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The Behind the Knee (BTK) as an Innovative Test Model for Mechanical and Chemical Irritation, Lotion and Dye Transfer and Testing on Compromised Skin

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Author's contribution

This whole work was carried out by author MAF.

Method Article

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ABSTRACT

Aims: The aim of this manuscript is to present innovative applications of the BTK model that can potentially contribute additional aspects of safety evaluations for a broader range of products and materials intended for prolonged skin contact.
Study Design and Methodology: The basic BTK protocol is one 6-h exposure per day for 4 days. Modification to the basic protocol were made for individual studies, as needed.
Results: Studies using fabrics, tissues and films indicate the BTK may be well suited to evaluating these materials for skin compatibility. The BTK test discriminated between; different fabrics, drying methods of the same fabric, similar toilet tissue products, and two similar topsheet films used as coverings on the surface of a range of absorbent consumer products. The method was used successfully to measure the transfer of lotion, and lotion skin benefits from lotioned absorbent products.
Conclusion: Studies demonstrate that the utility of the BTK goes beyond the original intent of evaluating the potential skin effects of feminine protection products. The ability to compare fabrics, tissues and films indicate the test model may be useful in the development broad range of absorbent consumer products and in textile development. The utility of the model in measuring the transfer of lotion and other materials from products to the skin surface has the potential to fill an important gap in the development of quantitative exposure assessments. Added endpoint measures, such as enhanced

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visual scoring and sensory effects further increase the ability to differentiate between very similar products without requiring other protocol modifications.

Keywords: BTK test system; behind the knee test system; skin irritation; mechanical irritation; feminine protection products; compromised skin; textile irritation.

1. INTRODUCTION

Consumer products manufacturers have a responsibility to evaluate their products and materials to ensure they will not cause adverse reactions once the products are on the market. Since many consumer products are expected to come into contact with skin, evaluations for skin compatibility are particularly important. The overall testing program to support skin safety of products is a multi-step process [1]. Initial stages include a complete evaluation of existing data on the ingredients or structurally similar ones, and may include additional testing of irritant potential using *In vitro* test methods such as those recently reviewed [2]. The result of these initial evaluations is that materials with known irritating properties or other unacceptable toxicological hazards are eliminated from consideration.

The next steps in an overall program include stepwise, ethical testing in human volunteer subjects [3]. Studies progress from extremely limited, controlled exposures, such as patch testing, to in-use studies that mimic actual use conditions for the specific product being tested. For example, consumers are exposed to laundry products via immersion of hands in solutions of the product to mimic hand laundering, or wear tests of laundered fabrics in close proximity to skin [4,5]. Personal cleansing products and baby wipes have been tested using modified wash or wipe tests on the relatively sensitive skin of the forearm [6]. Catamenial (feminine protection) products are typically tested in in-use clinical studies in which volunteer panelists use the product in place of their normal product, and gynecological and skin condition evaluations are conducted to determine potential effects [7].

Both the industry and the consumer have been served well for decades by this approach. However, relying on in-use testing protocols has certain practical limitations. Most modern products are inherently very mild to skin, and true adverse skin reactions to these products are extremely rare. Therefore, a meaningful in-use study must enroll a large group of subjects and incorporate some degree of exaggeration of real-life exposure in order to increase the possibility of detecting a rare adverse reaction. For some types of products, such as laundry products and personal cleansing products, this can be accomplished by increasing the duration of exposure, and/or increasing the concentration of product beyond normal consumer habits and practices. But for products such as facial tissues and diapers, this is not possible.

Feminine protection products present unique challenges for in-use testing [8]. In the real-life, consumer situation, these products are intended for continuous exposure for prolonged periods of time, therefore, increasing the duration of exposure is not feasible. Second, these products are intended to be used ‘as is’, so no increase in the test concentration is possible. Since the in-use tests are often designed so that start dates coincide with the panelists’ menstrual cycles, results may not be available for a minimum of 4–5 weeks from study initiation. Grading is done by visual assessment of the genitalia and is, therefore, intrusive for the panelists. Each panelist can test only one product at any one time, making side-by-side comparisons more difficult. In-use test results can be confounded by changing

conditions in the vulvar and vaginal regions due to microbiological and hormonal differences throughout the menstrual cycle. Panelists may have a broad range of pad wearing and hygiene habits. Finally, investigations into some areas, such as testing on compromised skin, are not possible due to the nature of the in-use test. Because of these special challenges, we developed an alternative to in-use testing, i.e., the Behind-the-Knee (BTK) test [9].

The BTK protocol utilizes the unique anatomic site of the popliteal fossa as a test model for evaluating potential skin effects, and solves many of the potential problems associated with in-use testing on feminine protection products [10]. As the panelistsgo about their everyday activities, normal movements generate friction between the test sample and the skin at the test site, thereby adding the element of mechanical irritation. This added element increases our ability to discriminate between products with very similar properties, especially when the nature of the product prohibits incorporating other exaggerations of normal exposure, such as concentration or duration of exposure.

The basic BTK test protocol allows for a number of opportunities for investigating potential irritation of products and materials under a variety of conditions. For example, prior to exposure to the test material, the integrity of the skin can be compromised by tape stripping. A sample of absorbent products can be tested ‘as is’, or wetted with saline. These protocol variations can be particularly useful for some product categories such as baby or adult diapers that are often in contact with skin when wet, and are commonly used on skin with some degree of irritant dermatitis.

Although it was initially developed as part of the overall skin compatibility and safety program for feminine protection products, this flexible test model can be adapted to provide information important to the overall safety assessment for a variety of products. The aim of this manuscript is to present innovative applications of the BTK model that can potentially contribute additional aspects of safety evaluations for a broader range of products and materials intended for prolonged skin contact.

