COMPARISON OF DERMATOLOGICAL COMPLICATIONS ASSOCIATED WITH REPEATED USE OF A HIGH ANIONIC VS A LOW ANIONIC SOAP TO DECONTAMINATE CANINES

A Thesis

by

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ABSTRACT

Harmful materials are released into the environment during emergencies and disasters. These materials pose a risk to animals involved in search and rescue efforts without the benefit of personal protective equipment. Search and Rescue (S&R) canines are often decontaminated multiple times during deployments to limit their potential exposure to toxic or harmful substances they come into contact with. Consecutive decontamination has the potential to induce epidermal irritation, decrease the natural protections associated with a healthy dermis and thereby increase the risk of absorption and internalization of hazardous material.

The focus of this study was to evaluate the efficacy of two soap products in the removal of oil-based contaminants and to determine the subsequent likelihood of inducing epidermal irritation and transepidermal water loss when used serially over a standard 14-day deployment.

The results of this study revealed that Dawn® Ultra is more effective than DermaLyte® at removing oil-based contaminants. The serial use of each of these products resulted in mild to moderate epidermal irritation within 4.9 to 15.8 days for Dawn® soap and 5.8 to 21.4 days for DermaLyte® soap. Transepidermal water loss did not quantify or predict visibly scored epidermal irritation. These results will guide the development of S&R dog decontamination protocols.

DEDICATION

I dedicate this thesis to my family for their unending guidance, support, and encouragement throughout my master's degree.

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I would like to extend my deepest thanks to Dr. Bissett for giving me the opportunity to work with the Texas A&M Veterinary Emergency Team, of which led me to starting my Masters. I would like to thank you for encouraging my research and for allowing me to grow as an individual. Your advice on both my research as well as on my career is irreplaceable. I would also like to thank my committee members, Dr. Zoran, Dr. Thompson, Dr. Dominguez, and Dr. Holub for serving as my committee members.

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NOMENCLATURE

CI	Confidence Interval
CADESI	Canine Atopic Dermatitis Extent and Severity Index
PPE	Personal Protective Equipment
TEWL	Transepidermal Water Loss
S&R	Search & Rescue

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INTRODUCTION

Natural disasters and emergencies are all too frequent realities. Search and Rescue (S&R) dogs play a critical role locating individuals who have become lost, displaced, injured, or victims during these incidents. S&R canines are effective assets for human detection; however, there are calculated risks associated with deploying canines into hazardous environments (Migala and Brown 2012). During searches, working canines commonly come into contact with materials and substances that could potentially possess hazardous, toxic, biological, or radioactive properties (Chan et al. 2013, Gwaltney-Brant et al. 2003). Exposure to these materials has the potential to result in direct health complications for the animal.

Disaster situations often result in the intentional or unintentional release of hazardous materials into the environment (Young, Balluz and Malilay 2004). The Agency of Toxic Substances and Disease Registry estimated that, in 2012, more than 15,000 chemical incidents occurred in the United States (Agency for Toxic Substances and Disease Registry). In addition to industrial accidents, disasters such as the World Trade Center terrorist attack, Hurricane Katrina, and the West, Texas explosion all resulted in the release of hazardous materials into the environment. In addition to the debris created, the destruction of the World Trade Center resulted in substantial amounts of asbestos, polycyclic aromatic hydrocarbons, metal compounds, dioxins, and volatile organic compounds liberated in the wreckage (Banauch, Dhala and Prezant 2005). The

flooding associated with Hurricane Katrina led to the mixture of hazardous materials from damaged chemical plants, petroleum refining facilities, and commercial establishments into the environment (Reible et al. 2006). The fertilizer explosion in West, Texas resulted in the spread of ammonium nitrate into the surrounding area (Pittman et al. 2014). In each of these situations, the introduction of hazardous materials into areas occupied by survivors, S&R teams, and resident animals created a significant risk to human and animal health.

The use of canines to search in contaminated areas can result in internal and external contamination of the dogs. External contamination occurs when hazardous materials come into contact with an animal's skin or hair coat, while internal contamination arises when materials are introduced into the body through absorption, ingestion, or inhalation (Murphy 2011). Regardless of type, the presence of contaminants may result in potential health complications. To mitigate both the exposure to and potential health consequences from contaminants, frequent decontamination is performed on search canines throughout a deployment period (Murphy 2011).

Decontamination is the process of removing contaminants from people, animals, equipment, structures, and the environment (Kumar et al. 2010). Decontamination protocols are devised to eliminate exposures to hazardous materials and reduce the spread of contamination. Mechanisms utilized in decontamination include: physical removal, solvation, emulsification, chemical alteration, absorption, adsorption, and friction (Chan et al. 2013).

While decontamination is currently utilized to reduce S&R canines' exposure to contaminants, there are many challenges associated with this process including efficacy, safety, and frequency of decontamination. As a result, the remainder of this manuscript will focus on external decontamination of S&R canines exposed to oil-based contaminants in a simulated disaster situation.

LITERATURE REVIEW

Introduction

Emergency and disaster situations result in an increased risk for release of hazardous materials into the environment and subsequent exposure to these contaminants by victims and first responders. Decontamination protocols for humans are well defined and reviewed. Animal decontamination protocols are less defined and primarily based on anecdotal evidence. This knowledge gap is particularly important for canine first responders as they are at an increased risk for exposure to hazardous materials due to the lack of protective equipment. It has been assumed that these dogs will require daily decontamination throughout a response period (Gordon 2012).

Search & Rescue Canines

For the past 200 years, Search & Rescue (S&R) canines have been a critical asset in the assistance of human detection (Jones et al. 2004). It is well documented that canines surpass human's ability to effectively search areas (Migala and Brown 2012). Canines not only have the ability to locate individuals based on their sense of smell, but also are able to search and work within confined spaces more effectively than their human counterparts (Jones et al. 2004). The ability for canines to work efficiently has been proven across multiple deployments including the Bastrop Complex Wildfires of 2011, as six canines searched a total of 15,598 acres of structures in five days (Migala and Brown 2012).

Although S&R canines are viewed as a critical partner in human detection during disaster situations, deployable S&R dogs have been known to have an elevated health risk associated with responding to disaster when compared to their human counterparts (Migala and Brown 2012). All human first responders normally wear Personal Protective Equipment (PPE) to ensure their safety and prevent exposure to possible unknown hazardous materials during a disaster response (Wenzel 2007). Canines however cannot wear PPE, nor have any additional protection against natural hazards, man-made hazards, or toxic chemicals (Gwaltney-Brant et al. 2003).

Exposures during Disasters for Search & Rescue Canines

The inadvertent or accidental introduction of hazardous materials into the environment during an emergency or disaster can contaminate victims, animals, and first responders. Contamination on-site during a disaster or emergency can increase the likelihood of S&R canines becoming exposed (Gwaltney-Brant et al. 2003). Exposures may include a wide range of hazardous materials such as biological fluids and tissues, industrial and household chemicals, petroleum products, and radioactive substances (Leary et al. 2014). A report on the general toxicological hazards and risks for S&R canines responding to urban disasters (**Table 1)** revealed a large diversity of hazards that canines can potentially be exposed to while searching collapsed buildings (Gwaltney-Brant et al. 2003).

Table 1: General Toxicological Hazards for S&R Canines

Physical Form	*Type of Exposures*	*Examples*
Solids & Liquids	Ingestion, Inhalation, Dermal, Ocular	Hydrocarbons, Polychlorinated Biphenyls, toxic metals, Acids, Alkalis, Glycols, Phenols, Alcohols
Particulates	Inhalation, Dermal, Ocular	Fiberglass, Asbestos particles, Mold Spores, Hydrocarbons, Glycols, Nontoxic Dust
Gases	Inhalation	Hydrogen Cyanide, Nitrogen Dioxide, Hydrogen Chloride, Hydrogen Fluoride, Hydrogen Bromide

(Gwaltney-Brant et al. 2003)

The consequences associated with working in areas that contain hazardous substances and the concurrent lack of protection present serious health concerns for canines and thus require decontamination for mitigation (Murphy et al. 2003).

Canine External Decontamination

There are anecdotal protocols designed for veterinary clinics and disaster situations based on a single contaminant incident. In current literature, animal decontamination protocols are based on the basic principles of leading animals through multiple stations involving the removal of contaminated articles (halters, collars, leashes), washing and rinsing the animal, and finally drying and performing a veterinary evaluation (Murphy 2009, Murphy 2011). These practices have been utilized for both large and small animal incidents for a vast range of chemical, biological, and radioactive exposures (Houston and Hendrickson 2005, Kumar et al. 2010).

Canine decontamination procedures use both gross decontamination and technical decontamination techniques. Gross decontamination removes the majority of surface contamination by using large amounts of water to rinse off loose particles from the animal's coat (Murphy 2009). Technical decontamination is a multi-step process that encompasses a detailed removal of the hazardous material from all external aspects of the animal's body. Methods utilized include brushing, vacuuming, and washing, to eliminate the contaminant from the animal (Houston and Hendrickson 2005, Murphy 2009, Murphy 2011, Soric, Belanger and Wittnich 2008). In short, technical decontamination is an extensive process and may require repeating steps to ensure the complete removal of the hazardous materials or toxic agents. Historically, liquid dish soap has been the agent of choice for external technical decontamination (Murphy 2011). The physical properties found in liquid dish soap allow for binding and emulsification of particles: however, liquid dish soap has been known to contain surfactants which can introduce epidermal irritation following consecutive uses (Heyer 2011). Other agents such as hypochlorite solutions and chlorhexidine solutions have been utilized for biological decontamination, but have the drawback of time dependency. Hypochlorite solution typically requires fifteen minutes and chlorhexidine requires six minutes of contact time with the skin to effectively denature biological agents (Heyer 2011). This in itself may result in a skin irritation effect if used consecutively over a period of time.

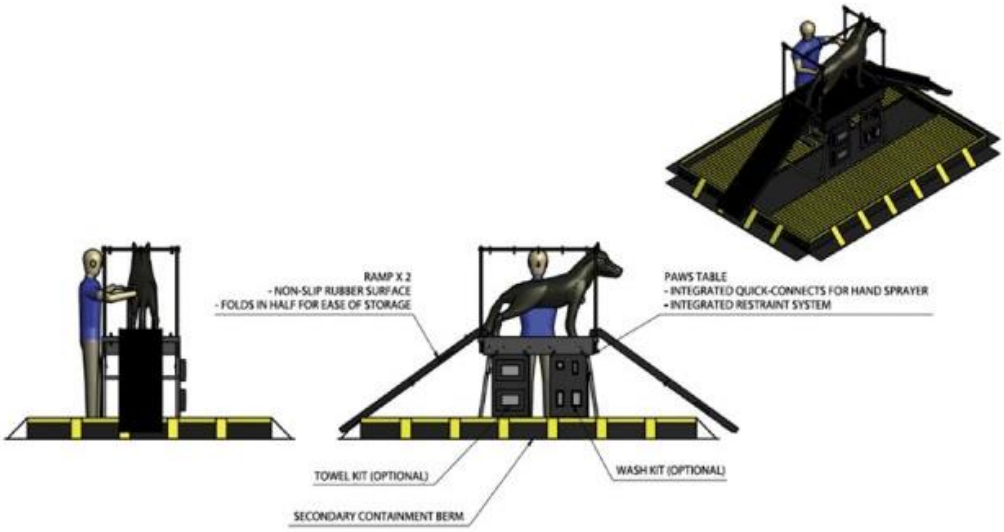
Canine Decontamination Challenges

The challenges associated with canine decontamination directly influence the effectiveness and efficiency of decontamination. The efficiency of canine

decontamination varies and is dependent on factors such as anatomical site of contamination, chemical property and amount of the contaminant, and the timing and duration of decontamination (Chan et al. 2013).

With currently utilized decontamination protocols, there is lack of containment to ensure safety for both the animal and individual decontaminating the animals. Based on anecdotal evidence, small animal decontamination systems utilize small containment pools to progressively wash and move the animal through the decontamination process. This concept captures the contaminants and waste water, but consequently introduces secondary exposures to animals that are to follow through the decontamination process. As a result, commercial canine decontamination units were developed that allow animals to be washed on a raised platform thereby preventing animal contact with waste water **(Figure 1)**. In addition an elevated platform also allows for an increase in human efficiency by allowing decontamination personnel to work at a comfortable level.

Figure 1: Rapid Pro K9Decontamination Station



(Versar Industries)

While the commercial canine decontamination units provide increased efficacy and waste water management, the systems do not increase the safety factor for the animal or decontamination personnel. Contaminated animals are often disoriented or frightened, and for this reason, responder safety is equally as important as animal safety during the decontamination process.

External decontamination is considered a valuable tool in the removal of contaminants and in prevention of secondary exposures. However, decontamination has the potential to cause secondary health complications that are not related to the initial contamination issue. From a dermatological standpoint, decontamination performed consecutively can result in an increased occurrence of epidermal complications. Anecdotal reports following the 2014 Washington State mudslide acknowledged that the

use of dish soap to remove mud and contaminants from S&R canines resulted in increased epidermal irritation (Gordon 2014). Epidermal irritation developed after three consecutive days of performing decontamination with liquid dish soap. Based on this observation, it has been hypothesized that the frequent use of dish soap may be a contributing or causal factor that resulted in compromised skin. It is known that dish soap, and many other skin cleansing products, contain surfactants that allow for a reduction in surface tension between two liquids or between a liquid and a solid (Liem, Nater and Groot 1983). Although these factors allow unwanted materials to be removed from the skin, subsequent epidermal irritation has been identified with the use of surfactant-based products (**Table 2).**

Table 2: Types of Surfactants

Type of Surfactants	*Frequently Utilized Compounds*	*Use*	*Irritation Potential*
Anionic	Sodium Laury Sulfate, Sodium Laureth Surfate, TEA-Lauryl Sulfatre	Emulsifying, solubilizing, wetting agent, detergent	High potent irritants to human and animal skin
Cationic	Quaternium-15, Quaternium-19, Stearalkoniumchloride	Hair conditioning, antimicrobial, preservatives	High potent irritants to human and animal skin
Amphoteric	Cocoamidopropyl Betaine, Coco-betaine, Disod, Cocoamphodiacetate	Foam boosters, emulsifying, detergent	Less irritation potential for eyes and skins
Non-ionic	Polysorbate 20, Cocamide DEA, Lauramide DEA	Emulsifying, solubilizing, suspending agent, foam boosters, detergent	Lowest irritant potential

(Liem, Nater and Groot 1983)

A soap's surfactant properties are an important attribute with regards to decontamination but with repeated use have been found to cause damage to skin proteins and lipids, dryness, barrier damage, erythema, and irritation (Ananthapadmanabhan et al. 2004). Thus, repeated decontamination has the potential to decrease the inherent protection provided by healthy skin, and may increase the potential for the absorption of hazardous materials across the epidermis and into deeper skin layers, thereby increasing potential exposure to the rest of the body through the dermal blood supply. These risks have not been tested nor modeled to determine the effects of daily decontamination during long-term deployments.

