STUDY REPORT: 23SCB01S50

In vitro assessment of skin irritation potential of Mu-conotoxin TIIIA following the ET_{50} method

REPORT APPROVAL

	Name	Signature	Date
Study Director	Dr Fiona Jacobs		18AUG23

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:	28 July 2023
Experimental start date:	31 July 2023
Preliminary test date:	31 July 2023
Main test start date:	01 August 2023
Experimental end date:	03 August 2023

CONTENTS

EXECUTIVE SUMMARY

No ET_{50} could be calculated for **Mu-conotoxin TIIIA** as the viability of exposed tissues did not fall below 50%. The ET_{50} will be greater than 18 hours, which would correspond to an expected *in vivo* irritancy between **Very mild and non-irritating**.

NATURE AND PURPOSE OF THE STUDY

Scientific Objective

In Vitro Assessment of the Skin Irritation Potential following ET_{50} method

Technical parameters of the study

The study was performed using the EpiDerm™ reconstructed tissue model EPI-200 (MatTek Corporation) and procedures based on the current MatTek protocol MK-24-007-0001.

This *in vitro* risk assessment assay is based on the effective time at which a test item causes a 50% reduction in tissue viability (ET_{50}).

QUALITY AND COMPLANCE

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 is a non-regulatory study.

TEST ITEMS

XCellR8 code	SCB0001
Test code	TA2
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 was 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. Test item was treated as a liquid for the main test.

CONTROL ITEMS

Negative control	Untreated tissues
Test code	NC

Positive control	Triton X-100
Test code	PC
Supplier	MatTek Corporation
Lot number	062723NMA
Expiry	28 June 2024
Concentration to be tested	1%
Administration method	Topically to the apical surface of the tissue.

TEST SYSTEM

Description

The EpiDerm™ tissue model is a reconstructed human epidermis 3D tissue model from a single donor provided by the MatTek Corporation. It consists of normal human-derived epidermal keratinocytes cultured on specially prepared tissue culture inserts to form a multi-layered highly differentiated model of the human epidermis: organised basal, spinous, and granular layers and a multi-layered *stratum corneum* containing intercellular lamellar lipid layers arranged in patterns analogous to those found *in vivo*.

Justification

With multiple ECVAM validations and OECD accepted test guidelines, EpiDerm™ is a proven in vitro model system for chemical, pharmaceutical and skin care product testing.

Characterisation

Each lot of tissues are quality assured by MatTek according to specific QC standards. All biological components of the epidermis and the culture medium are tested by MatTek for viral, bacterial, fungal and mycoplasma contamination and results must fall within an established range based on a historical database of results. Quality control data for the tissues used in this study are summarised below.

Criterion	Result	Outcome
Optical density at 540-570 nm of untreated tissues within the range 1.0-3.0	1.471±0.181	PASS
ET_{50} of tissues treated with 1% Triton X-100 within the historic range (4.77-8.72 hours)	4.96 hours	PASS
Tissues are free from contamination or infection.	None detected	PASS

Table 1 Summary of QC results for EpiDerm™ tissue lot 38756.

MAJOR COMPUTER SYSTEMS

CS002 FLUOstar Omega Spectrophotometer

Use and maintenance follows SOP 144.

EXPERIMENTAL DESIGN

Methods described in XCellR8 SOP L0030 "EpiDerm™ Skin Irritation Potency Assessment-ET_{50} Method" were followed. Materials, suppliers, catalogue numbers, lot/batch numbers and expiry dates were documented in study data.

Preliminary test

MTT interference for each test item was assessed.

Main Test

Day 1: Tissues received and conditioned overnight.

Day 2: Exposure to and removal of test and reference items, MTT viability test.

- Topical application of neat test items (100 µL liquid test items or 25 µL DPBS followed by nominal 100mg of solid test items) or 100 µL PC to the surface of the EpiDerm™ skin model.
- Test item was applied for 2h, 5h or 18h ± 5 min.
- Positive Control was applied for 3h, 5h and 7h.
- Negative Control tissues were left untreated for 5h.

Day 3: End of MTT, absorbance optical density (OD) at a wavelength of 570 nm, background at 690 nm.

Data Analysis

Results from the MTT assay were entered to a Microsoft Excel workbook (Form F0062) containing formulae to process the raw data as per the MatTek protocol.

The percentage viability value for EpiDerm™ tissue models exposed to the test item relative to the negative control set to 100% were plotted against time (mins) on a semi-log graph and the ET_{50} is determined.

Assay Validity Acceptance

The ET_{50} of the positive control generated during the study was used to categorise the control item, and the ET_{50} compared to historic data.

Prediction model

Correlation of *in vitro* and *in vivo* results: as a general guideline, the following groupings were used in assigning expected *in vivo* irritancy responses based upon the ET_{50} results obtained using EPI-200.

ET50 (h)	Expected *in vivo* irritancy	Example
< 0.5	Strong/Severe, possible corrosive concentration	Nitric acid
0.5 - 4	Moderate	1% Sodium Dodecyl Sulfate
4 - 12	Moderate to Mild	1% Triton X-100
12 - 24	Very Mild	Baby Shampoo
24	Non-Irritating	10% Tween 20

Table 2 Skin irritation ET_{50} Prediction Model

RESULTS

Preliminary Tests

No MTT interference was observed for Mu-conotoxin TIIIA.

