#207

COMPARISON BETWEEN HET-CAM PROTOCOLS AND A PRODUCT USE CLINICAL STUDY FOR EYE IRRITATION EVALUATION OF PERSONAL CARE PRODUCTS ACCORDING TO THEIR SURFACTANT COMPOSITION

https://www.metodos-alternativos.com/

https://www.ebal.com.ar/

Martín Nicolás Rivero[1], Mariela Lenze[1], Mercedes Izaguirre[12], Martina Benedetti[12], Silvia Pérez Damonte[3], Silvia Wikinski[12], María Laura Gutiérrez[12]

[1]Institute of Pharmacology - University of Buenos Aires, [2]CONICET, [3]CLAIM CRO

Corresponding author: mlgutierrez.ebal@gmail.com

Introduction

The hen's egg test on chorioallantoic membrane (HET-CAM) is one of the most frequently used alternative tests for prediction of ocular irritation of cosmetic products. There are different HET-CAM protocols widely accepted, but there is no information about which of the protocols better correlates with the results obtained in product use clinical study under the conditions of use.

The aim of this work was to determine which HET-CAM protocol better correlates with product use clinical results considering surfactant type composition, duration of use in case of rinse-off and leave-on products and the physical appearance that can interfere with the quantification of the phenomena on the CAM (solid and non transparent products).

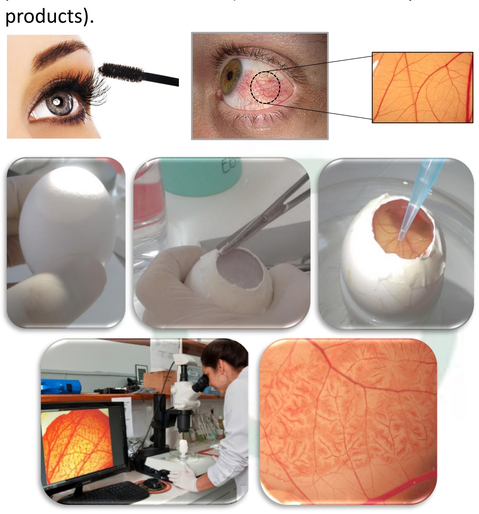


Table 1. Description of cosmetic products tested in this study

Code #	Physical appearance	Surfactants composition	Surfactant type	Cosmetic
1	L-T	0.5% polysorbate 20 0.4% disodium cocoamphodiacetate	Non-ionic Amphoteric	Micellar water cleansers
2	L-T	3% poloxamer 184 1.2% disodium cocoamphodiacetate	Non-ionic Amphoteric	
3	S-NT	1% cetearyl alcohol 1% sodium lauryl sulfate	Non-ionic Anionic	Eyelid sera
4	S-NT	3.5% cetyl alcohol 2.5% cetearyl alcohol 2.5% ceteareht 20	Non-ionic Non-ionic Non-ionic	
5	S-NT	1 % PEG-40 hydrogenated castor oil	Non-ionic	
6	L-T	35% coco glucoside	Non-ionic	Shampoos
7	L-T	37% lauryl glucoside	Non-ionic	
8	L-T	25% decyl glucoside	Non-ionic	
9	L-T	40% coco glucoside + glyceryl oleate	Non-ionic Non-ionic	
10	L-T	20% sodium lauroyl lactylate	Non-ionic	
11	L-T	25% coco glucoside	Non-ionic	
12	L-T	23% CAPB	Amphoteric	
13	L-T	25% CAPB 2% cocamide DEA	Amphoteric Amphoteric	
14	L-T	25% CAPB 2% cocamide DEA	Amphoteric Amphoteric	
15	L-T	19% CAPB 1.5% cocamide DEA	Amphoteric Amphoteric	
16	L-T	28% sodium laureth sulfate 6% CAPB 3% sodium lauryl sulfate 2.5% polyquaternium-7	Amphoteric Amphoteric Anionic Cationic	
17	L-T	1.5% sodium lauryl sulfate	Anionic	Eyelid sera
18	S-NT	0.5% TEA lauryl sulfate	Anionic	

L: liquid. S: solid. T: transparent. NT: non-transparent. TEA: triethanolamine, CAPB: cocamidopropyl betaine, DEA: diethanolamine

Materials and Methods

Table 2. FTM quantification and classification systems for A- Lüepke and B- ICCVAM HET-CAM protocols.

