STUDY REPORT: 23SCB01E50N

In vitro assessment of eye irritation potential of Mu-conotoxin TIIIA following the ET_{50} method (neat)

STUDY REPORT APPROVAL

	Name	Signature	Date
Study Director	Dr Tom Ward		25AUG23

SPONSOR DETAILS

Name:	**Science4Beauty**
Address:	ul. Ząbkowska 30/6, 03-735 Warsaw, Poland
Contact Name:	Magdalena Janczewska
Contact email:	magda@science4beauty.net

TEST FACILITY DETAILS

Name:	**XCellR8 Ltd**
Address:	Techspace One, Sci-Tech Daresbury, Cheshire, WA4 4AB, UK
Telephone:	+44 (0)1925 607134
Senior Scientist:	Dr Tom Ward PhD
Contract email:	tom.ward@x-cellr8.com

STUDY DATES

Study initiation date:	28 July 2023
Experimental start date:	31 July 2023
Main test start date:	08 August 2023
Experimental end date:	10 August 2023

CONTENTS

EXECUTIVE SUMMARY

Mu-conotoxin TIIIA was evaluated for eye irritation potential, based on the effective time at which the test item caused a 50% reduction in tissue viability (ET_{50}). Based on the ET_{50}, the test items can be ranked and compared to other previously published data.

The ET_{50} value obtained after test item was applied neat to the surface of EpiOcular tissues for 3, 30 and 60 minutes was as follows:

Test item name	ET_{50}	Classification
Mu-conotoxin TIIIA	> 60 mins	Non-irritating to minimal

Table 1 Executive summary.

NATURE AND PURPOSE OF THE STUDY

This study will assess the eye irritation potential of Rose De Mai Camomile Cleansing Butter following the *in vitro* ET_{50} neat method. The study will be performed using the EpiOcular™ reconstructed tissue model OCL-200 (MatTek Corporation) and procedures based on the current MatTek protocol MK-24-007-0030.

QUALITY AND COMPLIANCE

This study is being performed to comply with:

- The 7th Amendment to the Cosmetics Directive.
- REACH legislation and EU Directive 2010/63/EU on animal protection.

This study was a non-regulatory study.

TEST ITEMS

XCellR8 code	SCB0001
Test code	TA1
Test item name	Mu-conotoxin TIIIA
Supplier	Sponsor
Lot number	N/A
Expiry date	01 December 2024
Physical state	Solid
Colour and appearance	White crystals
Storage conditions	-17-25°C
Concentration to be tested	5 µg/mL in sterile tissue culture grade water[1].
Administration method	100 µL topically to the apical surface of the tissue

[1]A stock of 5 µg/mL Mu-conotoxin TIIIA will be made and stored in aliquots at -20°C. Aliquots were thawed thoroughly before use. Once thawed, the aliquot was not re-frozen to avoid freeze-thaw damage.

CONTROL ITEMS

Positive Control	Triton X-100
Test Code	PC
Supplier	MatTek Corporation
Lot Number	041823LHC
Expiry Date	07 February 2024
Concentration to be tested	0.3%
Administration method	Applied directly to the apical surface of the tissue

Negative Control	Sterile Water (tissue culture grade)
Test Code	NC
Supplier	Sigma-Aldrich
Lot Number	RNBL9915
Expiry Date	09 September 2023
Concentration to be tested	As supplied.
Administration method	Applied directly to the apical surface of the tissue

TEST SYSTEM

Description

The EpiOcular™ tissue model is composed of normal, human-derived epidermal keratinocytes that have been cultured to form a stratified, squamous epithelium, similar to that found in the cornea. Cultured on specially prepared cell culture inserts using serum-free culture medium, the cells differentiate to form a multi-layered structure that closely parallels the corneal epithelium.

Justification for use

These reconstructed tissues are used to test chemicals for the assessment of ocular toxicity. The recently implemented 7th Amendment to the EU Cosmetics Directive and the EU REACH legislation have heightened the need for *in vitro* ocular test methods.

