) tested different concentrations of DNCB on Caucasians and on blacks with varying degrees of skin pigmentation. They showed that 85% of Caucasians responded to 0.025%, 0.1%, or 0.25% with at least minimal erythema, whereas 52% of light-skinned blacks required a 1% dose and 58% of dark-skinned blacks required a 1% or 2.5% dose in order to respond. After tape stripping, the minimal perceptible erythema-inducing dose of DNCB was reduced to approximately 0.09% and was the same for all 3 subpopulations, suggesting barrier function as the key factor in the differential response. Also, since the tape stripping did not alter pigmentation, this latter result suggests that the differences in irritation seen in normal skin was not simply due to a greater difficulty in visualizing erythema in darkly pigmented skin. In contrast to the above findings, in a study by Gean et al. (51), blacks and Asians were compared to Caucasians for their visual response to methyl nicotinate. No racial differences among any of the subject populations were noted in the erythematous response profile.

These results based on traditional visual grading all suggest an equal or lesser susceptibility of blacks versus Caucasians to the skin irritation effects of chemicals. Use of alternative evaluative techniques, including photoplethysmography (PPG), laser Doppler flowmetry (LDF), and trans-epidermal water loss (TEWL) generally tend to support this conclusion. A few studies have shown slight (but significant) increases in methyl-nicotinate-induced blood flow in Caucasians versus blacks (48) or non-significant differences in sodium lauryl sulfate (SLS) induced blood flow in blacks versus Caucasians (52). Overall, the studies with LDF measurements have supported the visual grading studies showing reduced (47, 53) or equivalent (48, 52) responses among black versus

Caucasian subjects. The same is true of comparisons between Hispanics and Caucasians (54, 55).

The comparison of Caucasian and Asian subpopulations is more complex due to the fact that little comparative data exist in the literature and that which does exist gives conflicting information. Using traditional clinical grading of standard irritation patch test studies, Rapaport measured skin irritation in Caucasian versus Japanese subjects (56). He used a 21-day cumulative irritation test protocol to compare the irritation of SLS and several consumer product formulations (cleansers, sunscreens, facial creams, and moisturizers). Regardless of the level of inherent irritancy of the SLS or product formulation, Rapaport reported a generally greater cumulative response among the Japanese subjects. He stated that these results tended to confirm apparent, but undocumented, findings from other studies that Asian subjects were more reactive than Caucasians as a whole. Unfortunately, few experimental details and little data were provided. No new information has been published since, in spite of statements in the paper that similar evaluations were ongoing.

Similar descriptive papers have also appeared in the Japanese literature. Ishihara (57) reported that female Japanese subjects showed greater patch test reactivity to cosmetic products than female Caucasian subjects. The testing was conducted in separate geographical locations (Kanagawa, Japan, and New York) and no experimental details are avail-

able to enable one to examine other potential influencing factors. Tadaki et al. (58) reported a higher level of intolerance among Japanese subjects to topical tretinoin cream. Here, the greater reactivity among the Japanese was based upon a higher incidence of adverse skin effects (and resulting dropouts) in a Japanese clinical trial versus the incidence reported in prior studies among Caucasian subjects. Again, comparing irritant reactions in studies not conducted concurrently, and across different geographic locations, make it difficult to attribute differences in response patterns to the racial variable alone.

Recently, other investigators have begun to examine this question in more direct comparative evaluations. Unilever scientists have developed a human patch test method designed to assess chemical irritation in human test subjects as an alternative to animal irritation (Draize method) testing (59, 60). Irritation assessment is ethically conducted in human subjects using a gradual exposure protocol beginning with short duration exposures and terminating exposure at the first signs of mild irritation (11, 19, 61). They have been able to confirm the irritation potential of many chemicals using this method. Very recently, an initial interlaboratory collaboration has been completed verifying the robustness of the procedure (62).