In an effort to identify potential irritation, two dermatological modalities have been identified in multiple studies as a way to track and quantify epidermal irritation. The Canine Atopic Dermatitis Extent and Severity Index (CADESI) is a qualitative approach that applies a visual scoring system to evaluate the extent and severity of lesions seen in canine atopic dermatitis (allergic skin disease) (Olivry et al. 2007). This validated scoring system is utilized primarily for clinical trials in allergic canine patients. The system is designed to evaluate 62 body areas for signs of erythema, lichenification, excoriation, and self-induced alopecia. A severity-based scoring system (0-5) is used to grade each location for the above criteria. Following the completion of the visual scoring at each of the 62 body areas, a collective score is calculated. The CADESI scoring system allows veterinarians (primarily veterinary dermatologists and researchers) to track severity of canine atopic dermatitis cases during anti-inflammatory therapy (Olivry et al. 2007). In addition to the qualitative CADESI, quantitative assessments of epidermal irritation can be conducted by measuring Transepidermal Water Loss (TEWL). The skin is a barrier that prevents the loss of water, but when damaged or compromised it results in an increased loss of water through the epidermis (Shimada et al. 2008). TEWL measurements have been found to be an index that represents the barrier function of the epidermis. To ensure proper measurements and exclude environmental factors, a closed chamber system is recommended for measuring TEWL. Even with influential factors being limited, variability can be found from site-to-site, day-to-day, and patient-to-patient with regards to TEWL measurements (Lau-Gillard et al. 2010, Marsella 2012).

Conclusion

The potential release of harmful materials into the environment during an emergency or catastrophic event can contaminate the disaster site that S&R teams work in. This release establishes an immediate health risk for S&R canines due to the lack of PPE. Decontamination should provide an effective method for the mitigation of the exposure risk. However, the outcomes of consecutive daily decontamination have not been evaluated to determine to what extent they produce dermatological adverse effects. Identifying the potential dermatological effects from sequential daily decontamination allows S&R teams a better understanding of how long S&R canines can be expected to work and receive daily decontamination without risk of secondary complications.

Objectives and Aims

The objectives of this study were to determine the number of washes required for a high anionic versus a low anionic surfactant-based soap to remove oil-based contaminants and to compare the risks for development of transepidermal water loss and epidermal irritation from repeated decontamination.

Hypothesis

Based on the literature reviewed, the following working hypotheses were proposed: 1) high anionic soap will require fewer washes to completely remove oil-based contaminants from an animal's coat than a low anionic soap; 2) transepidermal water loss will increase prior to visible epidermal irritation; 3) repeated decontamination with a high anionic soap will result in an increase of visible epidermal irritation as

compared to decontamination with a low anionic soap when compared for fourteen consecutive days.

MATERIALS AND METHODS

Sample Size

A Bayesian statistical model was developed to analyze the daily risk for epidermal irritation resulting from high and low anionic soap exposure. Simulated datasets with sample sizes 10, 14, and 20 were generated, and each represented both extreme and slight differences in irritation resulting from the two treatments. Bayesian analysis of the simulated datasets was performed and the computed results compared to the expected results **Table 3**.

Table 3: Results of Simulated Datasets

	Treatment A at day 11		*Treatment B at day 12*	
N, Sample	Median	CI	Median	CI
10	3.68	[1.9, 8.0]	2.138	[0.3, 6.1]
14	4.184	[2.3, 8.5]	2.487	[0.3, 10.3]
20	4.165	[2.5, 7.4]	3.318	[1.3, 9.2]

Analysis of simulated databases at a sample size of 10 validated the Bayesian model's ability to detect slight and extreme differences in epidermal irritation with a 95% confidence and minimal sample size. An increased sample size of fourteen or twenty did not provide a significant advantage in the detection of both extreme and slight differences in epidermal irritation associated with each treatment.

Animals

Ten Coonhounds were obtained from a commercial breeder. The animals used in this study were approved by the Texas A&M Institutional Animal Care and Use Committee (IACUC) under AUP # 2013-0185. Inclusion criteria for the study were: healthy Coonhounds 2 years of age of similar in weight (45-75 lbs.) and receiving the same diet. Physical examinations were conducted upon arrival to determine each animal's suitability for inclusion in the study. In addition, a dermatological evaluation was performed by a veterinary dermatologist to identify dogs with current dermatological complications. Seven dogs were classified as short coarse hair coat, 2 dogs as short fine hair coats, and 1 dog as normal hair coat. Dogs were individually housed during the study period in a climate controlled building with environmental conditions maintained at a temperature of 22 °Celsius and 58 % humidity.

Experimental Design

All dogs underwent three different trials over a nine-week study period. Trial 1 was a single day observational study that evaluated the efficacy of high and low anionic surfactant-based soaps in the removal of oil-based contaminants. Ten dogs were randomly assigned to treatment groups A or B as shown in **Table 4.** A non-toxic fluorescent impregnated oil-based liquid, GloGerm™[1], was utilized to replicate an oil-based contaminant. Visual detection of GloGerm™ required the use of ultraviolet light in a dark room. Sixty mls of GloGerm™ was applied externally to each canine's coat

[1] GloGerm was purchased from GLOGERM. The product was composed of USP white miner oil and Synthetic Organic Colorant

and allowed to dry for 10 minutes. Following the application and drying of GloGerm™, dogs were bathed with Dawn Ultra®[2] or DermaLyte®[3] and rinsed thoroughly.

Table 4: Trial 1 Study Design

Group A				*Group B*			
Dog	Exposure	Treatment	*Washes	Dog	Exposure	Treatment	*Washes
1	GloGerm	Dawn®		6	GloGerm	DermaLyte®	
2	GloGerm	Dawn®		7	GloGerm	DermaLyte®	
3	GloGerm	Dawn®		8	GloGerm	DermaLyte®	
4	GloGerm	Dawn®		9	GloGerm	DermaLyte®	
5	GloGerm	Dawn®		10	GloGerm	DermaLyte®	

* Washes: represent the total number of washes required for each treatment to remove GloGerm™

At the completion of each wash, dogs were evaluated under an ultraviolet light to detect the presence of GloGerm™. If fluorescent material was detected on the canine's coat or skin, additional washing was performed. The washing and rinsing process was repeated until all fluorescent material was completely removed externally from the dog. The total number of washes required to remove the fluorescent material was recorded for each dog. The median quantity amount of washes determined in Trial 1 for

[2] Dawn Ultra is manufactured by Procter & Gamble Co. and classified as a high anionic surfactant-based product

[3] Dermalyte is manufactured by Dechra Veterinary Products and classified as a low anionic surfactant-based product

both treatments was utilized as the standard for the number of washes for both treatment groups in Trial 2 and 3.

Trial 2 and 3 utilized a crossover study design that modeled and evaluated epidermal irritation and transepidermal water loss (TEWL) resulting from consecutive daily bathing with a high anionic or low anionic soap. The crossover allowed for each study participant to be exposed and evaluated for each treatment. For Trial 2, 10 dogs were randomly assigned to treatment groups and washed daily for 14 consecutive days. Dogs in treatment Group A were washed twice with DermaLyte® dog shampoo, while the dogs in treatment Group B were washed once with Dawn® Ultra® **Table 5.** Each day, prior to washing all dogs received full body dermatological evaluations by a veterinary dermatologist and Transepidermal Water Loss (TEWL) measurements were taken at 19 locations. The 19 generalized locations are based on a modified version of the CADESI scoring system. All visual epidermal evaluations were assessed by a veterinary dermatologist daily to ensure the proper identification of irritation resulting from both treatments.

Table 5: Trial 2 Study Design

Group A					*Group B*				
Dog	Tx	*Wash	Derm	TEWL	Dog	Tx	*Wash	Derm	TEWL
1	DermaLyte®	2			6	Dawn®	1		
2	DermaLyte®	2			7	Dawn®	1		
3	DermaLyte®	2			8	Dawn®	1		
4	DermaLyte®	2			9	Dawn®	1		
5	DermaLyte®	2			10	Dawn®	1		

*Wash- Median number of washes required to remove GloGerm™ externally from an animal's coat for each treatment. Data was derived from Trial 1.

Trial 3 replicated the procedures in Trial 2 with the exception that the dogs in treatment Group A were washed once with Dawn® Ultra®, and dogs in treatment Group B will be washed twice with DermaLyte® dog shampoo as seen in **Table 6**.

Table 6: Trial 3 Study Design

Group A					*Group B*				
Dog	Tx	*Wash	Derm	TEWL	Dog	Tx	*Wash	Derm	TEWL
1	Dawn®	1			6	DermaLyte®	2		
2	Dawn®	1			7	DermaLyte®	2		
3	Dawn®	1			8	DermaLyte®	2		
4	Dawn®	1			9	DermaLyte®	2		
5	Dawn®	1			10	DermaLyte®	2		

*Wash- Median number of washes required to remove GloGerm externally from an animal's coat for each treatment. Data was derived from Trial 1.

Washout Period

Upon completion of Trial 1, there was a seven-day recovery period to allow dogs to fully recover from any epidermal complications resulting from Trial 1. Dogs were monitored daily to record progress of recovery and evaluated by a veterinary dermatologist prior to initiating additional trials. The completion of Trial 2 called for a 30 day recovery period to ensure all previous dermatological complications from the proceeding trial had resolved and to prevent carryover bias between Trials 2 and 3.

Canine Decontamination Protocol

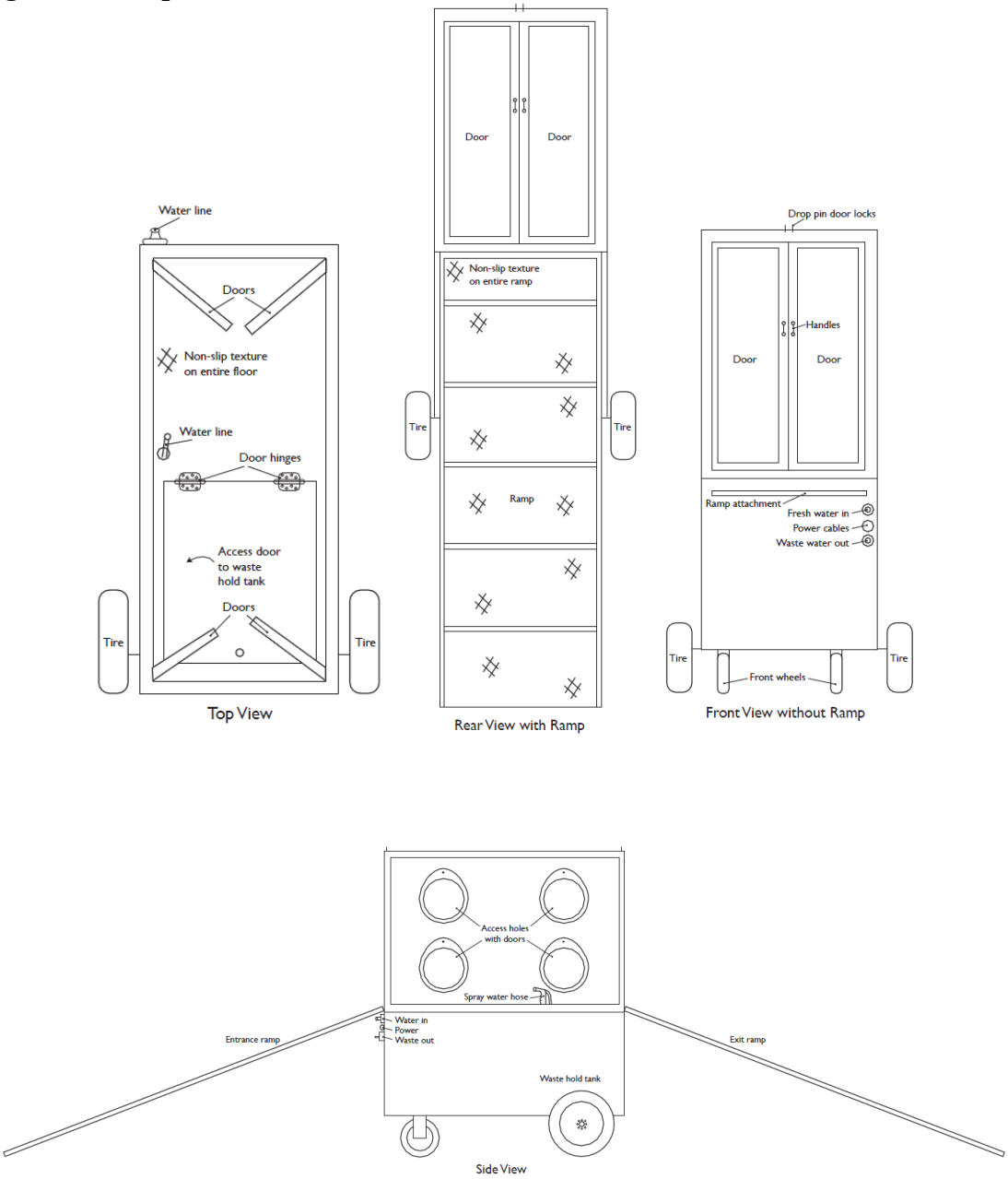
For this study a standardized 10 minute washing procedure was utilized for all trials. All washing took place in a companion animal decontamination unit designed to provide containment for the animal and waste material during the washing process. Dogs were first rinsed with water for one minute, followed by a scrubbing period of five minutes with one of the treatment soaps. An application of 120mls of soap per wash was spread evenly over the animal's body and worked into the coat. Dogs were then rinsed from head to tail and dorsum to ventrum for four minutes. Following the completion of washing, each dog was removed from the decontamination unit, toweled dry, and returned to the pen.

Canine Decontamination Unit

A mobile companion animal decontamination unit was designed and built by the Texas A&M Veterinary Emergency Team to facilitate the washing for this project (**Figure 2**). The steel/plexiglass structure allowed animals to be fully contained within a closed chamber system while being washed (**Figure 2**). After reviewing anecdotal evidence, the development of an enclosed system was favored as a means to provide safety and mitigate exposure to contaminants for both the animal and the decontamination personnel. The unit provided a physical barrier of protection between the animal and personnel performing the wash. The individual performing washing would reach though the access holes and wear arm length rubber gloves to scrub and rinse the animal. In addition, all wastewater and materials were captured within a recessed reservoir that prevented the animal from standing in potentially contaminated

water. Entrance and exit ramps were installed to ensure safety and ease of moving animals in and out of the unit.

Figure 2: Companion Animal Decontamination Unit



Epidermal Evaluations

Epidermal irritation was visually scored on a condensed modified version of the Canine Atopic Dermatitis Extent and Severity Index (CADESI), (Appendix A). The CADESI evaluates and scores 62 locations on the dog's skin for signs of erythema, lichenification, excoriation, and self-induced alopecia. The 62 locations were grouped and generalized into 19 locations, in order to simplify the evaluation system and provide the ability to classify the occurrence of epidermal irritation into broad body regions. The modified version of the CADESI evaluated the 19 generalized locations for visual signs of erythema, excoriation, and self-induced alopecia. The modified CADESI utilized a scale of zero to five (0: None, 1: Mild, 2-3: Moderate, 4-5: Severe) to score each location. Guidelines for scoring erythema, excoriation, and self-induced alopecia were developed to ensure consistency of scoring during the study (Appendix B). All skin evaluations were performed by a veterinary dermatologist to ensure accurate and consistent scoring according to dermatology standards. During the course of the study, the dermatologist remained blinded to both treatment groups.

Participant Withdrawal

Any dog with a score of 2 or greater for erythema or excoriation based on the CADESI scoring system resulted in the dog being removed from the study and from further treatments (washing) for the remainder of the trial. This protocol was in place to ensure that animals were not washed to the point that dermatological irritation was inhumane or resulted in potential health complications. Animals that were withdrawn

from the trial still received daily epidermal evaluations and TEWL measurements to monitor the progression/resolution of epidermal irritation and recovery.

Transepidermal Water Loss

Transepidermal water loss measurements were taken at the nineteen locations evaluated by the modified-CADESI. A closed-chamber VapoMeter®[4] was utilized to collect measurements on the nineteen locations. The probe required 10-second contact time to acquire a measurement at each location. All data were transferred wirelessly to the Delfin management software on a laptop computer.