2. METHODOLOGY

2.1 Subjects

Study participants were healthy adult volunteers aged 18 or older who had reviewed and signed an informed consent prior to the study. Subjects were excluded if: (1) they took anti-inflammatory corticosteroids or other medications that may interfere with test results, (2) they had participated in a similar test within the last 4 weeks or (3) they had diabetes, kidney, heart or circulatory disease, or were currently pregnant of breast-feeding.

2.2 Basic Protocol

2.2.1 Application of test materials

The BTK is a comparative test designed to evaluate potential skin effects of a new product or product innovation compared to a control product with a history of safety in testing programs and in the marketplace. In the basic BTK protocol [9,11,12], test material is placed horizontally on the popliteal fossa and held in place by an elastic knee band, as shown in Fig. 1. Fresh test material is applied to one, randomly chosen side and control material is

used on the opposite side. Typically, samples are applied daily for 4 or 5 consecutive days and left in place for 6-hours each day. The duration and number of exposures can be modified to meet the needs of a particlar investigative program.

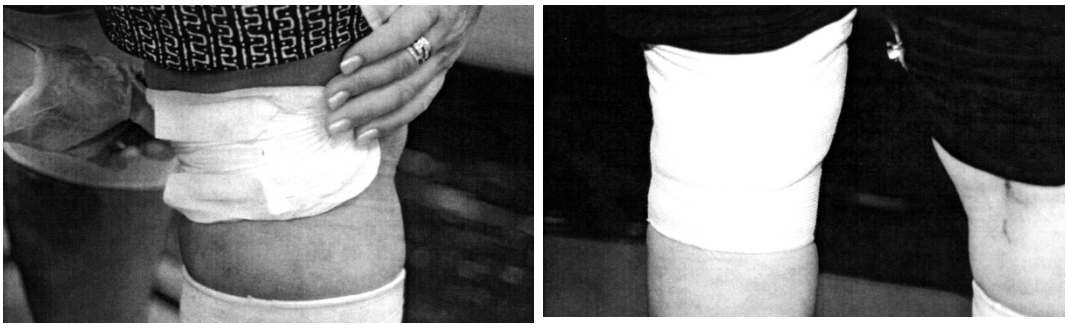

Fig. 1. Test sample application in the BTK test. Test materials are placed horizontally on the popliteal fossa, and held in place by an elastic knee band of the appropriate size

Other materials (i.e., tissues, films and fabrics) were applied in a manner identical to that described above. When tampons were tested, they were applied as uncompressed samples since the finished product is marketed in a highly compressed, cylindrical configuration that would make poor contact with the skin.

Identification of the specific test materials used in each study is contained in the figure captions.

2.2.2 Evaluations and analyses of data

Test sites are scored for visible signs of irritation on a scale of "0" to "4", where "0" is no apparent cutaneous involvement and "4" is moderate-to-severe, spreading erythema and/or edema [9,12]. Scoring is conducted at study commencement prior to any treatment (i.e., the baseline score), each afternoon 30-60 minutes after sample removal (PM scores), and each morning before sample application (AM scores). Afternoon scores can be used to evaluate the cumulative irritant effects with repeated exposure, and the average afternoon post-baseline (PBA) score can be used as an indication of overall irritation. Morning scores can be used to evaluate recovery of test sites. Specific statistical analyses used in each study are contained in the figure captions.

2.3 Protocol Variations for Evaluating Specific Endpoints

Compromised skin: The integrity of the skin can be compromised by tape stripping using Blenderm(R) tape (3M, St Paul, MN) prior to the first application [8,13]. In some experiments, this was done by repeatedly applying tape to the area up to 20 times, or until the skin exhibited an erythema score of 1.0–1.5 as per the grading scale described in the previous paragraph.

Wet products: When products are tested ‘wet’, they are saturated with saline prior to application [14].

Material transfer measurements: Small sections of a thin film dressing tape (Tegaderm™ tape, 3M™, St Paul, MN, USA) were applied directly to the skin under test samples. Sections of collection tape were removed after specified durations of exposure. Lotion was extracted and quantified by gas chromatography with flame ionization detection. Behenyl alcohol, a component of all the lotion formulations, served as a marker for lotion transfer. A calibration curve was generated based on the three standard concentrations of behenyl alcohol. Further details on lotion quantification were previously published [15]. Transferred dye material was extracted in methanol. Extracts were subjected to high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC/MS/MS), and dye content was quantified by comparing to standard dye solutions.

Enhanced visual scoring was conducted using cross-polarized light which allows visualization of reactions approximately 1mm below the skin surface (Syris v600™ Visualization System, Syris Scientific, LLC, Gray, ME, USA or syrisscientific.com) [16].

2.4 In-use Clinical Protocol

The in-use clinical was conducted among 43 subjects using a crossover design as part of an overall program to evaluate an experimental tampon product. Half the subjects wore the control tampon for one menstrual cycle and the experimental tampon for the second. The remaining subjects used products in the reverse order. Six anatomic sites were graded separately for erythema via colposcopic examination after each leg of the experiment: labia minora, introitus, lower and middle vaginal walls, upper vagina and cervix. In addition, the overall mean was calculated for all sites combined.

3. RESULTS AND DICUSSION

3.1 Utility in Evaluating Fabrics, Tissues and Films

Unlike the standard 24h patch test that evaluates the inherent irritation associated with a chemical or material (i.e., chemical irritation), the BTK also evaluates the mechanical irritation due to friction. Previously, we published results of a study directly comparing the irritation scores for the same test materials in the BTK (6h sample applications) and in a standard 24h patch test on the upper arm. In these studies, several protocol variations were used including: Exposures on intact skin and compromised skin, and the use of dry and wet test samples [13,14]. With one exception, scores from the standard patch test were 65-85% of those from the BTK test. The higher overall scores in the BTK reflect the contribution of mechanical irritation to the overall skin reaction. Since the BTK evaluates mechanical irritation, we evaluated the test model for examining other materials and products that come into prolonged contact with skin.

