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Clinical Skin Mildness Evaluations of Direct and Indirect Exposure to Two Commercial Laundry Detergents with Markedly Different pH Designed for Sensitive Skin Using a Hand-Laundering Model

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Synopsis

The skin mildness of two commercial laundry detergents designed for sensitive skin, Tide Free and Gentle® (TFG) versus All Free Clear® (AFC), was compared in clinical studies, and the role of marked product pH differences was assessed. Two double-blind randomized human studies were conducted. Study 1 was a 1-day repeat insult forearm test, in which four exposures to solutions of TFG or AFC were performed to mimic direct exposure to dilute detergent during hand-laundering. Corneometer, erythema and dryness grading, transepidermal water loss (TEWL), and skin surface pH evaluations were carried out. Study 2 was a 21-day arm patch test of fabrics washed with TFG or AFC to mimic indirect contact to skin of detergent residues, with erythema grading. Separately, pH and reserve alkalinity were determined for each detergent. In Study 1, TFG was significantly milder than AFC in all measures except TEWL (no significant difference). In Study 2, the detergents were approximately equivalent in erythema grading. Analysis showed AFC was substantially more alkaline (pH 10.8) than TFG (pH 7.9) with higher reserve alkalinity. TFG was significantly milder than AFC in Study 1, which may be due in part to the increased skin surface pH seen with direct exposure to AFC's high alkalinity.

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INTRODUCTION

Sensitive skin is prevalent among consumers. In surveys in the United States, Europe, and Asia, almost half of people reported having sensitive skin (1–4). The symptoms of sensitivity are varied, including stinging, redness, roughness, scaling, and itching (5–8). Certainly, direct contact to skin irritants can trigger sensitivity responses. Also, airborne materials such as smoke and chemicals can become trapped in fabrics and, thus, elicit skin responses when the fabrics contact the skin (9–12). Thus, frequent cleaning of clothes is important to dramatically reduce exposure of the skin to these irritating materials (9–11).

Although clean clothes are important to help avoid skin sensitivity issues from environmental irritants, indirect exposure through detergent residues on fabrics may also contribute to the problem (5,13). In addition, extensive direct exposure to detergent may occur during hand-laundering, and during prewashing and pretreating of fabrics before using a commercial washing machine. Ingredients such as dyes and perfumes are frequently reported triggers of sensitive skin, and as such, several commercially available laundry detergents designed for sensitive skin (known as "free detergents") are formulated without these ingredients. Notably, 80% and 97% of dermatologists in the United States and Canada, respectively, recommend the use of free detergents to their patients with sensitive skin. (US data are based on IQVIA (Durham, NC) ProVoice January 2018 cumulative 12-mo data. Canadian data are based on an Internet survey conducted in January 2018 of 150 dermatologists licensed to practice in Canada.) Although outright contact dermatitis from laundry detergents is a rare event, occurring in less than 1% of sensitive-skin patients (14,15), it is still important to understand the potential for irritation from detergent products because these products do come into direct and indirect contact with the skin.

Although there are many methods to evaluate detergent effects on the skin, from simple laboratory tests to *in vitro* evaluations such as cell culture, the most relevant to real-world skin responses are observations in controlled clinical studies (5). Recently published work (16,17) proposes using a specific set of *in vitro* methods (zein protein denaturation test, cell culture cytokine release test, and corneosurfametry measure of protein and lipid degradation) as sufficient to assess product mildness of laundry detergents designed for sensitive skin. By contrast, in this report, we describe clinical mildness comparisons of direct and indirect exposure to the two U.S. market-leading, commercially available free laundry detergents, both of which are designed for sensitive skin and which have markedly different pH values. (U.S. market leadership is based on 2017 retail sales, Nielsen laundry detergent category.) Both products are formulated as liquid detergents and as laundry detergent pacs encased in a dissolvable membrane. We conducted two clinical studies: one a mini-immersion, repeat insult test modeling direct exposure to the skin during hand-laundering of garments using dilutions of the liquid formulations of the two detergents and the other a patch test modeling prolonged exposure to detergent residues on fabrics after machine washing using either the liquid or pac formulations of the two detergents. Although laundry formulations are complex and many factors and ingredient components can play a role in skin mildness, pH has been cited as a concern for irritation in sensitive-skin individuals (18,19). Therefore, we explored whether pH differences may help explain the skin mildness effects observed in the clinical studies by analyzing the pH and reserve alkalinity of the liquid formulas and the pH of fabrics washed with the liquid formulas.

MATERIALS AND METHODS

CHEMICALS

Commercial laundry detergents All Free Clear® (AFC), All Free Clear Mighty Pacs® (AFCMP), Tide Free and Gentle® (TFG), and Tide Pods Free and Gentle® (TPFG) were purchased at a U.S. retail outlet in the United States in 2017. Tide® and All® are manufactured by the Procter & Gamble Company (P&G, Cincinnati, OH) and Sun Products Corp. (Wilton, CT), respectively. Active Wheel® (AW; Hindustan Unilever Ltd., Mumbai, India) was purchased in India in 2017. Ivory Bar® soap (IB; P&G) was purchased at a U.S. retail outlet in 2017. ReagentPlus® (≥98.5%) grade sodium lauryl sulfate (SLS; which is also known as sodium dodecyl sulfate) was manufactured by, and purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals were United States Pharmacopeia (USP) grade.