FTM - Fix Time Method				
Irritancy quantification				
A and B	Effect	time (seconds)		
		30	120	300
	Hyperemia (**A**)/ Lysis (**B**)	5	3	1
	Haemorrhage	7	5	3
	Coagulation	9	7	5
Classification				
A	*Score*	*Irritation category*		
	0 - 0.9	non-irritant (NI)		
	1-4.9	weak or slight irritation (WI)		
	5-8.9	moderate irritation (MI)		
	9.0 - 21	strong or severe irritation (SI)		
B	*Score*	*Irritation category*		
	> 9	severe irritant (SI)		
	< 9	non-severe (NS)		

Table 3. RTM quantification and classification systems for C- ECVAM protocol N°47and D- ECVAM protocol N°96

RTM - Reaction Time Method				
Irritancy quantification				
C and D	IS= [((301-H).5/300) + ((301-L).7/300) + ((301-C).9/300))] H, L y C are the times in seconds of the first appearance of Haemorrhage, vascular Lysis and Coagulation respectively			
Classification				
C	*Threshold (TH) concentration*	*IS (10%)*	*Severity*	*Irritation category*
	TH < 1%			severe/corr
	1.0 < TH < 2.5	> 16		severe/corr
	2.5 < TH < 10.0	< 16	severe reaction after 1 min.	severe/corr
	1.0 < TH < 2.5	< 16		Irritant
	2.5 < TH < 10.0	> 16		Irritant
	2.5 < TH < 10.0	< 16	severe reaction after 5 min.	Irritant
	2.5 < TH < 10.0	< 16	weak or no reaction	Moderate
	10 < TH	> 16		Moderate
	10 < TH	< 16	severe reaction	moderate
	10 < TH	< 10		no/slight
D	*model of irritation score*			
	Score	*Irritation category*		
	0 - 0.9	Non-irritant (NI)		
	1-4.9	weak or slight irritation (WI)		
	5-8.9	moderate irritation (MI)		
	9.0 – 21	strong or severe irritation (SI)		
	model of Q-Score			
	Qa-Score Range	*Qb-ScoreRange*	*Irritation category*	
	< 1.5	-	non-irritant (NI)	
	-	≤ 0.8	slight irritation (WI)	
	1.5 ≤ Q < 2	0.8 < Q < 1.2	moderate irritation (MI)	
	-	1.2 ≤ Q < 2	irritant (I)	
	≥ 2	≥ 2	severe irritant (SI)	

Table 4. Scale of symptoms reported by volunteers and signs observed by ophthalmologist in the product use clinical trial

score	Symptoms reported by volunteers	Signs observed
0	absent	absent
1	present but does not produce discomfort	barely visible
2	present with discomfort but does not interfere with daily activity	clearly visible but not severe
3	present, produces discomfort and disrupts daily activity	clearly visible and severe

Table 5. Transformation of irritation classification according to the original protocol and the new one used in this study.

Level	A	B	C	D	Qa	Qb	Clinical
1	NI	NS	NI	NI	NI	WI	G
2	WI		MI	WI		MI	R
3	MI		I	MI	MI	I	
4	SI	SI	SI	SI	SI	SI	B

NI: non irritant, NS: non severe, WI: weak irritant, MI: moderate irritant, I: irritant, SI: severe irritant, G: good, R: regular, B: bad.

Results

Table 6. Scores obtained by the different HET-CAM protocols compared. Mean values of irritation scores (IS) and standard deviations (SD) of 4-6 independent replicates are shown.