3.1.1 Fabrics

Original development work on the test method used burlap and satin as control materials for mechanical irritation (9). We comparted the irritation potential of these two fabrics in the BTK protocol (4 consecutive days for 6h each day) and in the standard upper arm patch test with 6h and 24h exposures. In the BTK protocol the burlap fabric produced a significantly higher level of mean erythema after a single application. The two fabrics were not significantly different with a comparable duration of exposure (i.e., 6h) in the standard upper arm patch

test. A longer duration exposure of 24h was required in the patch test to differentiate between the two materials

In a follow-up study, we compared the potential irritation in the BTK from two very similar fabric samples: 100% cotton terry cloth samples subjected to two different drying methods Fig. 2. The tumble-dried sample produced significantly less erythema after two and five exposures (p=0.05), and significantly less dryness after three exposures (p=0.05). Thus, the BTK test system diferentiated between two very similar samples.

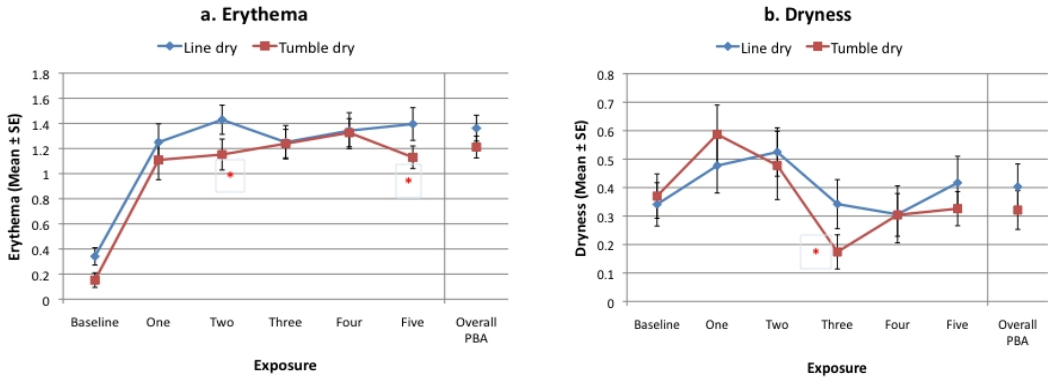

Fig. 2. Evaluating fabrics in the BTK. Subjects wore samples of 100% cotton terry fabrics washed twice in a prototype liquid laundry detergent and either line dried or tumble dried. Samples were worn for 6-h per day for 5 consecutive days. After each exposure, skin sites were scored visually for erythema and dryness. Treatment comparisons performed using generalized estimating equations (GEE) with baseline, treatment block, treatment, day, and subject (random) in the model (* p=0.05)

3.1.2 Tissues

This test used a single exposure of 6h in the BTK to compare the potential for mechanical irritation of two toilet tissue products. Test samples were worn for only 3h before test sites were graded. After grading fresh samples were applied to the same sites for an additional 3h (i.e., 6h total). Results of the study are shown in Fig. 3.Significant differences between the products were observed at both the interim (3h, p=0.0005) and final (6h, p=0.0013) grading point.

3.1.3 Films

Topsheets are nonwoven (fabric-like) coverings on the surface of absorbent consumer products such as feminine protection products, diapers, and incontinent containment systems. They provide a soft layer for skin contact while still allowing moisture to pass into the absorbent material in the core of the product. The BTK was used to compare the potential skin effects from two similar topsheets [13]. The duration of the exposures were lengthened to 16-h in this study due to the inherent mild nature of the test materials. With 16h exposures, there were significant differences between these very similar materials Fig. 4.

The studies presented above confirm that the BTK may be well suited to evaluating fabrics and similar materials for skin compatibility; a feature of potential importance to the textile industry. The test discriminated between different drying methods of the same fabric, between similar toilet tissue products, and between two similar topsheet films used as coverings on the surface of a range of absorbent consumer products

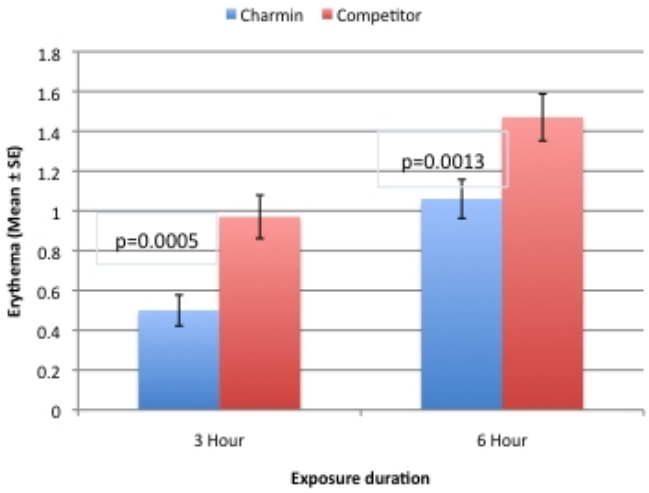

Fig. 3. Evaluating tissues in the BTK. Subjects (N=18) wore samples of toilet tissues in a single day BTK. Products were applied at equivalent weights of 4.8 g, which required 8 sheets of Charmin® Ultra Soft bathroom tissue and 26-28 sheets of a commercially available competitor's product. Samples were worn for 3-h then replaced by fresh samples for an additional 3-h (i.e., 6-h total). Mean erythema is plotted for the 3-h interim scoring and the 6-h final scoring. Comparisons between treatments were performed using Analysis of Variance (ANOVA)

3.2 Evaluating Lotion Transfer

A growing trend for absorbent consumer products is to incorporate lotions and emollients on the surface to improve overall consumer comfort during product use. A reliable means to measure the relative quantities of various lotions that transfer from absorbent products, and the potential effects of other product characteristics, is key for the development of lotion-containing products that provide skin benefits and are appealing to consumers. Lotion transfer has traditionally been evaluated in a clinical in-use test, by applying small sections of thin film dressing tape to the labia as collection surfaces for the lotion. Lotion transfers to the collection tape in the course of normal product wear. Sections of tape are removed after 3h (single pad usage) and 24h (multiple pad usage).