CLINICAL STUDY METHODS

Protocols for the two randomized (right–left and site), balanced, double-blind arm studies described in the following paragraphs were reviewed and approved by independent institutional review boards. The studies were supervised by an independent dermatologist, and were monitored by P&G personnel to ensure that they were conducted in compliance with the protocol and with Good Clinical Practices as specified under 21 Code of Federal Regulations (CFR) 321.66. Before participating in the studies, each subject signed a written informed consent that contained all the basic elements outlined in 21 CFR 50.25.

REPEAT INSULT FOREARM TEST (RIFT)

This mini-immersion, repeat insult method is a 1-d study modeling the skin effects of exposure to detergents during hand-laundering. It was conducted in Beijing, China, in August of 2017. For the study, 80 female subjects (ages 20–45 years) in general good health with self-described sensitive skin were recruited, and all of them completed the study without adverse events.

The subjects were instructed to not use lotions or cosmetic products on their forearms for 3 d before the start of the study and for the duration of the test. During that time, they were provided with commercial IB soap for washing and bathing. They were also instructed not to expose their forearms to soap or water in the 3 h before the visit on the day of the study.

On the day of the study, subjects were acclimated for at least 15 min under controlled temperature and humidity conditions (21° ± 2°C and 45–55% relative humidity) immediately before the start of the study and remained in that environment for the duration of the study (approximately 5 h). After the 15 min of acclimation, visual grading was carried out by two trained graders using grading scales: erythema (0–4 scale) and skin dryness (0–5 scale). Then, noninvasive instrumental measurements were carried out in the following order: corneometry with a Corneometer® CM 825 (Courage and Khazaka Electronic, Cologne, Germany), transepidermal water loss (TEWL) with a Delfin VapoMeter (Kuopio, Finland), and pH with a Hanna Instruments HI99181 skin pH meter (Woonsocket, RI). These measurements were repeated 40 min after each of the subsequent four exposures to test treatments, which was just before the subsequent treatment.

In the study, the corners of three 3.5 × 3.5-cm^2 sites on each inner (volar) forearm were marked (using a black permanent marker) for a total of six treatment sites. To simulate real-world conditions, all the treatments (except the water negative control) contained minerals (4:1 ratio of $CaCl_2$:$MgCl_2$) to create water hardness. The typical units for hardness are grains per gallon (gpg): 1 gpg = 1 part in 58,000 parts of water = 17.1 mg/kg (also reported as parts per million or ppm) of minerals. The six treatments were distilled deionized water (negative control) containing 0 gpg water hardness, 0.5% SLS containing 16 gpg water hardness (positive control), 1% SLS containing 16 gpg water hardness (positive control), 4,200 mg/kg solution of AW containing 16 gpg water hardness (positive control), 4,200 mg/kg solution of TFG containing 7 gpg water hardness, and 4,200 mg/kg solution of AFC containing 7 gpg water hardness. SLS is a demonstrated irritant for volar forearm skin (20), and AW has been used as a positive control in other studies with Procter and Gamble (P&G manufacturer data on file). Water containing 16 gpg is considered hard water, which is harsher to the skin (21), thus making the SLS and AW solutions high harshness controls. Seven grains per gallon is the average water hardness for tap water in the United States, and the investigators wanted to mimic hand-wash conditions in the United States. Solutions of 4,200 mg/kg TFG and AFC (equivalent to one manufacturer-recommended dose of liquid detergent in 5 gallons of water) were chosen as reasonable doses of detergent for handwashing of laundry. TPFG and AFCMP were not included in this study as laundry detergent pacs are less likely to be used than liquid detergents in hand-laundering, prewashing, or pretreating of garments.

The treatment solutions were prepared at room temperature (approximately 22°C) on the morning of the study. Before application to the skin, the solutions were warmed to 35° ± 2°C in a water bath and mixed on a magnetic stirrer to ensure homogeneity. Each subject had a total of four exposures to each of the test treatments for 15 min on the marked sites on the inner forearm, with a 1-h interval between the start of exposures. For exposure, subjects placed their forearms flat on a clean draped surface with the inner forearm facing upward. Glass exposure cups (1.9-cm-tall × 2.1-cm-inside-diameter polished glass cylinders) were held against the forearms with Velcro® bands (Velcro China Fastening Systems Comp. Ltd., Zhangjiagang, Jiangsu Province, China). Three milliliters of treatment solution was introduced into the exposure cup for 15 min of exposure, after which the treatment solution was removed by pipette, the skin was rinsed with running water (22° ± 2°C) for 15 s (with gentle rubbing with gloved fingers to remove residual test material for the last 5 s of the rinse), and the skin was patted dry with a commercial paper towel. Rinse water for each test site had the same water hardness (gpg) as that used for the test solutions. For the subsequent exposure, exposure cups were repositioned on the same forearm sites in the skin indentations that remained from the previous exposure.