Characterisation

Lot-specific QC information is provided by the MatTek Corporation for the EpiOcular™ tissue model used and will be checked for compliance with QC acceptance criteria (Table 2).

Control Measure	Acceptance Criteria	Result	Outcome
Tissue viability optical density	≥ 1.1 and ≤ 3.0	1.721±0.029	PASS
Barrier function - time taken by 0.3% Triton X-100 to reduce the viability of the ocular model to 50% relative to the negative control	12.2 - 37.5 min	22.89 min	PASS
Sterility	Confirmed	Confirmed	PASS
Biological contaminants	None	None	PASS

Table 2 Quality control acceptance criteria for tissue lot 38541.

MAJOR COMPUTER SYSTEMS

CS002 FLUOstar Omega Spectrophotometer

Use and maintenance follows SOP 144.

EXPERIMENTAL DESIGN

Preliminary Tests

All test items were assessed for MTT interference.

Main Test

Item	Volume	Application method	Exposure time
TA1	100 µL neat test item	Directly to the tissue surface	3, 30 and 60 minutes
PC	100 µL	Directly to the tissue surface	4, 15 and 45 minutes
NC	100 µL	Directly to the tissue surface	60 minutes

Table 3 Test conditions.

1. Tissues were equilibrated at 37°C and 5% CO_2 overnight.
2. Media was changed for all tissues.
3. Test or control item was applied directly to the surface of the tissue.
4. Tissues were incubated at 37°C and 5% CO_2 for the times specified in Table 3.
5. Tissues were harvested for MTT.
6. MTT reads were performed at a wavelength of 570 nm to assess viability of the tissue.
7. All test conditions were performed in triplicate (n=3).

Data Analysis

Results from the MTT assay were entered into a Microsoft Excel workbook containing formulae to process the raw data as per the MatTek protocol.

The percentage viability value for EpiOcular™ tissue models exposed to the test item relative to the negative control set to 100% were plotted against time and the ET_{50} is determined.

Assay Acceptance

The ET_{50} of the positive control generated during the study should correspond to values specified (between 15 and 45 minutes).

Prediction Model

Correlation of *in vitro* and *in vivo* results: as a general guideline, the following groupings will be used in assigning expected *in vivo* irritancy responses based upon the ET_{50} results obtained.

ET_{50} (min)	Expected *in vivo* irritancy	Example
<3	Severe, Extreme	5% Benzalkonium Chloride
3-30	Moderate	1% Triton X-100
30-60	Mild	Pareth 25-12
>60	Non-irritating, minimal	PEG-75, Tween 20

Table 4 Eye irritation ET_{50} (neat) prediction model

RESULTS

Preliminary test

The preliminary test confirmed that the test item did not interfere with MTT.

Viability and ET_{50} determination

Raw data are described in Annex I.

0.3% Triton X-100 (PC)

Tissues were exposed to 0.3% Triton X-100 for 4, 15 and 45 minutes, and the viability assessed relative to the negative control.

	Time (min)		
	4	15	45
1	92.70	70.47	43.87
2	91.87	76.45	51.34
3	94.05	75.66	48.85
Mean	**92.87**	**74.19**	**48.02**
SD	**1.10**	**3.25**	**3.81**

Table 5 Viability data for tissues exposed to 0.3% Triton X-100 (PC).

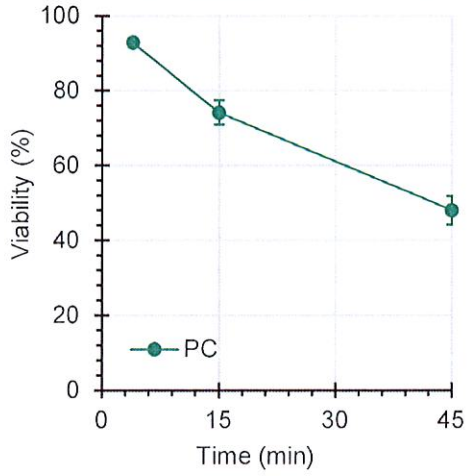

Figure 1 Viability of tissues exposed to the PC.

