

A Novel Multifactorial Approach to Developing Mild Laundry Detergents and Assessing Their Relative Mildness

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ABSTRACT

Introduction: Dermatologists are becoming more aware of the irritant and allergic potential of laundry detergents that incorporate harsh surfactants and potentially sensitizing ingredients. It is difficult however for the physician to distinguish one laundry detergent from another because the only distinguishing feature advertised tends to be the lack of dyes and fragrances.

Design: A new objective method was developed for measuring the harshness of laundry detergents using a three-pronged laboratory testing approach consisting of zein, corneosurfametry, and cytokine testing. Combing these methods, a Detergent Mildness Index was created which conveniently provides a single value by which products can be compared.

Results: A new mild laundry detergent was formulated with ingredients carefully selected by dermatologists who are experts in contact dermatitis. The irritancy potential of the formula was measured using the Detergent mildness index score. Compared to 11 other commercial laundry detergents marketed for sensitive skin, the new formula is measurably the mildest formula.

Discussion: The Detergent Mildness Index provides dermatologists with an objective method to compare commercial laundry detergents. Currently the only method available is patch testing, this new test is able to more finely differentiate between products and thus enables more informed recommendations on laundry detergent choices for their patients with sensitive skin.

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INTRODUCTION

Dermatologists often recommend laundry detergents that are free of harsh chemicals, fragrances and dyes.[1] New methodologies have enabled the development of a laundry detergent with a new level of mildness.

Chemistry of Surfactants

All detergents contain surface-active agents (surfactants) which possess cleaning properties and help lift dirt from fabric.[2]

Surfactants may unintentionally and negatively interact with skin when in contact with it for long periods of time.[3,4] Through direct contact with clothing containing detergent residue, there is the potential for irritant and allergic contact dermatitis, which is likely to be exacerbated in patients with existing dermatological disorders. One publication suggests that the amount of laundry residue deposited on fabric is 2.5%.[5]

Effects of Surfactants on Skin

Upon prolonged contact with the skin, surfactants can adsorb to keratin, and cause subsequent denaturation, transient swelling and hyperhydration.[6,7] Swelling increases the risk of surfactant penetration into deeper layers of the skin, and may lead to long-term skin dryness. Lipids are also susceptible to solubilization by surfactants, leading to increased permeability and destabilization of lipid bilayers.[2,6]

Persistent and unresolved stratum corneum-surfactant exposure can lead to long-term deleterious damage in some patients.[8] At first, the skin mounts a local immune response. Over time, a systemic response may occur. The inflammatory cascade is stimulated when keratinocytes in the epidermal layer secrete key cytokines, such as interleukin 1α (IL-1α) and tumor necrosis factor α (TNFα).[4,9] Initial symptoms include pruritus, erythema, and xerosis with a glazed, parched appearance.[2,10,11] Over time, frequent contact with surfactants can change skin permeability and texture.[3] Symptoms then progress to eczematous dermatitis, resulting in skin thickening, hyper- or hypopigmentation, and scaling, fissuring, and lichenification.[8]

Patch Testing

New mild detergent formulations are expected to be thoroughly evaluated for safety and mildness.[12] Patch testing on normal healthy volunteers is commonly used in the industry to demonstrate the expected safety profile during normal consumer usage. New methodologies have the potential to provide dermatologists with additional information and enable more

informed recommendations on laundry detergent choices for their patients with sensitive skin.[3]

OBJECTIVE

The objective of this work was to create an especially mild laundry detergent formulation through development in partnership with dermatologists (DPD formulation) and concurrent comparison of its mildness profile with existing mild laundry detergents. During formulation development, the ingredients were carefully selected and the surfactant composition was optimized. The potential mildness of the final formulation was further supported by utilizing unique scientific tests including the zein solubility assay, corneosurfametry (CSM), and Cytokine Assay of Il-1α production. These tests evaluate protein denaturation, overall stratum corneal damage, and keratinocyte-derived IL-1α response, respectively. The Detergent Mildness Index (DMI) was developed, which establishes a composite score to rank the irritancy potential of laundry detergents that are marketed for patients with sensitive skin.