Noninvasive instrumental measurements (corneometry, TEWL, and pH) and visual grading (redness and dryness) were carried out at baseline and at 40 min after each exposure to test treatments, and before the subsequent treatment. Erythema (0–4) and skin dryness (0–5) scores were measured separately by two trained graders, and those scores were averaged. The order of measurements was as follows: visual grading, corneometer, TEWL, followed by skin surface pH testing.

For statistical analysis of the data, the skin grade values for erythema and dryness from both graders were averaged for further analysis. For variables that have a continuous distribution, the posttreatment evaluations (each exposure after baseline) were analyzed using mixed-

effects regression models, which include a random subject effect and fixed effects for covariates: treatment, exposure, side, site, baseline value, and appropriate design parameters. A normal plot of the residuals was examined to assess the assumptions of the models. Moreover, analyses were performed separately for each posttreatment exposure (exposure after baseline measure) using analysis of covariance with covariates: treatment, baseline value, and appropriate design parameters. The baseline visit was analyzed similarly but without the baseline value as a covariate. Differences are considered significant at 0.05 (two-sided) for all comparisons.

21-D CUMULATIVE IRRITATION TEST (21DCIT)

This occlusive patch test study was performed in Cincinnati, Ohio, in November–December of 2017 in an independent clinical testing facility (North Cliff Consultants, Inc., Cincinnati, OH) and models the mildness effect of prolonged exposure to detergent residues on washed fabrics. For the study, 35 subjects (male and female, ages 18–65 years) in general good health with self-assessed sensitive skin were recruited, and 28 of them completed the study. Three subjects dropped from the study because of skin irritation from the tape used in patching. The irritation was followed up to resolution. Partial data were collected on four other subjects before they dropped from the study because of illness (not related to the study) or for personal reasons. The partial data from these four subjects were used in the analysis; therefore, data were collected from a total of 32 subjects.

Seven materials were tested under completely occlusive patches in the study: distilled water, 0.05% w/v SLS, and fabric washed in tap water, TFG, TPFG, AFC, or AFCMP. Fabrics (Hanes 100% cotton T-shirts; Winston-Salem, NC) were washed three times in Whirlpool Duet high-efficiency (HE) washing machines (Benton Harbor, MI) on the normal cycle at 77°F wash/60°F rinse with tap water alone or with 4,200 mg/kg of TFG, TPFG, AFC, or AFCMP (4,200 mg/kg is also the concentration of laundry detergent in a commercial HE washing machine based on manufacturer-recommended use levels) using 7 gpg water. After each wash, fabrics were dried in commercial clothes dryers. After the third and final wash/dry cycle, fabrics were cut into $2 \times 2\text{-cm}^2$ for use in the study.

At baseline, the skin was graded for erythema (0–4 scale) by a trained grader. Before application of the first patch, the skin of the outer upper arm was gently wiped with isopropyl alcohol on a cotton ball to remove skin surface oils for better adherence of patches. The corners of the skin test sites were marked with a permanent black marker: four sites on the left upper arm and three on the right upper arm. For patching, machine-washed test fabric was placed on a $2 \times 2\text{-cm}^2$ nonwoven cotton Webril patch, the test fabric was wetted with 0.1 mL of distilled water, and then the patch was affixed to the skin with an occlusive hypoallergenic tape. For patching with the control materials, 0.3 mL of distilled water or 0.3 mL of 0.05% w/v SLS was applied per patch. Patches were applied to the upper arms, and subjects were instructed to keep the patches dry.

Test subjects returned to the clinical site 23 h after patch application, where the patch was removed, and the skin site was wiped with water, the site graded (0–4 scale), and the site repatched. This schedule was repeated for 21 consecutive days. Patch application would have been discontinued on any skin site that had an erythema score of 2 or higher.

Statistical analysis of the data was performed with SAS Proc Mixed procedure (SAS, Cary, NC). The average score for each panelist and treatment across 21 d were used in a mixed effect model with panelist as a random effect, and treatment and site as fixed effects. The least squares means were calculated for each treatment along with the differences for each pair of treatments.

ANALYTICAL pH EVALUATIONS

The authors of this study wished to determine if pH differences between diluted detergent solutions or detergent-washed fabrics in contact with sensitive skin could play a role in the skin mildness effects of TFG compared with AFC.

Treatment solutions for the RIFT were prepared as described earlier, and pH was measured using a SevenCompact S210 pH meter (Mettler-Toledo, LLC, Columbus, OH).

The pH values of the highly viscous undiluted TFG and AFC products were also measured using a Thermo Electron (Waltham, MA) system: gel-filled pH/Automatic Temperature Compensation (ATC) triode with an epoxy body electrode. The pH values of detergent solutions and titrations of the solutions to determine their reserve alkalinity (22,23) were measured using a Metrohm AG (Tampa, FL) system: Titrando titration system with a Unitrode glass electrode. For titration to determine reserve alkalinity, solutions (10% w/v) of TFG or AFC were prepared in distilled-deionized water, and then 0.2 N HCl was used to titrate the 10% detergent solutions from their basic pH values down to pH 5.5, the approximate pH of the normal skin surface (24). The result is expressed as % free alkaline in the detergent using published equations (22,23).

