CONSUMER PRODUCT TESTING COMPANY
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FINAL REPORT

CLIENT: Smooth-On Inc.
5600 Lower Macungie Road
Macungie, Pennsylvania 18062

ATTENTION: Tim Boyer

TEST: The MatTek Corporation *In Vitro* EpiDerm™ Skin Irritation Test (EPI-200-SIT) (OECD 439)
Protocol: *In Vitro* EpiDerm™ Skin Irritation Test (EPI-200-SIT)
Protocol Date: 10/2/19

TEST ARTICLE: Dragon Skin 15 Lot Number: A 2204001 B 2204001

EXPERIMENT REFERENCE NO.: V22-3253.01

7/18/22
Steven Nitka
Vice President
Laboratory Director

FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJD982726648
ISO/IEC 17025:2017 Accredited

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QUALITY ASSURANCE UNIT STATEMENT

CPT Study Number: V22-3253.01

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice principles to the extent applicable, and in accordance with CPT Standard Operating Procedures and applicable Standard Protocols. The QAU has reviewed and approved this study on the date indicated below.

Approved by CPT Quality Assurance Unit:

Christine Vomela 7/25/2022
Signature/Date

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Objective:

"To predict skin irritation potential of neat test substances in the context of identification and classification of skin irritation hazard according to the EU classification system. The Modified EpiDerm SIT allows discrimination between irritants of category 2 and non-irritants."[1] The protocol used is in compliance with OECD 439 (Adopted 18 June 2019).

Introduction:

"The reconstructed human epidermal model EpiDerm consists of normal, human-derived epidermal keratinocytes which have been cultured to form a multilayered, highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multilayered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found *in vivo* . . . The EpiDerm tissues are cultured on specially prepared cell culture inserts and shipped worldwide as kits, containing . . . tissues on shipping agarose . . ."[1]

EpiDerm, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

Test Article: Dragon Skin 15 Lot Number: A 2204001 B 2204001

Reference Articles: Sodium dodecyl sulfate (5%) (Positive Control)
Dulbecco's Phosphate Buffered Saline (DPBS) (Negative Control)

Experimental Interval: July 13, 2022 to July 15, 2022

Method:

Prior to initiation of the irritation assay, 2 tests were conducted to determine if the test article would interfere with the assay:

1) It was determined that the test article would not change in aqueous conditions and then stain EpiDerm tissue during the exposure period.
2) It was determined that the test article would not directly reduce MTT and thus interfere with the assay.

[1]MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721.
Consumer Product Testing Company, Inc., 70 New Dutch Lane, Fairfield, NJ 07004

Method (continued):

After the appropriate tissue preparation, circular pieces of test article, 8 millimeters in diameter, 30 microliters of the positive control article and 30 microliters of the negative control article were each added to each of three Millicells containing the EpiDerm samples. Then the nylon mesh, supplied by MatTek, was applied to each control tissue surface. The articles were applied with a one minute interval between each application. After dosing the last tissue, the six (6) well plates containing the dosed EpiDerm samples were incubated at 37°C, five (5)% carbon dioxide and $\geq$ 95% humidity for 35 $\pm$ 1 minutes.

After the 35 $\pm$ 1 minute exposure period, the six well plates were removed from the incubator and placed in the sterile hood until the 60 minute exposure period was reached for the first dosed tissue. After the 60 $\pm$ 1 minute test article exposure, each insert was individually removed from its plate and rinsed with Dulbecco's Phosphate Buffered Saline (DPBS), from a wash bottle, to remove any residual material. Each insert was filled and emptied 15 times to remove the nylon mesh, if present, and any residual article. After the 15th rinse from the washing bottle, each insert was submerged 3 times in 150 ml DPBS and lightly shaken to remove any remaining article. Finally, each insert was rinsed once inside and out with sterile DPBS. Any excess DPBS was removed by gently shaking the insert and blotting the tissue. Each insert was then transferred to a new 6-well plate, with each well containing 0.9 ml of fresh assay medium. After all inserts were washed, the surface of each tissue was carefully dried using sterile cotton tipped swabs. The EpiDerm samples were then returned to the incubator for 24 $\pm$ 2 hours.

At the end of the 24 $\pm$ 2 hour incubation period, the unused wells of the 6-well plates were filled with 0.9 ml of fresh assay medium. The inserts were then transferred to these fresh wells and replaced in the incubator for an 18 $\pm$ 2 hour post incubation.

The following day, 24 well plates were appropriately labeled. The MTT media was prepared and placed into the appropriate wells (300 microliters per well). After the 18 $\pm$ 2 hour post incubation period was complete, each insert was removed from the 6-well plate. The bottom of each insert was blotted and transferred to the appropriate well of the 24 well MTT plate. When all inserts were transferred, the 24 well plate was placed back in the incubator for 3 hours $\pm$ 5 minutes.

