IFSCC 2025 full paper (**IFSCC2025-1162**)

Safety assessment of ingredients for pet care formulations: development of an in vitro canine skin tolerance model

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1. Introduction

The number of households with pets is increasing, with dogs and cats being the most popular globally [1-3]. More than half of the global population was estimated to have a pet at home with one dog in every three households in 2016 [4]. Around 45.5% of US households own a dog (2024) [1], 20% in the EU [2] (2022) and 32% in Asian countries (2021) [3]. Assessing the safety of ingredients and topical formulations for pet animals presents challenges, as it requires consideration of both physiological and behavioural specificities of the animal and the human that apply the products. For example, the analysis needs to take into account the propensity of dogs and cats licking themselves, which could lead to ingestion. The lack of their reflex to close their eyes to avoid contact with surfactants could serve as another illustration. This work aimed to develop a new *in vitro* experimental model as a part of a complete safety approach to select the most suitable ingredients and formulations for both humans and dogs.

Since the groundbreaking publication of a comprehensive protocol for producing *in vitro* reconstructed human epidermis [5], this 3D biological system has emerged as a favored alternative to animal testing, especially within the cosmetic industry. Indeed, a fully differentiated epidermis can be generated in two weeks using cultured keratinocytes. This model is valuable for evaluating ingredient and final product effects whether applied topically or systemically. Adapting this model to non-human keratinocytes is needed for identifying species-specific effects. There are few studies exploring such adaptations, such as canine epidermis [6, 7] and equine epidermis [8]. Previous study on canine epidermis focused on reproducing atopic canine skin to investigate the relationships with inflammatory mechanisms [6]. Another study addressed skin corrosion and irritation assessment using human-validated protocols based on cellular viability [7]. Assuming mechanistic parallels in skin irritation responses between canines and humans, even at non-cytotoxic concentrations, this work sought to investigate a more complete approach. The method extends beyond cellular viability assays to include assessments of early-stage perturbations within the irritation cascade. This study aimed to develop an *in vitro* experimental model using a reconstructed canine epidermis and protocol to evaluate canine skin tolerance by assessing the effects of ingredients and formulations on tissue viability, skin barrier properties, and inflammation, thereby gaining a better understanding of the mechanisms of action of applied products. Furthermore, this study aimed to provide essential information for the safety assessment strategy of ingredients in finished dog products contributing to more ethical, accurate research in veterinary dermatology and One Health approach.

2. Materials and Methods

2.1. Safety approach for ingredient selection

Substances intended for pet care, as well as those for cosmetic use, are subject to chemical regulations in various global regions. A parallel approach was adopted to ensure ingredient safety for both human and animal applications (summarized in Figure 1).