To measure the pH of detergent-washed fabrics, cotton fabric was washed with water or liquid detergent as described earlier for the 21DCIT. Five gram of the washed fabric and 50 mL of distilled-deionized water were placed in a glass jar and capped, and the content stirred with a magnetic stir bar at 500 rpm for 2 h at room temperature. The water was removed from the container and analyzed for pH determination using a Metrohm AG Titrando system with a Unitrode glass electrode. Four replicates of fabric under each wash condition were carried out.

Statistical analysis was carried out as follows. Fabric pH data were obtained by taking the average of two internal replicates to obtain two external replicates for each treatment (water, TFG, and AFC). The pH data were modeled separately using a one-way ANOVA with a fixed effect for each treatment. Treatments were considered statistically different based on a type I error rate of 0.05.

RESULTS

RIFT

This study contained three positive controls. AW is a commercially available detergent which has been used as a positive control in previous RIFT studies (P&G manufacturer data on file). Because of concerns that AW formulations may be subject to changes, which could impact its skin effect profile, 0.5% SLS and 1% SLS were evaluated as alternate positive controls for this and future studies.

AW detergent was significantly harsher than water, TFG, and AFC in four of the five measures at all four posttreatment time points: lower corneometer reading (less hydration) ($p < 0.0001$), higher dryness score ($p < 0.0001$), higher erythema score ($p < 0.01$), and increase in skin surface pH ($p < 0.0001$) (data not shown). AW was also significantly less mild than both concentrations of SLS at all time points in dryness grade ($p \leq 0.0003$) and increased skin surface pH ($p < 0.0001$). Yet, AW was milder than both concentrations of SLS at all time points in the corneometer reading ($p < 0.0001$).

For TEWL (data not shown), average baseline values for all treatments were approximately 6.0–6.5 g/m^2 h. There was substantial variation in the values within each of the treatment groups, making treatment differentiation difficult. Thus, there were no significant differences among water, AFC, or TFG at any of the measurement time points. SLS (0.05%) significantly ($p \leq 0.02$) increased TEWL at only the first and third posttreatment time points versus the water control. Yet, neither 1% SLS nor AW significantly altered TEWL at any time point.

Although numerically the 0.5% and 1% SLS treatments were nearly equivalent in their effects on skin (data not shown), there were some small but significant differences. Compared with 0.5% SLS, the 1% SLS treatment led to less skin hydration (based on corneometer readings) at the last three of the four posttreatment time points ($p \leq 0.03$), and higher skin surface pH at the last three of the four posttreatment time points ($p \leq 0.02$). For simplicity in presenting the data, only the 1% SLS data are presented as the positive control in the figures.

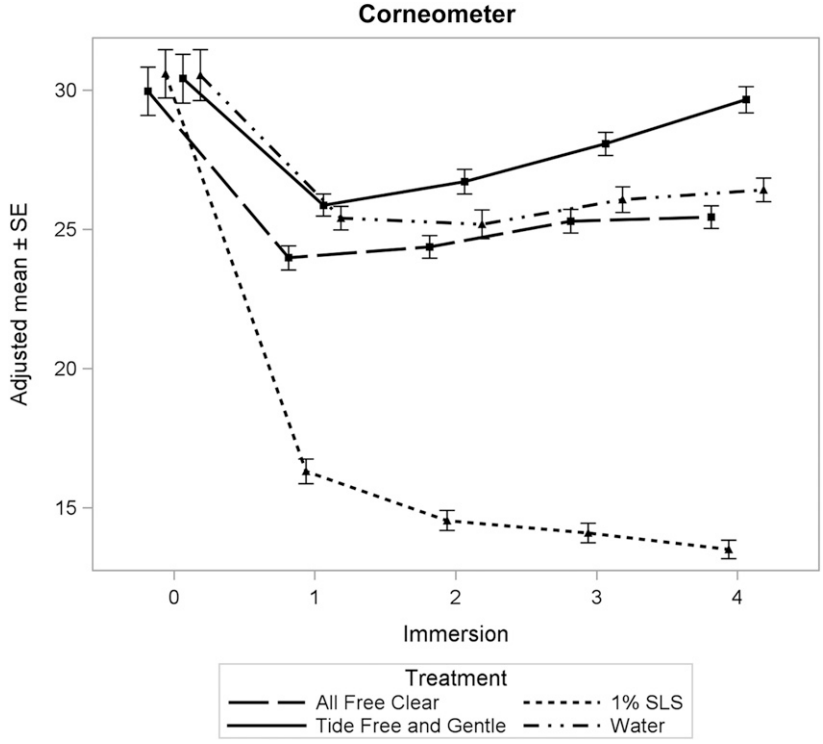

Figure 1. Forearm corneometer measurements for hydration after each of the four exposures to the test materials in the 1-d RIFT study.

Data from the four postexposure time points in this test revealed that whereas AFC and TFG are overall milder than the 1% SLS positive control, TFG was significantly milder on the skin than AFC, based on corneometer measures (Figure 1) and visual grading of dryness and erythema (Figures 2 and 3).