Product code	A - Lüepke		B - ICCVAM (NIH)		C- ECVAM DB–ALM Prot. N°47				D- ECVAM DB–ALM Prot. N°96				
#	IS ± SD	Category	IS ± SD	Category	TH (%)	IS (10%)	Severity	Category	IS ± SD	Category	Q-Score	Cat. Q(a)	Cat. Q(b)
1	0 ± 0	**non irritant**	0 ± 0	**non severe**	TH > 10	< 10	-	**no/slight**	0 ± 0	**non irritant**	0	**non irritant**	**no/slight**
2	0 ± 0	**non irritant**	5,2 ± 0,5	**non severe**	TH > 10	< 10	-	**no/slight**	4,7 ± 1,6	**weak***	0,42	**non irritant**	**no/slight**
3	0,2 ± 0,4	**non irritant**	-	-	TH > 10	< 16	severe	**moderate**	-	-	-	-	-
4	0 ± 0	**non irritant**	-	-	TH > 10	< 16	severe	**moderate**	-	-	-	-	-
5	0 ± 0	**non irritant**	-	-	TH > 10	< 16	severe	**moderate**	-	-	-	-	-
6	3 ± 2	**weak**	3 ± 0	**non severe**	TH > 10	< 16	severe	**moderate**	3,0 ± 1,3	**weak**	0,27	**non irritant**	**no/slight**
7	4 ± 0	**weak**	3 ± 0	**non severe**	TH > 10	< 16	severe	**moderate**	2,1 ± 0,17	**weak**	0,18	**non irritant**	**no/slight**
8	2,6 ± 2,3	**weak**	1,5 ± 1,7	**non severe**	TH > 10	< 16	severe	**moderate**	0,5 ± 0,6	**non irritant**	0,05	**non irritant**	**no/slight**
9	4 ± 0	**weak**	3,5 ± 1	**non severe**	TH > 10	< 16	severe	**moderate**	2,1 ± 0,9	**weak**	0,19	**non irritant**	**no/slight**
10	3 ± 2	**weak**	3,4 ± 0,9	**non severe**	TH > 10	< 16	severe	**moderate**	2,7 ± 0,2	**weak**	0,24	**non irritant**	**no/slight**
11	5,3 ± 2,3	**moderate***	4,5 ± 1,6	**non severe**	TH > 10	< 16	severe	**moderate**	3,9 ± 1,9	**weak***	0,35	**non irritant**	**no/slight**
12	8 ± 0	**moderate**	5 ± 0	**non severe**	1 < TH < 2,5	< 16	-	**irritant**	4,2 ± 0,1	**moderate**	0,38	**non irritant**	**no/slight**
13	8 ± 0	**moderate**	6 ± 0	**non severe**	2,5 < TH < 10	< 16	severe	**severe**	5,6 ± 0,9	**moderate***	0,50	**non irritant**	**no/slight**
14	8 ± 0	**moderate**	6 ± 0	**non severe**	TH < 1	< 16	-	**severe**	7,2 ± 0,2	**moderate**	0,65	**non irritant**	**no/slight**
15	15 ± 0	**severe**	14,3 ± 1,1	**severe**	2,5 < TH < 10	< 16	severe	**severe**	15,3 ± 2,8	**severe**	1,36	**non irritant**	**irritant**
16	8 ± 0	**moderate**	15,2 ± 3,5	**severe**	TH < 1	< 16	severe	**severe**	14,3 ± 3,5	**severe**	1,28	**non irritant**	**irritant**
17	8 ± 0	**moderate**	11 ± 2	**Severe**	TH > 10	< 16	severe	**moderate**	9,0 ± 1,2	**severe***	0,8	**non irritant**	**moderate**
18	0,2 ± 0,5	**non irritant**	-	-	TH > 10	< 10	-	**no/slight**	-	-	-	-	-
Texapon® ASV50 5%	12 ± 0	**severe**	12 ± 0	**severe**	TH < 1	-	-	**severe**	11,2 ± 0,4	**severe**	1	**non irritant**	**moderate**
SLS 1%	15,3 ± 3,5	**severe**	10 ± 0	**severe**	TH < 1	-	-	**severe**	9,6 ± 0,6	**severe**	-		
NaCl 0.9%	0 ± 0	**non irritant**	0 ± 0	**non severe**	TH > 10	10 >	-	**no/slight**	0 ± 0	**non irritant**	-		
NaOH 0,1 N	21 ± 0	**severe**	21 ± 0	**severe**	TH < 1	-	-	**severe**	18,4 ± 0,1	**severe**	-		

Q-Sore was obtained calculating the ratio between the IS product/IS Texapon® ASV50 5%. * indicates cases with mean ± SD in the limit between categories. IS±SD: mean value of irritation score and standard deviation; TH (%): threshold (maximum concentration that produces minimal irritation); IS (10%): irritation score at 10% dilution.