Products

Twelve detergents, including the new DPD formulation, were tested. They comprise 85% market share of the existing Sensitive Skin laundry detergent category. The total surfactant percentage in the category ranges from 10% – 28%.

METHODS

Protein Denaturation

The zein solubility assay is an in vitro method. Zein protein is structurally similar to keratin. Both are insoluble in aqueous solution unless they are denatured. Irritancy is determined by measuring the amount of solubilized zein after detergent exposure.[3]

In this study, zein powder was mixed with detergent solutions for about one hour. Blank surfactant solutions were prepared in the same manner. Undissolved zein were removed by filtration and the filtrate dried. The total percentage of solubilized zein was calculated by measuring the amount of zein solubilized by the detergent solution. The percentage of solubilized zein corresponds to the degree of surfactant-induced protein denaturation.[13]

Overall SC Damage

Corneosurfametry (CSM) is an ex vivo method, which measures the level of interaction between the SC and detergent.[4] Superficial layers of the SC are collected from healthy volunteers using tapes that are then placed in detergent solution. They are then stained using a dye solution to assess the degree of SC damage. A high degree of staining reflects a highly irritating detergent.[3]

In this study, samples were collected using Book tape strips. Volunteers were recruited by passing a pre-screening survey for inclusion and exclusion guidelines. Tapes were placed on volunteer's forearms and gently rubbed onto skin before removal. Tapes were then immersed in detergent solutions, dried, and then placed in Basic Fuschin Blue Dye. Once dry, samples are placed on transparency films and analyzed using a spectrophotometer.

The colorimetric index of mildness (CIM) was calculated using the following formula:

CIM = (L* - C*), where L* corresponds to mean luminancy and C* to Chroma.[14]

This calculation measures the degree of dye saturation, and consequently, the level of damage to SC proteins and lipids.[12]

Inflammatory Response

In this study, the EpiDerm™ Skin Model (MakTek Corporation) assessed potential dermal irritation secondary to detergent exposure. Samples were analyzed using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay after exposure to detergent solutions in sterile, deionized water. Each sample was added to 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) containing 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. This measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate. Samples were incubated for 24 hours, and gently agitated to evenly mix cytokine released into the medium.

Samples were added to microtiter plates which were previously coated with IL-1α detection antibodies. After incubation for 2 hours at room temperature, samples were rinsed with wash solution. Enzyme conjugate (IL-1α Conjugate) secondary antibody was added to the samples and incubated for another 20 minutes. Stop solution was added to halt the reaction. Finally, samples were read at 450nm using a Molecular Devices' Vmax plate reader within 30 minutes of stopping the reaction to measure the amount of IL-1α released in response to each detergent.[13]

RESULTS

The new DPD formulation was ranked mildest across each measure tested in this study: zein, CSM, cytokine, and DMI.

Zein Solubility Assay

As shown in Figure 1, a smaller percentage of zein was solubilized when placed in the new DPD formulation, compared to other mild detergents. The harshest detergent, Product 11 solubilized more than twice the amount of zein solubilized by DPD formula. The difference between DPD formula and other samples were found to be statistically significant, with the exception of Product 3. The similarity of zein protein to keratin allows us to

FIGURE 1. Percentage of zein solubilized by detergent solution.