For corneometer (a measure of skin hydration), average baseline values for all treatment groups were approximately 30 (a unit-less capacitance measure that can range from 0 for no water to 120 for on water, according to the manufacturer's description). One percent SLS significantly ($p < 0.0001$) reduced skin hydration at all four postexposure measurements (Figure 1) versus the other treatments, an expected effect for this positive control. By contrast, although neither AFC nor TFG was shown to be harsh in terms of an effect on corneometer values, there were significant differences. Corneometer readings for TFG were significantly higher (greater hydration) versus the water control ($p < 0.0006$ to $p < 0.0001$) for the last three time points, and significantly higher versus AFC ($p < 0.0001$) at all four posttreatment time points. These results indicate a mildness advantage for TFG versus AFC.

For visual grading of skin dryness (Figure 2), average baseline values for all treatment groups were approximately 0.4 (on a 0–5 grading scale). SLS was numerically the most drying of the treatments, although it reached significance from the water control only at two of the four (the first and the last) posttreatment measurements ($p = 0.018$ and $p = 0.038$, respectively). Whereas AFC was equivalent to the water control, TFG was significantly less drying at the last three time points ($p < 0.0001$) versus both AFC and water.

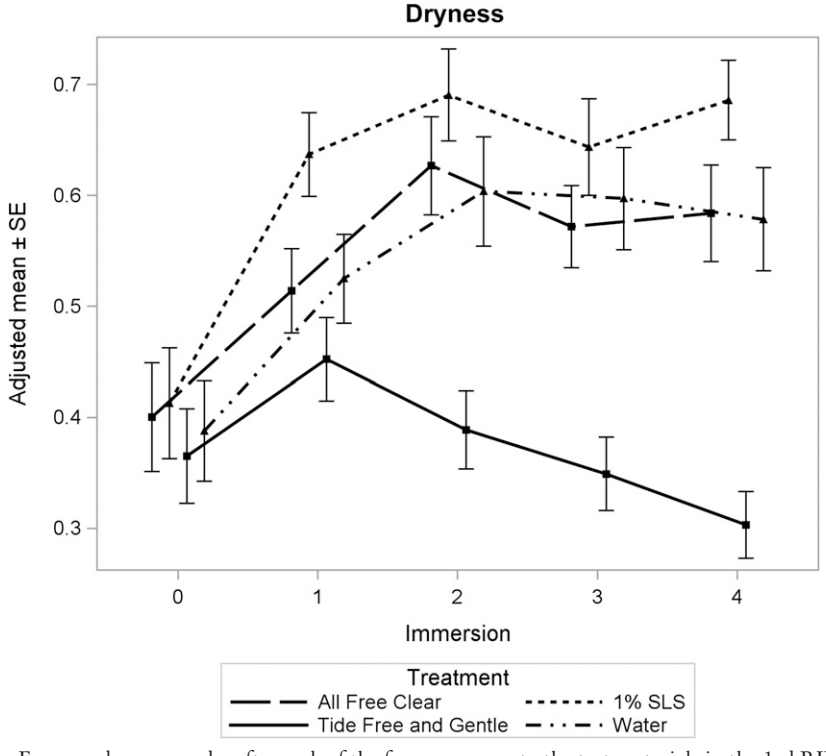

Figure 2. Forearm dryness grades after each of the four exposures to the test materials in the 1-d RIFT study.

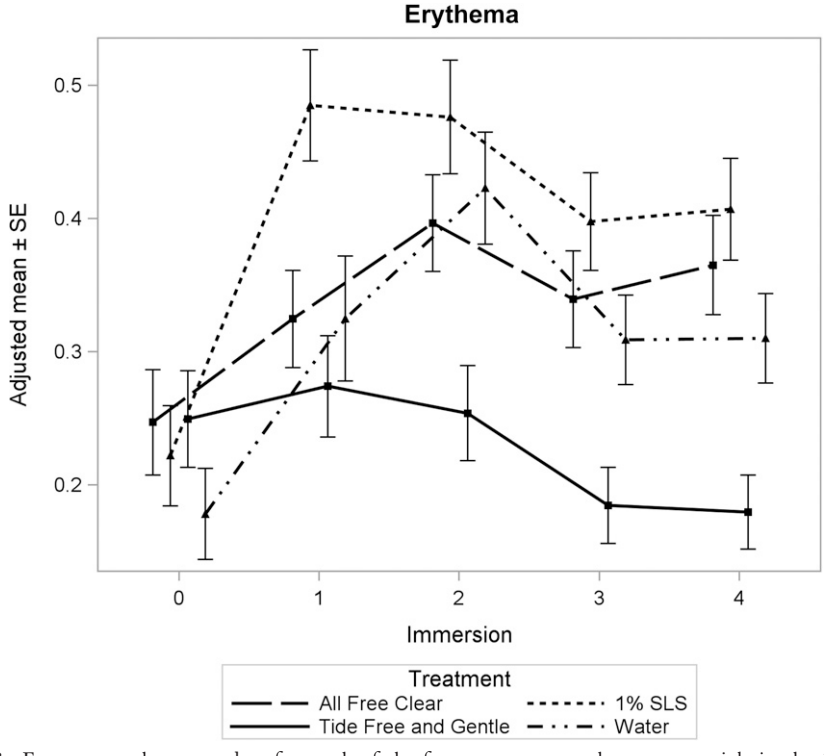

Figure 3. Forearm erythema grades after each of the four exposures to the test materials in the 1-d RIFT study.