Table 7. Scores obtained by product use clinical study.

Code #	Ophthalmological clinical evaluation			Tearscope
	24 hours	7 days	21 days	
1	0 0 0	0 0 0	G	S
2	0 0 0	0 0 0	G	S
3	0 0 0	0 0 0	G	S
4	0 0 0	0 0 0	G	S
5	0 0 0	0 0 0	G	S
6	0 0 0	0 0 0	G	S
7	0 0 0	0 0 0	G	S
8	0 0 0	0 0 0	G	S
9	0 0 0	0 0 0	G	S
10	0 0 0	0 0 0	G	S
11	0 0 0	0 0 0	G	S
12	0 0 0	0 0 0	G	S
13	0 0 0	0 0 0	G	S
14	0 0 0	0 0 0	G	S
15	0 0 0	0 0 0	G	S
16	0 0 0	0 0 0	G	S
17	1 1 0	Suspended	B	U
18	0 0 0	0 0 0	G	S

G: good response; B: bad response, S: stable and U: unstable.

Figure 1. Eye irritation results of the 18 cosmetics and personal care products evaluated by the different protocols (Lüepke, ICCVAM, INVITTOX No. 47, INVITTOX No. 96 and its variants Qa and Qb, and product use clinical study). The scores are represented in the range of 0 - 4 and growing circle size where: 0 = not tested; 1 = non-irritant and 4 = severe irritant. Scores 2 and 3 indicate intermediate levels of irritation. Each color corresponds to a type or mixture of surfactants, as shown in the legend. The red dashed line indicates the limit between mild irritation levels (scores 1 and 2) and moderate or severe irritation levels (scores 3 and 4).