One of the studies conducted under Unilever's direction was an international dose-response study with the anionic surfactant sodium lauryl sulfate (63). Laboratories in Germany, China, and the UK

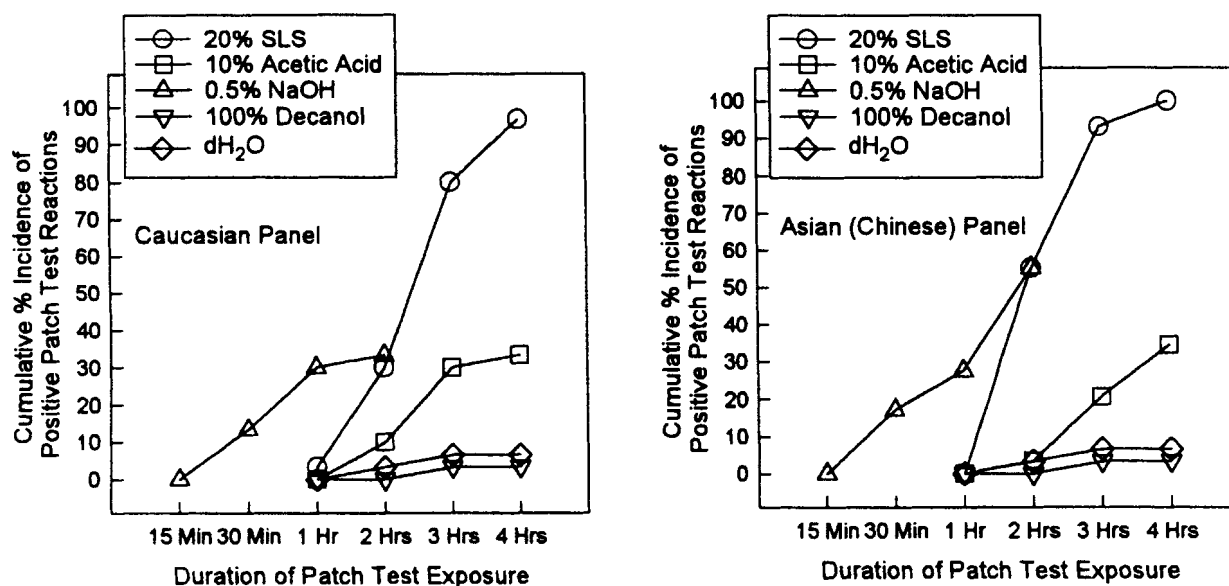


Fig. 1. Separate panels of Caucasian and Asian test subjects were exposed to the indicated irritant materials in a single side by side study (29–30 subjects per panel). A subject was considered a positive responder as soon as they showed at least a slight erythema response under patch. Based on the cumulative 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 (8).

tested SLS at concentrations ranging from 0.1% to 20%. There was considerable overlap in the dose-response pattern among the regional populations. The German population tended to be the most responsive and the Asian population was no more reactive than the Caucasians. This does not support an increase in reactivity among Asian (Chinese) versus Caucasian test subjects. The authors' view was that inherent variability within any given human test population as a whole will mask any interethnic variability.

The only difficulty with the Unilever study was the fact that the study populations were tested in different geographic locations at different times of the year. This introduces many additional variables into the interpretation of the data. To address this, they recently conducted direct comparison studies of different skin types (types I-VI; including Asian (type V) and black (type VI) subjects). Using the

acute irritation protocol, they were unable to discern significant differences in SLS dose-response across the skin types (64). In addition, we also recently conducted a patch test study, using the Unilever protocol (62), in which Caucasian and Asian (Chinese) subjects were tested at the same facility and at the same time (8). The irritation responses to SLS and 3 other model irritants are shown in Fig. 1.

No significant differences were seen between the Caucasian and Asian test populations for any of the 5 test and control materials used in this study. Whether the irritation response began to appear after relatively brief exposure (0.5% NaOH) or relatively late (10% HAC), and whether the cumulative incidence of positive responders was small (10% HAC) or large (20% SLS), both populations showed the same overall pattern. The same test materials were applied in a 2nd study conducted