For erythema grading (Figure 3), average baseline values for all treatment groups were approximately 0.2 (on a 0–4 grading scale). SLS was again numerically the most irritating of the treatments, although it reached significance versus water only at the first ($p = 0.0019$) and last ($p = 0.049$) postexposure measurements. AFC was not significantly different from water at any of the time points. TFG was significantly less irritating (based on erythema) than all other treatments: versus SLS at all four time points ($p \leq 0.0001$), versus water at the last three time points ($p = 0.0004$ to $p < 0.003$), and versus AFC at the last three time points ($p < 0.0001$ to $p = 0.0009$).

For skin surface pH (Figure 4), average baseline values for all treatment groups were approximately pH 5.5. Versus water, TFG significantly ($p < 0.0001$) increased skin pH at all posttreatment measurements, although the numerical increase was 0.25 pH units or less. SLS also increased pH significantly ($p < 0.0001$) by approximately 0.5 pH units. Of note, AFC significantly increased pH versus the other treatments ($p < 0.0001$), including the 1% SLS control, and by as much as 1 pH unit versus water.

21DCIT

Data from this 21-d patch study are presented in Table I. As expected, the positive control (0.05% w/v SLS) was significantly ($p < 0.05$) more irritating than all other treatments. However, TFG, TPFG, AFC, and AFCMP were determined to be equivalent (with

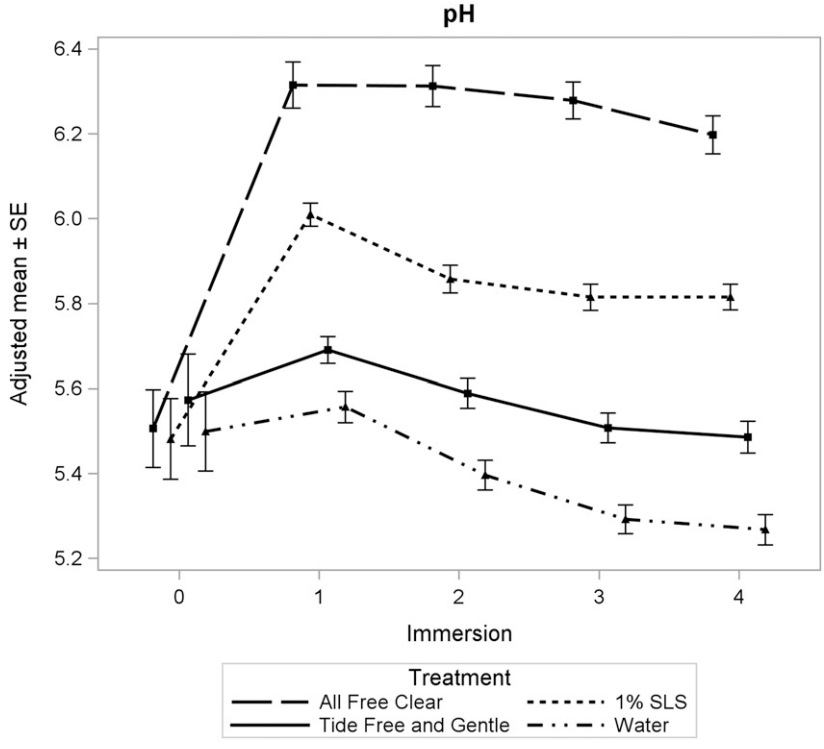

Figure 4. Forearm skin surface pH measurements after each of the four exposures to the test materials in the 1-d RIFT study.

95% confidence) as their score differences (including confidence limits) were not greater than the non-inferiority margin of 0.4. Because the mean erythema scores for the fabrics washed with any detergent product were less than 0.3 units different (on a 4-point scale) from the fabric washed with water, all of the fabrics washed with the detergent products tested can be considered mild.

Table I
Upper Arm Erythema Scores (21 D) with 95% Confidence Limits from the 21DCIT

Treatment	Mean skin score	Lower confidence limit 95	Upper confidence limit 95
Distilled water[a]	1.60	1.41	1.78
Fabric[b] washed in water	0.94	0.76	1.13
Fabric[b] washed in TFG liquid detergent	1.11	0.93	1.29
Fabric[b] washed in AFC liquid detergent	1.08	0.90	1.26
Fabric[b] washed in TPFG laundry pac	1.21	1.02	1.39
Fabric[b] washed in AFCMP laundry pac	1.07	0.89	1.25
0.05% SLS[a]	1.95	1.77	2.14

[a]For the control materials, 0.3 mL was applied directly to the patch.
[b]Fabrics are 2 × 2 cm^2 of 100% cotton T-shirt washed three times in a HE washing machine with the indicated treatment.

pH VALUES AND RESERVE ALKALINITY

The unadjusted pH values of the RIFT solutions are shown in Table II. The 4,200 mg/kg solution of AFC had a pH value of 9.81, substantially higher than the pH 7.03 value for the 4,200 mg/kg TFG solution and similar to the pH value of the 4,200 mg/kg AW solution (pH 10.61).