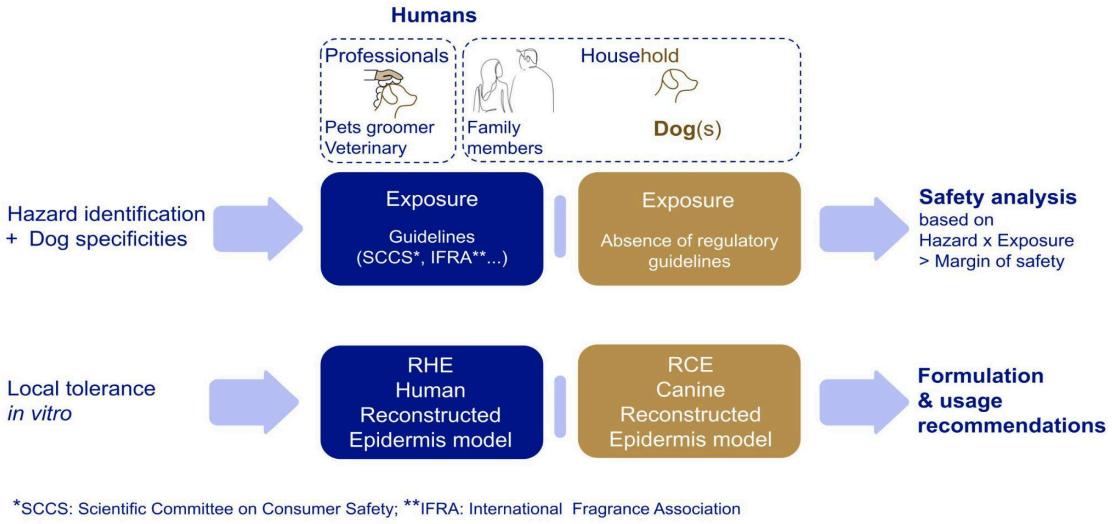

Figure 1. Safety strategy

While the assessment methodology was conceptually aligned, a critical disparity existed. Established cosmetic regulatory guidelines govern the safety evaluation of ingredients for human use (Scientific Committee on Consumer Safety - SCCS - guidance [9] or International Fragrance Association - IFRA - standards). However, equivalent standardized guidelines for pet care are currently absent, substantially increasing the complexity of the safety assessment process of ingredients for pet care formulations. Consequently, it became necessary to formulate research hypotheses and extrapolative assumptions to address the lack of prescriptive standards for dogs. This necessitated a risk-based approach informed by comparative physiological and toxicological principles. The absence of standardized weight and size metrics for dogs complicates the calculation of safety margins. Market analysis of existing products and their usage patterns was conducted to estimate exposure levels (application quantity/frequency of use). Dermal absorption was conservatively estimated at 100%. Given the propensity of dogs to groom themselves via licking, oral toxicity was systematically investigated. A key component of this risk analysis was the selection of ingredients with a toxicological profile appropriate for canine use, specifically excluding substances known or suspected to be toxic to dogs. This included identifying such substances, as ingredients tolerated in humans may pose a risk to canines. Examples of substances documented to be toxic to dogs include glycols (e.g., propylene glycol, xylitol) and cocoa butter [10-12]. This comprehensive risk assessment facilitated the selection of established ingredients possessing potential benefits for canine applications and enabled the determination of a safe testing dosage regimen. Dosage selection and testing conditions for formulations were determined based on the intended final application, differentiating between leave-on and rinse-off products by taking into consideration a retention factor, and accounting for specific anatomical sites of application, namely the periocular region, ears, nasal planum, and paw pads.

Safety evaluations for humans, including household members and professionals, were conducted adhering to SCCS and IFRA guidelines. The chemical structures of the ingredients and their known hazards to humans serve as the starting point for this analysis. Exposure was estimated by adjusting the frequency of use based on the product type.

Although IFRA Standards may apply to certain finished pet products, their scope is limited to evaluating human exposure during product administration, and they do not encompass the evaluation of exposure in the pet. For example, it was postulated that human exposure to pet shampoos would be comparable to exposure to hand dishwashing liquids.

Local tolerance data complement the safety assessment of ingredients and contribute to guide recommended use levels in formulations. *In vitro* models have been developed for humans to address specific conditions such as impaired skin barrier [13]. The *in vitro* canine local tolerance model developed in this study aims to further supplement these data.

2.2 Tested materials

Sodium Dodecyl Sulfate (SDS), a commonly used positive control, was tested at various concentrations (Table 1) and exposure durations to assess the model's sensitivity and refine the experimental protocol.

Table 1. Ingredients description

Identification	INCI name	Tested conditions (% w/w active matter)
PBS	Phosphate Buffered Saline	pure
SDS: Positive Control	Sodium Docecyl Sulfate	0.5%, 0.15% in demineralized water
TSPol: Thickening-Stabilizing Polymer	Polyacrylate crosspolymer-6	0.5% in demineralized water

Formulation benchmarks, designed for canine care and encompassing different applications such as cleansing, paw pad protection, and nose and pad moisturizing, were evaluated under conditions reflecting their intended use as either leave-on or rinse-off products (Table 2).

Table 2. Formulation benchmarks description

Identification	Application	Formulation type	**Composition** (main ingredients)
Benchmark 1	Rinse-off	Shampoo	*Surfactants*: Sodium Lauryl Glucose Carboxylate, Lauryl Glucoside, Coco-Glucoside, Sodium Cocoamphoacetate, Cocamidopropyl Betain *Preservative system:* Sodium Citrate, Citric Acid, Sorbic Acid, Sodium Benzoate
Benchmark 2	Leave-on	Cream-gel for paw pads	*Oil:*Jojoba *Stabilizer:* Xanthan gum *Preservative system:* Benzyl alcohol
Benchmark 3	Leave-on	Oil-in-Water emulsion (O/W) for nose and paw pads	*Emulsifiers:* Cetearyl alcohol, Glyceryl Stearate *Oils & fats*: Coco-Caprylate/Caprate, Shea butter *Stabilizers:* Acacia Senegal Gum, Xanthan gum *Preservative system:* Ethylhexylglycerin *Active ingredients*: Urea, *Centella asiatica* extract

Benchmarks 1 and 2 were employed to optimize the protocol, identify key parameters, and calibrate the tolerance assessment. Benchmark 1, representing a typical rinse-off formulation (Shampoo), was evaluated at a 1% dilution. This dilution factor was derived from the recommended retention factor of 0.01, as specified by the Scientific Committee on Consumer Safety (SCCS). Preliminary histological analysis revealed that the *in vitro* model exhibited

complete structural disruption when exposed to a 10% concentration of this benchmark (data not shown). In addition, the substantial volume of water necessary for generating foam and thoroughly rinsing canine fur supported the selection of this dilution [14]. Benchmark 2, representing leave-on formulations, was tested undiluted. Benchmark 3 (Table 2) and a thickening-stabilizing polymer with established use in human applications (Table 1) were subsequently evaluated using the optimized protocol. For the latter, a moderate concentration representative of typical current usage was selected for this initial assessment. The formulations were devoid of both fragrance and essential oils which can interfere with the animals' sense of smell.