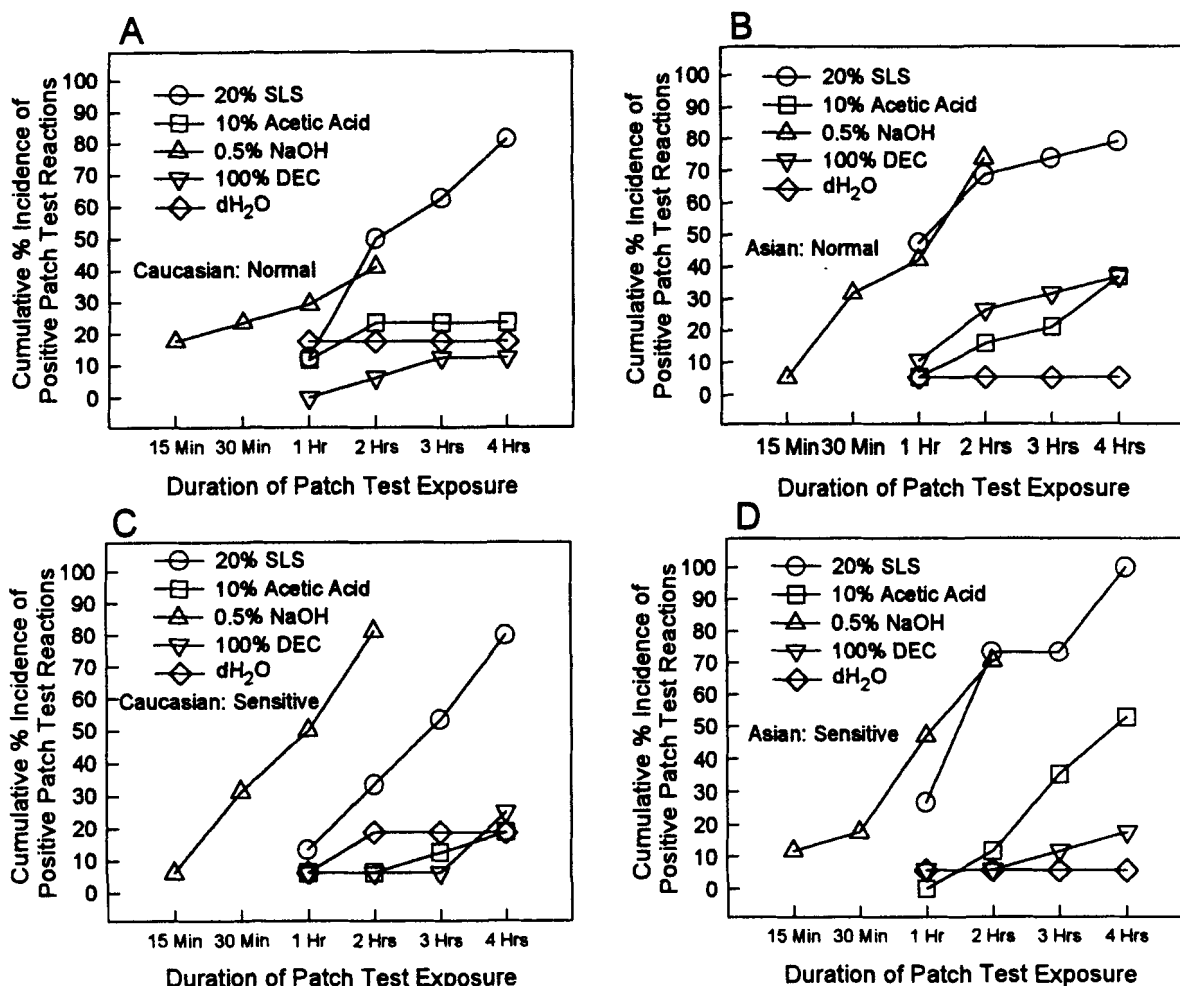


Fig. 2. Caucasian and Asian test subjects were screened for neurosensitivity to lactic acid (based on quantification of a stinging response to 10% lactic acid applied to the face) and subdivided into "sensitive" and "normal" subpopulations. The 4 test populations were then exposed to the indicated irritant materials in a single side-by-side study (13–20 subjects per panel). A subject was considered a positive responder as soon as they showed at least a slight erythema response under patch. Cumulative irritation responses to all test materials were similar among all 4 test subject populations (8).

among Caucasian and Chinese test subjects. In the 2nd study, the test subjects were further subdivided on the basis of normal or enhanced neurosensitivity to facial application of 10% lactic acid (stinging response). The results, shown in Fig. 2, again indicate no consistent difference between the Chinese and Caucasian populations in their responses to any of the test materials. This lack of difference was also apparent when comparing neurosensitive and normal test subjects across either racial population. These results, from direct side-by-side testing, agree with the Unilever results showing no significant differences between Chinese and Caucasian subjects in the acute objective SLS dose response.