Statistical Analysis

Descriptive statics were used to quantify and summarize data collected from Trial 1. Ninety-five percent confidence intervals were calculated for the median number of washes for Dawn® and DermaLyte® to remove GloGerm™. In addition descriptive statics were utilized to summarize hair coat classification, summary of visual dermatological effects, and patient withdrawal.

Bayesian statistical methods were employed to analyze the visual dermatological evaluations and the TEWL data collected in Trials 2 and 3. Two independent models were composed and the parameters were estimated using a Markov Chain Monte Carlo (MCMC) method using OpenBugs version 3.2.3 software (Lunn et al. 2009).

A time-to-event model was developed to generate median survival times at a 95% confidence interval to test the hypothesis that repeated decontamination with a high

[4]VapoMeter manufactured by Delfin technologies

anionic soap will result in an increase of visible epidermal irritation as compared to decontamination with a low anionic soap over fourteen consecutive days (Congdon 2007). Time-to-event analysis accounts for both the period of observation and whether an event occurred or not. The approach for this study was to model the events excoriation, erythema, and self-induced alopecia that were the basis of the modified CADESI scoring system. Scoring for erythema and excoriation were summarized into a single score that represented the highest identified dermatological complication for any given location. The likelihood survival time for each level of epidermal severity was modeled as a Weibull distribution. The logit of the Weibull parameter was modeled as a linear function of an intercept, a random effect for dog, treatment effect, and the effect of treatment order. Vague priors were used for the model coefficients (Congdon 2007).

Two separate Bayesian statistical models were utilized to model the measurements of TEWL. The first model used a Poisson distribution to evaluate the change in TEWL over the 14 day treatment period. were modeled The log of the Poisson parameter was then modeled as a linear combination of: a treatment-specific intercept, random effects for both dog and location and the effect of treatment as a day-specific effect (Congdon 2007). To evaluate any potential effect of treatment order, in the cross-over design, the effect of treatment order was included in the original model and when it was confirmed that there was no effect of order, the modeling was completed without a treatment order parameter. A Bayesian implementation with vague or minimally informative priors was used for analysis. Specifically, the priors where uniform (-infinity, +infinity) for the treatment specific intercepts (Congdon 2007). Both dog and

location were assigned vague normal random priors that included a mean of zero and gamma precision parameter of (0.01, 0.01). For the effect of (treatment and day) specific to time, a random-walk prior was used (Lunn et al. 2009). The effect of each treatment was assigned a normal prior with zero mean and a precision of 0.001

The second model evaluated the possibility that treatment effects were different among locations. Model 1 was adapted by removing the random effect for location and then providing separate random walk priors for treatment and day combinations, allowing autoregressive smoothing across days. Models 1 and 2 were compared by evaluating the posterior for the temporally smoothed treatment effects among locations and by evaluating the Deviance Information Criterion for the two models.

The implementation was performed within OpenBugs version 3.2.3 (Lunn et al. 2009). A burn-in of 5,000 iterations and a sampling of 100,000 iterations were used for the MCMC simulation. The Bayesian estimate was taken as the posterior median of the parameter. The confidence interval at 2.5% and 97.5% percentiles were taken directly from the posterior distribution. Statistical significance was defined as 95% confidence interval for the survival times and changes in TEWL that excluded <1.

RESULTS

Effectiveness of a High and Low Anionic Soap

The median number of washes and amount of soap to effectively remove oil based contaminants was determined for each treatment (**Table 7 and 8 respectively**). Tabulated data is provided in Appendix C.

Table 7: Median Number of Washes

Treatment	*N*	*Mean*	*STDEV*	*Median*	*Min*	*Max*
Dawn®	5	1.4	0.89	1	1	4
DermaLyte®	5	2.4	0.55	2	2	3

Table 8: Median ml of Soap

Treatment	*N*	*Mean*	*STDEV*	*Median*	*Min*	*Max*
Dawn®	5	168ml	107.33	120ml	120ml	360ml
DermaLyte®	5	288ml	65.73	240ml	240ml	360ml

Epidermal Irritation

One hundred percent of dogs exposed to Dawn® and 90% of dogs exposed to DermaLyte® developed clinical signs of epidermal irritation during the study period. The individual daily dermatological summary score (maximum combined severity score for erythema, excoriation, and self-induced alopecia) for each treatment group and

tabulated data is provided in Appendix D. **Figures 3 and 4** display the frequency of dogs that reached each severity level of excoriation based on location while **Figures 5 and 6** express the frequency of dogs that reached each severity level of erythema based on location. Two dogs exposed to DermaLyte® showed signs of alopecia at the sternum region. Alopecia was not identified on any of the 10 dogs exposed to Dawn®. The patient withdrawal rate is provided in **Figure 7.**

Figure 3:Erythema Occurrences by Location with DermaLyte® Exposure

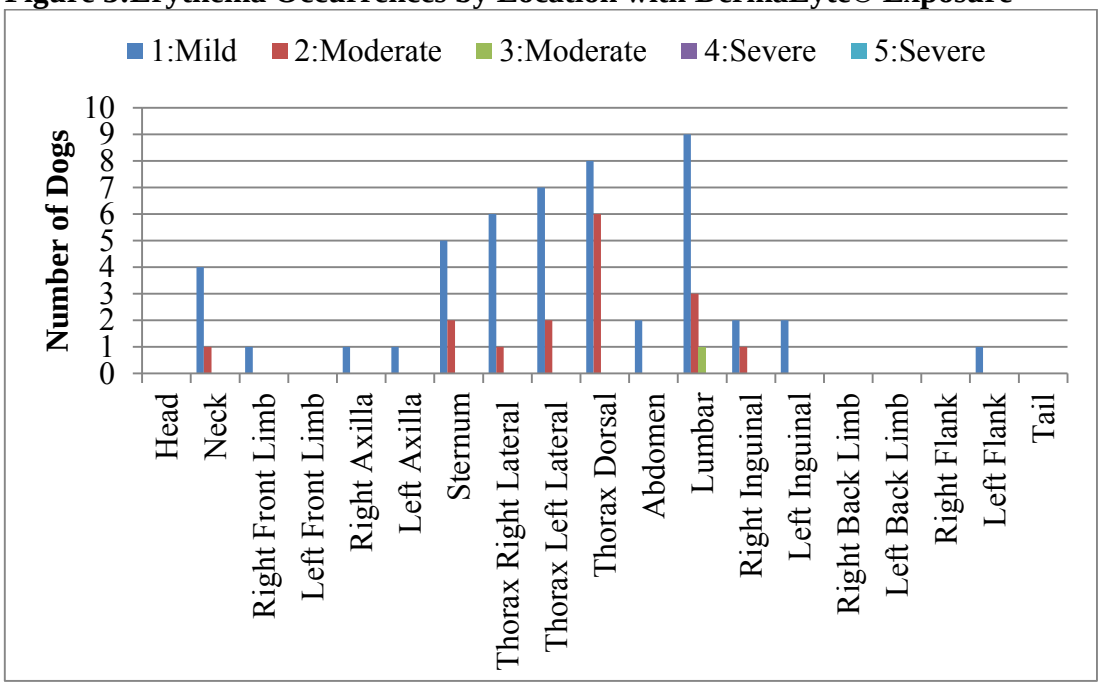


Figure 4: Erythema Occurrences by Location with Dawn® Exposure

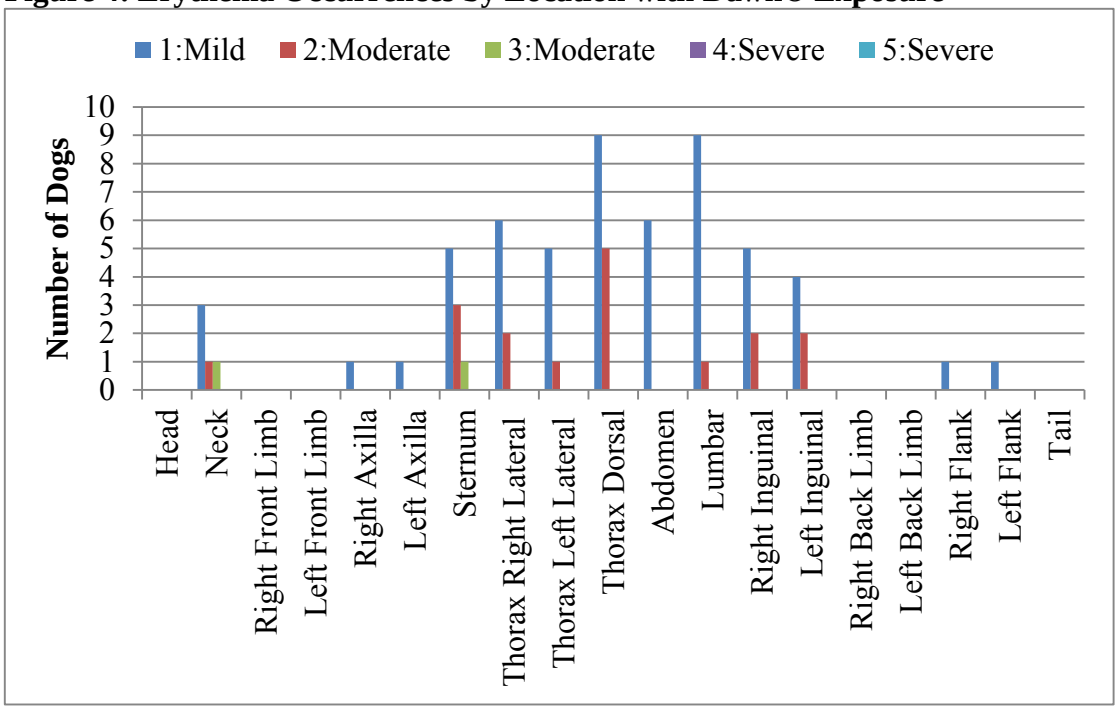


Figure 5: Excoriation Occurrences by Location with DermaLyte® Exposure

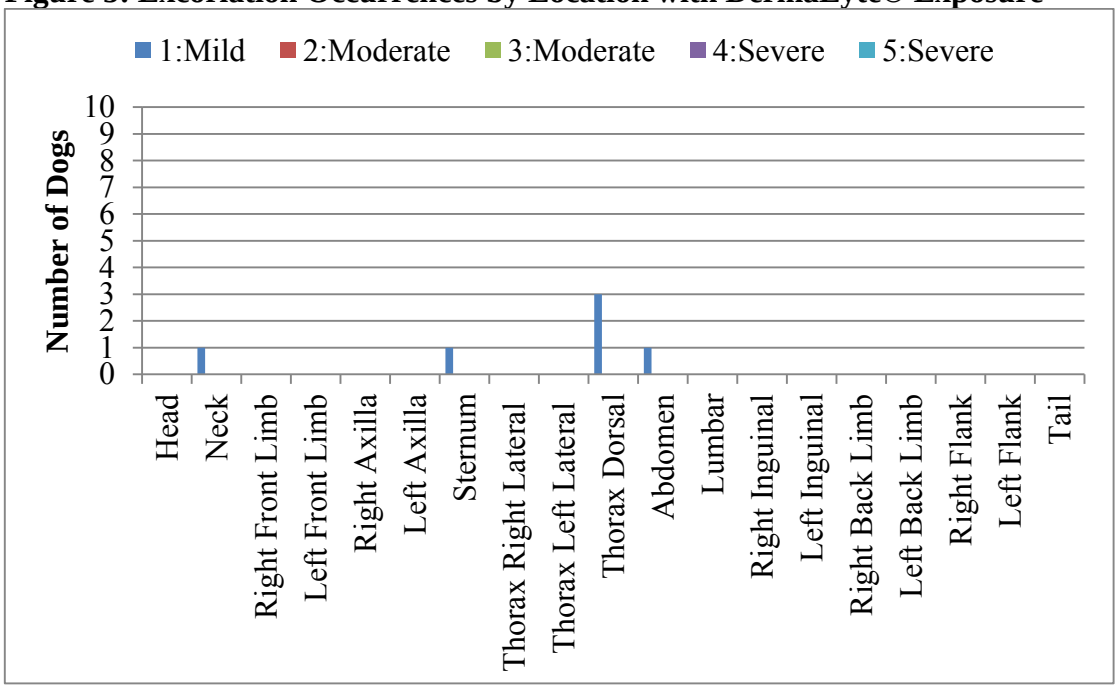


Figure 6: Excoriation Occurrences by Location with Dawn® Exposure

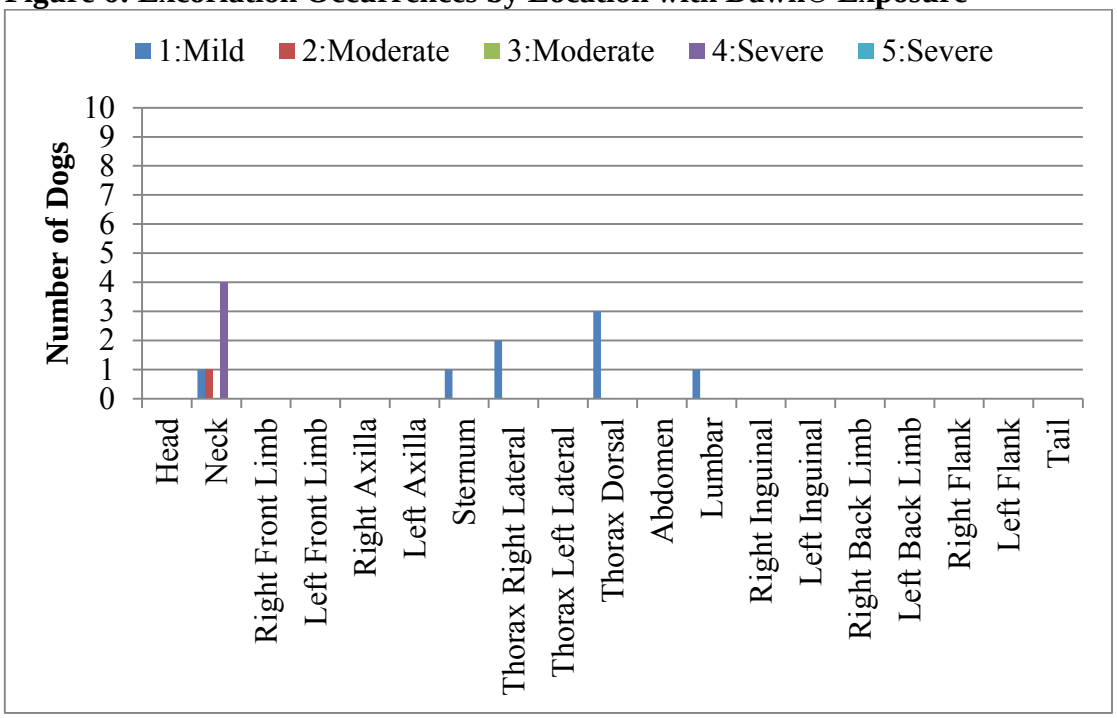
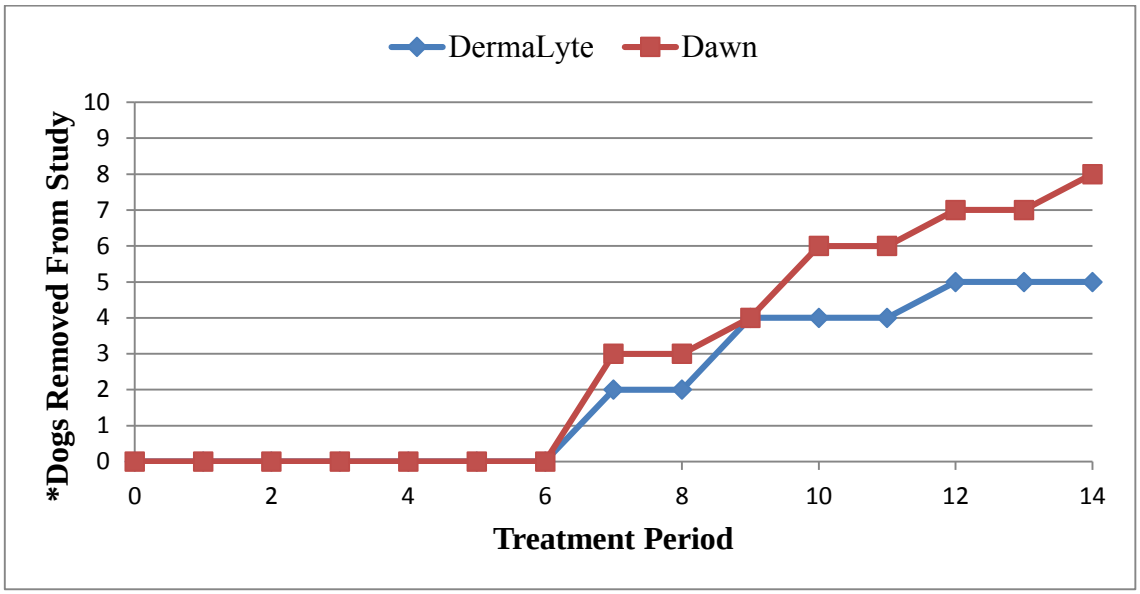


Figure 7: Participant Withdrawal From Study as a Result of Moderate Epidermal Irritation



*Patients receiving a score of 2 or greater for erythema or excoriation based on the CADESI scoring system resulted in the animal being removed from the study.