2.3 In vitro assessment of ingredients and formulations effects

Evaluations were performed utilizing a ten-day-old Reconstructed Canine Epidermis (RCE) model. The RCE exhibited a well-defined histological structure comprising a basal layer, spinous layer, granular layer containing keratohyalin granules, and stratum corneum. A volume of 50 µL of each test compound was applied to the RCE and distributed using a brush, followed by overnight incubation (*i.e.* 16 or 12 hours) at 37°C with 5% CO_2. RCE cultures were terminated on day 11. The initial experimental contact time was 16 hours, subsequently adjusted based on preliminary findings. Several biological parameters indicative of skin tolerance were assessed, including cellular viability, barrier function via Trans-Epidermal Water Loss (TEWL) [15] or Trans-Epithelial-Electrical-Resistance (TEER) measurements, morphological analysis, and inflammatory response by monitoring Interleukin-8 (IL-8) release [16]. The number of RCE samples and measurements per sample varied throughout the experiments and are detailed with the corresponding results.

- Cellular viability: RCEs were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which was reduced to blue formazan crystals by mitochondrial succinate dehydrogenase. This reduction is directly proportional to enzyme activity. Following cell lysis and formazan crystal solubilization using isopropanol/HCl, the optical density (OD) of the solubilized extracts was measured at 550 nm using a spectrophotometer. Absorbance is proportional to viable cell count and metabolic activity. Viability (%) was calculated as 100 - ((Untreated OD - Test OD) / Untreated OD x 100. Compounds with >50% viability were considered non-cytotoxic; <50% were considered cytotoxic.
- Trans-Epidermal Water Loss (TEWL): measurements were conducted using a Tewameter TM300. Thirty measurements were acquired per reading at a rate of one measurement per second. Results were reported in g/m²/h.
- Trans-Epithelial-Electrical-resistance (TEER): TEER was measured across the epithelium (paracellular pathway), in comparison to an untreated control, using a Voltmeter-Ohmmeter and the results were expressed in Ohms. This measurement, which reflects the regulation of ion movement by polarized plasma membrane surfaces and cell-to-cell tight junctions, indicates the overall integrity of the epidermal barrier.
- IL-8 release: IL-8 levels, involved in inflammatory mechanisms, were quantified in the culture supernatants, using a specific Enzyme-Linked Immunosorbent Assay (ELISA) kit, following the manufacturer's instructions (Canine IL-8 R&D Systems). Results were expressed in pg/mL.
- Tissue morphology: RCE were fixed in formalin before being dehydrated in multiple baths with increasing concentrations of ethanol and then embedded in paraffin. Transverse sections, 5 µm in thickness, were obtained using a microtome (several sections per slide, two slides per RCE) and maintained at room temperature until hematoxylin and eosin (H&E) staining. Sections were deparaffinized, stained with hematoxylin, rinsed, and then stained with eosin. After washing, sections were mounted in aqueous medium for microscopic examination using a Pannoramic slide scanner. Two to three images were captured per replicate.

Results for cellular viability, TEWL, TEER, and IL-8 release were expressed as mean ± standard error of the mean (SEM).

3. Results

3.1. Protocol optimization and model calibration

Initial experiments focused on determining optimal operating conditions and calibrating the model using a standard positive control, Sodium Dodecyl Sulfate (SDS), and Benchmarks 1 and 2. In this preliminary phase, a maximum contact time of 16 hours with the test item was applied. Two experimental series were conducted.

The first series, represented by the hatched bars in Figures 2 and 3, aimed to establish a suitable SDS concentration that would avoid complete tissue destruction and allow for meaningful assessment of other parameters, specifically barrier function and inflammatory response. Tissue viability (Figure 2, hatched bars) and Trans-Epidermal Water Loss (TEWL) (Figure 3, hatched bars) were affected in a dose-dependent manner by increasing SDS concentrations. A 0.5% SDS concentration induced strong tissue damage, resulting in cellular viability below 20%. Conversely, a 0.15% SDS concentration was deemed suitable as a positive control, maintaining cellular viability above 75%. Despite statistically significant variations, the TEWL values remained relatively close across the tested doses of SDS (Figure 3).