Lotion transfer can also be evaluated in the BTK. As in the in-use clinical studies, small sections of collection tape are applied to the test area. Sections of tape are removed after 3h (single application) and 6h (two applications). The study presented in Fig. 5. shows the previously published results of lotion transfer studies comparing transfer from products with absorbent cores comprised of cellulose and absorbent gel materials(AGM)(15). This study demonstrated lotion transfer measured with BTK exposure Fig. 5a. was more consistent, and resulted in statistical differences that were not found when the transfer was measured in an In-use study exposure Fig. 5b.

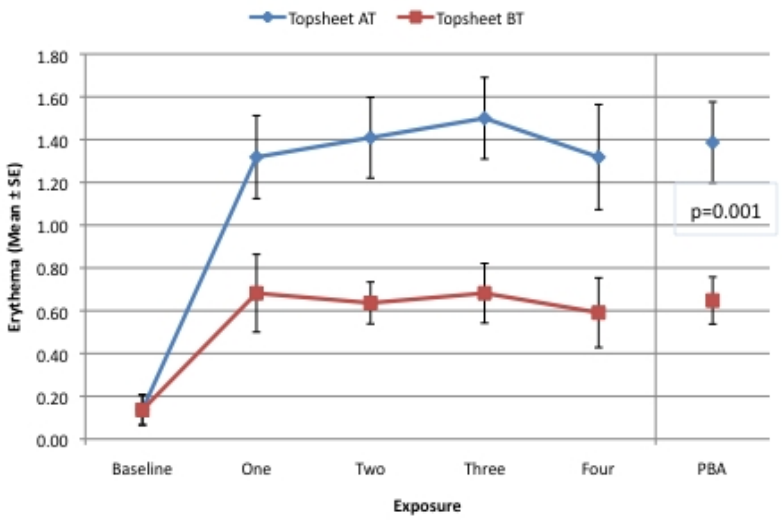

Fig. 4. Using the BTK to evaluate topsheet films. In this study, 11 subjects wore two similar menstrual pad topsheets in a BTK for 16-h per day for 4 applications. Daily afternoon scores and the PBA are plotted. Treatment comparisons were conducted for the PBA values using Wilcoxon's Signed Rank Test. These studies have been detailed in an earlier publication [13]

Lotion transfer and skin condition can be determined on identical samples in order to evaluate the visual benefits of lotion on absorbent products [17]. Fig. 6 presents the results of BTK studies for skin effects conducted in parallel with BTK-lotion transfer studies. A series of feminine protection products were prepared that were identical in all respects except for the amount of lotion applied to the surface, and lotion transfer was measured using a BTK protocol. In parallel, BTKs were conducted to evaluate the skin effects of the lotioned products. The degree of erythema produced in the BTK by these products did not differ consistently with the amount of lotion transferred to the skin Fig. 6a. However, the degree of dryness was inversely proportional to the lotion transfer amount Fig. 6b demonstrating a skin benefit to increasing lotion concentrations on product.

Measuring lotion transfer from absorbent consumer products in a traditional in-use study has proven to be challenging. Differing use patterns and habits among panelists often leads to a high degree of variability in the results. As with other endpoints, the BTK has been shown to produce more consistent results, thereby increasing the ability to detect statistically significant differences between products. When coupled with a standard BTK for skin effects, the visual benefits from lotioned absorbent products can be demonstrated.

3.3 Quantifying Transfer of Materials from Consumer Products to Skin

Quantitative risk assessment (QRA) techniques have become increasingly important in the overall evaluation of consumer product safety. This approach involves a comparison of the lowest observable or no effect doses generated from experimental test systems to the actual doses that a consumer may receive during normal use of the product. QRA approaches have been developed for contact dermatitis for products and ingredients that come into contact with normal skin [18,19] and mucosal surfaces [20] under a variety of usage

conditions. We hypothesized that the BTK may provide an effective model for quantifying the amount of materials that may transfer to skin during the use of consumer products. A convenient method to measure material transfer would help fill an important gap in the safety evaluation process, i.e., quantifying the potential dose to a consumer.