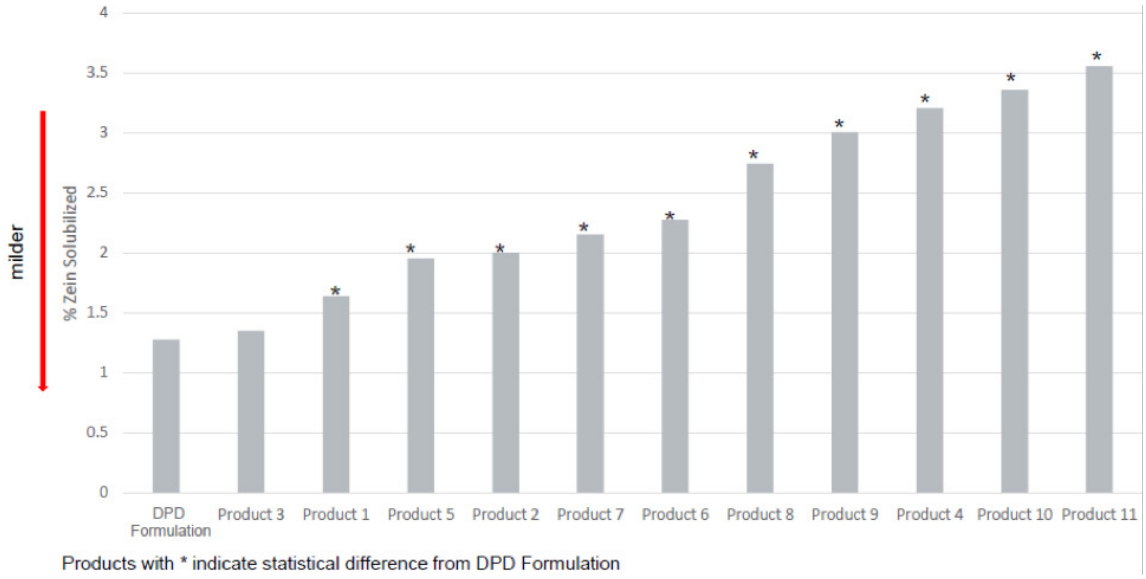
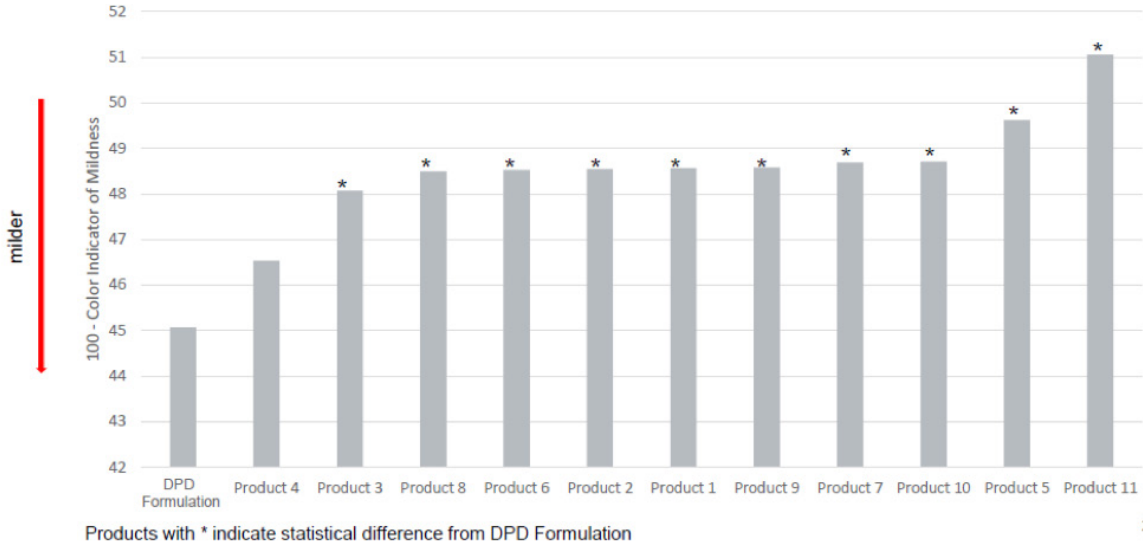


FIGURE 2. Corneosurfametry test results.



extrapolate these test results to potential SC irritancy. Therefore, the DPD formula is expected to cause the least damage to the SC proteins, when compared with other mild detergents.

Corneosurfametry

The results of the CSM test showed a lower degree of staining after exposure to DPD formula compared to other mild detergents. The 100-CIM value is proportionately related to the degree of SC damage and subsequent staining. Therefore, DPD formula was shown to cause the lowest level of SC damage compared to other mild laundry detergents. In contrast, Product 11 was associated with the greatest amount of SC damage. With the exception of Product 4, these differences compared to DPD formula were found to be statistically significant.