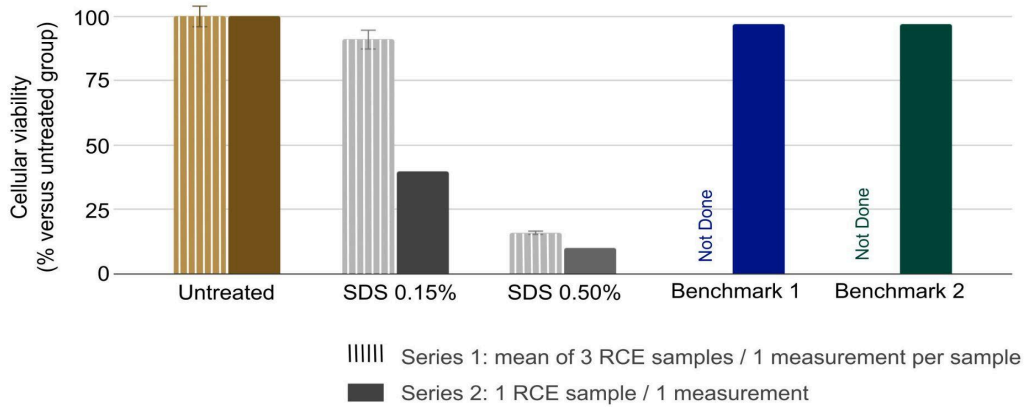

Figure 2. Cellular viability / 16 hours of contact (in % versus untreated group)

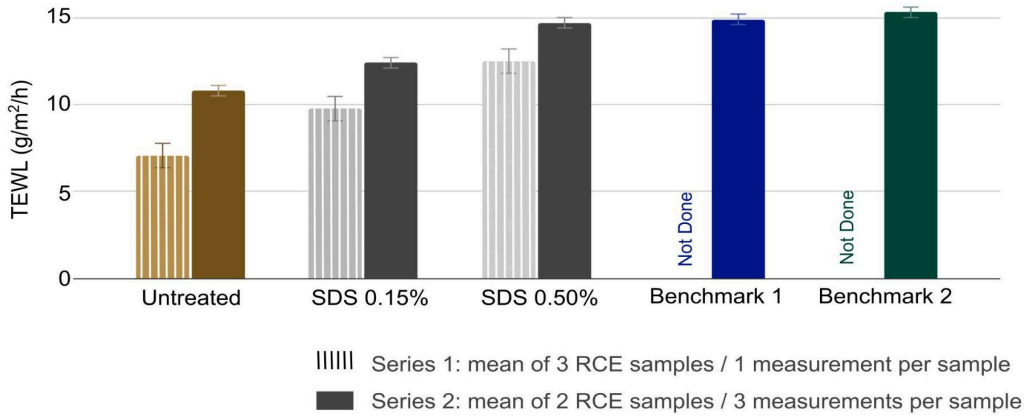

Figure 3. TEWL (in g/m^2/h) / 16 hours of contact

In the second experimental series, Benchmarks 1 and 2 were incorporated to assess the reproducibility of SDS effects and to calibrate the model based on the benchmarks'

responses. Interleukin-8 (IL-8) release was measured to further refine the assessment. The significant adverse effects of 0.5% SDS on cellular viability were corroborated. However, 0.15% SDS exhibited substantial variability in tissue viability compared to the initial experiment (Figure 2, represented by solid-colored bars), suggesting that this concentration may be at the upper limit of acceptable exposure. This interpretation warrants caution due to the analysis of a single replicate. However, it is further substantiated by the significant morphological alterations depicted in Figure 4b, including basal layer disorganization, detachment from the cell culture insert membrane, and the presence of large vacuoles within the granular layer.

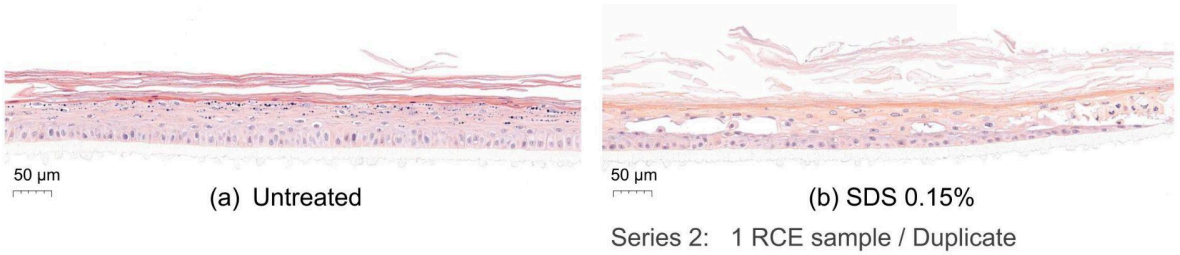

Figure 4. Morphology of RCE: (a) Untreated; (b) Treated with SDS 0.15% (scale bar 50 µm)

Benchmarks demonstrated no significant impact on tissue viability and morphology (data not shown), consistent with their anticipated low irritation potential. Conversely, Trans-Epidermal Water Loss (TEWL) values (Figure 3) were comparable to those induced by 0.5% SDS, and elevated IL-8 release (Figure 5) was observed, suggesting a marked effect on skin barrier function and inflammatory response. This outcome could come from surfactants contained in Benchmark 1, but was unexpected for leave-on Benchmark 2. The reduction in IL-8 release after exposure to 0.5% SDS, compared to 0.15% SDS, was attributed to the death of IL-8 secreting cells.

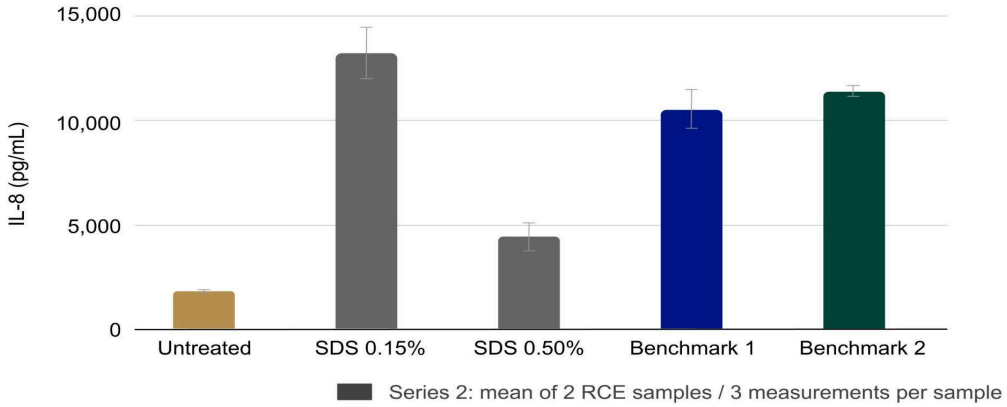

Figure 5. IL-8 release (in pg/mL) / 16 hours of contact

The results obtained with 0.15% SDS and the benchmarks suggested that a reduced exposure time was necessary for subsequent experiments.

3.2. Results with refined Protocol for Canine Skin Tolerance Model Optimization

The contact time with the test item was reduced to 12 hours. Given the limited discriminatory capacity of Transepidermal Water Loss (TEWL) observed in the initial two experimental series, it was replaced with Trans-Epithelial Electrical Resistance (TEER) measurement to assess barrier functionality.

The shortened contact time resulted in decreased cytotoxicity of the positive control (Figure 6), correlating with reduced effects on tissue organization, particularly in the basal layer,

where detachment from the cell culture insert membrane was no longer evident. This reduced cytotoxicity was intentionally sought to enable accurate measurement of other parameters. The benchmarks, as well as the Thickening-Stabilizing Polymer, demonstrated no significant cytotoxicity, indicating a low irritation potential. This finding was supported by damage limited to the superficial epidermal layers (histology not shown). However, the reduced viability percentage compared to the untreated epidermis highlighted the high sensitivity of the model.

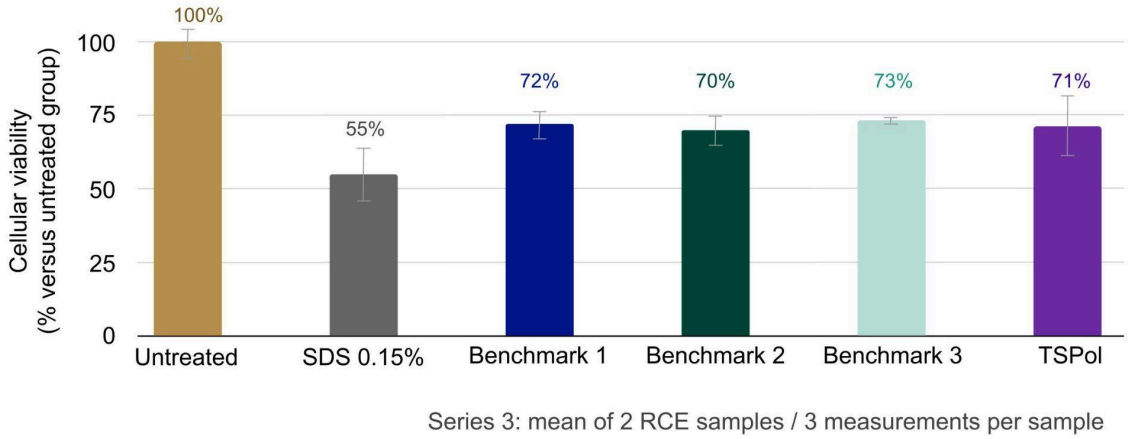

Figure 6. Cellular viability / 12 hours of contact (in % versus untreated group)

In contrast to TEWL, Transepithelial Electrical Resistance (TEER) effectively differentiated the positive control from the benchmarks and the tested polymer, as illustrated in Figure 7. SDS significantly compromised barrier functionality. Benchmarks 1, 2, and the tested polymer exhibited moderate effects. Benchmark 3 and Phosphate Buffered Saline (PBS) treatment did not impact barrier functionality, correlating with maintained tissue organization and an intact stratum corneum. The observed results for Benchmark 3 may be attributed to the presence of shea butter and active ingredients, namely urea, known for its moisturizing properties, and Centella asiatica extract, recognized for its skin repair-promoting properties [17].

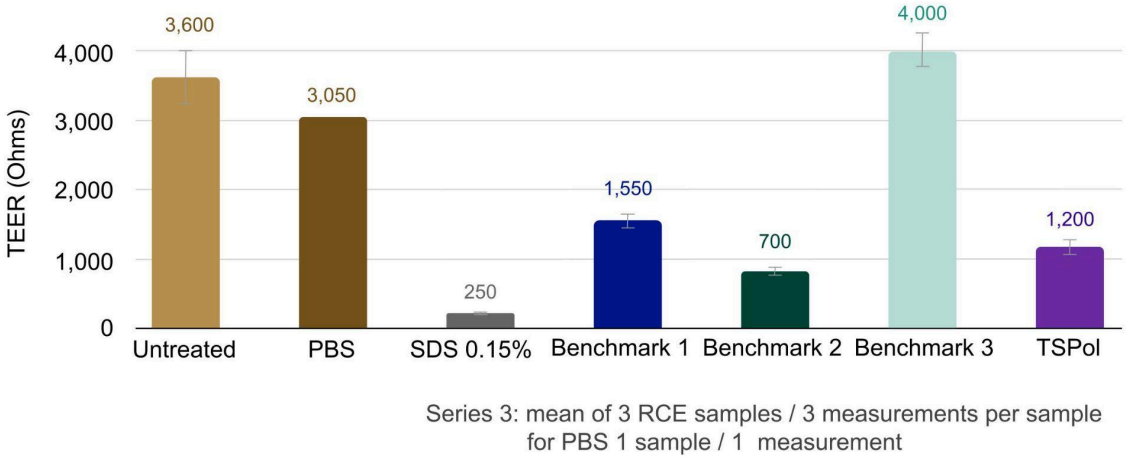

Figure 7. TEER (in Ohms) / 12 hours of contact

Positive control SDS induced a significant pro-inflammatory response (Figure 8). A non-negligible release of Interleukin-8 (IL-8) was observed in the untreated epidermis. Moreover, the increase in IL-8 release after contact with Phosphate Buffered Saline (PBS) demonstrated the physical effect of the treatment and the sensitivity of the model to

manipulations. Benchmarks 1 and 2 exhibited no significant difference compared to the PBS treatment. The polymer slightly increased the inflammatory response. Benchmark 3 decreased IL-8 release, which can likely be attributed to the presence of Centella asiatica extract, also known for its inhibitory effects on pro-inflammatory cytokines [18].