Time to Event for Visual Dermatological Effect Indices

The median and 95% Confidence Intervals (CI) for the expected median survival time for each of the severity indices compiled from the modified CADESI is provided in **Table 9.** Severity index 4 for Dawn® treatment and severity index 5, for both DermaLyte® and Dawn® treatments, were not computed as a result of the absence of data for these indices.

Table 9: Expected Median Time (days) for Severity Occurrence

Severity Levels	*DermaLyte® Median (95%CI)*	*Dawn® Median (95% CI)*
1 (Mild)	7.7 (5.6-10.6)	6.6 (5.0-8.9)
2 (Moderate)	13.9 (10.1-21.4)	10.9 (7.9-15.8)
3 (Moderate)	24.3 (15.5-45.7)	25.6 (15.2-54.3)

Transepidermal Water Loss

Table 10 represents the median relative daily risk for TEWL over the 14 collection times for both treatment groups. **Table 11** shows the median TEWL daily relative risk for the difference between treatment groups over the collection period. **Table 12** represents the expected differences between 2 consecutive days within a treatment group, the expected difference between 2 dogs, and the expected difference between 2 collection locations on a single dog. Calculated location specific median relative risks for each treatment group are provided in Appendix E.

Table 10: Median Relative Daily Risk for TEWL

	DermaLyte®			*Dawn®*		
Day	*Mean*	*Median*	*95% CI*	*Mean*	*Median*	*95% CI*
1	1.094	1.094	1.049-1.141	1.013	1.013	0.9705-1.057
2	0.9482	0.9481	0.9064-0.9904	0.9271	0.9269	0.8869-0.9679
3	1.053	1.053	1.009-1.098	1.011	1.011	0.9702-1.054
4	1.085	1.084	1.04-1.13	1.099	1.098	1.055-1.144
5	1.057	1.057	1.014-1.102	1.065	1.065	1.023-1.109
6	1.05	1.049	1.006-1.094	1.032	1.032	0.99-1.074
7	1.143	1.142	1.096-1.19	0.9739	0.9738	0.9332-1.015
8	0.9991	0.9989	0.9564-1.042	0.9599	0.9598	0.92-1
9	0.9983	0.9981	0.9561-1.041	0.969	0.9689	0.9291-1.01
10	0.8579	0.8578	0.8184-0.8977	0.9714	0.9712	0.9306-1.013
11	0.9038	0.9037	0.8644-0.9444	1.087	1.087	1.043-1.134
12	0.8894	0.8893	0.85-0.93	0.9424	0.9422	0.9019-0.9833
13	0.8974	0.8972	0.8573-0.938	0.9635	0.9634	0.9234-1.005
14	1.082	1.082	1.037-1.129	1.007	1.007	0.9645-1.05

Table 11: Median TEWL Relative Risk Between Treatment Groups

Day	*Median*	*95% CI*
1	1.08	1.017-1.146
2	1.023	0.961-1.088
3	1.041	0.9815-1.104
4	0.9874	0.9321-1.046
5	0.9925	0.9368-1.051
6	1.017	0.9595-1.078
7	1.173	1.107-1.244
8	1.041	0.98-1.105
9	1.03	0.970-1.094
10	0.8833	0.8297-0.9398
11	0.8314	0.7821-0.8834
12	0.9437	0.8869-1.005
13	0.9314	0.8756-0.9904
14	1.075	1.012-1.141

Table 12: Standard Deviation of Collection Days, Dog, & Collection Location

	Mean	*Median*	*95% CI*
Day: DermaLyt	0.11	0.11	0.07-0.18
Day: Dawn®	0.09	0.09	0.06-0.14
Dog	0.18	0.17	0.11-0.34
Collection Location	0.24	0.23	0.17-0.34

DISCUSSION

While contamination of canines working in hazardous environments is a concern, the decontamination process used to remove these contaminants has the potential to cause secondary health complications. The effectiveness of decontaminating dogs exposed to oil-based substances has not been assessed, nor have the dermatological effects associated with repeated daily decontamination. The results of this study showed that Dawn® required a single wash to remove an oil-based contaminant vs two washes with DermaLyte® to achieve the same effect. The increased efficiency of Dawn® will allow for a reduction in the time required to decontaminate an animal and potentially conserve resources such as water, soap, and labor force.

Although Dawn® was found to be more effective than DermaLyte® at removing oil-based substances; both soaps induce dermatological complications with sequential daily use. The initiation of epidermal irritation as measured by visual dermatological assessment and scoring was found to be associated with repeated treatments of both Dawn® (high anionic surfactant) and DermaLyte® (low anionic surfactant) soaps over 14 consecutive days. The time-to-event analysis revealed there was no significant difference, based on a 95% CI, in time to occurrence for each of the severity indices between treatment groups. Regardless, it is importance to acknowledge the time periods for which each severity indices is expected to occur as a result of continuous treatment. Dawn® resulted in mild irritation between days 4.9 and 8.8, and between days 7.8 to 15.8 for moderate irritation. Daily use of DermaLyte® resulted in mild irritation

between days 5.8 and 10.6 and moderate irritation between days 10.1 and 21.4. With daily use of Dawn®, dogs developed moderate irritation at a faster rate than dogs exposed to DermaLyte®.

A summary of the modified CADESI revealed areas of location-dependent risk for development of erythema, excoriation, and self-induced alopecia. Areas of increased location-dependent risk accounted for 5 or more dogs that presented in each of the severity indices. The lumbar, dorsal thorax, right and left lateral thorax, and sternum regions were all locations that represented an increased risk of mild erythema that were consistent between the two treatment groups. However, exposure to Dawn® did result in two additional regions (abdomen and right inguinal) for high risk of mild erythema. The dorsal thorax was the only location that resulted in a high risk for moderate erythema and was consistent between the two treatment groups. No locations were found to be of high risk for severe erythema between both treatment groups. Location-dependent regions for the presence of excoriation included: head, neck, sternum, right lateral thorax, dorsal thorax, abdomen, and lumbar regions. These regions represented areas were excoriation was detected; however these regions were not classified as high risk. When comparing the location specific erythema and excoriation incidences, erythema occurrences were significantly higher than excoriation occurrences. Two occurrences of self-induced alopecia were identified in the DermaLyte® treatment group on the sternum region, but the data did not support an association between hair loss and treatment. Based on these findings, erythema is the most commonly noted dermatological complication appreciated in canines as a result of serial decontamination.

In this study, the assessment of TEWL revealed there was an increased risk for TEWL on days 1, 3, 4, 5, 6, 7, and 14 as a result of exposure to DermaLyte®, and days 4, 5, and 11 as a result of exposure to Dawn®. However these increased risks in TEWL cannot be credited exclusively to individual treatments. An increased variability was identified between dogs and measurement locations consecutively over the 14 day treatment period. Also the expected differences in TEWL between consecutive days were similar between the two treatment groups. Based on this study TEWL cannot provide an accurate early detection for the onset occurrence of visual epidermal irritation.

The companion animal decontamination unit was used a total of 215 times throughout the study. All animals were properly contained in the unit during the washing period. The enclosed unit provided a safety barrier that ensured the animal could not exit the unit during the washing process. The ramps allowed the patients to easily be loaded and removed from the unit. In addition, a minimal amount of water and soap escaped the unit around the arm holes where the individuals performing the washing reached through.

Study Limitations

Skin and hair quality variability can be found among animal species, breeds, and individuals within a breed. In this study, 7 dogs were classified as short coarse hair coat, 2 dogs as short fine hair coats, and 1 dog as normal hair coat. The dog that classified with a normal hair coat was the only dog that did not develop visual signs of moderate epidermal irritation for both Dawn® and DermaLyte® treatments. As a result skin and

hair quality should be included in the inclusion criteria, to ensure a uniform sample population. Furthermore, there is individual variability due to age, gender, and between evaluation locations. Areas that contain the thickest skin are found on the forehead, dorsal neck, dorsal thorax, rump, and base of the tail, while the pinnal, axillary, inguinal, and perianal areas possess the thinnest skin. Areas with thin skin have a higher potential of absorption and permeability of substances due to the decreased number of skin cells present within the epidermal layer. Therefore thickness of skin is a potential confounding factor that prevents uniformity throughout the animal's body. This means that not all areas of a given canine have the same risk for the development of epidermal irritation and potential absorption of hazardous materials.

The CADESI uses a total of 62 locations to adequately score and assess a canine's entire body. In this study, we created a modified version of the CADESI by grouping the 62 regions into 19 individual locations. The19 locations identified for the visual detection were also employed for the TEWL measurement sites. By generalizing the visual scoring and TEWL measurements locations, we limited our ability to accurately assess TEWL based on location specific areas. The CADESI organizes the visual assessment areas of a canine's forelimb into 4 different areas (the medial, lateral, cubital flexor and carpal flexor) and the modified CADESI we used for this study grouped these 4 areas into one single location/measurement site. This means that during our visual assessment of a canine's forelimb we looked at medial, lateral, cubital flexor and carpal flexor as one whole area, but when we measured TEWL the probe was placed in the general vicinity of the forelimb. A Vapometer is designed to measure a 1cm

diameter area per measurement. Due to the small area captured by the Vapometer there is an increased variability between collection locations within a generalized area. By generalizing all these regions to represent a single location, the TEWL measurement could potentially not get collected in the same region as the presence of the epidermal irritation. One way to reduce the location TEWL variability is to apply the original 62 locations specified in the CADESI. Using 62 locations rather than 19, would allow for TEWL measurement to be taken within the actual area and not a large generalized region. In addition, triplicate TEWL measurements within the 62 locations would allow for an improved sampling of TEWL for a given location rather than basing the findings on a single measurement.

The participant withdrawal was an important aspect to insure that inhumane health complications did not arise from prolonged exposure to either treatment throughout the study period. Moderate (severity index 2) at any location was defined as the treatment withdrawal point for the remainder of the trial. Consequently by removing dogs at a moderate severity level 2, we were unable to detect a significant amount of data that support moderate (severity 3) and severe (severity 4 & 5) dermatological complications based on treatment exposure. Although treatment ceased, visual evaluations and TEWL measurements were still taken following a patient's withdrawal from the trial. Continuing TEWL measurements after a patient was removed from the trial introduced fallout bias. Our statistical model for TEWL did not take into account when an animal was removed from the trial. This introduced a mixing of data between animals on the trial and animals removed from the trial and reduced the accuracy of

assessing the change in TEWL based on treatment. In addition, two animals developed lacerations and abrasions on neck, thorax dorsal, and forelimb regions as a result of injuries obtained during the treatment period. These injuries were not directly related to the treatment and resulted in the moderate and severe epidermal irritation at the respective locations. Fortunately, prior to receiving these injuries, the two animals were removed from the treatment groups as a result of the detection of moderate epidermal irritation.

From a statistical analysis perspective, a Bayesian Model was developed to assess the daily changes in visual epidermal complications and was validated using random generated data sets. The initial model and data sets determined the sample size for the study. After completing the study, we found that the initial model did not account for time-to-event analysis. As a result, we generated a new model to assess the visual epidermal irritation as a time-to-event. The new model potentially could have required a different sample size to conclude significance.

A final limitation to the study was standardizing the washing protocol for time and amount of soap utilized per wash. In setting the wash standard, we did not account for animal surface area and number of gallons of water needed or used to wash each dog. Calculating the appropriate dosage of each soap for the animal's surface area would potentially prevent the over exposure of soap to the animal, which could reduce the occurrences of epidermal irritation. This data would have provided significant insight into the logistical requirements for decontaminating canines that range in size for future deployments.

While we acknowledge these potential limitations, we still feel that this study provided a significant step forward in understanding the potential effects that could be introduced through sequential daily decontamination and for future research projects.

SUMMARY AND CONCLUSIONS

There are several relevant conclusions that can be drawn from the study. First, this study showed that Dawn®, a high anionic soap, has a higher degree of effectiveness when compared to DermaLyte®, a low anionic soap, to externally decontaminate canines with an oil-based contaminant exposure. Second, this study showed that search and rescue canines will not be able to work and receive daily sequentially decontamination for 14 days, regardless of being decontaminated strictly with Dawn® or DermaLyte®. Nonetheless, this study still provided ranges for guidance on potential epidermal irritation that could arise from consecutive daily decontamination: daily exposure to Dawn® will result in mild to moderate epidermal irritation between days 4.9 and 15.76, while DermaLyte® will produce the same results between days 5.8 and 21.4. Finally, this study showed that TEWL measurement cannot be used as an early detection of potential epidermal irritation. Visual epidermal evaluation remains the more accurate method for determining when secondary irritation from consecutive decontamination presents.