The ET_{50} of the positive control was calculated as 41.41 minutes.

Mu-conotoxin TIIIA (TA1)

Tissues were exposed to TA1 for 3, 30 and 60 minutes, and the viability assessed relative to the negative control.

	Time (min)		
	3	30	60
1	94.38	93.55	96.07
2	90.23	92.60	97.23
3	90.90	94.24	98.09
Mean	**91.84**	**93.47**	**97.13**
SD	**2.23**	**0.82**	**1.01**

Table 6 Viability data for tissues exposed to TA1.

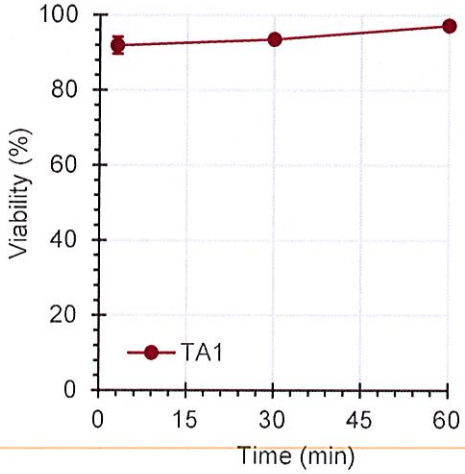

Figure 2 ET_{50} determination of TA1: Mu-conotoxin TIIIA.

The ET_{50} of Mu-conotoxin TIIIA could not be calculated as the viability did not fall below 50% at any timepoint tested.

Assay Acceptance Criteria

For the assay to be accepted as valid, the ET_{50} determination for the positive control must be between 15 and 45 minutes. We calculate the positive control ET_{50} to be 41.41 minutes in this assay and, therefore, the assay can be accepted as valid.

CONCLUDING REMARKS

The ET_{50} of **Mu-conotoxin TIIIA** was >60 minutes and, therefore, we classify this test item as **Non-Irritating to Minimally Irritating**.

The results of this study using the *in vitro* Human Corneal Model EpiOcular™ should be considered together with other sources of data regarding the ocular irritation potential of the test formulation. The *in vivo* response of the human eye to an irritant is a complex physiological process and the results of this test should always be considered alongside user trials.

The EpiOcular™ tissue model has been widely used as a successful *in vitro* model for human ocular irritation, and a large amount of published data using common ingredients and formulations is available, which may provide additional useful reference. Further information is available from XCellR8 upon request.

RECORD RETENTION

Study records are retained for a minimum of 5 years in the archive at XCellR8.

ANNEX I. RAW DATA

Raw data refers to the blank- and background-corrected absorbance.

$$Raw\ data = \left(A_{570} - A_{570\,(Blank)}\right) - \left(A_{690} - A_{690\,(Blank)}\right)$$

	Time (min)		Raw data (au) Aliquot 1	Aliquot 2	Mean	OD Mean (au)
NC	60	1	2.055	2.038	2.046	2.107
		2	2.126	2.183	2.154	
		3	2.099	2.143	2.121	
PC	4	1	2.091	1.816	1.953	1.957
		2	2.052	1.820	1.936	
		3	2.135	1.829	1.982	
	15	1	1.476	1.494	1.485	1.563
		2	1.591	1.631	1.611	
		3	1.591	1.598	1.594	
	45	1	0.950	0.899	0.924	1.012
		2	1.103	1.061	1.082	
		3	1.049	1.010	1.029	
TA1	3	1	1.979	1.999	1.989	1.935
		2	1.873	1.930	1.901	
		3	1.886	1.945	1.915	
	30	1	1.945	1.998	1.971	1.970
		2	1.915	1.988	1.951	
		3	2.008	1.964	1.986	
	60	1	2.010	2.039	2.024	2.047
		2	2.009	2.089	2.049	
		3	2.017	2.117	2.067	

Table 7 Raw absorbance data for tissues exposed to control or test items. OD. au; Arbitrary Units.

END