Cytokine

As Figure 4 shows, the EpiDerm™ Skin Model released a lower level of cytokine IL-1α after exposure to DPD formula when compared to the other mild detergents. Additionally, there was a marked difference between the amounts of cytokine IL-1α released after exposure to DPD formula compared to Product 11. With the exceptions of Products 1, 2, 4, and 5, the difference between the samples were found to be statistically significant. Therefore, DPD formula was shown to induce a lower

FIGURE 3. Epiderm™ Skin Model test results.

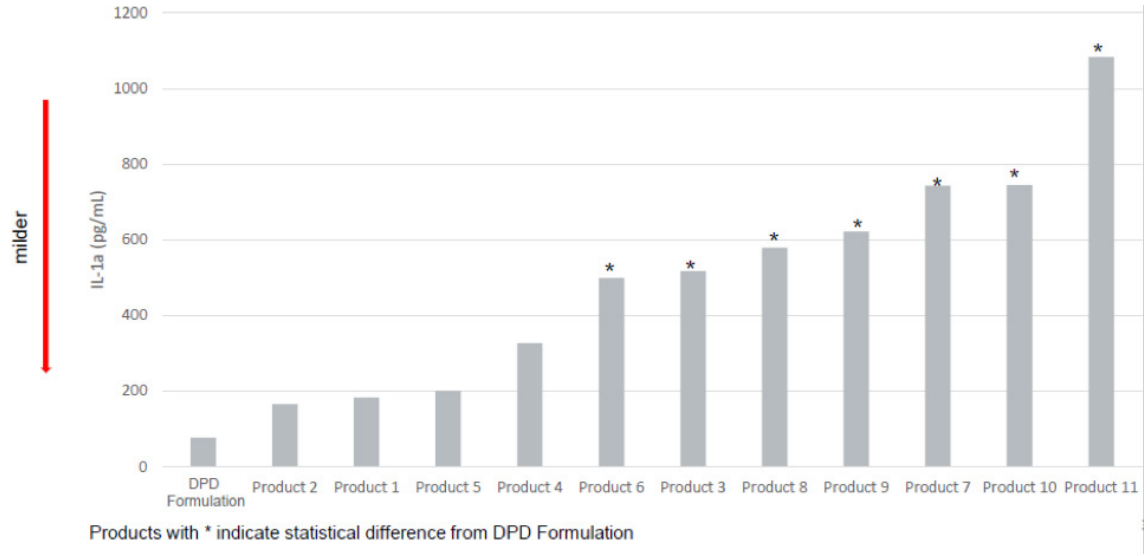
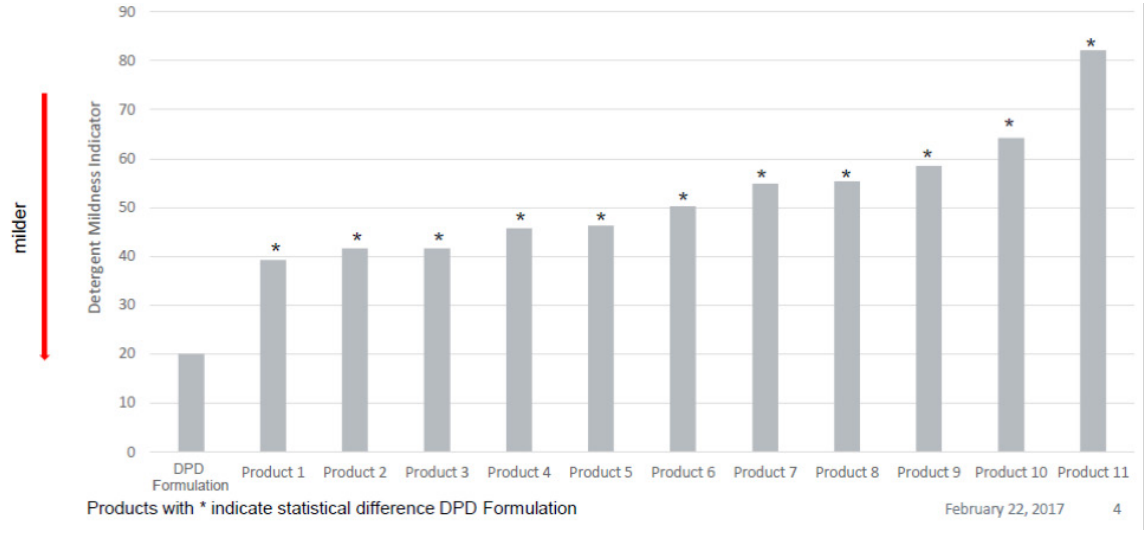