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APPENDIX A

CANINE ATOPIC DERMATITIS EXTENT AND SEVERITY INDEX

Figure A-1

CADESI-03.lv - © ITFCAD 2004 BODY AREAS				Erythema	Lichenification	Excoriations	Self-Induced Alopecia	TOTAL
Face		Preauricular	1					
		Periocular	2					
		Perilabial	3					
		Muzzle	4					
		Chin	5					
Head		Dorsal	6					
Ear Pinna	Left	Convex	7					
		Concave	8					
	Right	Convex	9					
		Concave	10					
Neck		Dorsal	11					
		Ventral	12					
	Lateral	Left	13					
		Right	14					
Axilla		Left	15					
		Right	16					
Sternum			17					
Thorax		Dorsal	18					
	Lateral	Left	19					
		Right	20					
Inguinal		Left	21					
		Right	22					
Abdomen			23					
Lumbar		Dorsal	24					
Flank		Left	25					
		Right	26					
Forelimb	Left	Medial	27					
		Lateral	28					
		Cubital Flexor	29					
		Carpal Flexor	30					
	Right	Medial	31					
		Lateral	32					
		Cubital Flexor	33					
		Carpal Flexor	34					
Forefoot	Left	Palmar Metacarpal	35					
		Dorsal Metacarpal	36					
		Palmar Phalangeal	37					
		Dorsal Interdigital	38					
	Right	Palmar Metacarpal	39					
		Dorsal Metacarpal	40					
		Palmar Phalangeal	41					
		Dorsal Interdigital	42					
Hind Limb	Left	Medial	43					
		Lateral	44					
		Stiffle Flexor	45					
		Tarsal Flexor	46					
	Right	Medial	47					
		Lateral	48					
		Stiffle Flexor	49					
		Tarsal Flexor	50					
Hind Foot	Left	Plantar Metatarsal	51					
		Dorsal Metatarsal	52					
		Plantar Phalangeal	53					
		Dorsal Interdigital	54					
	Right	Plantar Metatarsal	55					
		Dorsal Metatarsal	56					
		Plantar Phalangeal	57					
		Dorsal Interdigital	58					
Perianal			59					
Perigenital			60					
Tail		Ventral	61					
		Dorsal	62					

APPENDIX B

MODIFIED CANINE ATOPIC DERMATITIS EXTENT AND SEVERITY INDEX&

VISUAL SCORING SYSTEM

Figure B-1: Scoring Locations

	Location	Erythema (0-5)	Excoriation (0-5)	Alopecia (0-5)
1	Head			
2	Neck			
3	Right Front Limb			
4	Left Front Limb			
5	Right Axilla			
6	Left Axilla			
7	Sternum			
8	Thorax Right Lateral			
9	Thorax Left Lateral			
10	Dorsal Thorax			
11	Abdomen			
12	Lumbar			
13	Right Inguinal			
14	Left Inguinal			
15	Right Back Limb			
16	Left Back Limb			
17	Right Flank			
18	Left Flank			
19	Tail			

Figure B-2: Erythema Scoring

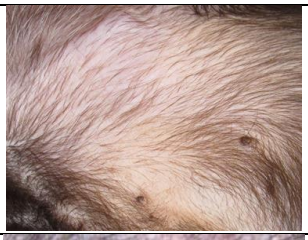
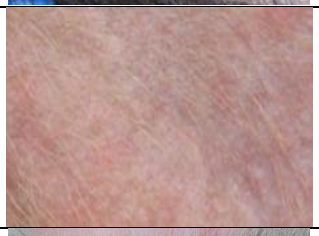
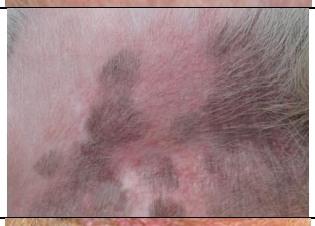
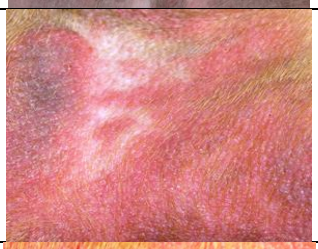
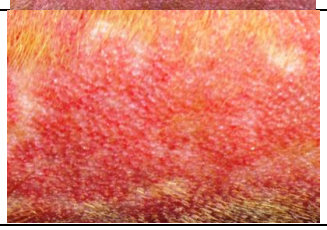
Scoring	Classification	Visual
0	None	
1	Mild	
2	Moderate	
3	Moderate	
4	Severe	
5	Severe	

Figure B-3: Excoriation Scoring

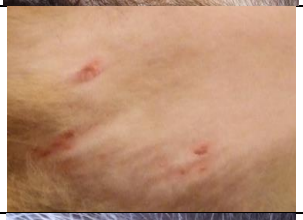
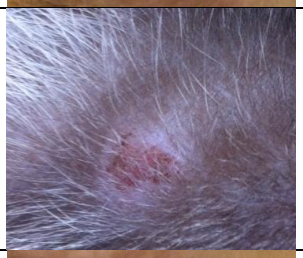
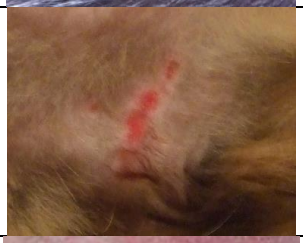
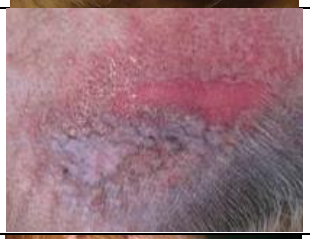
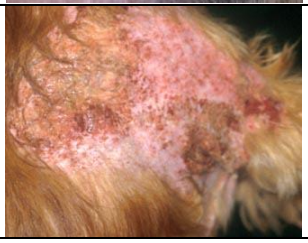
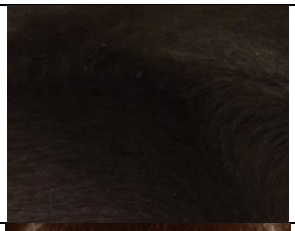
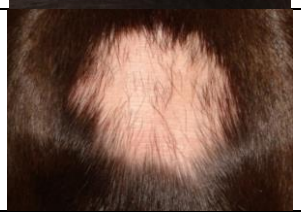
Scoring	Classification	Visual
0	None	
1	Mild	
2	Moderate	
3	Moderate	
4	Severe	
5	Severe	

Figure B-4: Alopecia (Self-Induced) Scoring

Scoring	Classification	Visual
0	None	
1	Mild	

APPENDIX C

EFFECTIVENESS OF A HIGH AND LOW ANIONIC SOAP FOR THE REMOVAL OF OIL-BASED CONTAMINANT

Table C-1

Dog ID	*Tx Group*	*Soap*	*Number of Washes**	*Soap Amount, ml*
1	1	Dawn®	3	360
2	1	Dawn®	1	120
3	1	Dawn®	1	120
4	1	Dawn®	1	120
5	1	Dawn®	1	120
6	2	DermaLyte®	2	240
7	2	DermaLyte®	3	360
8	2	DermaLyte®	2	240
9	2	DermaLyte®	3	360
10	2	DermaLyte®	2	240

APPENDIX D

DAILY DERMATOLOGICAL SUMMARY SCORE

Table D-1

		Evaluation Days														
Dog	*Treatment*	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
1	DermaLyte®	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
2	DermaLyte®	0	0	0	0	0	0	0	1	1	1	1	1	2	2	2
3	DermaLyte®	0	1	1	1	1	1	1	2	2	2	1	2	2	2	2
4	DermaLyte®	0	0	0	0	0	0	0	1	1	2	1	3	2	2	1
5	DermaLyte®	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
6	DermaLyte®	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1
7	DermaLyte®	0	0	0	0	0	1	1	2	2	2	1	1	1	1	1
8	DermaLyte®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	DermaLyte®	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1
10	DermaLyte®	0	0	0	0	0	0	1	1	0	2	1	1	1	1	1
1	Dawn®	0	0	0	0	0	0	0	1	1	1	2	2	1	1	1
2	Dawn®	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2
3	Dawn®	0	0	0	0	0	0	1	2	2	2	1	1	1	1	1
4	Dawn®	0	0	0	0	0	0	1	2	1	1	2	2	3	4	4
5	Dawn®	0	0	0	0	0	0	0	1	0	1	1	1	1	1	2
6	Dawn®	0	0	0	0	0	0	0	0	1	2	2	2	2	2	3
7	Dawn®	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
8	Dawn®	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
9	Dawn®	0	0	0	0	0	0	0	1	1	1	2	1	1	1	2
10	Dawn®	0	0	0	0	0	0	1	2	1	1	1	1	2	2	2

