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Assessment of ocular discomfort caused by 5 shampoos using the Slug Mucosal Irritation test

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ABSTRACT

Assessment of ocular discomfort caused by veterinary care products is less legitimately regulated than that caused by human care products. The Slug Mucosal Irritation (SMI) assay was adapted to evaluate canine hygiene shampoos to predict ocular discomfort.

Experiments were performed using four commercial canine shampoos, a baby care product, and two controls (ArtTear® and BAC1%). Groups of 3 slugs were tested with 5% dilution of the 7 test substances.

The negative control (ArtTear®) was the best tolerated. The baby care product Mixa bébé as well as Douxo Entretien Démêlant and Phlox Shampooing Entretien were classified to cause mild ocular discomfort. Together with the positive control (BAC 1%), Shampooing Physiologique Virbac and Physiovet Shampooing were considered to cause severe ocular discomfort.

Different intensities of ocular discomfort were measured for veterinary care products. The SMI model was considered as a reproducible and adaptable evaluation method for screening veterinary care products causing ocular discomfort.

1. Introduction

The safety assessment of cosmetic and personal care products is a requirement of the European's Cosmetics Directive 1223/CE/2009, which imposes industries to use the best and latest available research data to substantiate the safety of products before they are marketed. The aim of such study was to ensure that marketed cosmetic products do not cause damage to human health when applied under normal or reasonably foreseeable conditions of use.

For decades, commercial and political pressures have constantly led to a halt in the use of animals for safety evaluations. According to EURL ECVAM, in the EU, the Cosmetics Directive has prohibited the testing of finished cosmetic products and their ingredients on animals since 2004 and 2009, respectively. Therefore, substantial efforts have been made towards the development and international acceptance of alternative methods. Full replacement alternative methods are currently available for evaluation of skin corrosivity, skin irritation, dermal absorption, and phototoxicity, while analyses of eye irritation, acute toxicity, and mutagenicity/genotoxicity are only covered by partial replacement methods (Rogiers and Pauwels, 2008).

In terms of risk assessment procedures, eye tolerance is one of the tested criteria of finished cosmetics. The Draize Rabbit Eye Test has been the gold standard for testing eye irritation potency for many years (Draize et al., 1959, 1944). Owing to the concern for animal welfare and the limitations of this method, a strategic combination of validated alternative models has been considered a reliable alternative (Scott et al., 2010). Several validation studies are now used to assess eye irritation potential, such as Bovine Corneal Opacity & Permeability test (BCOP), Isolated Chicken Eye test (ICE), Isolated Rabbit Eye test (IRE), and the Hens Egg Test on the Chorioallantoic Membrane (HET-CAM) (Hartung, 2007).

Another alternative model known as the Slug Mucosal Irritation assay (SMI) has undergone a corporate prevalidation study and showed promising reproducibility at various laboratories (Adriaens et al., 2008). Based on a stinging, itching, and burning sensation (SIB), it assesses the eye irritation potential of test formulations by measuring the amount of mucus produced by slugs (*Arion lusitanicus*) in contact with the test substances. Several studies have reported the SMI as an alternative method for screening the local tolerance of nasal, buccal, and vaginal tissue to pharmaceutical formulations, and their ability to cause ocular discomfort (Adriaens and Remon, 1999; Callens et al., 2001; Dhondt et al., 2005; Lenoir et al., 2013).

Unlike cosmetic products developed for humans, veterinary care products are currently unregulated with regard to verification of their safety and tolerance. Therefore, the manufacturers are free to set their internal standards. The qualitative and quantitative compositions of veterinary care product (VCP), therefore, rely on the manufacturer's responsibility. Although the safety standards of topical agents have led to

Abbreviations: VCP, veterinary care products; SLES, sodium laureth sulfate.

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the development of products that are considered as both effective and non-irritable, it is not a zero-risk situation for potential skin irritation or ocular discomfort, particularly when these products are applied to abraded skin or mucosal surfaces.

A previous study involving a French toxicological monitoring register (Centre National d'Informations Toxicologiques Vétérinaires, Marcy l'étoile) indicates that 62.2% of declared canine ophthalmic emergencies are mainly related to detergents, pesticides, and care products (Cassiat-Hervé-Bazin, 2002). Together with different topical agents, shampoos are valuable parts of the therapeutic arsenal used by veterinary dermatologists for therapy or hygiene control (Halliwell, 1991). With or without active agents, shampoos included surfactants and other chemicals such as suspending agents, foam boosters, pH adjusters, thickening agents, solvents, preservatives, antioxidants, conditioners, coloring agents, and fragrances. Exposure to these agents can potentially cause ocular discomfort (Freeberg et al., 1984). The use of canine shampoos for daily care or as a preventive measure can expose dogs to potential ocular discomfort. Additionally, any owner or professional applying VCPs can be subjected to eye splashes. Although seemingly rare, adverse reactions to VCPs do occur by accidental or intentional exposure. They are often mild in nature and sometimes include adverse effects such as minor skin reactions or ocular discomfort (Woodward, 2005).