Additional pH measurements were made on the neat liquid detergent formulations and their solutions (Table III). The neat AFC formulation had a substantially higher pH (10.8) than that of the TFG formulation (7.9). Solutions (10%) of the same detergents showed similar differences in pH. Consistent with those observations, AFC was shown to have a higher reserve alkalinity value than TFG (1.55 vs. 0.81).

The pH values for the washed cotton fabric are shown in Table IV. The water-washed and AFC-washed samples yielded pH values near 9 and were not significantly different ($p > 0.05$) from each other. However, the TFG-washed cotton fabric yielded a pH of 8.26, which is significantly lower than the pH of either the water-washed fabric ($p = 0.0024$) or AFC-washed fabric ($p = 0.0011$).

DISCUSSION

Skin can be directly exposed to commercial laundry detergents, e.g., during hand-laundering of fabrics or during hand prewashing or pretreating (e.g., applying liquid detergent to shirt collars or to visible stains) before the use of a commercial washing machine for full wash. Recent research has found that a significant number of U.S. consumers wash at least some laundry loads by hand (unpublished P&G 2015 USA Habits and Practices Survey). For the survey, an Internet research supplier recruited 3,175 consumers nationwide (70% females, 30% males) who were over the age of 18 years and responsible for doing their household laundry and laundry product purchasing. The consumers replied to questions presented to them via an Internet survey. Their responses revealed that 20% of them do at least some loads of laundry by hand, indicating that there is substantial exposure of the skin to dilutions of liquid laundry detergents. The same survey shows that among people who do laundry by hand, 5% hand-wash daily, 14% hand-wash two to three times per week, and 20% hand-wash weekly. It takes approximately 5 min to hand-wash a garment. So, if we

Table II
Treatment Solutions in the Repeat Insult Forearm Test (RIFT) Study

Treatment solution (see Materials and Methods for details)	Water hardness ($CaCl_2/MgCl_2$)	pH of test solutions[a]
Water (negative control)	0	6.48
0.5% SLS	16	7.08
1% SLS	16	6.86
4,200 mg/kg[b] TFG	7	7.03
4,200 mg/kg[b] AFC	7	9.81
4,200 mg/kg[b] AW	16	10.61

[a]No pH adjustments were carried out in preparing these test solutions.
[b]Solutions of 4,200 mg/kg TFG, AFC, and AW (equivalent to one manufacturer-recommended dose of liquid detergent in 5 gallons of water) were chosen as reasonable doses of detergent for hand-washing of laundry.

Table III
Liquid Detergent Solution pH Values and Residual Alkalinity

Solution[a]	pH	Reserve alkalinity (%)[b]
TFG	7.9	—
10% TFG	8.2	0.81
AFC	10.8	—
10% AFC	11.0	1.55

[a]Ten percent or higher solutions of liquid detergent are typically used by consumers for hand-washing of fabric or for hand-prewashing of fabric before use of a commercial washing machine.
[b]The amount (expressed as %) of 0.2 N HCl required to titrate the solution down to pH 5.5, the approximate pH of the skin surface. Reserve alkalinity was only determined on the 10% dilution, the approximate concentration expected in a hand-fabric-prewash situation.

conservatively assume that these respondents are only washing one garment at a time, exposure to diluted detergent can range between 5 and 35 min/week, at minimum. Therefore, the 15-min exposure times in the RIFT study are relevant to real-life exposures. This also reinforces that being aware of the potential consequences through mildness testing is a necessary part of the evaluation of commercial laundry detergents.

This is particularly important considering the large and growing global population of people with sensitive skin. In the RIFT study reported here involving individuals with self-assessed sensitive skin, TFG was found to be significantly milder than AFC by several measures, including corneometer and visual grading of dryness and erythema. In fact, by these same measures, TFG was either as mild as or even significantly milder than water. And whereas erythema alone is often used as a measure of skin damage in surfactant studies, a battery of measures such as hydration (25) and particularly skin surface pH (26–28) can be even more revealing with regard to mildness on sensitive skin.

An important difference between TFG and AFC that might account at least in part for the mildness disparity between the two products in the RIFT study is the formulation pH. The pH of laundry detergents tends to be alkaline to improve cleaning performance and to avoid possible damage to fabrics from acidic conditions (19,29,30). Yet, the pH of AFC is particularly high (10.8), nearly 3 units higher than the pH of TFG (7.9).