APPENDIX E

RELATIVE DAILY RISK BY LOCATION &MEDIAN TEWL RISK DIFFERENCE BETWEEN LOCATION TREATMENT GROUPS

Figure E-1: Head median relative daily risks for TEWL

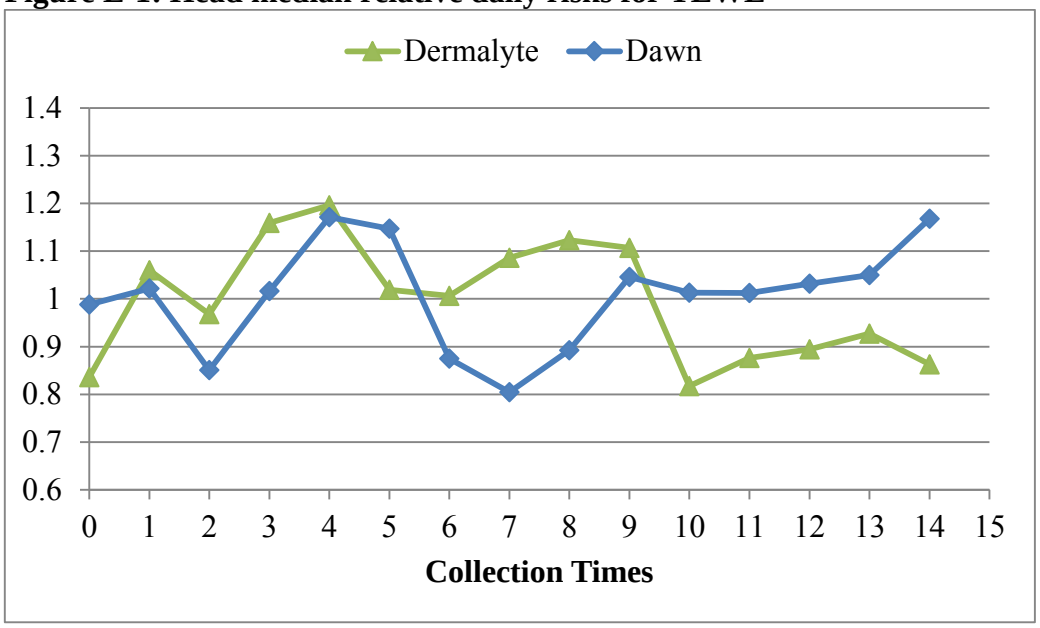


Figure E-2: Neck median relative daily risks for TEWL

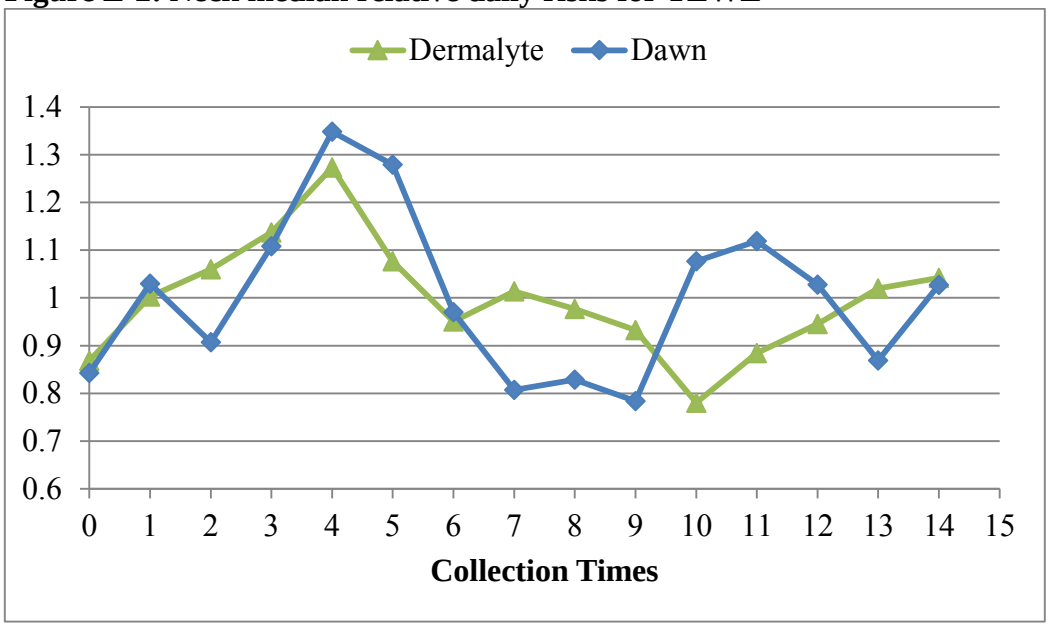


Figure E-3: Right Front Limb median relative daily risks for TEWL

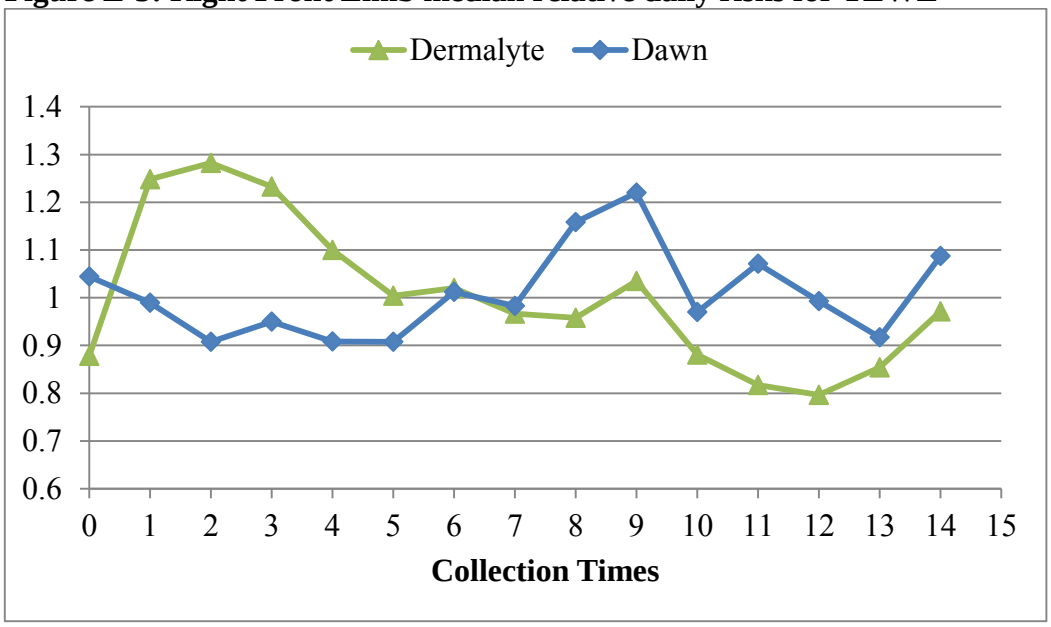


Figure E-4: Left Front Limb median relative daily risks for TEWL

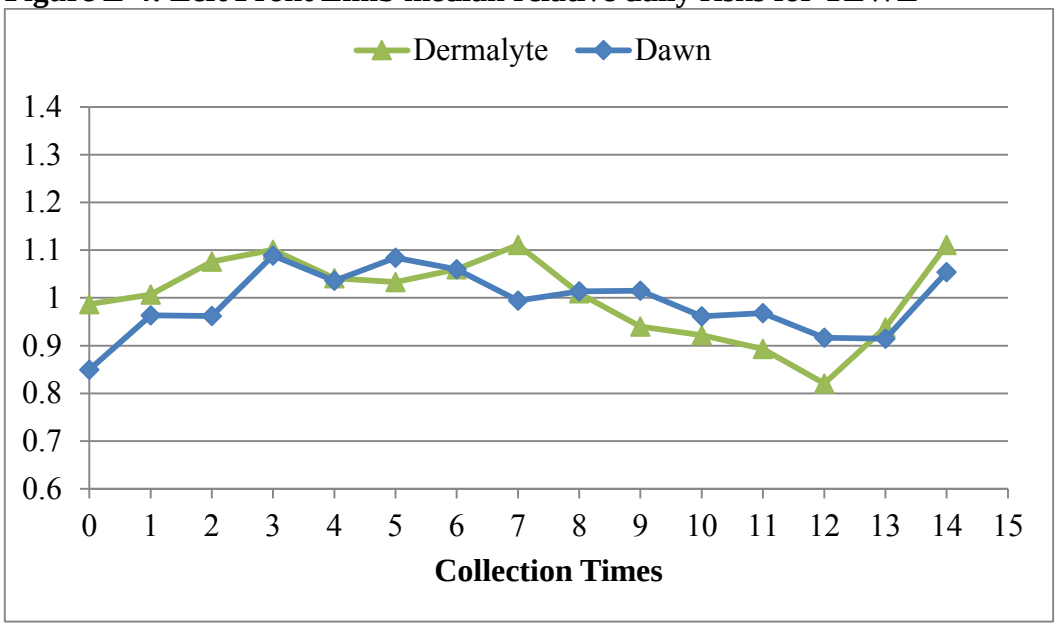


Figure E-5: Right Axilla median relative daily risks for TEWL

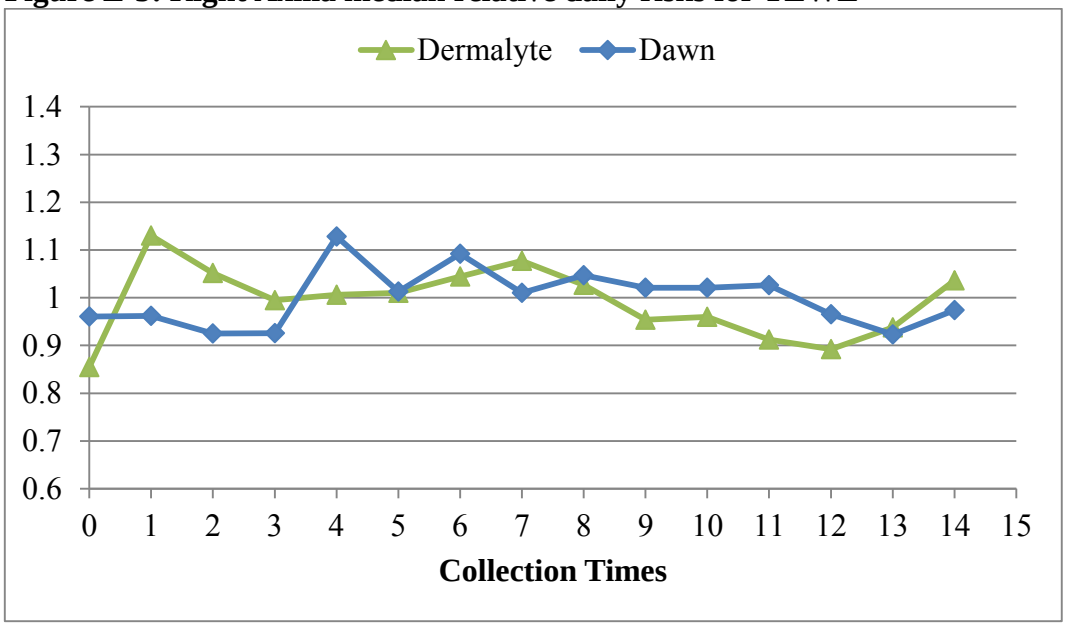


Figure E-6: Left Axilla median relative daily risks for TEWL

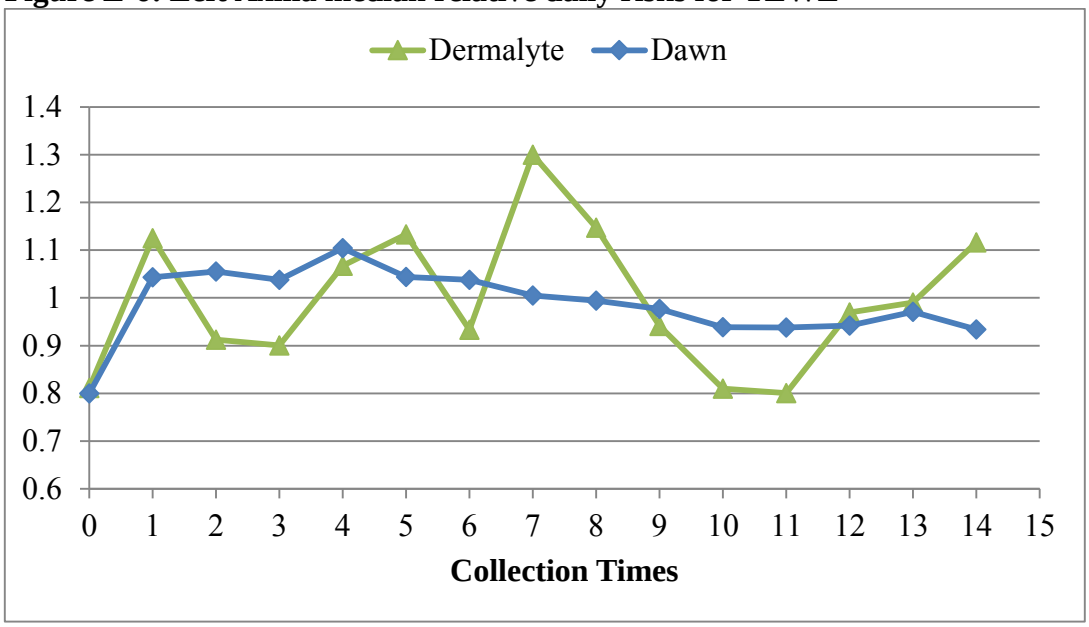


Figure E-7: Sternum median relative daily risks for TEWL

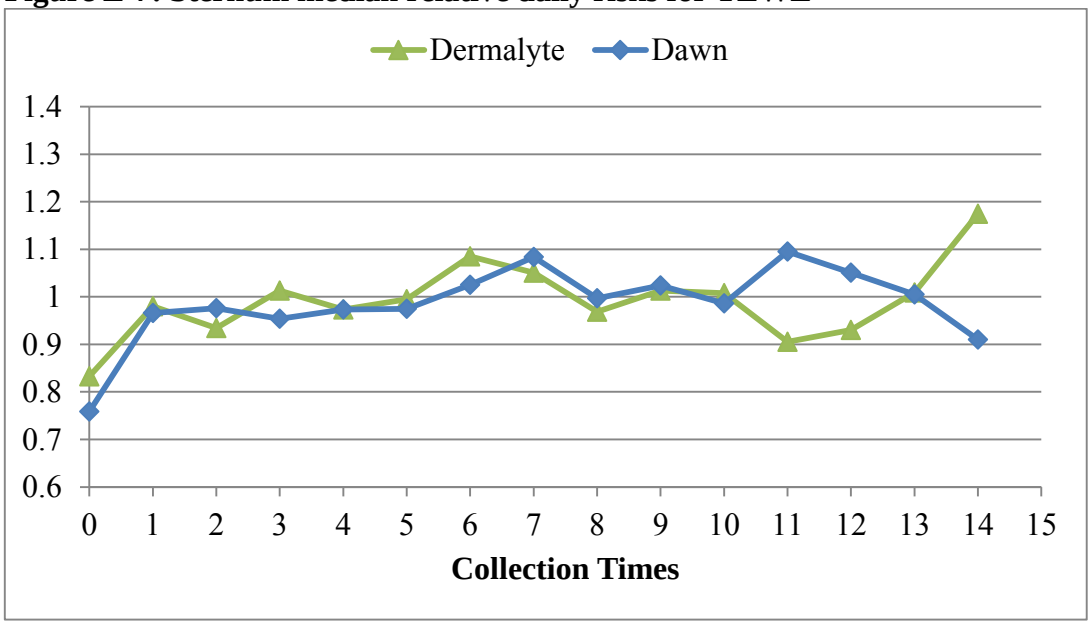


Figure E-8: Thorax Right Lateral median relative daily risks for TEWL

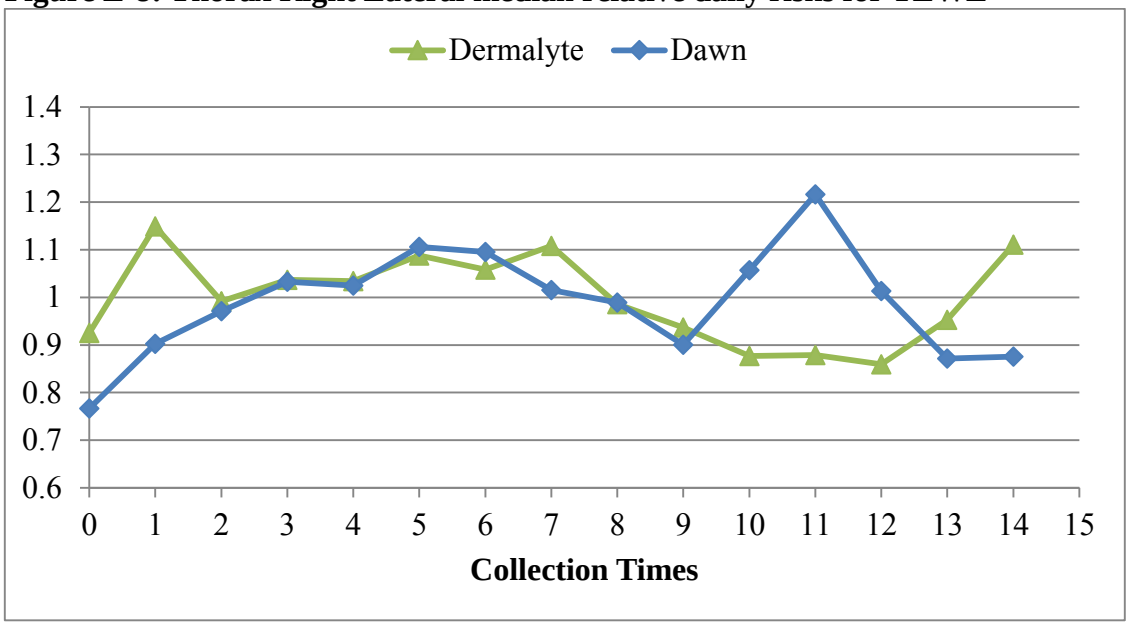


Figure E-9: Thorax Left Lateral median relative daily risks for TEWL

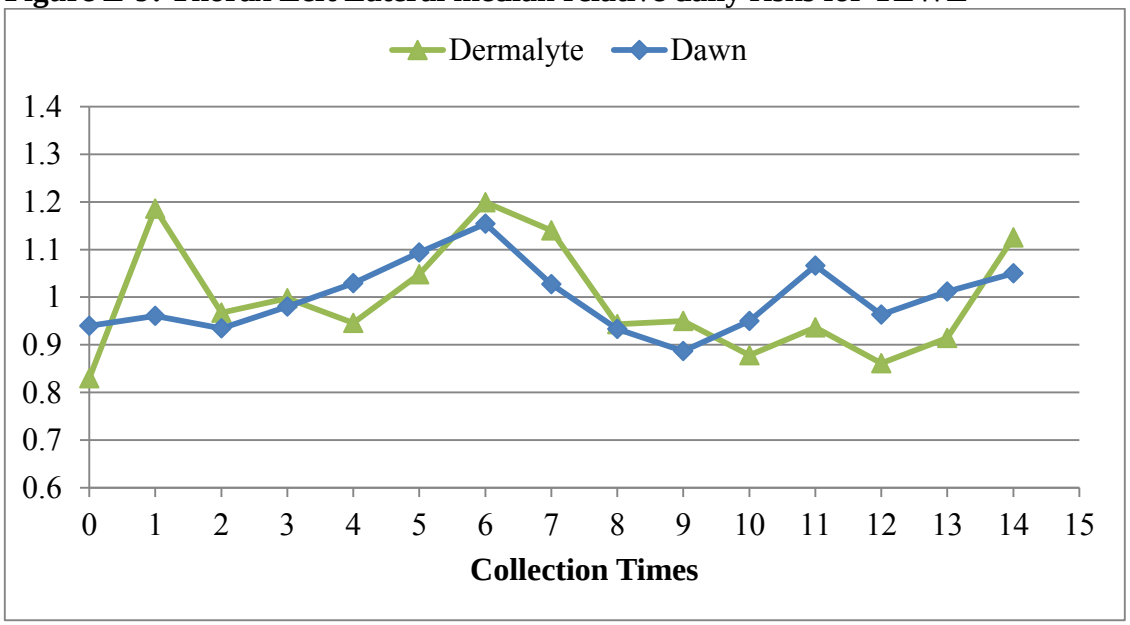


Figure E-10: Thorax Dorsal median relative daily risks for TEWL

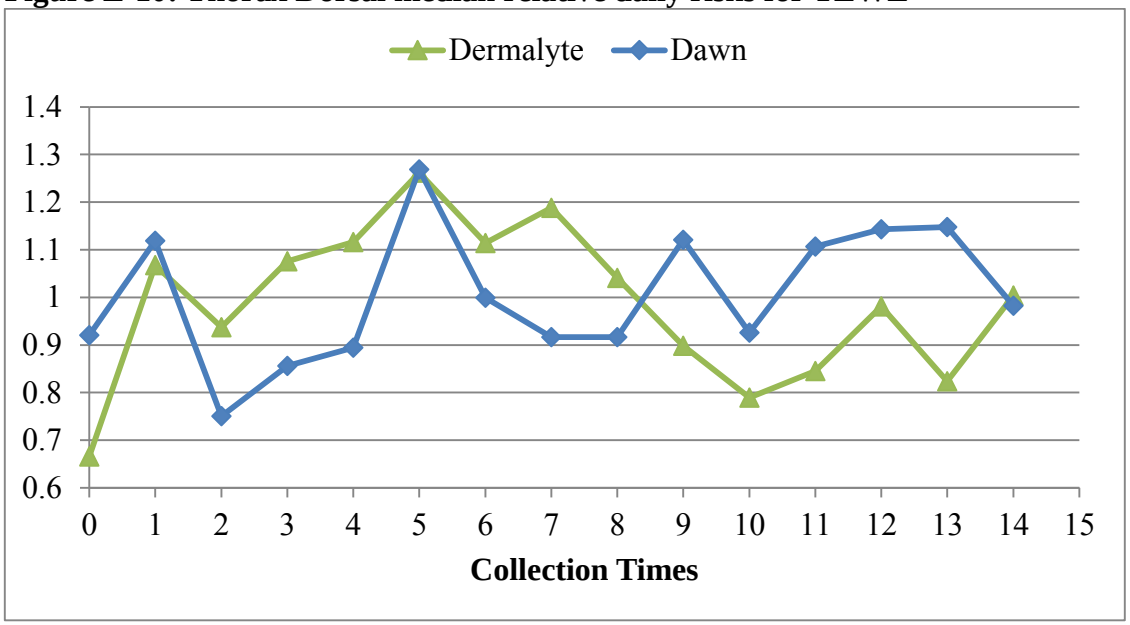


Figure E-11: Abdomen median relative daily risks for TEWL

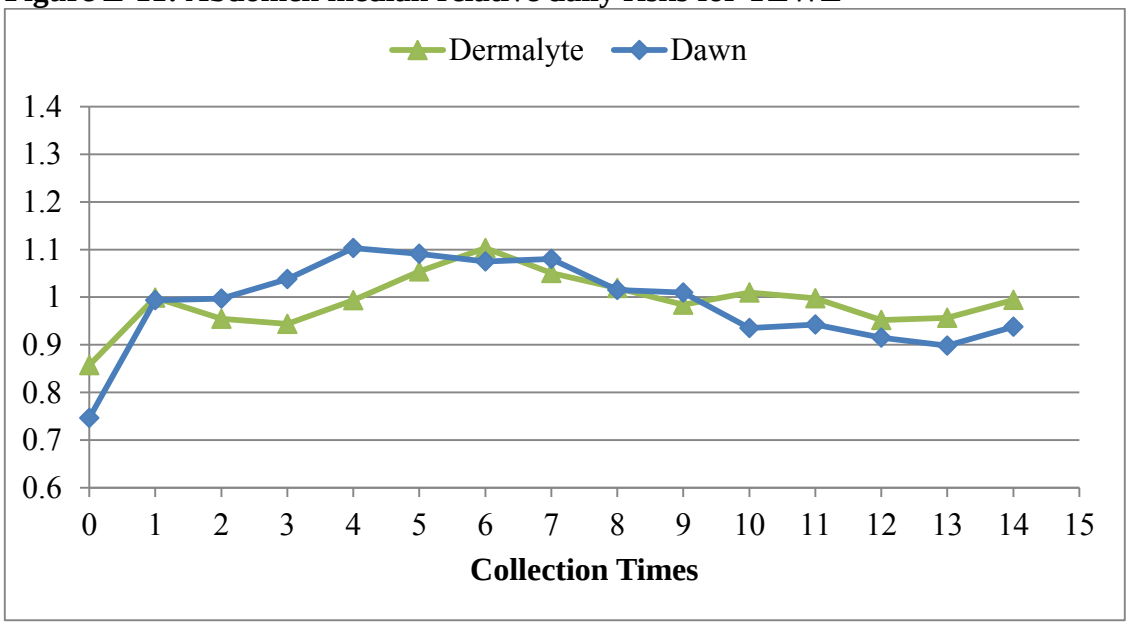


Figure E-12: Lumbar median relative daily risks for TEWL

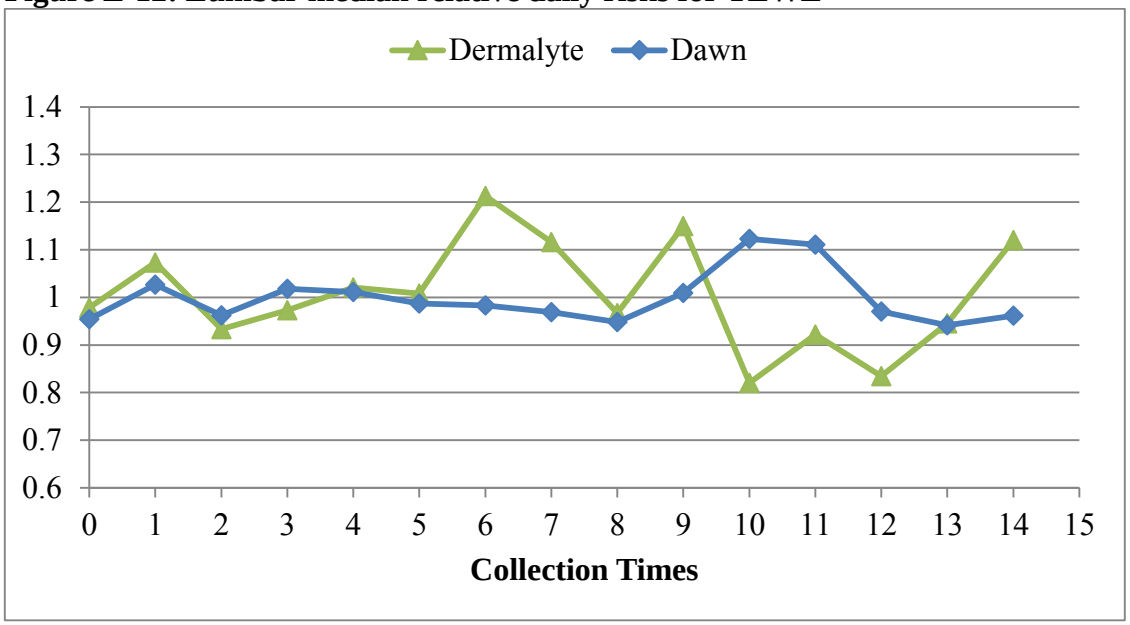


Figure E-13: Right inguinal median relative daily risks for TEWL

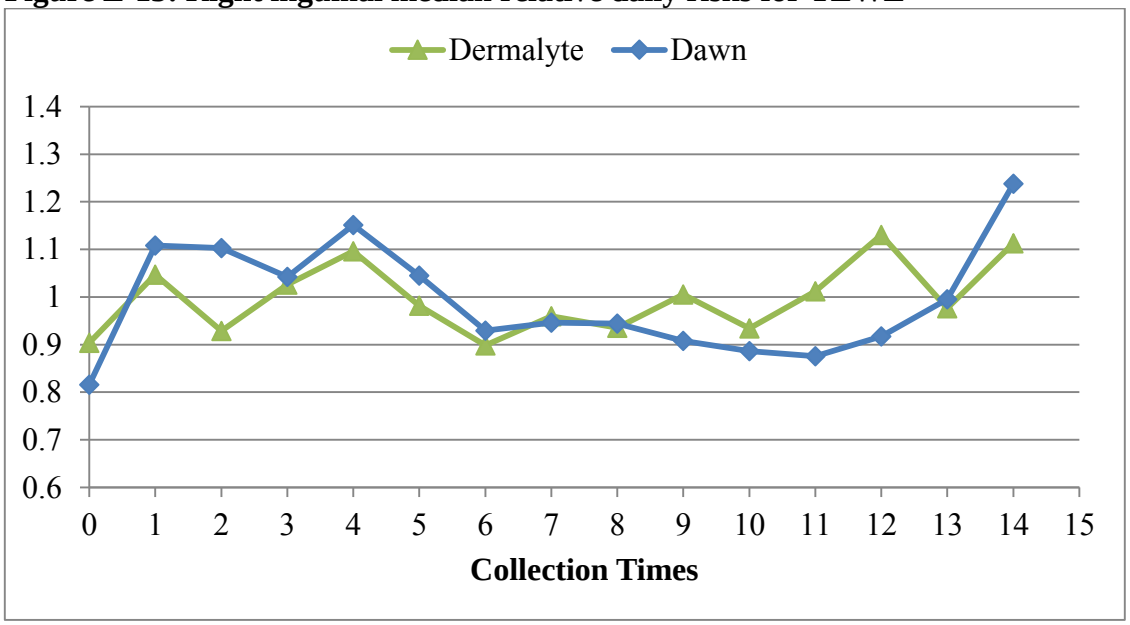


Figure E-14: Left inguinal median relative daily risks for TEWL

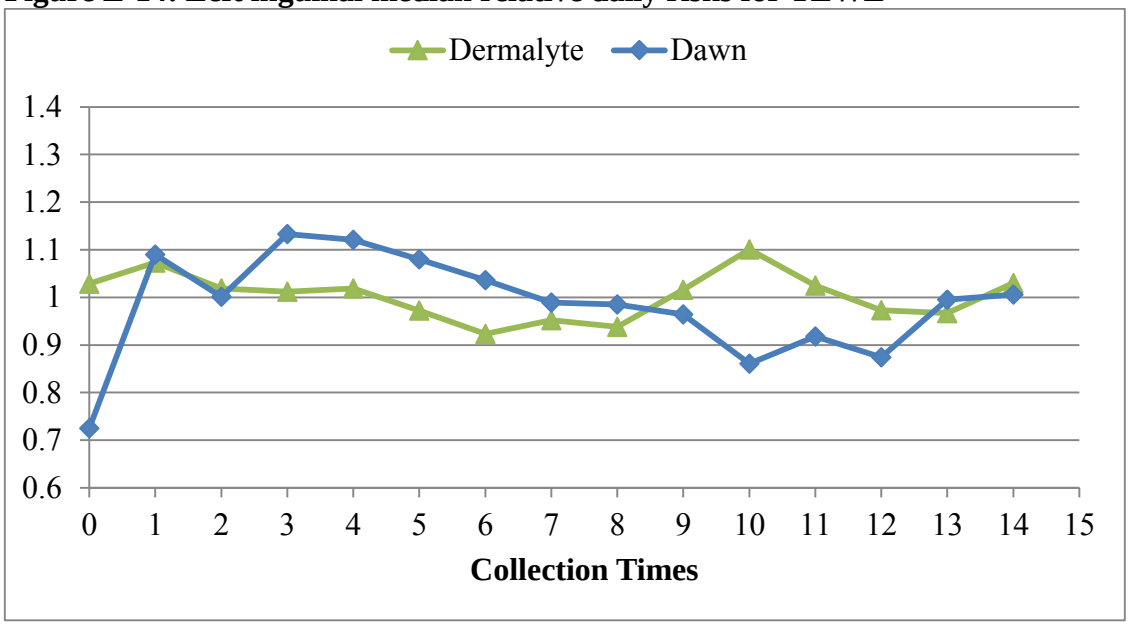


Figure E-15: Left inguinal median relative daily risks for TEWL

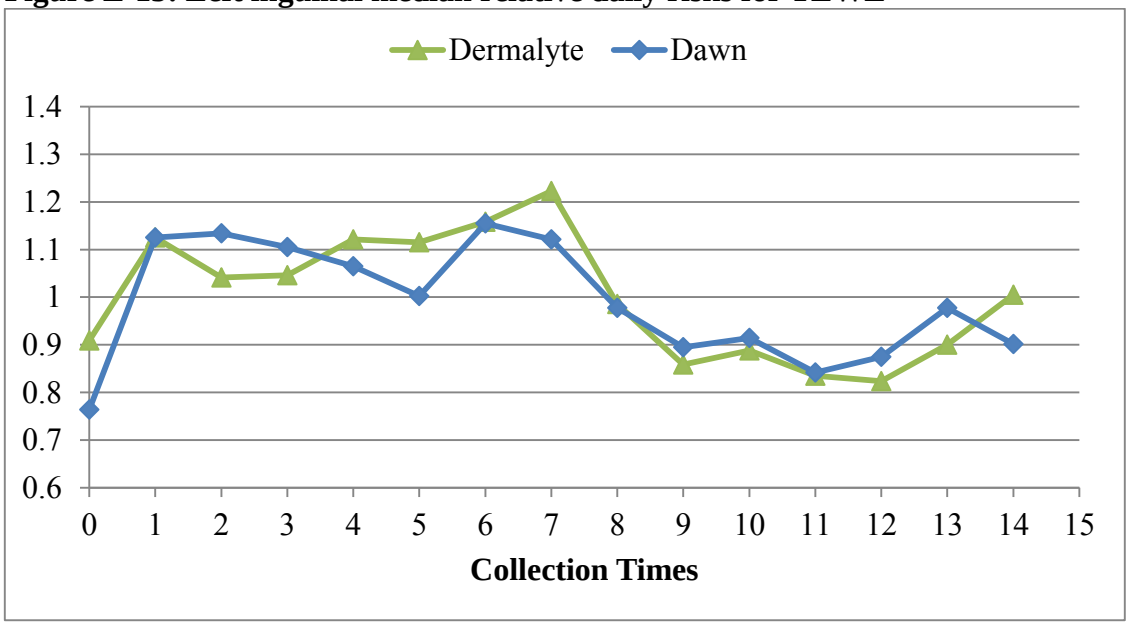


Figure E-16: Right back limb median relative daily risks for TEWL

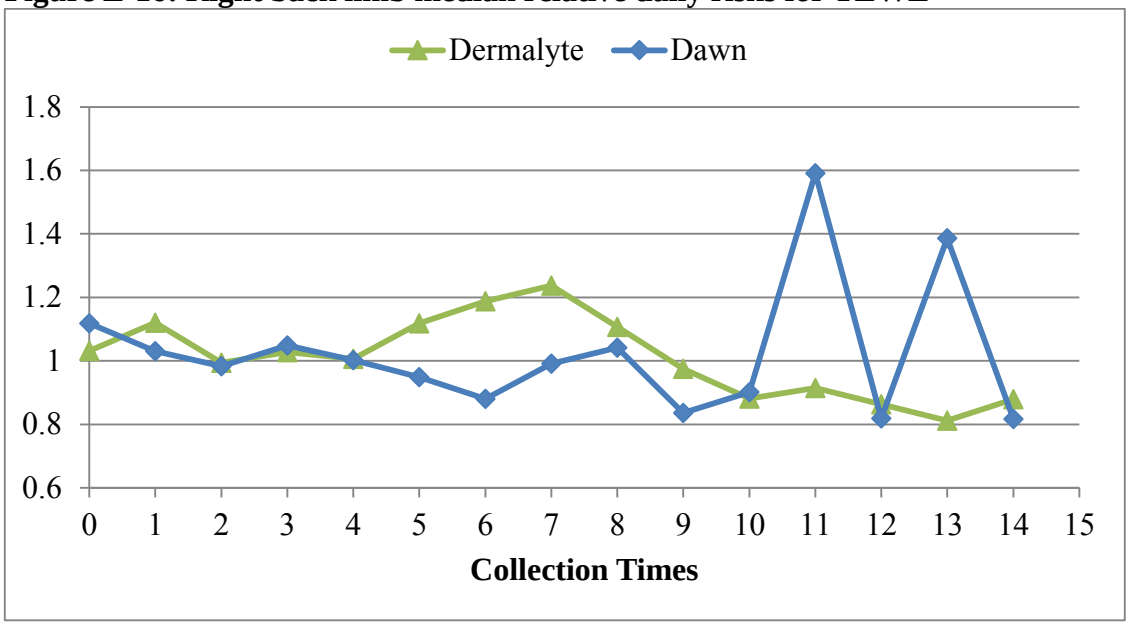


Figure E-17: Left back limb median relative daily risks for TEWL

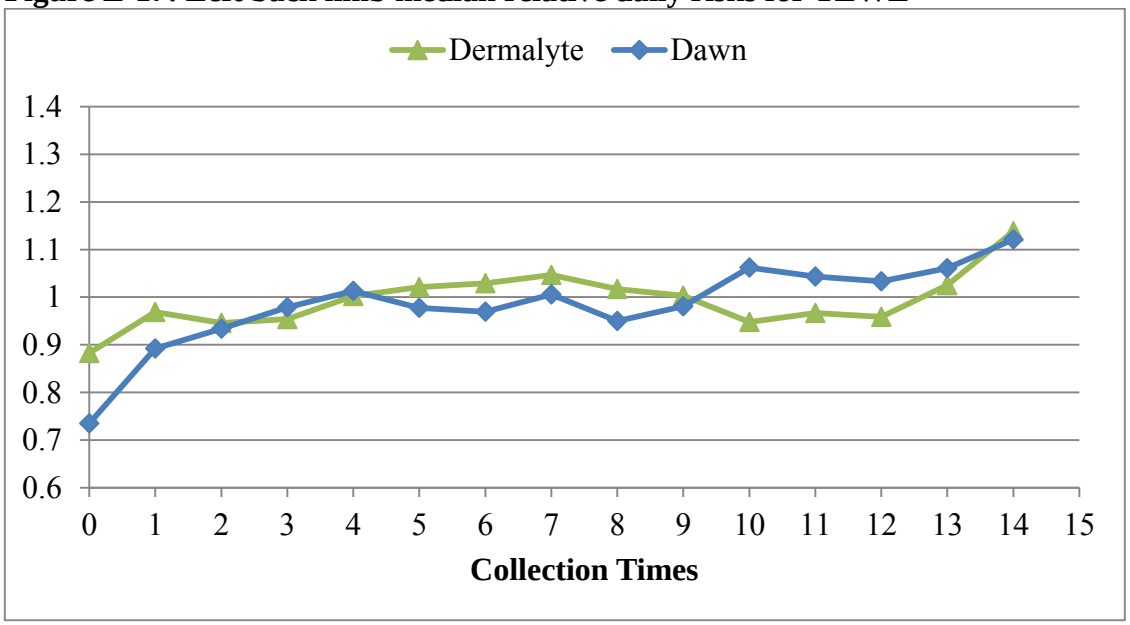


Figure E-18: Right flank median relative daily risks for TEWL

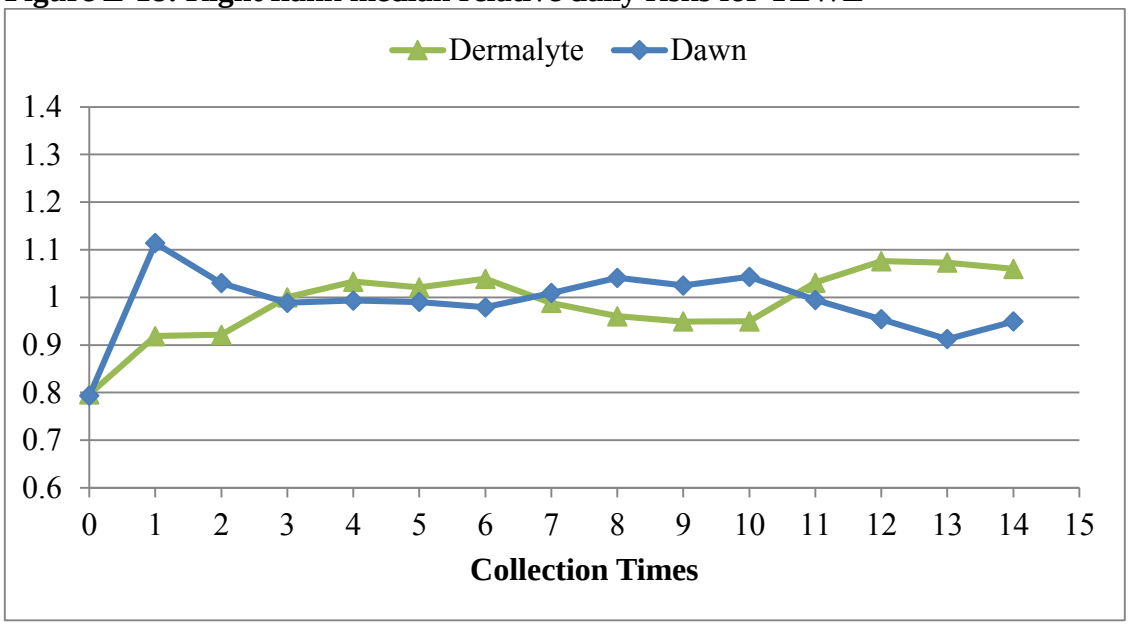


Figure E-19: Tail median relative daily risks for TEWL

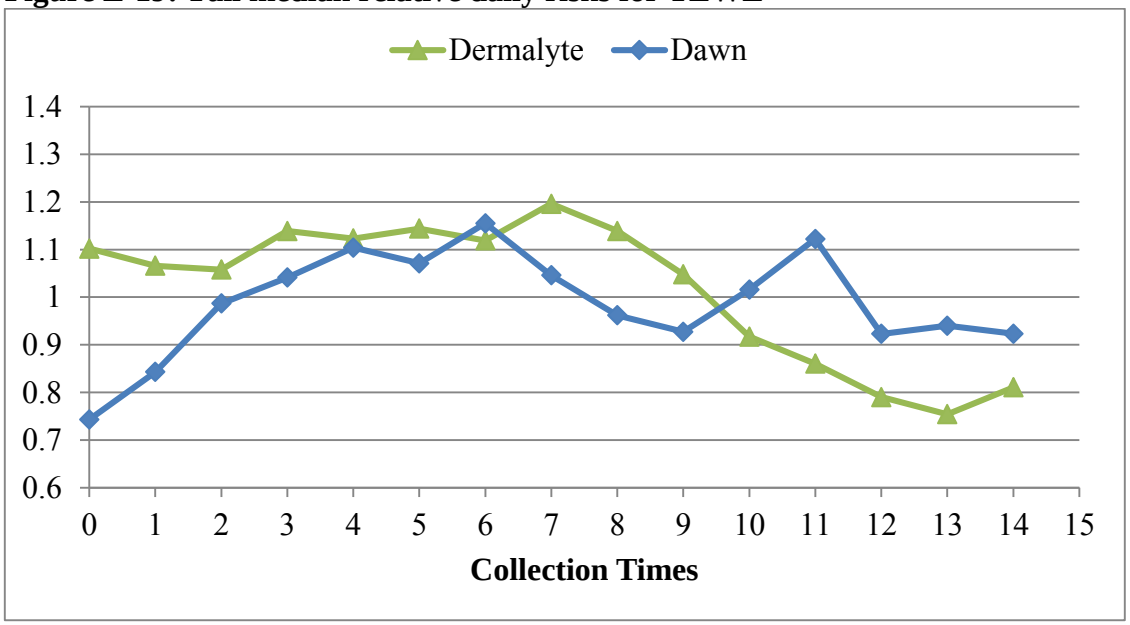


Table E-1: Median TEWL relative risk between locations treatment groups

	Head		*Neck*		*Right Front Limb*	
Day	Median	95 % CI	Median	95 % CI	Median	95 % CI
0	0.8448	0.6381-1.127	1.03	0.8063-1.299	0.835	0.6059-1.162
1	1.037	0.8344-1.298	0.974	0.7993-1.17	1.264	0.9887-1.585
2	1.138	0.9017-1.45	1.168	0.9755-1.409	1.415	1.145-1.781
3	1.141	0.9429-1.387	1.027	0.8678-1.22	1.298	1.056-1.599
4	1.023	0.8404-1.253	0.945	0.7868-1.129	1.21	0.9794-1.505
5	0.885	0.7188-1.085	0.842	0.708-0.9985	1.106	0.8946-1.381
6	1.153	0.9354-1.415	0.978	0.8071-1.167	1.006	0.8114-1.242
7	1.35	1.098-1.693	1.26	1.051-1.528	0.988	0.795-1.228
8	1.261	1.038-1.547	1.179	0.9855-1.418	0.826	0.6604-1.009
9	1.059	0.8686-1.298	1.192	0.9863-1.456	0.849	0.6747-1.067