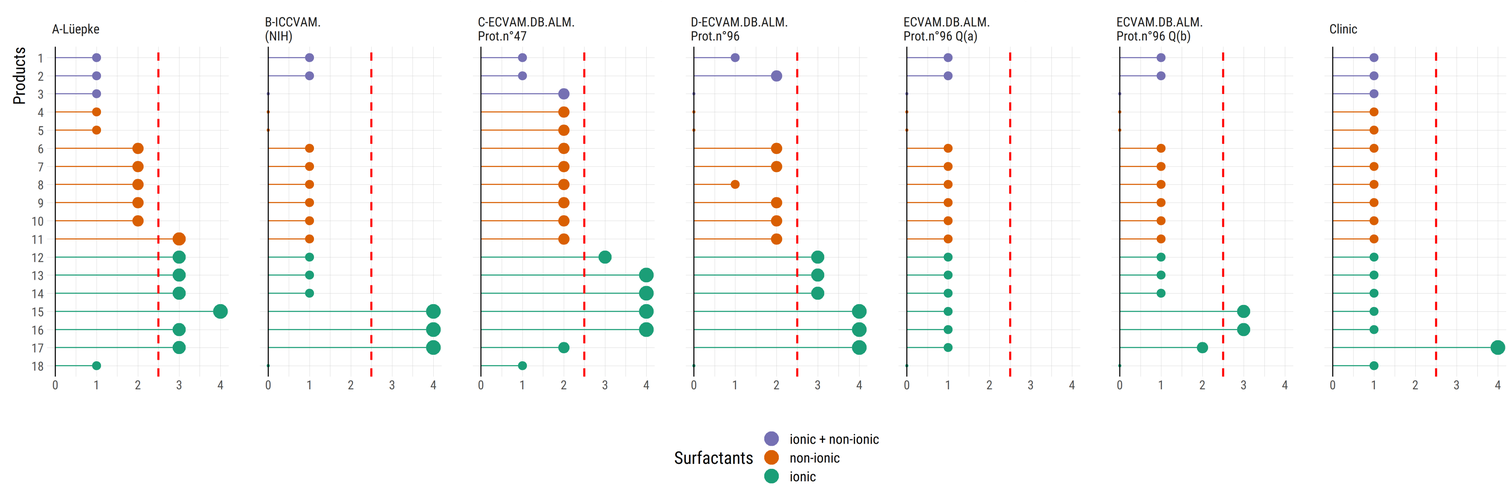
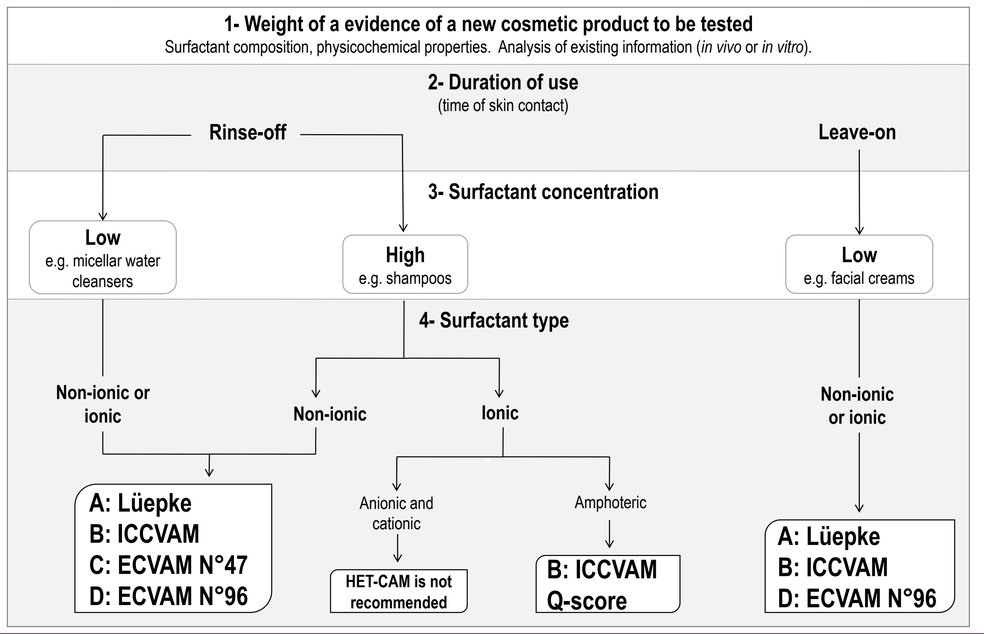


Figure 2: In-house strategy for testing personal care products including cosmetics by different protocols of HET-CAM methodology. A protocol is chosen taking into account the following points: 1- weight of the evidence, 2- duration of use, 3- surfactant concentration and 4- surfactant type.

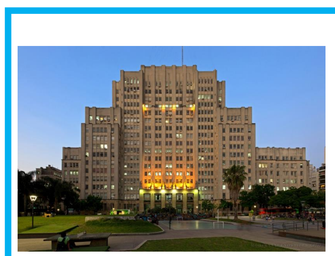


Conclusion

We establish an in-house strategy for testing personal care and cosmetic products using HET-CAM.

From the findings in this work, it can be concluded that: Method (A) was the one to be chosen for the evaluation of nontransparent products; methodologies (A), (B) and (D) had the sensitivity to evaluate irritating products with low surfactant concentration when they also were irritating to human eye. All protocols could be appropriate to evaluate 10% dilutions of rinse-off products with a high content of non-ionic surfactants as shampoos; protocol (B) was useful to test products containing high concentration of amphotheric surfactants. Finally, HET-CAM is not recommended to test products containing high concentration of anionic and cationic surfactants due to the highly irritating reaction obtained.

Funding



UNIVERSIDAD DE BUENOS AIRES

CONICET

Agencia I+D+i

Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación