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Effects of Depilatory Cream Formulation and Contact Time on Mouse Skin

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Depilatory creams are widely used in research to remove hair in preparation for surgery, imaging, and other procedures. However, few studies have evaluated the effects of these creams on mouse skin. We sought to determine the cutaneous effects of 2 different depilatory formulations of a widely used brand as related to the duration of exposure. We compared a standard body formula [BF] and a facial formula [FF] that is marketed as being more gentle on skin. The cream was applied to one flank for 15, 30, 60, or 120 s; hair on the contralateral flank was clipped and used as a control. Treatment and control skin were scored for gross lesions (erythema, ulceration, and edema), degree of depilation, and histopathologic changes. C57BL/6J (B6) and Crl:CD-1(ICR) (CD-1) mice were used to allow comparison of an inbred/pigmented strain to an outbred/albino strain. BF caused significant cutaneous injury to both strains of mice, whereas FF produced significant cutaneous injury only in CD-1 mice. Both strains showed gross skin erythema, with the most severe erythema seen in CD-1 mice treated with BF. Contact time did not affect histopathologic changes or gross erythema. Both formulations produced depilation comparable to clipping in both strains when left on for a sufficient duration. In CD-1, mice, BF required at least 15 s of exposure, whereas FF required at least 120 s. In B6 mice, BF required at least 30 s of exposure, whereas FF required at least 120 s. The 2 mouse strains did not show statistically significant differences in erythema or histopathologic lesions. Overall, these depilatory creams were comparable to clippers for hair removal from mice but they produce cutaneous injury that may affect research outcomes.

Abbreviations: BF, body formula; FF, facial formula; B6, C57BL/6J; CD-1, Crl:CD-1(ICR)

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Introduction

Depilatory creams have been used in human surgical procedures since the 1950s to remove hair preoperatively and have been found to help reduce surgical site infections in some instances.[1,11,23] While depilatory creams are no longer commonly used preoperatively in humans, they are still used to remove hair from mouse skin before surgery, imaging, and other procedures.[1,5,12,24] However, although the use of depilatory creams in rodent research is relatively common, few studies have evaluated the cutaneous injury caused by these creams even though they contain active ingredients known to be irritants and corrosives.[6,22]

A previous study found that the product Nair was the most commonly used depilatory cream in animal research, with 59% of questionnaire respondents using that brand.[14] The body formulation (BF) of Nair contains 3 active ingredients that break disulfide bonds in hair to facilitate removal: potassium thioglycolate, calcium hydroxide, and sodium hydroxide. Of these, potassium thioglycolate can cause irritation and sodium hydroxide (also known as lye) can be corrosive and damaging to skin.[22] In addition to the standard BF of Nair, a facial formula (FF) is marketed as being "gentler" on skin.[16] Despite these claims, the 2 formulations contain many of the same active ingredients (calcium hydroxide, sodium hydroxide, and calcium thioglycolate).

Another concern with using depilatory creams on mice is that the instructions for contact time were developed for human skin and hair and are typically 3 to 5 min. Contact times used in experimental studies in rodents range from 5 s to 10 min,[1,5,21] with 73% of respondents of a recent survey reporting contact times of 30 s to 2 min.[14] A previous study using a 10-s exposure to the BF in B6 mice identified epidermal hyperplasia, dermal fibroplasia, and infiltration of neutrophils.[11] In another study, application for 15 to 20 s led to epidermal hyperplasia and an increase in immune cells as compared with just clipping.[1] Both of these studies used relatively short contact times compared with the reported average of 30 s to 2 min,[14] suggesting that most application protocols may induce significant localized cutaneous injury. This hypothesis is supported by a recent study in rabbits (*Oryctolagus cuniculus*) in which the BF was applied for 3 to 10 min and resulted in histopathology results similar to those seen in mice, namely, increased inflammatory cell infiltrates, epidermal hyperplasia, and dermal necrosis.[12]

We sought to characterize the effects of body and facial depilatory creams and contact times to determine what combinations can achieve depilation yet minimize cutaneous injury in mice. Specifically, we evaluated the changes that occurred in the skin of 2 strains of mice after topical application of 2 different depilatory creams for 4 different contact times. We hypothesized that shorter contact times would result in less cutaneous injury and that the FF would cause less cutaneous injury than the BF. We also predicted that cutaneous injury would be more severe in the inbred strain B6 compared with the outbred stock CD-1 due to the propensity of B6 mice to develop skin disorders such as ulcerative dermatitis.[8,10]

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Materials and Methods

Animal housing and husbandry. Two strains of 4- to 6-wk-old mice (C57BL/6J, *n* = 64; Crl:CD-1(ICR), *n* = 64) were purchased from Charles River Laboratories (Wilmington, MA). Only females were purchased for this study to reduce the risk of skin injury due to fighting, which is more common among male mice.[3,19] All experimental procedures were approved by the IACUC at the University of Minnesota and all mice were housed in accordance with AAALAC accreditation and the *Guide for the Care and Use of Laboratory Animals*.[9] Mice were housed 3 to 5 per cage in static caging, with irradiated corncob bedding (Envigo Teklad 7902, Indianapolis, IN) and unlimited access to reverse-osmosis water and a standard rodent diet (Irradiated Laboratory Animal Diet 2919, Envigo Teklad; Indianapolis, IN). Macroenvironment conditions were 20 to 26 °C (68 to 79 °F), 30% to 70% humidity, and a 14:10-h light:dark cycle (on/off at 0600/2000).

Contact-bedding sentinel mice in the facility were free from mouse parvovirus, minute virus of mice, mouse hepatitis virus, mouse rotavirus A, Theiler murine encephalomyelitis virus, Sendai virus, pneumonia virus of mice, reovirus, mousepox, mouse adenovirus type 1 and 2, polyoma virus, lymphocytic choriomeningitis virus, mouse cytomegalovirus, *Mycoplasma pulmonis*, *Clostridium piliforme*, *Filobacterium rodentium*, *Encephalitozoon cuniculi*, fur mites, and pinworms.

Experimental design. Mice were randomly assigned to 1 of 2 depilatory cream treatments: BF (Nair Softening Baby Oil, Church and Dwight, Ewing, NJ) or FF (Nair Face Cream, Church and Dwight, Ewing, NJ). Within these groups, mice were randomly assigned to 1 of 4 contact times: 15, 30, 60, or 120 s,, with 8 mice in each group. Only BF or FF was applied to the treated flank (no prior clipping) while hair on the contralateral flank was clipped and used as a control. Mice were anesthetized for application of depilatory creams on day 0. Photographs of both treated and control skin were taken from unanesthetized mice on days 1, 2, and 3. On day 3, mice were anesthetized again and then euthanized. Anesthesia and euthanasia were as described in the next section. Skin samples were then collected. Skin samples were fixed in 10% buffered formalin and sent to IDEXX BioAnalytics (IDEXX Laboratories, Columbia, MO) for processing, sectioning, and staining. The slides were then analyzed by a board-certified veterinary pathologist who was blind to all treatment groups.

To ensure that clinical and histologic lesions were due to the active ingredients in the cream rather than to the vehicle, we performed a pilot study in B6 mice to evaluate the effects of the vehicle (an oil-water emulsion[6]) on injury to the skin. BF and the vehicle (an agitated 50:50 mineral oil–sterile water combination; Mineral oil, heavy; Sigma-Aldrich; St. Louis, MO) were left on the skin (on opposite, non-clipped flanks) for 2 min, then removed as described above. Samples were collected after 3, 5, 7, and 10 d (2 mice per time point) and analyzed by same pathologist who evaluated samples in the main study.

Skin preparation and collection. Mice were treated with 2 mg/kg of buprenorphine SR-LAB administered subcutaneously on the dorsum (ZooPharm, Fort Collins, CO) 2 to 4 h before skin preparation to reduce scratching related to pain or itch at the application site. Mice were anesthetized with isoflurane (Piramal Enterprises Limited, Telangana, India) to effect. One randomly-selected flank was clipped using electric clippers (WAHL Professional BravMini, Sterling, IL), and depilatory cream was applied to the contralateral flank. The same clippers were used for all mice and flanks were clipped first in the direction of hair growth, then against the hair growth. The creams were applied in an approximately 3 cm × 1.5 cm rectangle along the flank at a thickness of approximately 0.5 cm. After the defined treatment time in each group, the cream was gently removed with sterile saline-soaked gauze and the mouse was recovered from anesthesia.

On day 3, the mice were again anesthetized with isoflurane and were then euthanized by cervical dislocation. An area of approximately 1-cm^2 of skin was removed from both the treated and the control flanks by using a #10 scalpel blade. The tissues were placed in 10% neutral buffered formalin for a minimum of 48 h before being sent to IDEXX Laboratories (Columbia, MO) for tissue processing, sectioning, and staining. Some samples were lost during transport to IDEXX before they

Scoring Type	Criteria	Value
Erythema Score		
	No erythema	0
	Very slight erythema	1
	Well defined erythema	2
	Moderate to defined erythema	3
	Severe erythema (beet red)	4
Edema Score		
	No edema	0
	Very slight edema	1
	Slight edema (defined edges)	2
	Moderate edema (raised area ~1 mm)	3
	Severe edema (raised area > 1 mm)	4
Ulceration Score		
	No ulceration	0
	Small ulceration (< 3 mm area)	1
	Moderate ulceration (< 5 mm area)	2
	Large area of ulceration (> 5 mm area)	3

Figure 1. Skin scoring system (modified from reference 7) used by blind reviewers to provide qualitative values for gross skin photos.

Areas Evaluated	Parameters Evaluated*
Dermal Inflammation	Numbers of perivascular, perifollicular to interstitial mast cells, lymphocytes, and fewer granulocytic cells.
Dermal Fibroplasia	Increased numbers of spindle-shaped cells consistent with fibroblasts associated with increased dermal eosinophilic matrix (collagenous matrix).
Hair Follicles	Dermal perifollicular mixed inflammatory cells, follicular hyperkeratosis, luminal folliculitis, or follicular wall disruption (furunculosis).
Thickness of Stratum Corneum and Contents	Increased quantity of compact orthokeratotic hyperkeratosis and/or focal or multifocal parakeratosis (nuclear retention in the stratum corneum), intracorneal crust (s) with or without coccoid bacteria and/or hair shafts.
Thickness of Cellular Epidermis (Ancanthosis) and contents	Acanthosis (a form of epidermal hyperplasia), which consisted of an increase in the number of cells in the epidermis either focally, multifocally, or diffusely. Cellular crust (s) with or without coccoid bacteria, and/or hair shafts. Rare foci of degeneration
***Criteria for Evaluation:**	No lesions = 0 Minimal lesions = 1 Moderate lesions = 2 Robust (more extensive) lesions = 3

Figure 2. Areas of the skin and parameters evaluated histologically by a veterinary pathologist who was initially blind to treatments. This grading scale pertains to this study only.

could be processed. This resulted in different *n* values for some B6 treatment groups (BF15 *n* = 7; BF30 *n* = 7; BF60 *n* = 7; FF15 *n* = 7; FF60 *n* = 6; FF120 *n* = 5).

Gross evaluation. Depilated sites were photographed (Samsung Galaxy S10, Suwon-si, South Korea) immediately after cream removal and every 24 h thereafter for 3 d. Three reviewers who were blind to the treatment group used a modified Draize Skin Test scoring system[7] to assess the photographs (Figure 1). Each reviewer scored the treated skin for erythema (score 0 to 4) and ulceration (score 0 to 3). In addition, the skin was scored for edema (Figure 1) immediately after cream removal and once daily by the same researcher. The edema score was recorded without the use of photographs because the 3-dimensional nature of edema was not visible in photographs, as was deter-

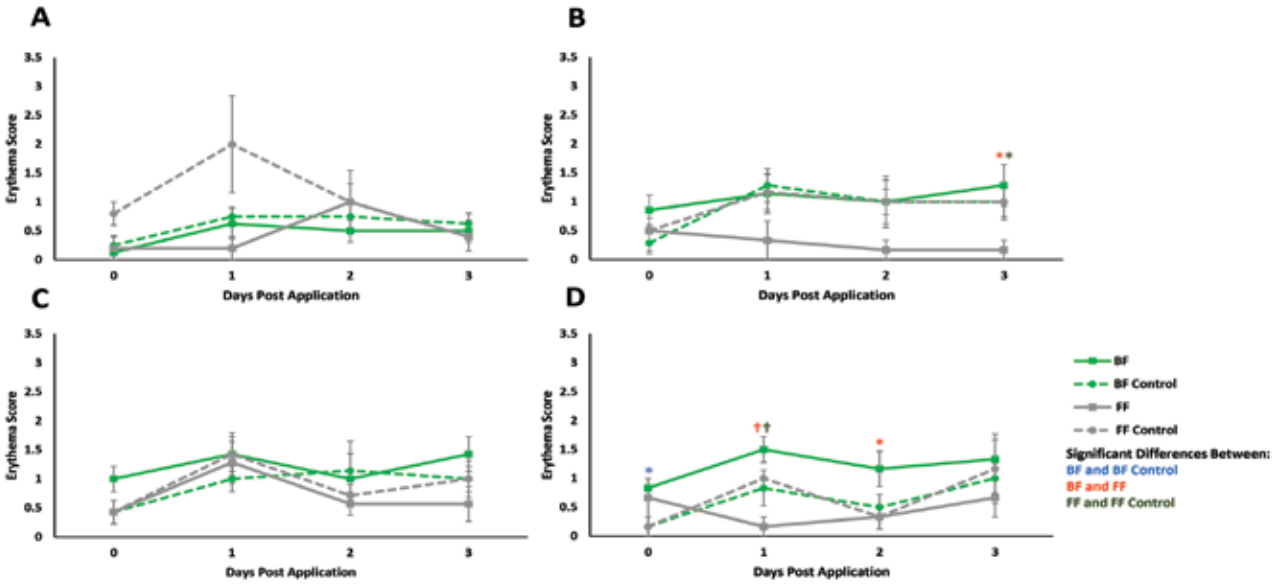

Figure 3. The effects of contact time on erythema scores in B6 mice. Average erythema scores ± SEM of B6 mice treated with BF or FF for 15 (A), 30 (B), 60 (C), or 120 s (D). Statistically significant differences between treatments and controls or between treatments (see legend) are as follows: *, $P \leq 0.05$; †, $P \leq 0.01$

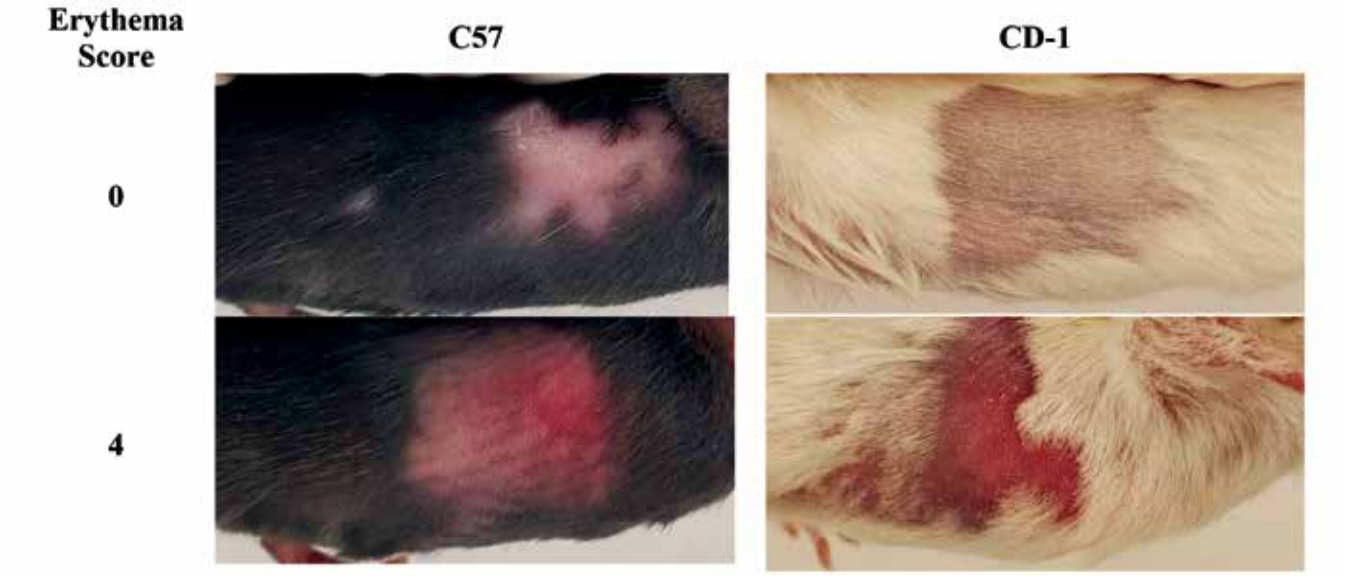

Figure 4. Representative photographs of the lowest (0) and highest (4) possible erythema scores in both B6 (left column) and CD-1 mice (right column). All photos are orientated with the head of the animal to the right and the tail to the left.

mined during data collection for the pilot study. The researcher who recorded the edema score was not blind to the treatments.

Photographs were also assessed for depilation to determine how well each treatment removed hair. Three reviewers who were blind to treatment groups evaluated a section of treated skin that was approximately 1-cm^2 section to estimate the degree of depilation, ranging from 0% to 100%, with 0% representing no depilation (no hair removed) and 100% representing total depilation (all hair removed).

Histopathologic evaluation. Two sections of skin from each of 116 mice were stained with hematoxylin and eosin (H and E) and examined histologically (sections from 12 B6 mice were lost during transport). One section of control skin and one section of treated skin were examined from each mouse. Dermal inflammation, dermal fibroplasia, follicular lesions, stratum corneal thickness, and epidermal hyperplasia (acanthosis) were evaluated on a subjective scale: no lesions = 0, minimal lesions = 1, moderate lesions = 2, and severe lesions = 3. The scoring was based on the portions of the sections that were most affected; the degree of damage often varied within the individual section. The grading scale (Figure 2) pertains only to the current study.

Statistical analysis. Histopathologic, depilatory, and Draize skin scores are shown as means ± SEM. Histopathologic and Draize skin scores are categorical data and hence cannot have

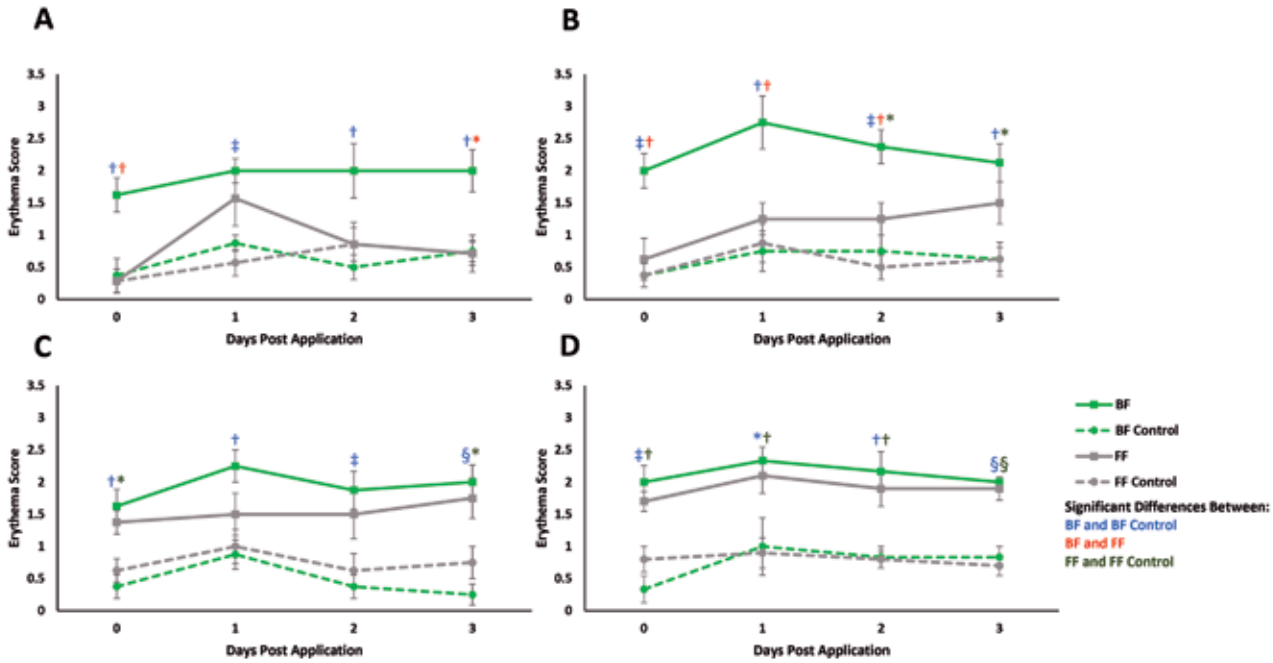

Figure 5. The effects of contact time on erythema scores over time in CD-1 mice. Average erythema scores ± SEM of CD-1 mice treated with BF or FF for 15 (A), 30 (B), 60 (C), or 120 s (D). Statistically significant differences between treatments and controls or between treatments (see legend) are as follows: *, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; §, $P \leq 0.0001$.

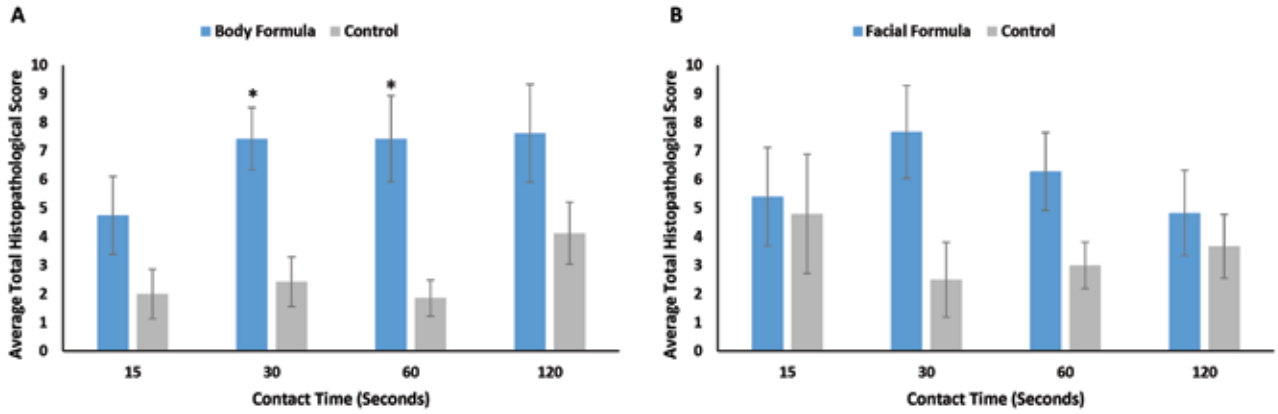

Figure 6. The effects of contact time on total histopathology scores in B6 mice 3 d after application of cream. Average total histopathology scores ± SEM of B6 mice treated with BF (A) or FF (B) for 15, 30, 60, or 120 s. Statistically significant differences between treated skin and control (clipped) skin are indicated as follows: *, $P \leq 0.05$.

normal distributions. Therefore, significance was tested for those measures by using the nonparametric Wilcoxon Method. Depilatory scores were analyzed by using repeated-measures ANOVA. Data were statistically analyzed using JMP Pro 16 (SAS Institute, Cary, NC), and $P \leq 0.05$ was used to determine statistical significance.

Results

Pilot study. Before the main study was conducted, a pilot study was performed to evaluate the effects of the Nair vehicle (an oil-water emulsion[6]) on injury to the skin The vehicle did not influence the variables measured as compared with control values. Indicators of dermal injury were highest in samples that were collected at 3 d after treatment as compared with those collected at 5, 7, or 10 days later. Therefore, a vehicle control was not included in the main study, and a timeline of 3 d was used for the main study.

Gross skin lesions. Erythema scores in B6 mice were not different between BF, FF, and controls in the 15-s treatment group (Figure 3 A). Scores were not significantly elevated at day 1 for the FF control, (Figure 3 A). Erythema scores of BF treated skin were higher ($P \leq 0.05$) than FF treated skin at day 3 in the 30-s group (Figure 3 B). No significant differences were present between any groups at the 60-s time point (Figure 3 C). Erythema scores were higher in BF than in FF treated mice on days 1 and 2 in the 120-s group ($P \leq 0.01$ and $P \leq 0.05$, respectively; Figure 3 D). On day 0 of the 120-s treatment, erythema scores were increased in BF treated skin compared with the control ($P \leq 0.05$; Figure 3 D). Representative photos of erythema scores 0 and 4 in B6 mice are shown in Figure 4. Time had no statistically significant effects on erythema scores in any treatment groups. No ulcerations or edema were noted in any group.

In CD-1 mice, BF caused a significant increase ($P \leq 0.05$) in erythema scores in all treatment groups at all time points as compared with the control (Figure 5). BF caused significantly higher erythema scores than did FF at several time points in the 15- and 30-s groups ($P \leq 0.05$; Figure 5 A and B). Both formulations caused significant erythema compared to control skin at 60 and 120 s ($P \leq 0.05$; Figure 5 C and D). Longer application times did not result in statistically significant changes in erythema scores for any treatment group. Representative photos of erythema scores 0 and 4 in CD-1 mice are shown in Figure 4. None of the groups showed edema. Ten mice had ulceration scores greater than 0; however, the ulcers were all

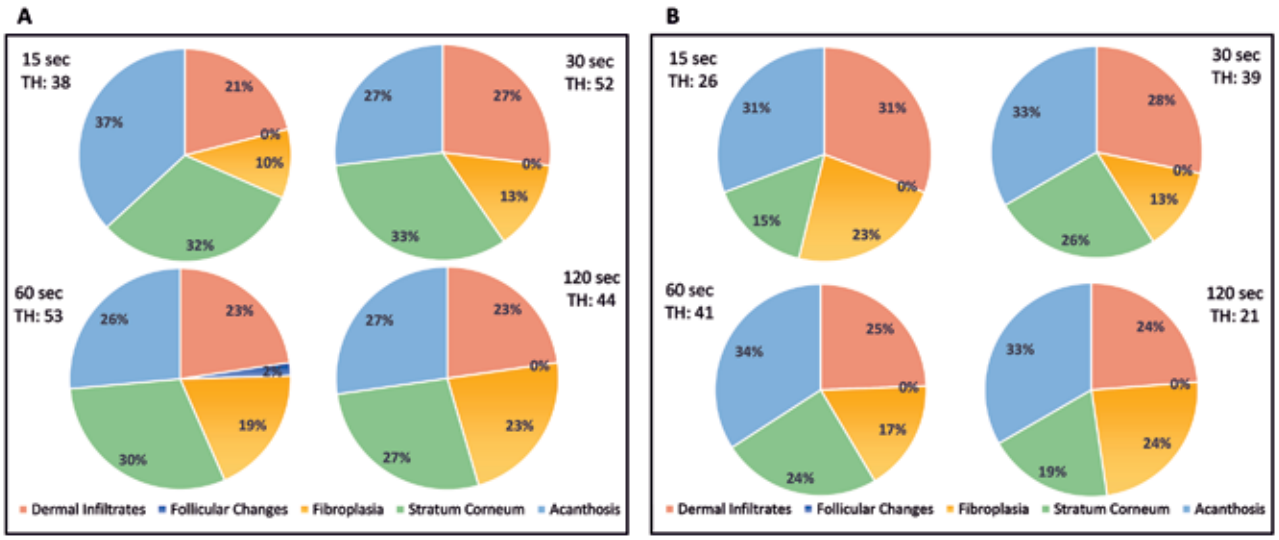

Figure 7. Total proportions of the 5 criteria that make up the total histopathology scores (TH) in B6 mice treated with BF (A) or FF (B) at all contact times tested.

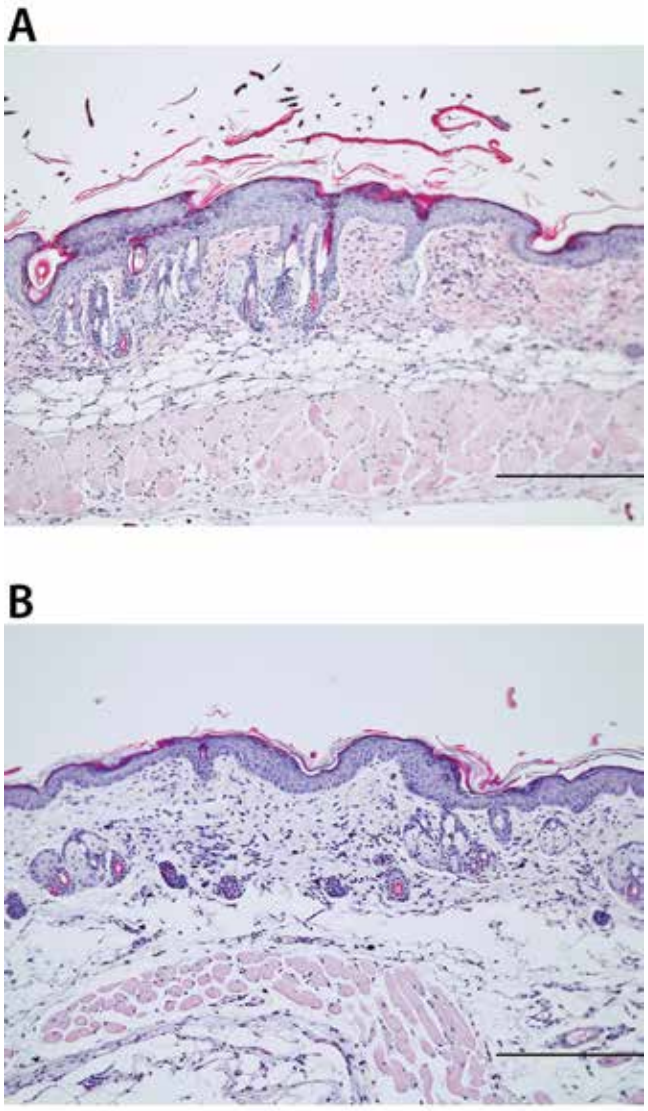

Figure 8. (A) Photomicrograph of B6 (#50) mouse treated with FF for 30 s. The epidermal surface is thickened by layers of compact stratum corneum lifting from the surface and forming scales that can be noted clinically. The epidermis is extensively thickened by acanthosis, and the dermis contains numerous mixed inflammatory cells and moderate to extensive fibroplasia. Hematoxylin and eosin staining, magnification 100×, bar = 200 µm. (B) Photomicrograph of CD-1 (#93) mouse treated with the body formulation for 15 s. The epidermal surface is thickened by moderate compact and basketweave stratum corneum, and moderate to extensive acanthosis. The dermis contains numerous mixed inflammatory cells and moderate fibroplasia. Hematoxylin and eosin staining, magnification 100×, bar = 200 µm.

found immediately after clipping and were clipper cuts rather than ulcerations.

An unexpected observation during data collection and the blind review of gross skin lesions was dry skin with scaling (flaking); this was observed repeatedly after treatment with both formulations in both strains. No controls were noted to have dry and/or flaking skin. Overall, 34% of B6 mice and 31% of CD-1 mice developed flaking skin.

Skin histopathology. Average total histopathologic scores in B6 mice treated with depilatory cream were higher than controls at all time points for both BF and FF (Figure 6 A and B). This effect was only significant ($P < 0.05$) when BF remained on the skin for 30 or 60 s (Figure 6 A). Histopathologic scores between contact times or formulation types did not differ significantly. Figure 7 illustrates the proportions of each of the 5 histopathologic criteria (dermal cellular infiltrates, dermal fibroplasia, follicular changes, stratum corneal changes, and epidermal hyperplasia [acanthosis]) that made up the total histopathologic score for both BF and FF treated mice (Figure 7 A and B, respectively). Follicular changes were the least observed histologic change across all groups. Dermal cellular infiltrates, stratum corneal changes, and acanthosis scores made up the majority (> 75%) of the total histopathologic score in B6 mice (Figure 8 A).

Average total histopathologic scores in CD-1 mice were significantly higher ($P < 0.05$) for every contact duration for both formulations as compared with controls (Figure 9 A and B). No significant changes in histopathologic scores were detected when comparing between formulations and across contact times. Figure 10 shows the proportional distribution of the histopathologic criteria that comprise the total score. Like B6 mice, most of the changes were dermal cellular infiltrates, stratum corneal changes, and acanthosis, with follicular changes making up the smallest proportion of the score for all treatment groups. Figure 8 B shows an example of histopathologic changes in CD-1 mice.

Depilation scores. Depilation scores were expressed as a percentage of depilation in an area of skin that was approximately 1-cm^2 (0% = no depilation, 100% = total depilation). Depilation in B6 mice varied between treated and control flanks (Figure 11). When left on the skin for 15 s, both BF and FF removed significantly less hair than did clipping ($P \leq 0.05$; Figure 11 A). When left on for 30 (Figure 11 B) or 60 s (Figure 11 C), FF still removed significantly less hair than clipping while BF depilated the skin to a degree comparable to clippers ($P \leq 0.05$). At 120 s (Figure 11 D), FF performed no differently than clippers while BF removed significantly more hair than clippers at Days 0 and 3 ($P \leq 0.01$).

In CD-1 mice, BF provided significantly more depilation ($P < 0.05$) at all time points in all treatment groups than did clipping (Figure 12). Conversely, FF provided significantly more depilation than clippers only when left on for 120 s ($P \leq 0.05$; Figure 12 D). Anecdotally, we found CD-1 hair to be thicker and more difficult to clip, which may have contributed to the lower depilation scores. When comparing formulations, BF produced significantly more depilation than FF in the 15- and 30-s treatment groups ($P \leq 0.05$; Figure 12 A and B) and at most time points in the 60- and 120-s treatment groups ($P \leq 0.05$; Figure 12 C and D).

Fragrance. Both depilatory formulations had a strong and noticeable fragrance. The odor of the FF cream seemed to be much stronger scent than that of the BF.

Discussion

The depilatory creams caused significant cutaneous injury to both B6 and CD-1 mice as compared with hair clipping. Of the 2 formulations tested, BF caused significant cutaneous injury including dermal inflammation in both strains while FF produced significant injury only in CD-1 mice. Erythema also developed in both strains, with the most severe erythema seen in CD-1 mice treated with BF. In B6 mice, BF produced the same depilation scores as clipping only at contact times of ≥ 30 s while FF was comparable to clipping only after 120 s of contact time. Conversely, in CD-1 mice, BF was superior to clipping at all times tested, whereas FF only outperformed clippers with 120 s of exposure.

Our original hypothesis was that shorter contact times would result in less cutaneous injury. However, this was not the case. Contact time did not significantly affect the total histopathologic scores for either formulation or mouse strain, indicating that

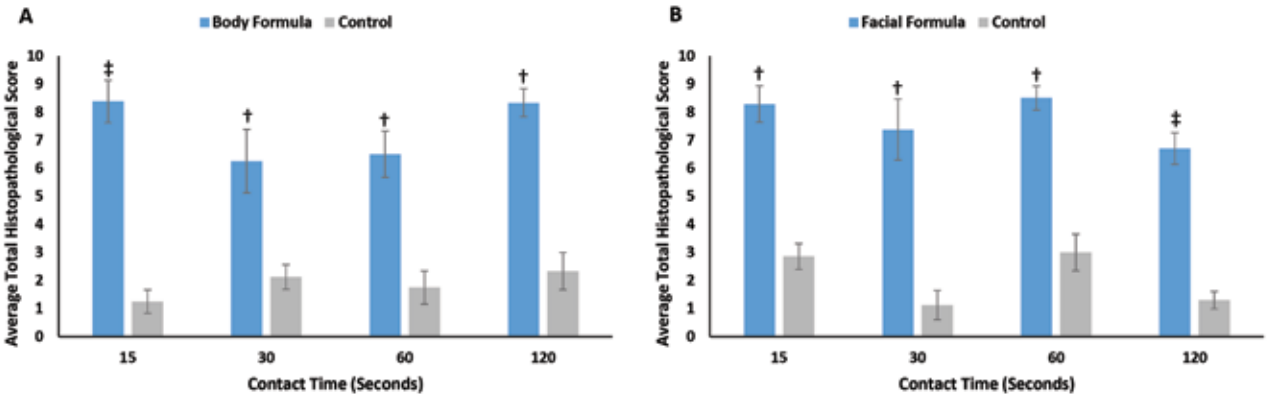

Figure 9. The effects of contact time on total histopathology scores in CD-1 mice at 3 d after application. Average total histopathology scores ± SEM of CD-1 mice treated with BF (A) or FF (B) for 15, 30, 60, or 120 s. Statistically significant differences between treated skin and control (clipped) skin are as follows: †, $P \leq 0.01$; ‡, $P \leq 0.001$.

BF and FF produce cutaneous injury regardless of how long the creams remain on the skin and that a contact duration that will produce adequate depilation without also damaging the skin may not be achievable. Whether the local cutaneous injury seen here significantly affected systemic inflammatory markers was not determined. Further studies into the potential systemic effects of depilatory creams are warranted due to the potential for systemic inflammation to confound other study goals.

Our second hypothesis was that the facial formulation would cause less cutaneous injury than the body formulation. This hypothesis was not supported in CD-1 mice, which had significant injury scores at all time points for both formulations. Conversely, B6 mice had significantly higher injury scores after exposure to BF as opposed to FF. While this outcome may indicate that FF produces less injury in B6 mice than does BF, an alternative reason for this difference may be related to the different sample sizes. Twelve B6 skin samples were lost during transport to the processing laboratory, with the majority of lost samples belonging to FF-treated mice. This loss of samples resulted in sample sizes ranging from $n = 6$ to 8 mice in various treatment groups, perhaps resulting in more variation that masked a real effect. For example, in Figure 3 A at day 1, the FF control erythema score has large error bars and is not significantly higher than that of the FF treatment group. This result may have been due to a combination of a lower FF15 group size ($n = 7$) together with clipper rash in 1 or 2 control mice. Further studies of FF effects on B6 skin are warranted before a conclusion can be drawn regarding differences in BF- and FF-induced skin damage.

The proportions of the 5 histologic criteria that made up the total histopathologic scores of the depilatory-treated skin also did not differ significantly based on formulation. In CD-1 mice, dermal cellular infiltrates, stratum corneal changes, and acanthosis scores made up over 80% of the total histopathologic scores for both BF and FF at all time points. Total histopathologic scores of B6 mice were also mostly made up of dermal cellular infiltrates, stratum corneal changes, and acanthosis scores (over 75% of the total score), but in several treatment groups fibroplasia scores made up an equivalent or larger portion of the total. These slight differences further suggest that the depilatory cream may produce different reactions in different strains of mice. This difference in reactions may variably affect research outcomes depending on the mouse strain.

From a clinical standpoint, BF and FF both produced significant erythema in CD-1 mice, although the erythema scores were only statistically significantly elevated in B6 mice that were

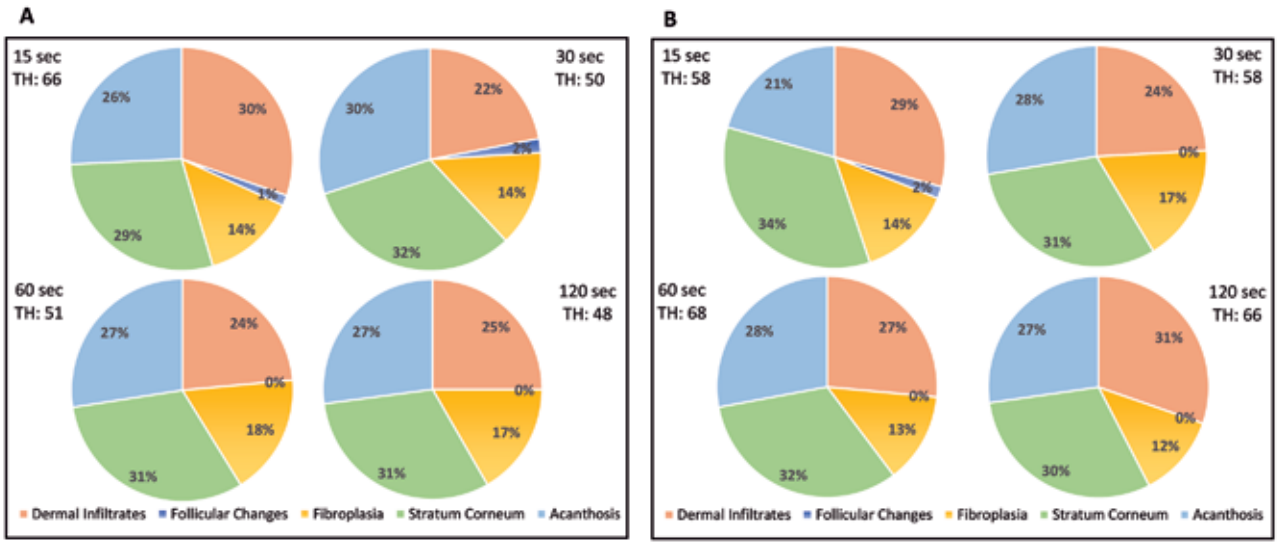

Figure 10. Total proportions of the 5 criteria that made up the total histopathology scores (TH) in CD-1 mice treated with BF (A) or FF (B) at all contact times tested.

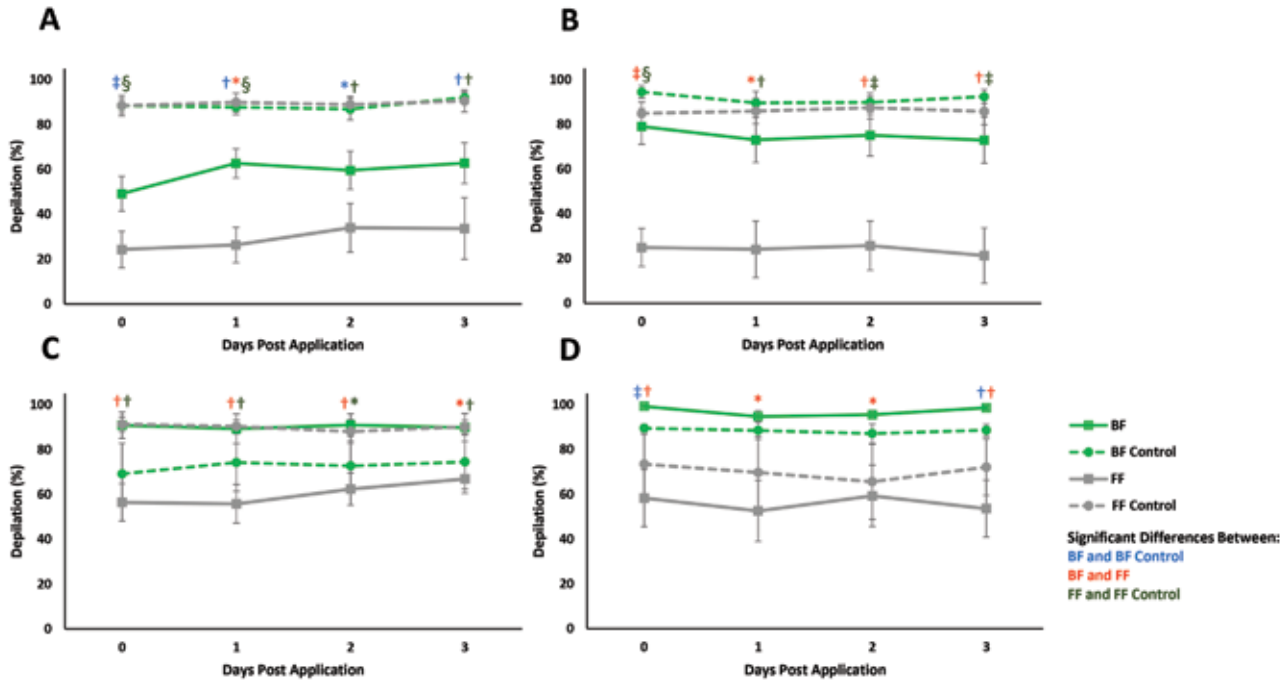

Figure 11. The effects of contact time on depilation over time in B6 mice. Average depilation ± SEM of B6 mice treated with BF or FF for 15 (A), 30 (B), 60 (C), or 120 s (D). Statistically significant differences between treatments and controls or between treatments (see legend) are as follows: *, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; §, $P \leq 0.0001$.

exposed for 120 s. None of the mice developed edema, suggesting that the skin injury likely did not involve mast cells and/or an allergic mechanism, as these effects are often responsible for foci of dermal edema.[17,20] An unexpected clinical finding was dryness and flaking of the exposed skin surface, with 34% of B6 mice and 31% of CD-1 mice developing flaking skin. Scaling (flaking) is a manifestation of a hyperplastic stratum corneum that may separate into layers forming scales. Stratum corneum changes leading to scaling are a common nonspecific response to chronic stimuli such as superficial trauma and inflammation. The dry skin could have caused discomfort to the mice and may have affected other measurements (erythema, histopathology scores, etc).