10	0.8056	0.6423-1.001	0.723	0.5897-0.8749	0.911	0.7322-1.16
11	0.8664	0.7103-1.058	0.789	0.6585-0.942	0.762	0.6072-0.9408
12	0.8658	0.7075-1.052	0.918	0.7689-1.1	0.799	0.6353-0.9924
13	0.8843	0.7189-1.089	1.173	0.9754-1.421	0.934	0.7476-1.191
14	0.7379	0.5896-0.9118	1.014	0.8388-1.231	0.894	0.6988-1.165

Table E-1 continued

	Left Front Limb		*Right Axilla*		*Left Axilla*	
Day	Median	95 % CI	Median	95 % CI	Median	95 % CI
0	1.165	0.8473-1.607	0.8912	0.8063-1.299	1.012	0.7505-1.395
1	1.044	0.828-1.324	1.174	0.7993-1.17	1.079	0.8746-1.333
2	1.121	0.9221-1.398	1.139	0.9755-1.409	0.865	0.7048-1.052
3	1.011	0.8267-1.236	1.074	0.8678-1.22	0.869	0.7074-1.05
4	1.002	0.8264-1.218	0.8905	0.7868-1.129	0.965	0.7952-1.16
5	0.9508	0.7766-1.157	0.9967	0.708-0.9985	1.084	0.8978-1.317
6	1.002	0.8264-1.209	0.9547	0.8071-1.167	0.897	0.7193-1.09
7	1.123	0.9266-1.383	1.069	1.051-1.528	1.296	1.069-1.563
8	0.9964	0.8201-1.209	0.9807	0.9855-1.418	1.155	0.9611-1.395
9	0.9239	0.7523-1.122	0.932	0.9863-1.456	0.962	0.7906-1.161
10	0.9584	0.7829-1.185	0.9385	0.5897-0.8749	0.864	0.7001-1.056
11	0.9215	0.7528-1.122	0.8866	0.6585-0.942	0.853	0.6948-1.037
12	0.8942	0.7004-1.116	0.9234	0.7689-1.1	1.03	0.8547-1.25
13	1.027	0.8345-1.276	1.016	0.9754-1.421	1.019	0.8303-1.231
14	1.053	0.8219-1.349	1.066	0.8388-1.231	1.198	0.972-1.48

Table E-1 continued

	Sternum		*Thorax Right Lateral*		*Thorax Left Lateral*	
Day	Median	95 % CI	Median	95 % CI	Median	95 % CI
0	1.102	0.807-1.517	1.204	0.8063-1.299	0.886	0.6245-1.264
1	1.015	0.824-1.256	1.272	0.7993-1.17	1.236	0.9592-1.583
2	0.9567	0.7824-1.16	1.019	0.9755-1.409	1.033	0.8176-1.311
3	1.067	0.8939-1.278	1.002	0.8678-1.22	1.017	0.8167-1.27
4	1.001	0.8339-1.19	1.008	0.7868-1.129	0.918	0.731-1.143
5	1.021	0.8512-1.221	0.9842	0.708-0.9985	0.957	0.7696-1.181
6	1.06	0.8834-1.265	0.966	0.8071-1.167	1.035	0.8168-1.312
7	0.9702	0.7939-1.165	1.094	1.051-1.528	1.111	0.9008-1.391
8	0.9707	0.7992-1.169	0.9944	0.9855-1.418	1.013	0.8043-1.277
9	0.9916	0.8256-1.192	1.042	0.9863-1.456	1.076	0.8568-1.37
10	1.022	0.8556-1.241	0.83	0.5897-0.8749	0.924	0.7329-1.16
11	0.8247	0.6705-0.9831	0.7224	0.6585-0.942	0.878	0.6855-1.098
12	0.8842	0.7276-1.052	0.8464	0.7689-1.1	0.894	0.7038-1.139
13	1.003	0.8274-1.201	1.096	0.9754-1.421	0.904	0.7173-1.118
14	1.296	1.05-1.645	1.271	0.8388-1.231	1.077	0.8341-1.395

Table E-1 continued

	Thorax Dorsal		*Abdomen*		*Lumbar*	
Day	Median	95 % CI	Median	95 % CI	Median	95 % CI
0	0.7202	0.547-0.9655	1.147	0.8063-1.299	1.026	0.7601-1.372
1	0.9555	0.7737-1.183	1.006	0.7993-1.17	1.043	0.8285-1.31
2	1.247	0.9902-1.572	0.9559	0.9755-1.409	0.974	0.7867-1.194
3	1.256	1.028-1.535	0.9093	0.8678-1.22	0.955	0.7789-1.155
4	1.248	1.023-1.534	0.8991	0.7868-1.129	1.01	0.8267-1.228
5	0.9963	0.8197-1.219	0.9658	0.708-0.9985	1.019	0.8345-1.249
6	1.116	0.9128-1.361	1.028	0.8071-1.167	1.235	1.014-1.502
