

Hygiene Products for Pets: innovative approaches using the human cosmetics' experience

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ABSTRACT

This R&D project consisted in the development of different hygiene products for pets (dogs and cats), through an approach similar to "quality by design", assuring the efficacy for pets and safety for both pets and owners. Throughout the development process different current market trends were considered and followed.

OBJETIVE

The objective of this R&D project was to develop hygiene products for pets, using the experience and knowledge created with human cosmetics products, supporting the creative process in trends like natural ingredients and environmentally friendly production.

METHODS

The different hygiene products were developed following the steps described bellow.

Benchmark products evaluation

- Ingredients used;
- Package;
- Content net;Claims.



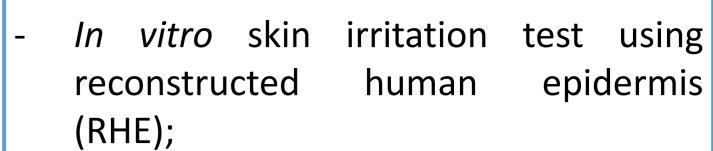
Product Development

- Selection of functional ingredients, know to be safe and efficient;
- Prioritisation of natural or naturally derived ingredients;
- Prioritisation of cold process production method;
 Minimalist approach, excluding
- unnecessary ingredients;
 pH adjustment to correspond to dogs and cats' skin pH value.

Product safety evaluation (theorical)

- Safety evaluation model for veterinary products based on the margin of safety (MoS) calculation used for human cosmetic products, with adaptations based on pets characteristics (weigh and area of exposure);
- Consideration of worst-case scenario of product exposure (pet vs owner).

Product safety evaluation (in vitro)



- *In vitro* eye irritation test - Hen's Egg Test (HET) Chorioallantoic Membrane (CAM).

Skin Irritation_[1] in vitro – OCDE TG439 Incubation of the tissues for 1h to release transport-stress related compounds and debris Tissues transfer to fresh medium Treatment on 3 tissue replicates per substance (study substances, negative control (DPBS) and positive control (SDS 5% w/v))30μL of substance Incubation for 60 minutes (37°C, 5% CO₂, humidity 90±10%) Rinsing with DPBS, immersing in DPBS and rinsing again Incubation for 42 h ± 2 h (37°C, 5% CO₂ and saturated humidity 90±10%) Incubation of 0.3mL of the MTT solution (1mg/mL) per tissue for 3 hours (37°C, 5% CO₂ and saturated humidity 90±10%)) Extraction with 2mL isopropanol, in prefilled wells in a 24 well plate for 2h (RT), under mild agitation (~120rpm) MTT endpoint measurement, in duplicate for each tissue, through spectrophotometry (570nm) using isopropanol as blank. Table 1 - Classification of the test substance regarding its irritancy based on the Prediction Model of the OECD TG 439 In vitro results on mean tissue cell viability *In vivo* prediction ≤ 50% Irritant

In vitro eye irritation test – Hen's Egg Test Chorioallantoic Membrane (HET-CAM) Test_[2] – ICCVAM Leghorn chicken eggs (9 days incubated: 37.8±0.3°C , RH 58±2%) 0.3mL of each testing product

Chorioallantoic membranes of embryonated eggs (3 eggs per product)

(not previously diluted)

Irritant effect monitored during 5 minutes (3 time points) by macroscopic alterations in the structure of the embryo vascular system (hemorrhage, coagulation and/or vessel lysis)



Negative control (NaCl 0.9%)

Table 2 - Scoring scheme for irritation testing with the HET-CAM test method

Scoring

Table 3 - Classification of the test substance with the HET-CAM test method.

est method.	
Results	Classification
≤9	Non-severely irritating
>9	Severely irritating

Reactions	Scoring		
	0.5 min	2 min	5 min
Vascular Lysis	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5
	9	7	5

Results

- Seven hygiene products for pets (cats and dogs) were developed: three shampoos, one leave-on spray conditioner, two wet wipes and one ear solution. These products include in their composition different natural ingredients such as *Pelvetia canalicula* extract, an ingredient with hydrating properties derived from the brown seaweed know as channeled wrack and an extract from *Cistus ladanifer*, an endemic Portuguese plant.

Table 4 – Results of in vitro safety evaluation **HET-CAM Skin Irritation** Non-irritant ND Leave-on spray conditioner ND Wet wipes for fur and paws hygiene Non-irritant Non-severely irritating \\ Wet wipes for periorbital and eyelid hygiene Non-irritant Ear solution ND Non-irritant Legend: ND - Not determined

> 50%

All of these products present a pH value similar to the pH presented by dogs and cats' skin (pH 6-7.5);

Four of the seven products are produced using a completely cold processable method, and the remaining three are produced using minimal heat (maximum 45°C for 10 minutes), allowing the reduction of energetic impact;

The products were all considered safe with MoS >100 for all the ingredients, with results obtained from both RHE and HET-CAM supporting that safety (table 4).

Conclusions

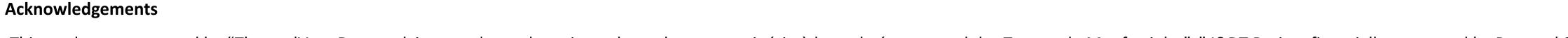
This project allowed the development of seven, safe and efficient hygiene products for pets, based on trends of the market like natural ingredients, minimalism, safety, environment friend industrial process.

References

[1] Test Guideline No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Methods," 2021. [Online]. Available: http://www.oecd.org/termsandconditions/.

[2] ICCVAM-Recommended Test Method Protocol: Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) Test Method." [Online]. Available: http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm;





Non-irritant