As previously reported, we conduct a variety of human patch tests for assessment of skin irritation and allergy prior to marketing new product formulations (12, 14, 15). In reviewing skin irritation data from human repeat insult patch test (HRIPT) studies (65), we were able to identify a test in which the study population was comprised of a few Asian test subjects among the majority of Caucasians. This study was conducted to assess the safety of a fragrance ingredient in a body soap formulation. During the induction phase of the HRIPT, mild to moderate skin irritation was observed at each patch reapplication. These individual skin grades were summed to determine a cumulative skin irritation response for each subject during the 3-week repetitive exposure induction phase of the study. The data for the Caucasian and Asian subjects are shown in Fig. 3. There was wide variation seen among the Caucasian test subjects in the degree of cumulative irritation. The pattern showed a very

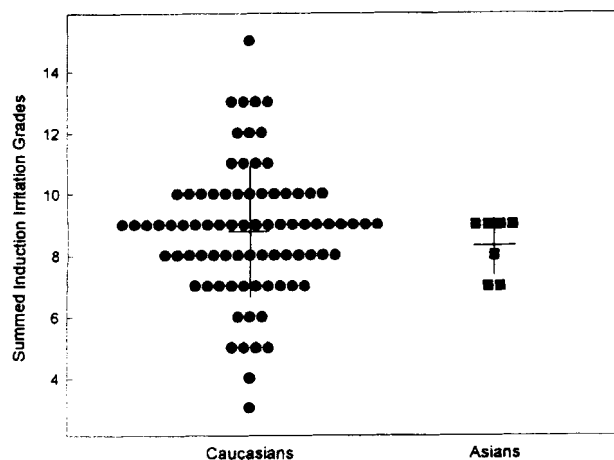


Fig. 3. Data from P&G human repeat insult patch test (HTR Study 93-6374-76; Material #BP0008-031 (fragrance) in BP0008-030 (body soap)). Irritation grades during 9 patch induction phase were summed to provide a total cumulative grade for each test subject.

normal distribution. Interestingly, the small Asian population showed a mean irritation grade almost identical to that of the much larger Caucasian population.

All of these recent study data tend to run counter to the earlier observations of Rapaport and the Japanese investigators and fail to support the validity of any premise that there is a *fundamental* difference in objectively measured skin irritation responsiveness between Caucasians and Asians. While the more recent studies comparing Caucasian and Asian populations have focused mainly on Chinese panels, we are also in the process of developing data on Caucasian versus Japanese test subjects. These latter results will provide a more direct comparison to those data previously developed by Rapaport and the Japanese investigators.

The acute irritation patch test studies conducted to date have the advantage of producing a relatively predictable level of irritancy to known chemicals. Still, there is a need to extend this direct comparative testing database to longer term cumulative irritation test protocols and the testing of product formulations as well as chemicals such as described above for the HRIPT study. This will also enable us to develop data to more directly address this question.

The issue of neurosensory irritation (e.g., itch, sting, burn) and racial differences in susceptibility to these symptomatic responses is even more difficult to resolve than the question of objective irritation responses. Part of this difficulty stems from a lack of well-validated clinical procedures for assessing neurosensory irritation (5, 7, 20). There is widespread speculation that Asians are more susceptible to neurosensory irritation than Caucasians (66, 67), but virtually no data to substantiate the speculation (67). This is an area of critical need for valid methods development and application and, subsequent to that, carefully controlled inter-racial comparison studies.

Racial Differences in Susceptibility to Skin Allergy

The most common type of skin allergy is allergic contact dermatitis, a cell-mediated (Type IV) immunological response driven by T lymphocytes in response to allergenic small molecules (haptens). Allergic contact dermatitis is a relatively common dermatologic disorder, with heavy metals, fragrances, preservatives, and topical medicaments comprising the major classes of causative chemicals. However, the overall incidence of allergic contact dermatitis is less than that of irritant contact dermatitis.

Experimental sensitization studies

The literature on racial differences in susceptibility to allergic contact dermatitis is sparse, largely because few investigators are willing or able knowingly to sensitize test subjects in order to prospectively study etiologic or mechanistic factors in the development of this condition. In terms of the potential for induction of allergic contact dermatitis (ACD), 2 studies in the historical dermatology literature demonstrated a reduced sensitivity for black versus Caucasian subjects. Rostenberg & Kanof (68) studied black and Caucasian subjects for incidence of induced sensitization to dinitrochlorobenzene (DNCB) and parnitrosodimethylaniline (PNDA). Each material was tested at a concentration of 1% via open application to a uniform area of skin (2.54 cm diameter circle). 1 month after the induction exposure, the subjects were challenged with the chemical to determine if sensitization had occurred. If not, the process was repeated for a 2nd month and so on, until 4 attempts had been made to induce sensitization.