7	1.298	1.058-1.602	0.9728	1.051-1.528	1.153	0.9506-1.4
8	1.135	0.9284-1.392	1.004	0.9855-1.418	1.02	0.8322-1.258
9	0.8019	0.6496-0.9797	0.9722	0.9863-1.456	1.143	0.9277-1.418
10	0.8507	0.6759-1.062	1.082	0.5897-0.8749	0.725	0.5712-0.9024
11	0.7624	0.6214-0.9359	1.058	0.6585-0.942	0.829	0.6675-1.016
12	0.8585	0.705-1.051	1.04	0.7689-1.1	0.86	0.6911-1.069
13	0.7172	0.5751-0.8744	1.068	0.9754-1.421	1.005	0.822-1.244
14	1.022	0.8211-1.282	1.062	0.8388-1.231	1.163	0.9359-1.461

Table E-1 continued

	Right Inguinal		*Left Inguinal*		*Right Back Leg*	
Day	Median	95 % CI	Median	95 % CI	Median	95 % CI
0	1.111	0.7946-1.576	1.414	0.8063-1.299	1.189	0.7964-1.745
1	0.9452	0.7486-1.212	0.9845	0.7993-1.17	1.002	0.7792-1.275
2	0.8403	0.6576-1.044	1.021	0.9755-1.409	0.918	0.7275-1.14
3	0.9876	0.8019-1.225	0.892	0.8678-1.22	0.945	0.755-1.162
4	0.9533	0.7714-1.184	0.9123	0.7868-1.129	1.051	0.849-1.308
5	0.94	0.7645-1.155	0.9	0.708-0.9985	1.114	0.8999-1.419
6	0.9692	0.7665-1.205	0.8895	0.8071-1.167	1.001	0.7976-1.251
7	1.015	0.8227-1.248	0.9622	1.051-1.528	1.089	0.8587-1.403
8	0.9864	0.7941-1.219	0.9513	0.9855-1.418	1.007	0.8052-1.267
9	1.107	0.9067-1.387	1.054	0.9863-1.456	0.958	0.7469-1.215
10	1.052	0.8379-1.3	1.283	0.5897-0.8749	0.971	0.77-1.216
11	1.158	0.9466-1.428	1.114	0.6585-0.942	0.993	0.7783-1.272
12	1.239	1.005-1.555	1.116	0.7689-1.1	0.944	0.7301-1.193
13	0.9797	0.7748-1.213	0.9703	0.9754-1.421	0.918	0.7193-1.145
14	0.8978	0.7047-1.146	1.025	0.8388-1.231	1.118	0.8615-1.471

Table E-1 continued

	Left Back Limb		*Right Flank*		*Left Flank*	
Day	Median	95 % CI	Median	95 % CI	Median	95 % CI
0	0.9147	0.6508-1.283	1.204	0.8063-1.299	1.01	0.7041-1.45
1	1.089	0.8188-1.437	1.086	0.7993-1.17	0.823	0.6569-1.024
2	1.01	0.7689-1.324	1.01	0.9755-1.409	0.893	0.7399-1.073
3	0.9806	0.7513-1.27	0.9722	0.8678-1.22	1.015	0.8488-1.218
4	1	0.7667-1.306	0.9895	0.7868-1.129	1.044	0.8761-1.245
5	1.177	0.909-1.547	1.044	0.708-0.9985	1.035	0.8676-1.227
6	1.351	1.041-1.788	1.061	0.8071-1.167	1.06	0.8955-1.281
7	1.251	0.9681-1.631	1.042	1.051-1.528	0.979	0.814-1.17
8	1.064	0.8245-1.384	1.073	0.9855-1.418	0.921	0.7619-1.091
9	1.165	0.8874-1.542	1.023	0.9863-1.456	0.925	0.7669-1.101
10	0.9763	0.7376-1.303	0.8902	0.5897-0.8749	0.908	0.7471-1.077
11	0.5744	0.4536-0.7439	0.925	0.6585-0.942	1.037	0.8644-1.24
12	1.053	0.7917-1.43	0.9283	0.7689-1.1	1.13	0.9507-1.37
13	0.5845	0.4427-0.756	0.9629	0.9754-1.421	1.18	0.9851-1.446
14	1.077	0.7958-1.49	1.016	0.8388-1.231	1.119	0.9038-1.396

Table E-1 continued

	Tail	
Day	Median	95 % CI
0	1.483	1.12-1.985
1	1.268	1.028-1.593
2	1.073	0.8889-1.273
3	1.094	0.9099-1.309
4	1.016	0.8512-1.205
5	1.068	0.9012-1.285
6	0.9692	0.8041-1.146
7	1.145	0.9617-1.361
8	1.186	0.9954-1.433
9	1.13	0.9437-1.365
10	0.9032	0.7519-1.083
11	0.7667	0.628-0.9257
12	0.8572	0.706-1.043
13	0.8013	0.654-0.9628
14	0.8773	0.7057-1.086