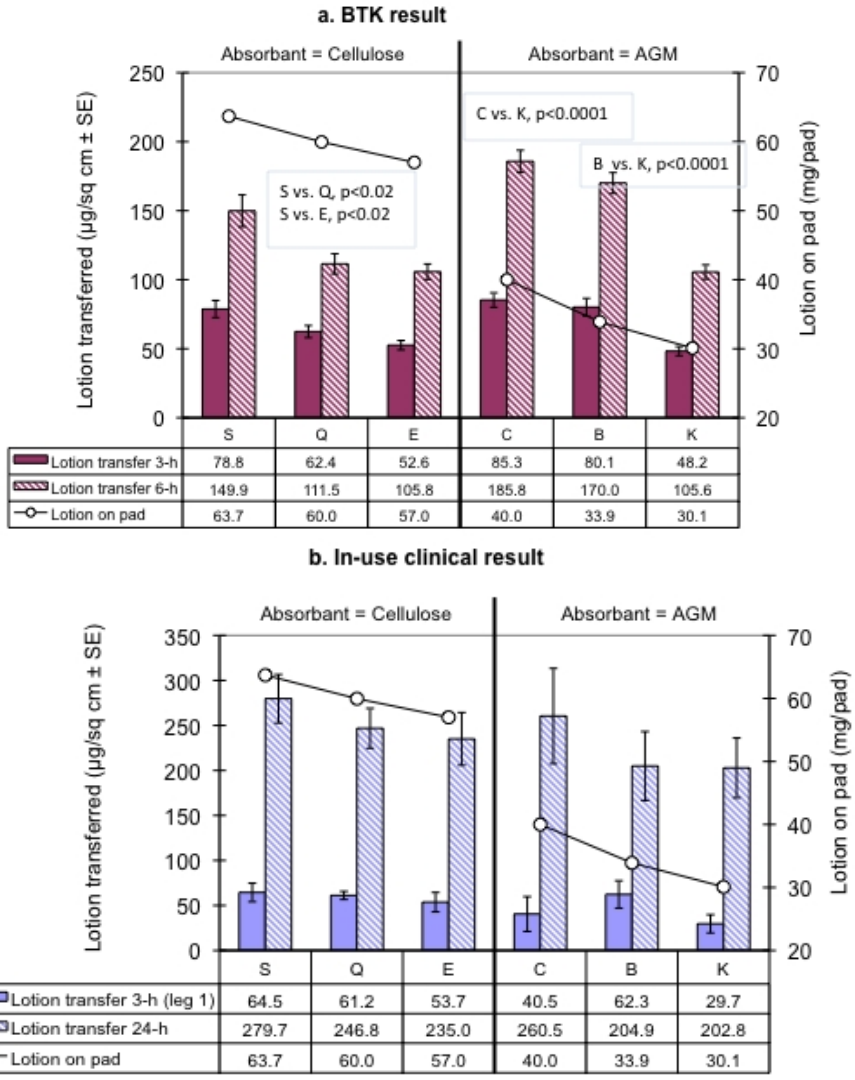

Fig. 5. Measuring lotion transfer in the BTK. Lotion transfer from a series of test products was evaluated using the BTK and the clinical in-use protocols. The figure shows the amount of lotion transferred (in µg/cm^2) compared with the starting amount of lotion on the pad (in mg/pad) for both types of absorbent cores: cellulose and AGM. (A) Lotion transfer after three and six hours in the BTK protocol. Pair wise comparisons resulted in the significant differences at both time points, as shown. (B) Lotion transfer after 3 and 24 hours in the clinical in-use protocol. Pair wise comparisons resulted in no significant differences. These studies have been detailed in an earlier publication [15]

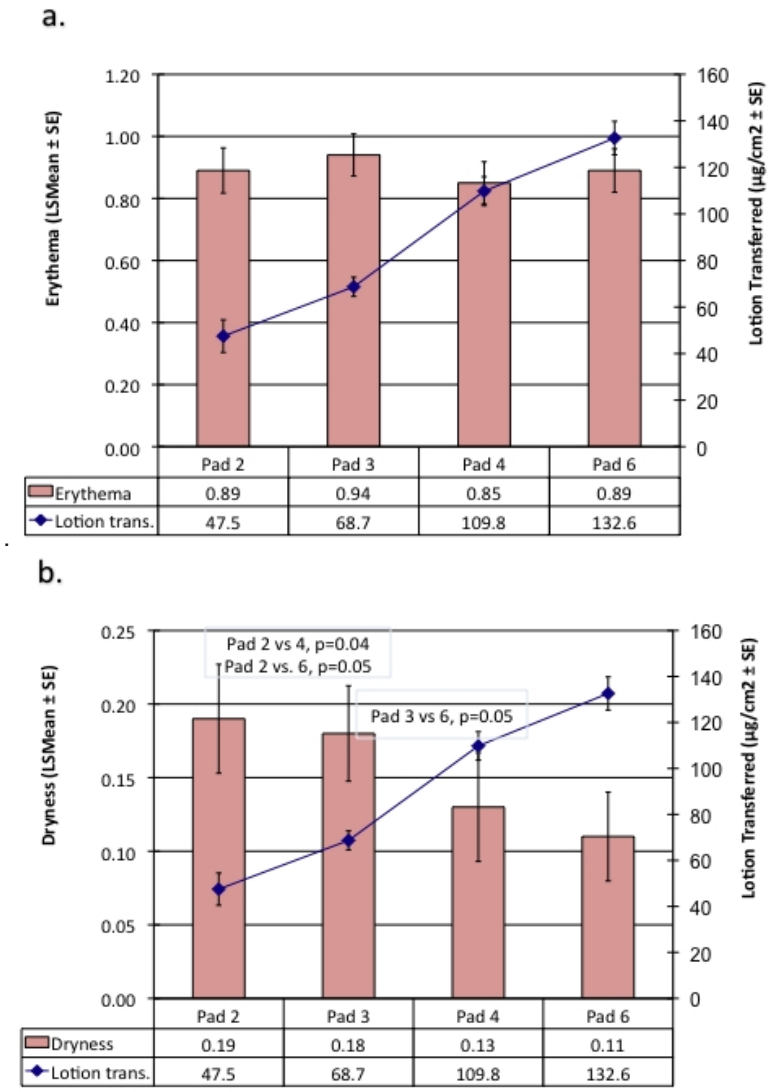

Fig. 6. Effects of various lotion concentrations on skin condition in the BTK. Four sample feminine hygiene pads were constructed identically in all respects except for the amount of lotion on the surface. For each pad, the resulting amount of lotion transfer to the skin was measured (as shown on the right vertical axis). These products were used in the BTK, and skin reactions were scored visually each day after removal of the samples. The graph shows the overall Least Square Means (LSMean ±SE) for the post baseline average scores for erythema (a) and dryness (b) for each product. Differences between treatments were compared using Analysis of Covariance (ANCOVA) on the LSMeans. Pair wise comparisons resulted in no significant differences in erythema. Significant differences in paired comparisons for dryness are shown in graph b. This study has been detailed in an earlier publication [17]

As an initial investigation, we chose to evaluate the transfer of a dye material from a prototype menstrual protection product using the basic protocol described above for the lotion transfer studies. The menstrual protection pads with dye were tested dry, and after wetting the area of the pad which contained the dye with saline. Samples were applied to 20 volunteer panelists with the dry sample on one side and wet sample on the other. Transferred dye material was extracted from collection tapes and determined by HPLC/MS/MS, as described in the Methods section.