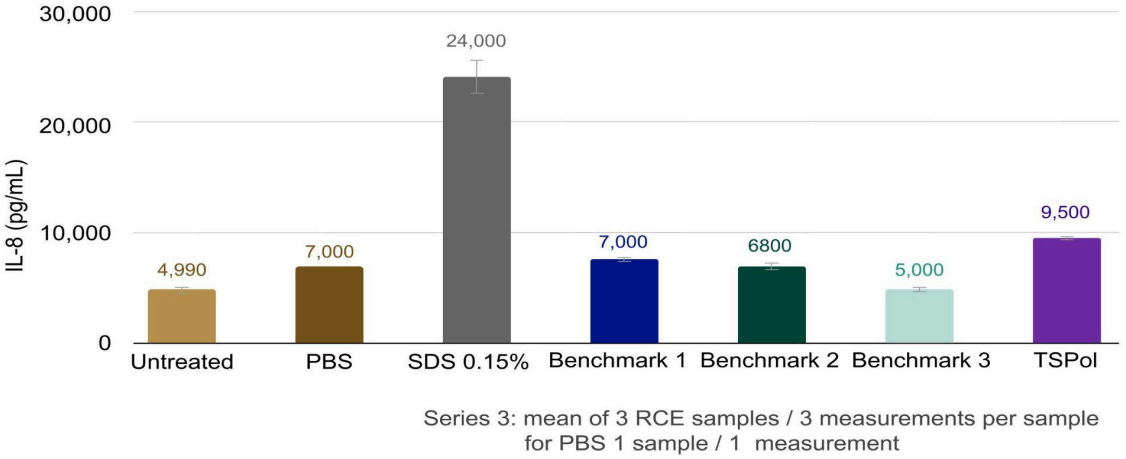

Figure 8. IL-8 release (in pg/mL) / 12 hours of contact

4. Discussion

Although the *in vitro* skin tolerance canine model remains experimental, the protocol was optimized to yield consistent results for a reference surfactant and formulated benchmarks applied in both leave-on and rinse-off conditions. Within individual experimental series, results demonstrated satisfactory repeatability. To achieve a more comprehensive analysis beyond cellular viability, multiple measurement parameters were assessed. TEER measurement demonstrated superior discriminatory capacity compared to TEWL for assessing effects on the canine skin barrier. Furthermore and despite being commonly used, *in vivo* TEWL measurements in dogs have been reported to exhibit greater variability compared to human measurements, regardless of the device used, primarily due to the presence of fur [19]. Additionally, *in vivo* TEWL measurements following shampoo application in dogs with atopic dermatitis showed only a minimal increase in TEWL [20]. This observation is similar to the *in vitro* findings in this study after SDS application. The measurement of Interleukin-8 (IL-8) release served as a second indicator of the model's sensitivity, revealing a significant observation: mechanical manipulation of the tissue, in the absence of PBS application, induced a notable effect, which was corroborated by morphological observation of the tissue. In future experiments, it will be necessary to routinely include Phosphate Buffered Saline as a negative control. TEER and IL-8 release readings provided additional information that appeared to correlate with formulation composition, as suggested by the results obtained with Benchmark 3. Model reproducibility and predictive capacity, assessed via statistical analysis, will require further validation across a greater number of experimental series and with an expanded dataset of ingredients and formulations. Additional ingredient types with potential relevance for pet care are currently under investigation. In anticipation of broadening ingredient testing scope, it will be necessary to incorporate the commonly used solubilizing solvents as additional control groups.

To conclude on canine local tolerance potential, some hypotheses can be based on results compared to the negative control (PBS ideally) and Benchmarks 1 and 2 according to cytotoxicity, TEER, IL-8 release and histological observations. The tolerance could be considered good when viability is superior to 50% and the other parameters are not

significantly affected compared with the negative control. The tolerance could be considered acceptable when viability is superior to 50% and other parameters do not exceed the results provided by the benchmarks. Tolerance could be considered poor when viability was below 50% indicating an irritant effect and when strong and consistent effects significantly superior to those obtained with the two benchmarks are observed on TEER, IL-8 release and histology. A tentative analysis is proposed in Table 3.

Table 3. Tentative analysis (currently hypothesized versus untreated)

Identification	Cellular viability (%)	TEER (Ohms)	IL-8 (pg/mL)	Results analysis
Untreated	100	3,600	4,900	-
NC: PBS:	Not done	3,050	7,000	Good tolerance
PC: SDS 0.15%	55	250	24,000	Poor tolerance
Benchmark 1 (1%)	70	1,550	7,000	Acceptable tolerance
Benchmark 2	72	700	6,800	Acceptable tolerance
Benchmark 3	73	4,000	5,000	Good tolerance
TSPol (0.5%)	71	1,200	9,500	Acceptable tolerance

NC: Negative Control; PC: Positive Control

The *in vitro* canine model demonstrated a suitable level of sensitivity for ingredient screening, helping in preventing adverse reactions on canine skin. However, it differs from the *in vivo* scenario. Specifically, the presence of fur, which provides an additional barrier over most areas of the body, the substantial variations in skin thickness observed both between different anatomical regions and across various dog breeds and the unique morphology of the nose and paw pads are not accurately represented in the model [21].

5. Conclusion

The study successfully developed an *in vitro* model using reconstructed canine epidermis (RCE) to evaluate the local tolerance of ingredients intended for pet care formulations. Assessing multiple endpoints, including cellular viability, TEER, Interleukin-8 release, and morphological observations, provided a better understanding of ingredient effects beyond just cellular viability. Multiple parameters improved the model's sensitivity for detecting reactions in reconstructed canine skin. A tentative tolerance analysis was proposed based on the different parameters. However, further validation of the model's reproducibility and predictive capacity through statistical analysis and increased experimental series is necessary. Although somewhat distant from the *in vivo* reality, the model represents a promising tool for selecting suitable ingredients and formulations for dogs. The integration of this *in vitro* model within a comprehensive approach, encompassing systemic toxicity assessment and historical human use data, ensures the safety of both dogs and their human caregivers, thereby contributing to a One Health perspective.

6. References

[1] European Pet Food Industry Federation (FEDIAF), European pet population and market data 2022, annual report 2024. https://europeanpetfood.comingsoon.site/about/statistics/.
[2] American Veterinary Medical Association, U.S. pet ownership statistics, 2024. https://www.avma.org/resources-tools/reports-statistics/us-pet-ownership-statistics.
[3] Rakuten insights, pet ownership statistics in Asia, Online Survey January 2021 https://insight.rakuten.com/pet-ownership-in-asia.
[4] GfK online survey in 22 markets, 2016. https://nielseniq.com/global/en/insights/report/2016/mans-best-friend-global-pet-ownership-and-feeding-trends/.
[5] U.S. Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine (CVM), Human User Safety in New and Abbreviated New Animal Drug