Main Test

Raw data is presented in Annex I. The percentage viability for EpiDerm™ models exposed to the PC or test item, relative to the negative control, was calculated.

Assay Acceptance

	Time (h) 3	5	7
1	53.24	27.90	4.65
2	54.92	28.30	3.50
3	39.84	16.99	5.62
Mean	**54.08**	**28.10**	**4.59**
SD	**1.19**	**0.28**	**1.06**

Table 3 Viability data for the PC. SD: Standard Deviation. Values in red were deemed to be outliers and were excluded from the analysis.

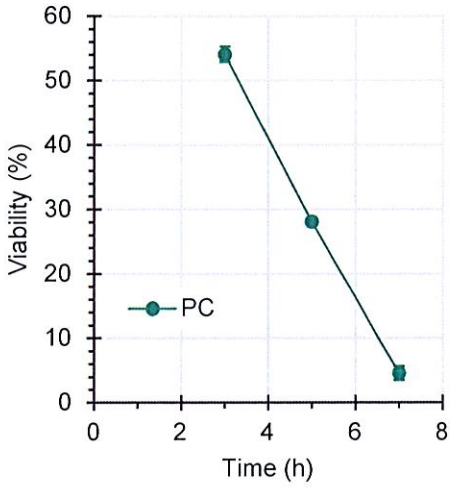

Figure 1 Viability data for the PC. Data points are mean viability and error bars are standard deviation.

The ET_{50} obtained with the Positive Control was **3.31 hours**. This is outside the typical ET_{50} range for Triton-X100 (3.7-7.0 h); however, as the difference is marginal (approx. 25 minutes) and the PC produces a dose response, we do not believe this difference in ET_{50} has affected the outcome of the study.

Mu-conotoxin TIIIA

	Time (h) 2	5	18
1	104.46	95.09	103.97
2	105.80	113.03	112.34
3	100.54	111.68	112.83
Mean	**103.60**	**106.60**	**109.71**
SD	**2.73**	**9.99**	**4.98**

Table 4 Viability data for SCB0001 Mu-conotoxin TIIIA. SD: Standard Deviation.

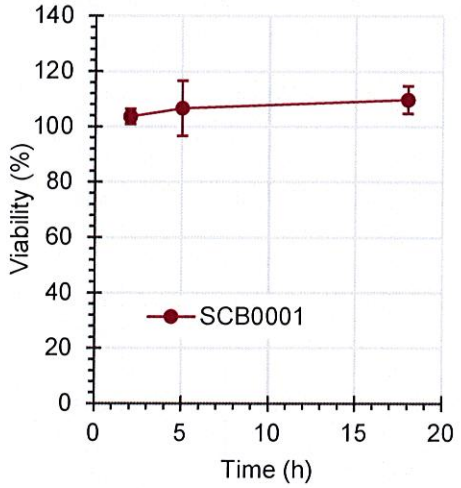

Figure 2 Viability data for SCB0001 Mu-conotoxin TIIIA. Data points are mean viability and error bars are standard deviation.

As the viability did not fall below 50%, an ET_{50} could not be calculated.

CONCLUSION AND RECOMMENDATIONS

No ET_{50} could be calculated for **SCB0001 Mu-conotoxin TIIIA** as the viability of exposed tissues did not fall below 50%. However, the ET_{50} will be > 18 hours, as no loss of viability was seen for tissues treated with the test item at that timepoint. This would correspond to an expected *in vivo* irritancy between **Very mild and non-irritating**. For a more accurate ET_{50} and classification, an XtraMild test should be considered. This modified skin irritancy test uses a broader range of timepoints especially suited for mild and non-irritating test items (1, 5, 18, 24 and 48 hours).

RECORD RETENTION

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

ANNEX I. RAW DATA

Condition	Tissue	Aliquot 1	Aliquot 2	Tissue mean	Condition mean
NC 5	1	1.813	1.740	1.776	1.750
	2	1.766	1.703	1.734	
	3	1.724	1.757	1.740	
PC 3	1	0.978	0.886	0.932	0.864
	2	0.988	0.935	0.961	
	3	0.713	0.682	0.697	
PC 5	1	0.485	0.492	0.488	0.427
	2	0.498	0.493	0.495	
	3	0.308	0.287	0.297	
PC 7	1	0.082	0.081	0.081	0.080
	2	0.063	0.060	0.061	
	3	0.099	0.098	0.098	
TA2 2	1	1.864	1.793	1.828	1.813
	2	1.857	1.847	1.852	
	3	1.744	1.776	1.760	
TA2 5	1	1.673	1.656	1.664	1.866
	2	2.114	1.843	1.978	
	3	1.970	1.940	1.955	
TA2 18	1	1.893	1.747	1.820	1.920
	2	1.975	1.958	1.966	
	3	2.034	1.916	1.975	

Table 5 Raw blank and background corrected absorbance data.

END