After the MTT exposure, the MTT medium was gently aspirated from each well and replaced with DPBS, which was then aspirated. This rinsing was repeated twice. Each tissue was examined to ensure that it was dry after the last rinse. The inserts were then transferred to a new 24 well plate. Two milliliters of the supplied isopropanol extractant solution was then gently pipetted into each well, completely covering the tissue in each well.

Method (continued):

The 24 well plate was then sealed and placed in a plastic bag. The formazan was then extracted for at least 2 hours, at room temperature, with gentle shaking on a plate shaker (120 rpm).

The plate was then opened and each tissue was pierced with a 20 gauge injection needle and the extract was allowed to run out into the well from which it was taken. Each insert was then discarded.

Two, 200 microliter aliquots were then pipetted from each well and placed into wells of a 96 well flat bottom microtiter plate. Each aliquot was pipetted up and down 3 times before being transferred. The isopropanol extract fluid was used as blanks (n = 6).

A BioTek 800TS Microplate Reader was used to determine the absorbance of each extract at 570nm. With the absorbance of the negative control defined as 100%, the percent absorbencies of the articles were determined.

The results are presented on the following page.

Results (Blank Average = 0.0402):

Article/% Tissue #/Aliquot	OD-570	Blank Corrected OD-570	Mean of Aliquot	Percent Viability
Dragon Skin 15 Lot Number: A 2204001 B 2204001 (100%)				
(1/1)	2.027	1.987		
(1/2)	2.080	2.040	2.014	100.4
(2/1)	2.086	2.046		
(2/2)	2.031	1.991	2.018	100.6
(3/1)	1.951	1.911		
(3/2)	1.996	1.956	1.933	96.4
DPBS (100%)				
(1/1)	2.111	2.070		
(1/2)	2.186	2.145	2.108	105.1
(2/1)	1.901	1.861		
(2/2)	1.966	1.926	1.893	94.4
(3/1)	2.104	2.064		
(3/2)	2.009	1.969	2.016	100.5
SDS (5%)				
(1/1)	0.101	0.061		
(1/2)	0.104	0.064	0.062	3.1
(2/1)	0.102	0.061		
(2/2)	0.104	0.064	0.063	3.1
(3/1)	0.103	0.063		
(3/2)	0.112	0.072	0.068	3.4

Results (continued):

Article	Mean of OD-570's	Deviation of OD-570's	Mean of Viability	Deviation of Viability	Coefficient of Variation
Dragon Skin 15 Lot Number: A 2204001 B 2204001	1.988	0.048	99.1	2.38	2.40
DPBS	2.006	0.108	100.0	5.37	5.37
SDS	0.064	0.003	3.2	0.14	4.53

Discussion:

As per MatTek and OECD 439, the assay meets the negative control criteria if the mean OD-570 for the negative control tissues is $\geq$ 0.8 and $\leq$ 2.8. The value is 2.006 and meets the acceptance criteria.

As per MatTek, the assay meets the positive control criteria if the mean viability of the positive control tissues, expressed as the percent of the negative control tissues is $\leq$ 20%. The value is 3.2 and meets the acceptance criteria.

As per MatTek and OECD 439, the assay meets the standard deviation criteria if the standard deviation calculated from individual percent tissue viabilities of the 3 identically treated replicates is < 18%. The values are 2.38, 5.37 and 0.14, and they meet the acceptance criteria.

Conclusion:

According to the EU and GHS classification (R38/ Category 2 or no label), an irritant is predicted if the mean relative tissue viability of three individual tissues exposed to the test substance is reduced below 50% of the mean viability of the negative controls.

***In vitro* result**	***In vivo* prediction**	
	EPI-200-SIT classification	**OECD/OCDE TG No. 439 (adopted and updated in 2019)**
Mean tissue viability ≤ 50%	Irritant (I) (R38 or GHS category 2)	Requiring classification and labeling (GHS Category 1 or 2)
Mean tissue viability > 50%	Non-Irritant (NI)	Non-irritant to skin (GHS No Category)
Test Article (@ 100%) Mean tissue viability = 99.1%	**Non-Irritant (NI)**	**Non-irritant to skin (GHS No Category)**

Therefore, under the conditions of this test, the test article's *in vivo* prediction is that the test article is a non-irritant, having elicited a mean tissue viability of 99.1%.

Record Retention:

All records and documents pertaining to the conduct of testing shall be retained in the CPTC archives for a period of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the CPTC archive period, with no further notice, in a manner that renders them useless.

Professional personnel involved:

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Vazquez, B.S.	-	Senior Analyst
Christine Vornehm	-	Quality Assurance Supervisor
Nicole Bowley, B.S.	-	Quality Assurance Auditor