The 2 chemicals differed in their sensitization potencies, with DNCB being the less potent of the two. With DNCB, the cumulative sensitization incidence rose gradually, with more Caucasian subjects showing positive responses at each treatment number. Sensitization to PNDA occurred more quickly; however, once again, the cumulative incidence rose more quickly with Caucasian subjects. Thus, similar to the "historical" skin irritation study of Marshall et al. (49), black subjects were more resistant than Caucasian subjects to induced sensitization to strongly sensitizing chemicals.

25 years after the Rostenberg study, Kligman compared the responses of black and Caucasian subjects to several commonly encountered skin allergens. Using his human maximization test procedure, Kligman (69) studied induction of sensitization to p-phenylenediamine (PDA), monobenzyl ether of hydroquinone (MBEH), nickel sulfate (NiSO_4), penicillin G (Pen G), and neomycin sulfate (NEO). With the strongest contact allergens (PDA and Pen G) there was little difference in the response among blacks versus Caucasians. However, as the potency of the allergens decreased, there was an increasing differential response, with Caucasians increasingly more susceptible. As in the Rostenberg study, this again supported a tendency for blacks to be more resistant to induction of contact sensitization. This may well be related to the penetrability of chemicals through black versus Caucasian skin, as noted above.

Epidemiology: incidence of allergic contact dermatitis from common allergens

Other investigators have approached the question of racial differences in skin allergy from an epidemiological standpoint. According to Fisher (70), there is some controversy. When exposure factors are considered, equivalent incidences of ACD among Caucasians and blacks have been noted in occupational dermatitis. Some dermatologists claim a lesser degree of poison ivy dermatitis in blacks though it is not clear whether this is due to sensitivity or exposure. Fisher notes a possible difference in the manifestation of ACD in blacks, being more commonly associated with lichenification and hyperpigmentation versus the vesicular, papular, erythematous responses seen in Caucasians. The North American Contact Dermatitis Group (NACDG) studied the incidence of patch test reactions among 1057 Caucasians and 143 blacks (55–60% female). They found no significant differences in prevalence rates among blacks and Caucasians (71).

The epidemiological studies are, by definition, retrospective and cannot be controlled for exposure. They suggest that, in the real world (assuming equivalent exposures – a large assumption), blacks and Caucasians do not differ in susceptibility to consumer-relevant ACD. These retrospective studies also monitor the elicitation phase of the sensitization process, whereas the earlier cited historical studies from Rostenberg (68) and Kligman (69) examined sensitivity to induction of ACD in previously naive subjects. Clearly, these latter studies suggest that, under controlled exposure conditions, blacks can be shown to be significantly less sensitive to induction of sensitization. A compromise situation exists for transdermal drug delivery systems, where sensitization data is retrospective, but exposures are more consistent among exposed populations. Here, the sensitization rate to clonidine, the transdermal drug with the highest known incidence of sensitization (72), is $2\times$ as high in Caucasians as in blacks (73), a finding consistent with the prospective studies.

While the literature comparing sensitivity to ACD in Caucasians and blacks is limited, the literature for other racial comparisons is virtually nonexistent. In the report of Rapaport (56) cited previously, it states that human maximization testing of standard cosmetic ingredients showed more severe reactions (though not a higher incidence) among Japanese versus Caucasian subjects. However these studies were done at different times, there was no indication of the materials tested or their concentrations, and no actual data were re-

ported. In retrospective studies of patch test subjects in Singapore, Goh has found little difference in the incidence of ACD among Chinese, Malays, and Indians within the indigenous population (74, 75). In our cumulative irritation test shown previously (Fig. 3) 5 test subjects developed reactions upon challenge that were suggestive of sensitization. None of these 5 subjects were from the small Asian subpopulation. The net of this limited collection of data is that there is no compelling evidence generated to date to suggest that, aside from a possible decreased susceptibility among blacks, there are any racial differences in susceptibility to ACD.

Age and Sex Differences in Susceptibility to Irritant and Allergic Contact Dermatitis

Age differences

The literature on age-related susceptibility to skin irritation generally shows a reduced sensitivity in older (i.e., >60 years) versus younger adults. A study by Lejman et al. (76) compared 2 widely different age groups (18–25; 65–84) for reactivity to a strong irritant stimulus (24 h patch exposure to 5% sodium lauryl sulfate, SLS). The response (0–5 visual grading scale) for the young subjects was 4.57 ± 0.76 and the response for the older subjects was 2.62 ± 1.66 ($p < 0.001$). Cua et al. (77) studied the response to a much lower SLS insult (0.25% patch exposure for 24 h). They exposed a variety of body sites in their study. The mean age of their 2 test populations was 25.9 and 74.6 years. The mean response and % positive responders was reduced in the older cohort. In some areas of the body (e.g., thighs) the response difference was quite dramatic. The decrease in visual scores in the elderly was matched by a decrease in the magnitude of SLS-induced changes in TEWL values.

Also, Grove et al. (78) studied different varieties of chemical-induced skin irritation in young and old cohorts. In a study of ammonium-hydroxide-induced blistering, they saw a more rapid initiation of the blistering response in the older cohort (65–75 years) versus the younger cohort (18–30 years), but a much slower development of the full blister response. They also examined the response to a variety of irritants (e.g., histamine, DMSO, 48/80, chloroform-methanol, lactic acid, and ethyl nicotinate). In all cases, the visual grades were greater in the younger cohort.

Age-related studies of skin sensitization are mainly limited to retrospective analysis of elicitation patterns and incidences across patch testing cohorts. Here the data are mixed and it is difficult to separate age-related *inherent* susceptibility from age-related patterns of *exposure*. An early study

by Prystowsky et al. (79) showed no age-related differences in the incidence of allergic patch test reactions across 4 common contact allergens (nickel, neomycin, ethylenediamine, and benzocaine). However, other investigators have seen other trends. Goh (75) studied the response incidence to common patch test allergens and saw a general increase in incidence in subjects >40 versus those <40. The trend was even greater when he compared subjects >59 versus those <20. Since these totals were cumulative, they may just represent a greater tendency to become sensitized to different allergens as one progresses through life, due to possible repetitive exposures. Young et al. (80) looked at the incidence of sensitization on an allergen-by-allergen basis. They found different patterns depending on the allergen; cobalt and nickel sensitivity was greater in those <30 versus those >50, but the reverse was true for wood tar. Again, this could be related to the exposure opportunities, but may also be a reflection of some diminution in response in older subjects, assuming that they would have had the potential to be sensitized as younger adults (see below).

Lejman et al. (76) studied the elicitation of Rhus reactivity in young and older subjects. In their 18–25 cohort they found a quick onset of reactivity and resolution by 9–15 days. The older cohort responded more slowly and the reaction resolved more slowly (12–36 days). Finally, Wantke et al. (81) looked at sensitization as a function of age from birth to >70 years of age. Incidences of patch test reactivity to standard series allergens was comparable (46–58%) across 0–7, 8–14, and 20–50 age groups, but dropped (34%) in the oldest cohort (>70). In a recent review, Kwangskustith & Maibach (82) suggested that the incidence of allergic contact dermatitis gradually increases from birth to 14 years of age, then holds steady in overall incidence but greatly varies by allergen based on exposure patterns. The incidence declines with advancing age in terms of both the severity of response (possibly related to similar reduction in irritation responses discussed above) and a waning of the allergic response in previously sensitized individuals.

The only way truly to assess allergic sensitivity is to evaluate the induction of allergic contact sensitization in previously naive subjects. Studies of this type are not generally done today, but one study from the literature does shed some light on age-related susceptibility. Back in the early 1950s, Schwartz (83) studied the induction of sensitization to DNCB in previously naive subjects of different ages. Approximately $1/2$ of 174 subjects became sensitized. Among 3 age cohorts (21–59, 60–79, >80) there was no significant difference in the

incidence of sensitization. Thus, for very potent sensitizers, there appears to be little age-related difference in allergic sensitivity.