Results are shown in Fig. 7.The dye material transferred an average of 10.4±1.4ng from dry product samples (shown at the bottom of the figure). The wet product samples resulted in the transfer of a significantly greater amount; 24.5±1.9ng ($p=0.0001$). Further, if we establish smaller ranges of transferred dye, i.e., 0-10ng, 10-20ng, etc., and plot the percentage of analytical samples falling into each category, we can visualize the distribution of the results. With the dry samples, 55% of samples showed transfer of 10ng or less of the dye material, with only 35% and 10% of samples transferring 10-20 and 20-20ng, respectively. None of the dry samples transferred more than 30%. In comparison, the distribution of values from the wet product is shifted to the right. The majority of the wet samples transferred between 10ng and 40ng, with one sample above 40ng.

The transfer of material measured in the BTK assay can be used to determine the potential dose per unit area in developing a QRA. In the experiment presented in Fig. 7 the exposed area of the collection surface was 4.5cmx4.5cm, or 20.25cm^2. Therefore, the measured amounts of dye expressed in dose per unit area are 0.51ng/cm^2 and 1.21ng/cm^2 for dry and wet product samples, respectively.

3.4 Evaluating Products Intended for Mucous Membrane Exposure

Tampons are an important part of the feminine hygiene market and, as with any other product, they must be evaluated for potential adverse skin effects. The mucous membrane is generally considered to be more susceptible to irritant effects than exposed skin. However, the highly exaggerated conditions of exposure, with the added component of mechanical irritation, make the BTK protocol useful in evaluating the relative potential for irritant effects on mucous membranes. We compared the erythema produced in the BTK test to that produced at various anatomic sites in an in-use clinical study to determine if the mucous membrane sites were more susceptible to irritation under these test conditions [21,22]. For both the experimental and control products, the mean severity of scores on mucosal sites in the in-use study was similar to the mean erythema in the BTK at the AM scores, i.e. after 18h of recovery Fig. 8. The only anatomic site in the in-use study which produced a higher mean erythema than the BTK at the AM scores was the labia minora, which is not mucosal tissue. The mean erythema for the afternoon scores from the BTK test were 3- to 15- fold higher than at any anatomic site scored in the in-use study.

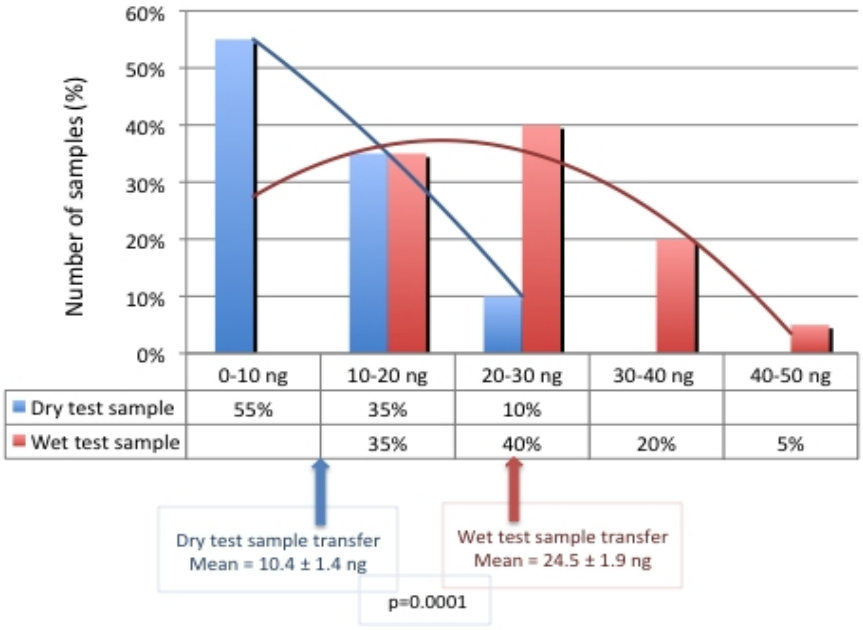

Fig. 7. Comparisons of distribution of dye transfer in the BTK using wet and dry samples. Prototype menstrual protection pads with surface dye were tested on 20 volunteer panelists to compare dye transfer from wet and dry samples. Dry pad samples were applied to one side, and wet pad samples were applied to the other. After 6 hours of exposure, tape sections were removed and transferred dye material was quantified. Ranges of transferred dye amount were established, as shown on the horizontal axis. The percentage of samples with transfer amounts within each range are plotted for wet and dry samples. A best fit polynomial curve was added to each data set. Overall mean transferred amounts for wet and dry samples are shown at the bottom of the graph with the result of a statistical comparison performed using GEE

The BTK offers many advantages as a test site for feminine protection products such as tampons that are intended for exposure to mucous membranes and transitional epithelium. As reviewed by Ledger [10], the epithelium in the area of the popliteal fossa is quite sensitive, but anatomically protected from the confounding factors that can influence the skin of the genitalia, especially during a woman's menstrual cycle. The BTK test site is easily accessible for repeated evaluations using non-intrusive methods. Unlike in-use testing, side-by-side comparisons are possible between the new product and FDA-approved commercial products.

The results shown in Fig. 8 demonstrate that the BTK test method produced erythema reactions that were similar to or more severe than those observed on mucosal sites in the in-use clinical study, indicating the BTK does not underestimate the potential for causing irritation to mucosal and transitional epithelium. Overall, the BTK clinical test showed a higher sensitivity, more rapid turnaround time, higher flexibility and easier implementation compared to the in-use clinical, making the BTK test a useful tool for evaluating products intended for mucous membrane exposure.