Inflicted eye epithelial aggressions stimulate the corneal nerve endings, leading to symptoms of ocular discomfort (Lemp and Foulks, 2007). The level of sensitivity to ocular discomfort had been previously assessed by the SMI and a correlation was demonstrated between the increasing mucus production (MP) and the human eye irritation test (HEIT) after different shampoo applications (Lenoir et al., 2011). Since ocular discomfort in dogs has not been extensively studied, we investigated canine shampoos using the same standard measuring scale adapted to the human care products.

The objective of our study was to verify if this method is suitable for the evaluation of ocular discomfort in animals. We also compared the relative ocular discomfort caused by VCPs with that caused by a referenced human product that claims to have minimal ocular discomfort.

2. Materials & methods

2.1. Shampoos tested and controls

Four hygiene commercial canine shampoos were selected: Shampooing Physiologique Virbac (Virbac, Carros, France), Phlox shampooing Entretien® (Phlox, Wambrechies, France), Douxo Entretien Démélant (Sogeval, Laval, France), and Physiovet® (MP Labo, Antibes, France). Additionally, one human care shampoo, Mixa bébé shampooing (Mixa, Saint-Ouen, France), was also investigated. Besides sodium laureth sulfate as a common surfactant, these shampoos differed in terms of color, fragrance, and some specific claim ingredients. The qualitative formula and pH of the tested formulations are listed in Table 1. In accordance to Lenoir et al. (2011), 5% aqueous solutions were prepared in distilled water. In the system, artificial tear (ArtTear®) (Teofarma, Valle Salimbene, Italy) was used as a negative control whereas 1% dilution of benzalkonium chloride (BAC) (Sigma, St Louis, USA) in phosphate buffered solution (PBS) was used as a positive control.

2.2. Methods

The experiment was conducted based on the methodology designed by Lenoir et al. (2011). In contrast to his protocol, two deviations were recorded. The weight of the slugs and applied location were different.

2.2.1. PH measurements

The pH of the diluted test substances was measured with a pH meter model HI 111 (Hanna Instruments, Tanneries, France). The measurements were performed in triplicate and the mean values were used for analysis.

2.2.2. Stinging, itching, and burning test procedure of the SMI-test

The slugs (*Arion lusitanicus*) were issued from a laboratory (Inverrtox, Bellemdorpweg, Belgium). They were kept in an acclimatized room (18°–20 °C), where they were housed in plastic containers and fed with vegetables such as cucumber, lettuce, and carrots.

Slugs weighing between 4 g and 12 g were isolated in large Petri dishes two days prior of the experiment. In Lenoir et al. (2011), the slugs weighed between 3 g and 6 g. Each Petri dish containing a paper towel was wet with PBS solution and placed at 18–20 °C.

The ocular irritation potency of the reference shampoos, negative and positive (BAC 1%) controls on the mucosal tissue of slug was investigated. Twenty-one slugs were first randomized and individually placed in fresh Petri dishes containing 1 ml of PBS. Seven groups of three slugs were made, corresponding to the shampoo or control solutions.

The experiment consisted of three contact periods (CP) of 15 min, with 1 h rest/recovery period in between the CPs. For each CPs, 100 μl of the test medium was pipetted onto the dorsal wall of the slug. The test media were pipetted underneath the foot of the slug in Lenoir et al. (2011) protocol.

The mucus produced during this 15-min contact period with the test items was measured by weighing the Petri dishes before and after the 15-min contact period and was expressed as percentage of the initial weight of the slug.

2.2.3. Data analysis

The total MP was calculated by adding the 3 MPs for each slug, and then the mean MP per treatment group was calculated and expressed in percentage. A classification prediction model established by Lenoir et al. (2011) was used to define the ocular irritation potency. The four categories of irritation are defined based on total MP:

1. ≤3% = Non irritant
2. <3–8% = Mild discomfort

Table 1
Overview of the tested products and their wash-active ingredients.

Affiliated samples	Tested products	Qualitative formula	pH
A	BAC 1%	Benzalkonium chloride	7,80
B	ArtTear®	Disodium edetate, sodium chloride, benzalkonium chloride, sodium hydroxide, purified water	7,08
C	Shampooing Physiologique Virbac	Sodium laureth sulfate, propanaminium-1, Cocamidopropyl-*N*-2-Hydroxyethylcarbamoyl methyl ammonium chloride	7,56
D	Mixa bébé shampooing	Dmdm hydantoin, Hexylene glycol, sodium cocoamphoacetate, sodium laureth sulfate, sodium methylparaben	7,40
E	Phlox shampooing entretien	Butylated hydroxytoluene, glycerin, methylisothiazolinone, panthenol, peg-40 hydrogenated castor oil, propylene glycol, sodium chloride, sodium cocoamphoacetate, sodium laureth sulfate, sodium methylparaben	7,85
F	Douxo entretien démêlant	Alkyl ether sulfate C10-16, sodium salt, 1-propanaminium, *N*-(3-aminopropyl)-2-hydroxy-*N*,*N*-dimethyl-3-sulfo-, *N*-coco acyl derives, hydroxides, inner salts, decyl glucoside, propylene glycol, phenoxyethanol-2, dodecan-1-ol, sodium hydroxide, D-limonene, pin-2(10)-ene	7,53
G	Physiovet®	Sodium laureth sulfate, cocamide dea, polyhexamethylene biguanide hydrochloride	7,55