FIGURE 4. Detergent Mildness Index.



inflammatory response compared to other mild detergents, and especially compared to Product 11.

A Comprehensive Analytical Methodology

The Detergent Mildness Index was developed to comprehensively rank the irritancy potential of the mild laundry detergents. Each detergent was individually analyzed using zein, CSM, and cytokine tests. A standardized score was obtained based on a 0-100 scale to rank these detergents in order of expected overall mildness.[13]

As shown in Figure 5, DPD formula was shown to have the lowest composite DMI score. In contrast, Product 11 was found to have the highest individual zein, CSM, and cytokine values, as well as the highest composite DMI score. In this way, the DPD formula demonstrated superior comprehensive mildness compared to other existing mild detergent formulations.

DISCUSSION

Laundry detergents have the potential to impact the skin in several ways. Carefully selecting the right mild detergent is particularly important for patients with sensitive skin, as up to 2.5% of laundry detergent may remain on fabric as residue. Traditional patch testing provides a first step in demonstrating safety in normal consumer usage, but does not provide practicing dermatologists with enough information to differentiate between the many choices of mild laundry detergents and enable them to confidently recommend more mild formulations for their patients with sensitive skin.

A three-pronged testing approach was developed with tests to correlate with the level of protein denaturation, the amount of overall SC damage, and the extent of cytokine IL-1α release after detergent exposure. In this way, multiple aspects of skin responses in sensitive patients could be considered. This led to the development of the DMI, to allow for the ranking of various mild laundry detergent formulations, by predicting potential mildness for patients with sensitive skin.

Results of this study showed that the DPD formula had the mildest effect on protein denaturation, SC damage, and IL-1α release, as measured by zein, CSM and cytokine testing, respectively, compared to all other commercially available sensitive skin formulas, resulting in the lowest DMI composite score.

It would be expected that lower surfactant concentration would be milder on skin. However, the DPD formula contains 18% surfactant and appears more mild, while Products 1, 2, 3, 5, and 6 all contain lower surfactant levels, yet produce harsher results. Thus, this study shows that thoughtful surfactant selection and composition, as guided by dermatologists, produces a product with superior mildness, while maintaining the required cleaning benefits.

These results demonstrate that experienced dermatologists have an important role to play in the selection of ingredients and the development of a mild laundry detergent formulation.

It is understood that laboratory testing is intended to provide guidance in understanding the relative mildness of laundry detergent formulations across this category and that these tests are not directly comparable to exact consumer usage. However, these methods can serve as important tools in the design of mild formulations. This objective scientific testing can help dermatologists to choose which mild laundry detergent to recommend to the patients with sensitive skin.

DISCLOSURES

Joseph F. Fowler, MD has acted as a consultant and received honoraria relevant to the topic from the Sun Products Corporation. Matthew J. Zirwas, MD has acted as a consultant and received honoraria relevant to the topic from the Sun Products Corporation. Lisa Napolitano, BS was an employee of the Sun Product Corporation during the development of this manuscript. Meghan Russell, BS is a current employee of the Sun Product Corporation. Janet Coope-Epstein, PhD employee of the Sun Product Corporation. **Funding/Support:** This work was supported by The Henkel Corporation.

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