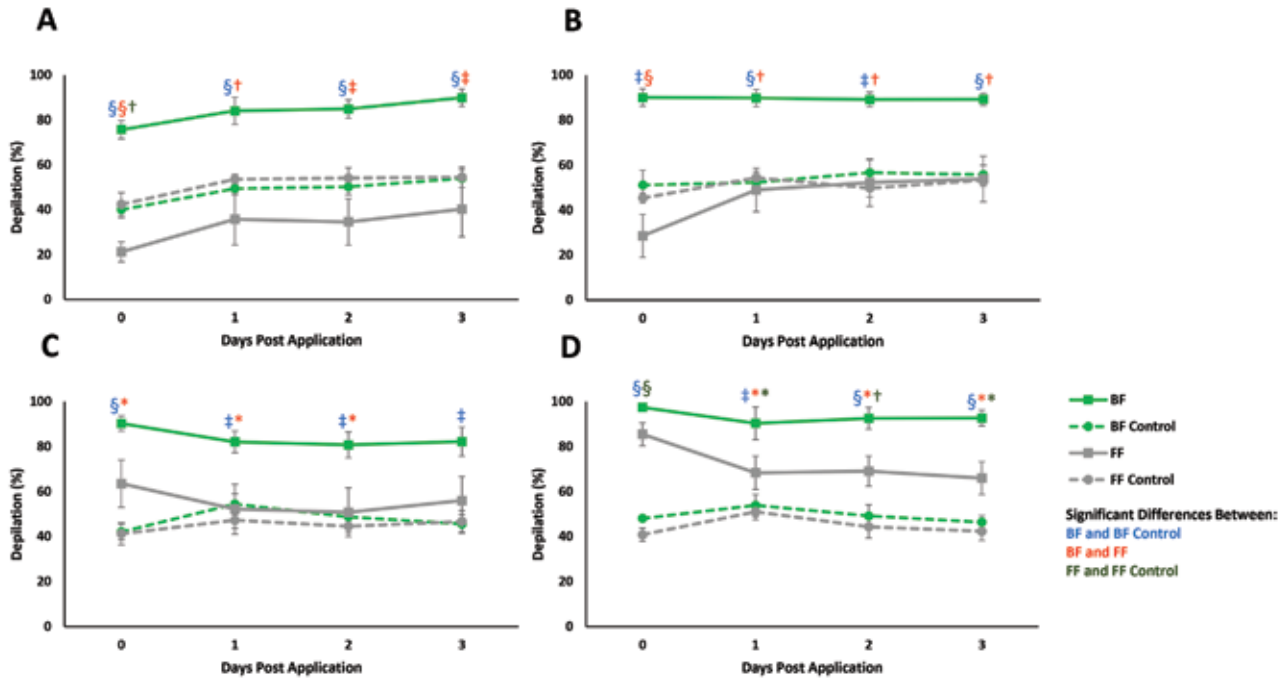

Figure 12. The effects of contact time on depilation in CD-1 mice. Average depilation ± SEM of CD-1 mice treated with BF or FF for 15 (A), 30 (B), 60 (C), or 120 s (D). Statistically significant differences between treatments and controls or between treatments (see legend) are as follows: *, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; §, $P \leq 0.0001$.

Our study did not evaluate pain. Before cream application, all mice were treated with buprenorphine SR-LAB, which is labeled as providing analgesia to rodents for 72 h.[25] Because an analgesic was used, cutaneous injuries caused by the creams may have been painful, but clinical signs of pain were not observed. Without the concurrent use of an analgesic, these creams may induce signs of pain such as overgrooming the area, more severe facial grimace scores, and reduced food intake. Clinical signs might also be worse if the creams are not carefully and completely removed after the exposure time. Future research into pain associated with depilatory creams in mice and how removal practices affect pathology and pain is necessary to determine if analgesics are required to ensure that pain does not go untreated.

Our final hypothesis was that cutaneous injury would be more severe in the B6 mice as compared with CD-1 mice due to their propensity of B6 mice to develop skin disorders.[8,10] However, we found no significant differences between these 2 strains. Past studies of the comparative sensitivity of albino and pigmented skin to various traumas have indicated that albino mice are more sensitive to photodynamic and mechanical injury.[15,18]

We unexpectedly found that depilatory scores using clippers (the control) were significantly lower in CD-1 mice than in B6 mice despite using the same equipment and personnel. We also unexpectedly found that 10 CD-1 mice had ulceration scores greater than 0 on their clipped skin. CD-1 hair subjectively seemed to be thicker and thus harder to clip, which may have led cuts in the skin that were later scored as ulcerations. Despite the difficulties in clipping CD-1 mice, both cream formulations produced a level of depilation that was appropriate for most procedures.

Another unexpected factor was the odor of the creams. Both formulations contain fragrances that may enhance them for human use, but we anecdotally noticed that the facial formula had a much stronger scent than the body formulation. The effect of these odors on the health and wellbeing of the mice is unknown, but the cream could have stimulated grooming of the depilated area to remove the foreign scent. An increase in grooming could have increased erythema and/or histopathology scores. The American Academy of Dermatology Association and the Canadian Dermatology Association both recommend using fragrance-free or unscented skin products in humans.[2,4] Further research could extend this recommendation to use in mice. To avoid negative dermatologic effects of added fragrance, we recommend testing an unscented depilatory cream on mouse skin to compare its effectiveness and safety to the products tested in this study. Unscented Nair depilatory creams are not currently available.

In conclusion, we found that the depilatory creams we tested can reliably remove hair from B6 and CD-1 mice but that they also induce gross and histopathologic injury. Because injury was not associated with the duration of contact time, we cannot recommend the appropriate duration of exposure for minimizing cutaneous damage. As depilatory creams produced significantly higher dermal injury scores as compared with clipping, we recommend using clippers in lieu of creams whenever possible. If depilatory creams are used (for example, for highly sensitive imaging that is not compatible with hair in the hair follicles[13]), we advise gentle and thorough removal of the cream after use so that residual product does not remain on the skin where it may cause further dermal damage and be consumed by the mice during grooming. Investigators should consult with veterinarians to discuss the potential research complications before using a depilatory agent n lieu of clippers in mice.

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