In some studies, exposure of the skin to solutions with a high pH was shown to induce physiological changes such as irritation, stratum corneum swelling, alterations in stratum corneum proteins and lipids, and barrier damage (31–34). These effects appear to be more pronounced as the pH approaches or exceeds 10. The measurements carried out here indicate that even a low concentration of AFC has a pH of nearly 10. The negative effects

Table IV
pH of Washed Cotton Fabric

Fabric washing treatment[a]	pH of washed cotton fabric extracted with water
Water	9.00
AFC	9.22
TFG	8.26

[a]Fabric is 5 g of 100% cotton T-shirt washed three times in a HE washing machine with the indicated treatment (water, TFG, or AFC liquid detergent) and then extracted with 50 mL of water for 2 h.

of this high pH, especially with repeat or chronic exposure, may be exacerbated by exposure to or challenge by surfactants (34), such as in laundry detergents and in other cosmetic products. Because surfactants are present not only in laundry detergents but also in many other consumer products that contact the skin, exposure to a variety of surfactants is inevitable.

In the 1-d RIFT study reported here, repeat exposure to AFC resulted in a significant increase in skin surface pH of nearly 1 unit. It has been reported that higher skin surface pH can be associated with skin conditions such as itching, dermatitis, acne, and microbial infections (35–40). Particularly for atopic dermatitis, the difference in skin surface pH between lesional skin (pH 6.1) and non-lesional skin (pH 5.5) is less than 1 unit (35). In most cases, it is not clear whether the increased skin surface pH in these disorders is a cause of the disorder or is an effect of an existing skin disorder. However, in the case of atopic dermatitis, a causal relationship has been observed, specifically that increasing skin pH may cause an atopic skin condition (40).

Maintenance of a normal pH is related to skin health. For example, the opportunistic yeast *Malassezia*, which resides on the skin, will release allergens as the pH rises, leading to increased risk of inflammation and possibly triggering atopic eczema (41).

In contrast to the effect of AFC, TFG maintained skin pH at a normal pH of 5.5 in the 1-d RIFT study after four exposures. This is a desired outcome for preservation of healthy skin properties, and it is likely also important for preservation of a balance in the skin's normal microbial flora.

Whereas the specific forearm chamber test protocol (RIFT) reported in this article is not a widely used method, forearm immersion and exposure chamber methods have long been used to evaluate skin responses to treatments, such as those with surfactants (24,42–45). In such testing, forearm responses are predictive of effects on hands (43).

Although the AFC product increased skin surface pH in the RIFT study, increased pH in and of itself does not necessarily cause skin irritation (46–48). In addition to pH of detergent formulations, specific components of the products likely play a role in skin mildness. Connecting specific product ingredients to the observed skin effects is difficult because formulations are very complex; however, it is recognized that use of certain types of materials such as anionic surfactants with long alkyl-chain lengths will yield milder formulations (49,50).

Skin is also indirectly exposed to detergent products or their components if there are any residual materials left behind on fabrics after they are washed. In fact, this is the most common route of indirect exposure to laundry detergents. Based on the results of the 21DCIT reported here, fabrics washed with TFG, TPFG, AFC, or AFCMP showed no significant increases in skin irritation as assessed by visual erythema grading among subjects with self-assessed sensitive skin. Although there were significant differences in the pH of fabrics washed with TFG and AFC liquid detergents, those differences did not result in an impact on skin mildness in the 21 DCIT, based on erythema as the end point. It is worth noting that these results support other work with these two laundry detergent products under patch on the human skin which revealed that both products are mild, with no significant differences between the two, again based on erythema as the end point (16).

In light of the work reported here, more research is needed to understand how a long-used method such as patch testing was not sufficient to detect the substantial product

differences observed in the RIFT, and to evaluate the significance of these new results. It is worth considering the value of additional measures in future studies, or recruiting individuals with very sensitive skin (e.g., the elderly or those with diagnosed atopy) (51,52).

Clearly, as demonstrated from the RIFT results, *in vivo* studies are critical for a full evaluation of detergent formulation mildness. Yet, other work (16,17) proposes using a specific set of *in vitro* methods (zein protein denaturation test, cell culture cytokine release, and corneosurfametry measure of protein and lipid degradation) as sufficient to assess and compare the mildness of different laundry detergents. In that testing, the AFC product was reported to be milder than TFG (16). Yet, the RIFT clinical results reveal the opposite: TFG is significantly milder than AFC in a model mimicking exposure of the skin to laundry detergents during hand-laundering of clothes.

Although *in vitro* methods can be useful tools for rapid screening of large numbers of surfactants and product formulations for skin mildness profiles, they can be limited in scope. It is particularly important to recognize that false negatives do occur with *in vitro* and other laboratory models (53–58), most likely because such methods do not replicate all the possible skin properties and reactions to treatments (erythema, allergy, hydration, barrier damage, skin surface pH, alteration of the surface microbial community, environmental effects, *etc.*). Thus, inclusion of the appropriate real-life human studies, using a battery of skin end points, is recommended for precisely defining mildness of surfactant-containing formulations, such as laundry detergents (5,24,53,54).

As a final note, in addition to using a milder detergent, consumers can improve the mildness of clothing on skin by using fabric softeners. Studies with softened fabrics have revealed less friction, better skin hydration, and gentler effects on sensitive and infant skin (51,59–61).

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