Sex differences

There is a common perception that women are more sensitive to skin irritation than men, a finding not generally borne out in direct testing (84). A recent review (85) cited specific incidences of irritant contact dermatitis as either greater in women versus men or about the same, depending on the study. In any event, the incidence is almost certainly exposure-related, as women are more often exposed to wet work, detergents, and cosmetics, but less to cutting oils and abrasives. In a study of barrier function, Reed et al. (44) showed no difference between men and women in terms of skin barrier integrity (i.e., loss in barrier function after tape stripping) or time for barrier recovery, again suggestive of a comparable susceptibility to skin irritation. Our HRIPT study (Fig. 3), was also examined in terms of sex of subject and showed no difference in irritant reactivity.

Differences in the susceptibility of men and women to contact dermatitis reactions have predominantly focused on allergic skin responses. Several studies have reviewed the epidemiology of elicited allergic responses to known allergens in men and women. An early study by Prystowsky et al. (79) showed that response prevalence among 4 common allergens was strictly allergen related. Women were far more reactive to nickel and men more reactive to ethylenediamine. Responses to the other 2 allergens (neomycin and benzocaine) were equal. Young et al., showed similar results (80); women having a higher incidence of responses to cobalt, nickel, and Peru balsam and men having a higher incidence of responses to chromate and carba mix. Goh (75) reported an overall equivalent incidence of positive patch test reactions among men (51%) and women (47%). Finally, a Finnish study by Kanerva et al. (86) showed a 60/40 preponderance of occupational ACD among Finnish women versus men. However, again the relative preferential responsiveness was allergen related. Women showed higher incidences of reactivity to rubber, nickel, and formaldehyde, and men higher reactivity to epoxy resin, chromate, and colophony. As in the age-related studies, the overall findings indicate that exposure is the primary factor in relative incidence of sensitization and that prospective studies are needed to assess better inherent differences in reactivity (82). A compromise between retrospective sensitization rates and controlled exposures, cited above for black versus Caucasian sensitization potential, relates to sensitization rates

to transdermal clonidine (73). Here, the sensitization rate for women (black or Caucasian) was 2× that of men.

A few studies have actually looked prospectively at the relative sensitivity of men and women to induction of allergic contact sensitization. Jordan & King (87) used the Draize human repeat insult patch test method to examine the relative incidences of induced sensitization to common (but not necessarily potent) contact allergens. Of 10 allergens studied, women reacted more often to 7 and the other 3 showed equivalent incidences of reactivity. Leyden & Kligman (88) used the human maximization test and studied allergens from all 5 severity classes. Only with the weakest sensitizers (class I) did women react more commonly than men. With the other 4 classes, the responses were the same. More recently, Rees et al. (89) studied the induction of sensitization to experimental exposures to the strong sensitizer DNCB. In this study, they used a single exposure to induce sensitization and then did a dose-response challenge. Women showed a significantly stronger response than men, both in terms of severity of response and steepness of the slope of the dose-response curve. Thus, from the collective data related to both induced sensitization and frequencies of sensitization in the population, the preponderance of evidence would suggest that women are, at least to a degree, the more sensitive responder population.

Implications of Population Differences in Skin Responsiveness for Safety Testing and Risk Assessment

In 1989, we published a summary of a risk assessment process (15) for allergic contact sensitization, updated since (13, 14), that emphasized the importance of clinical testing. The clinical assessment of the skin irritation and allergic contact sensitization potential of product ingredients and formulations is central to our overall safety assessment process and clearance for market (14, 15). A key question that we face, in an age of global marketing of products, is the relevance of regional skin safety testing for global consumers.

Skin safety testing is often conducted through internal laboratories or through selected contract clinical facilities. The most commonly used clinical labs are located in North America and Western Europe. For the most part, the volunteers for skin safety testing are Caucasian and predominantly female. Effort can be made to recruit special populations, usually at a substantial cost premium.