Applications, Draft Guidance for Industry, April 2023, ID FDA-2023-D-0592-0002. https://www.regulations.gov/document/FDA-2023-D-0592-0002.
[5] Poumay, Y., Dupont, F., Marcoux, S., Leclercq-Smekens, M., Hérin, M. and Coquette, A., 2004. A simple reconstructed human epidermis: preparation of the culture model and utilization in in vitro studies. Archives of dermatological research, 296, pp.203-211.
[6] Combarros, D., Brahmi, R., Musaefendic, E., Heit, A., Kondratjeva, J., Moog, F., Pressanti, C., Lecru, L.A., Arbouille, S., Laffort, C. and Goudounèche, D., 2025. Reconstructed Epidermis Produced with Atopic Dog Keratinocytes Only Exhibit Skin Barrier Defects after the Addition of Proinflammatory and Allergic Cytokines. JID Innovations, 5(2), p.100330.
[7] Marques, M., Nunes, J., Ustymenko, B., Fialho, L., Martins, L., Burke, A.J., Filho, C., Craveiro, A.C., Costa, A.R., Branco, S. and Antunes, C.M., 2022. cEpiderm, a Canine Skin Analog Suitable for In Vivo Testing Replacement. BioChem, 2(4), pp.215-220.
[8] Alkhilaiwi, F., Wang, L., Zhou, D., Raudsepp, T., Ghosh, S., Paul, S., Palechor-Ceron, N., Brandt, S., Luff, J., Liu, X. and Schlegel, R., 2018. Long-term expansion of primary equine keratinocytes that maintain the ability to differentiate into stratified epidermis. Stem cell research & therapy, 9, pp.1-12.
[9] Scientific Committee on Consumer Safety (SCCS). SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation - 12th revision [Internet]. European Commission, Directorate-General for Health and Food Safety; 2023 May 15.
[10] Claus, M.A., Jandrey, K.E. and Poppenga, R.H., 2011. Propylene glycol intoxication in a dog. Journal of Veterinary Emergency and Critical Care, 21(6), pp.679-683.
[11] Kovalkovičová, N., Šutiaková, I., Pistl, J. and Šutiak, V., 2009. Some food toxic for pets. Interdisciplinary toxicology, 2(3), p.169.
[12] Bates, N., 2024. Chocolate toxicosis in pets. The Veterinary Nurse, 15(8), pp.320-325.
[13] Roso, A., Puginier, M., Bergal, M., Nunzi, F. and Alonso, A., 2021. In vitro approach to assess local tolerance of ingredients dedicated to specific topical care applications. Journal of Dermatology and Skin Science, 3(1).
[14] Pet Care Professional guidelines. Washing correctly with shampoo.Online search April 24, 2025. https://transgroom.com/en/advice/thank-you-shampoo-and-conditioner-.html.
[15] Shimada, K., Yoshihara, T., Yamamoto, M., Konno, K., Momoi, Y., Nishifuji, K. and Iwasaki, T., 2008. Transepidermal water loss (TEWL) reflects skin barrier function of dog. Journal of veterinary medical science, 70(8), pp.841-843.
[16] White, A.G., Wolsic, C.L., Campbell, K.L. and Lavergne, S.N., 2014. Canine progenitor epidermal keratinocytes express various inflammatory markers, including interleukin-8 and CD 40, which are affected by certain antibiotics. Veterinary dermatology, 25(6), pp.493-e82.
[17] Arribas-López, E., Zand, N., Ojo, O., Snowden, M.J. and Kochhar, T., 2022. A systematic review of the effect of Centella asiatica on wound healing. International journal of environmental research and public health, 19(6), p.3266.
[18] Cohen, G., Jakus, J., Baroud, S., Gvirtz, R. and Rozenblat, S., 2023. Development of an effective acne treatment based on CBD and herbal extracts: preliminary in vitro, ex vivo, and clinical evaluation. Evidence-Based Complementary and Alternative Medicine, 2023(1), p.4474255.
[19] Marsella, R., Olivry, T., Carlotti, D.N. and International Task Force on Canine Atopic Dermatitis, 2011. Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. Veterinary dermatology, 22(3), pp.239-248.
[20] Taguchi, N., Onishi, I., Iyori, K. and Hsiao, Y.H., 2024. Preliminary evaluation of a commercial shampoo and fine bubble bathing in the treatment of canine atopic dermatitis: A single-blinded, randomised, controlled study. Veterinary Dermatology, 35(4), pp.400-407.
[21] Ardicli, S., Ardicli, O., Yazici, D., Pat, Y., Babayev, H., Xiong, P., Zeyneloglu, C., Garcia-Sanchez, A., Shi, L.L., Viscardi, O.G. and Skolnick, S., 2024. Epithelial barrier dysfunction and associated diseases in companion animals: Differences and similarities between humans and animals and research needs. Allergy, 79(12), pp.3238-3268.