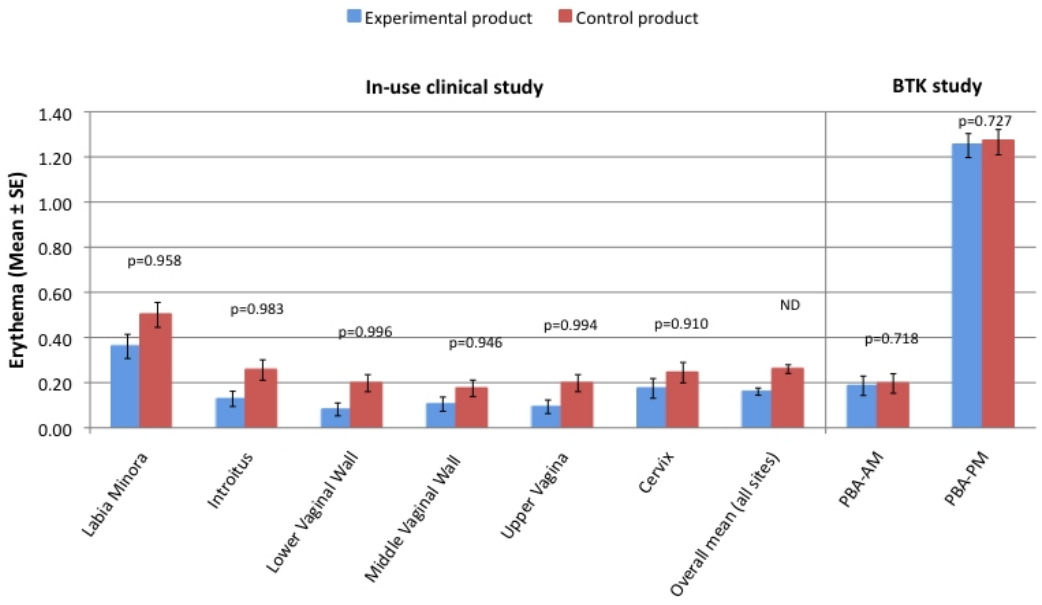

Fig. 8. Using the BTK for products intended for mucous membrane exposure. The in-use clinical was conducted as detailed in the methodology section. The mean erythema for six anatomic sites are presented, along with the overall mean (all sites combined). Results were compared to a BTK test in which a total of 17 subjects wore test samples 6-h per day for 4 consecutive days. Post-baseline averages are reported separately for the morning (i.e. PBA-AM) and afternoon scores (PBA-PM). In the in-use study, treatment comparisons between the two test products were performed using a Cochran–Mantel–Haenszel (CMH) test. In the BTK study, the PBAs were compared using analysis of variance (ANOVA). (ND=Statistical significance was not determined.) This study has been detailed in an earlier publication[21,22]

Importantly, the BTK test is not intended to be a model for mucosal tissue. It is a comparative clinical protocol comparing the skin effects from BTK exposure to a new or experimental product to those from a product with a known safety profile. In this way it is similar to standard patch tests. Products are routinely patched on the upper arm, even if the areas of intended contact are other body sites, i.e. shampoos that contact the scalp, diapers that contact the genital area, or cosmetics that contact facial skin. Although the products come into contact with a different type of tissue in the BTK (squamous cell epithelium) compared to the in-use study (squamous cell and transitional epithelium and mucous membrane), it does not appear that the BTK underestimates the overall potential for irritation. Therefore, the BTK can be used to evaluate potential effects on products intended for contact with mucous membranes.

3.5 Additional Endpoints in the BTK: Enhanced Visual Scoring and Sensory Effects

Visual scoring has been the mainstay of irritation testing for many decades. It is reproducible and non-invasive, and can reliably detect irritation in most circumstances [23,24]. Enhanced visual scoring using cross-polarized light can increase the sensitivity of the test even further by allowing visualization of subsurface changes [16]. Subjective sensory data collected from

panelists during the BTK can provide additional perspective in the overall assessment process [25,26]. This was illustrated by evaluating two menstrual pads (products A and B).

Standard skin irritation test protocols failed to detect differences in the potential skin effects of these two similar products. Yet, consumer surveillance consistently suggested that pad B was perceived as more comfortable and less irritating than pad A [16,22,22]. In a BTK conducted to compare these two products small but significant differences were detected that were consistent with consumer perception. At the morning (recovery scoring) time point Pad A produced a significantly higher mean erythema than Pad B after 6h exposures Fig. 9a. When visual scoring was enhanced using cross-polarized light a significant difference between these two products was detected earlier, after a single 6h exposure Fig. 9b. The ability of the enhanced visual scoring to detect subclinical irritation may indicate that the use of this tool has the potential to increase the sensitivity of standard tests.

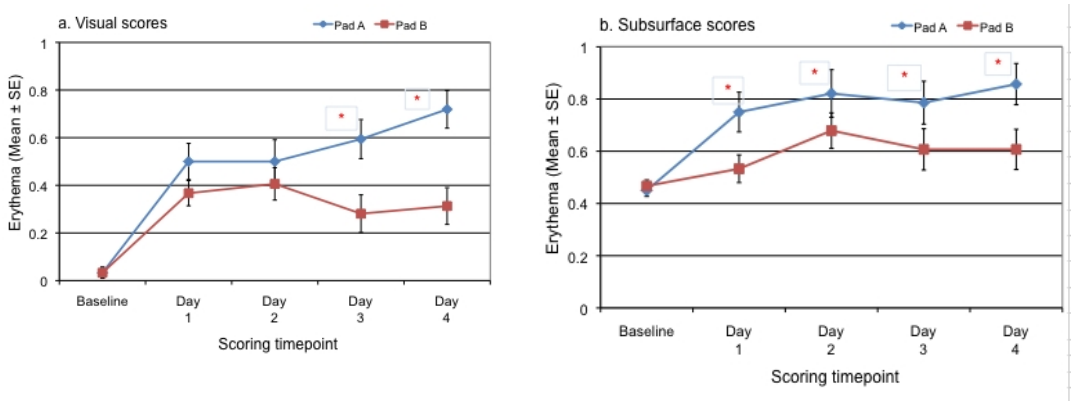

Fig. 9. Standard visual and enhanced visual grading using two similar products in the BTK. Two feminine protection products (pad A; open symbols, and pad B; closed symbols) were evaluated in the BTK. Samples were applied for 6-h per day for 4 consecutive days (14–16 panelists per group). The graphs plot mean erythema (± SE) at each scoring time point (a; visual scoring, and b; enhanced visual scoring for subsurface effects). Treatment comparisons for visual and subsurface scoring were evaluated using the stratified CMH test. (*Significant difference between pad A and pad B, p=0.05) [16]

In this same study, panelists were asked to keep a daily diary of perceived adverse sensations at the test sites [11,25]. Panelists were asked if they experienced one or more of eight specific sensations including: the sample rubbing against the skin, the sample sticking to the skin, chafing, burning, itching, pain, edema, or any other discomfort (described in [26]). Reports of adverse sensory effects were evaluated for a higher occurrence of each individual skin problem with one treatment vs. another. Results of the investigation showed a higher percentage of the subjects reporting a ‘‘burning’’ sensation at the test site for product A compared to product B Fig. 10.

The BTK with visual scoring has proven to be a versatile test methodology to evaluate skin effects that are barely discernable in other testing protocols. Protocol modifications such as the use of polarized light to enhance visual scoring and the addition of collecting sensory effects have expanded the utility of the BTK test protocol in safety and investigative programs.

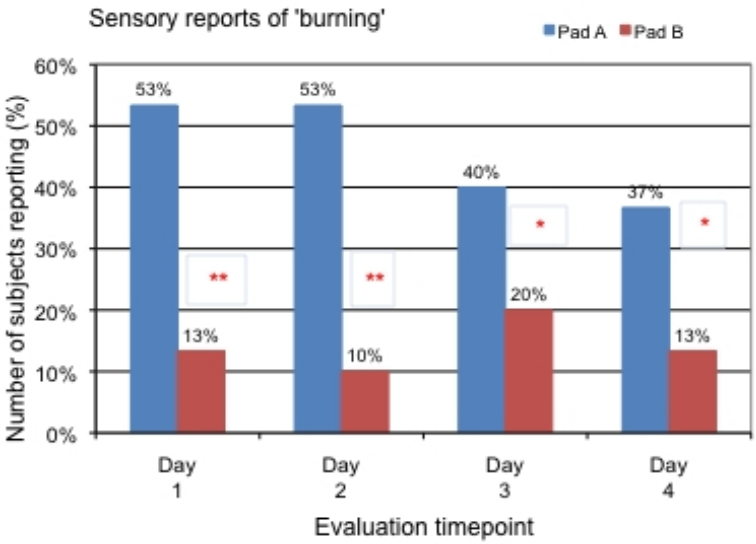

Fig. 10. Reported sensory effects using two similar products in the BTK. Each of the panelists participating in the experiment described in the previous caption was asked to keep a daily diary of skin problems experienced at the test sites. The graph plots the portion of the subject population reporting sensations of 'burning' at the test sites during each patch application. Treatment comparisons for the sensory effect were evaluated using McNemar's test. (*Significant difference between pad A and pad B, p=0.05, ** Significant difference between pad A and pad B, p=0.001) [16]

4. CONCLUSION

The BTK test was developed as a method for evaluating potential skin effects of feminine protection products intended for prolonged contact with the skin of the urogenital region that eliminates the difficulties of the in-use clinical test without compromising the quality of the results. For this application the BTK has demonstrated a number of specific advantages over clinical in-use testing. The ability to compare products side-by-side in the BTK, and the absence of many of the confounding factors that may impact in-use testing yields reliable, reproducible results. The highly exaggerated exposure in the BTK evaluates both chemical and mechanical irritation, and enables discrimination between very similar products. The BTK test results demonstrate that the model can be used successfully to evaluate products intended for mucous membrane exposure, such as tampons Fig. 8. For investigative studies the skin can be compromised prior to testing, and products can be applied wet to evaluate this extreme condition of exposure. These options are not available with in-use test protocols, but provide important information for some categories of products such as diapers and incontinent containment products. In addition, the BTK is relatively inexpensive and easy to conduct, and results can be available in days rather than weeks facilitating the overall safety evaluation process.

The usefulness of the BTK goes beyond the original intent of evaluating the potential skin effects of feminine protection products. We have successfully used the BTK to compare fabrics, tissues and films indicating the test model may be useful in textile development. Variations in skin effects from different laundry treatments on the identical fabrics can be detected in the BTK Fig. 2. as well as differences in similar tissue and film materials Figs. 3

and 4. The model can be used to measure lotion transfer and skin benefits from lotioned products Figs. 5 and 6. The transfer of other materials can be measured to fill an important gap in the development of quantitative exposure assessments Fig. 7.

The addition of endpoint measures, such as enhanced visual scoring Fig. 9 and sensory effects Fig. 10 can increase the ability to differentiate between very similar products without requiring other protocol modifications [16,25,26].

CONSENT

A consent to publish is not applicable for these investigations. All studies were conducted on healthy volunteers, and no personal information, medical or otherwise, was released on any of the subjects. The author declares that each study participant read and signed an informed consent document prior to study commencement regarding the study design and any potential adverse effects.

ETHICAL APPROVAL

All study protocols were conducted in accordance with the Declaration of Helsinki, and were reviewed and accepted by the Institutional Review Board of the research facility [27].

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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