Based on the information discussed in this review, the prevailing question is whether the testing for irritant and allergic contact dermatitis that is

currently done is sufficient to protect the global consumer. Asked differently, by testing predominantly in Caucasians (and females), are we underestimating the risk of inducing irritant or allergic contact dermatitis in users of our products? The prevailing evidence available to date would suggest that, overall, the testing approach is conservative. Why is that the case? First of all, Caucasians represent a generally responsive population at least when compared to blacks. Most of the literature on reactivity in blacks would suggest lesser reactivity due to reduced skin penetration of chemicals. While there is speculation that Asians are more reactive than Caucasians to skin irritants and allergens, most of the limited literature available on this subject does not bear that out. The simple fact is that most of the data supporting a true population difference in skin reactivity (e.g., blacks versus Caucasians) can be correlated to a degree with a lesser barrier permeability in the more resistant population (39). Little or no difference in barrier function of skin is seen comparing males and females or Caucasians and Asians (44) consistent with the limited or conflicting data on real differences in skin irritation. Interestingly, a difference in barrier function is seen across photoreactive skin types (44). Perhaps this classification will prove a more predictive indicator of susceptibility to irritation than race or sex differences per se. However, even here, there is preliminary data to suggest little difference among different photoreactive subpopulations to acute irritant challenge (64).

There is still work to be done in evaluating Caucasians and Asians in terms of relative skin reactivity. For skin irritation, this includes (a) better comparison of Caucasian and Japanese subjects (i.e., are Japanese a more reactive Asian subpopulation?), (b) better evaluation of neurosensory responses as well as objectively measured skin irritation responses in subjects that differ in neurosensory reactivity, (c) effects of chemical exposures on skin pigmentation, (d) examination of both acute and chronic exposure protocols, and (e) a better understanding of the effects environmental variables (e.g., sun exposure, temperature, humidity, altitude) or consumer habits (e.g., bathing, concurrent product use/skin treatments) or expectations (including cultural) have on the susceptibility to objective or sensory irritation. All of these are active areas of research within both industry and academia. Future research on racial differences in susceptibility to induction of allergic sensitization is restricted by ethical considerations in knowingly sensitizing test subjects to chemical allergens. Still, routine sensitization safety studies (HRIPT, use tests) conducted in the past, and that have incorporated racially diverse test populations,

have not shown any tendency for Caucasian subjects to be less responsive. Thus, there is no reason to suspect that current testing of predominantly female Caucasians in any way under-predicts the irritation or allergenic potential of chemical ingredients and products. Also, the predominance of female subjects in most skin safety testing panels likely provides added conservatism, given the evidence cited above that females are, if anything, more responsive to induction of allergic contact sensitization than males.

Are there ever reasons to do regional or population-specific testing? Certainly, there may be situations in which this is warranted; however, a clear rationale for this type of testing and flexibility in the approach are important. Certain product formulations may be specially formulated to be marketed in very specific and limited geographic regions or in specific populations (e.g., women). Under those circumstances, it is reasonable to consider conducting patch and use testing among panels of the anticipated race and/or sex. There may also be special circumstances, such as external relations issues or regulatory approvals, that may dictate special population testing. Scientifically, there may be little justification for this based on recognized population differences in skin responsiveness, and one would hope that regulatory authorities would base their approval criteria on valid published data rather than anecdotal information. Still, current regulations may require special population or regional testing. The decision to conduct any population-specific testing should be made on a case-by-case basis relative to the business or market need. Clearly, an important driver of additional research in this field is to help counter regulatory or regional requirements that mandate population-specific testing for which there is no scientifically based justification.

Finally, we must begin to consider the issue of neurosensory irritation in any discussion of skin safety evaluation. Consumers may often discontinue use of a product and complain of adverse skin effects based solely on symptoms (sting, burn, itch, tingle). We are beginning to see publications in the literature documenting subjective irritation and attempts to quantify the phenomenon better (5, 9, 20, 21, 90). However, we have little understanding of the relationship between subjective and objective indices of irritation nor how to assess subjective irritation on a predictive level. Moreover, we have no evidence, pro or con, as to whether there are true, consistent, and predictable differences between races, ages, or sexes in terms of subjective irritation responses. The neuroanatomy of the skin, certainly relevant to the issue of neurosensory irritation, indicates no racial differ-

ences (between Caucasians, Chinese, and Japanese) in epidermal innervation (37). Still speculation and assumption regarding differential neurosensory irritation abound (20, 66). Work is beginning in several laboratories to study the mechanistic and pathophysiological basis of subjective irritation, including the relationships between subjective responses to different neurosensory stimuli, the relationship between subjective and objective responses, and the incidence of neurosensory reactivity across different populations. These are critical areas of research that will only improve our safety testing and risk assessment capabilities in the future.

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Address:

Michael K. Robinson
Human & Environmental Safety Division
The Procter & Gamble Company
Miami Valley Laboratories
Cincinnati, OH 45253-8707
USA