SUPPLEMENT

Aluminum Starch Octenylsuccinate (Polysaccharide Octenylsuccinates)

Dialkyl Dimer Dilinoleates

Organo-Titanium Ingredients

Polyfluorinated Polymers

Polyol Phosphates

CIR EXPERT PANEL MEETING
JUNE 4-5, 2018

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Alice Akinsulie, Scientific Writer/Analyst
Date: May 23, 2018
Subject: Wave 2 – Aluminum Starch Octenylsuccinate – Polysaccharide Octenylsuccinates

Concentration of use data were received from Council. Attached herein, please find an updated Use Table (*starch062018wave2_use_table*) along with the raw concentration of use data for Aluminum Starch Octenylsuccinate and related ingredients (*starch062018wave2_data*).

In the original safety assessment, concentration of use data (1998/1999) indicated that Aluminum Starch Octenylsuccinate was used at up to 30% in leave-on products and at up to10% in products for which for incidental inhalation may result from spray or powder. The maximum concentration of use for Aluminum Starch Octenylsuccinate in the recent data set is also 30% in leave-on products. The highest concentration for spray or powder, however, is now up to 26%. Seven of the cosmetic ingredients that are proposed for inclusion in this re-review were not found to be in use.

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Table 1. Current(2018) and historical(1998) frequency and concentration of use according to duration and exposure

	# of Uses		*Max Conc of Use (%)*					
	Aluminum Starch Octenylsuccinate							
	2018[16]	**1998[1]**	**2018**	**1998/1999[1]**				
Totals*	**786**	**172**	**30**	**30**				
Duration of Use								
Leave-On	*744*	*158*	*0.087-30*	*0.5-30*				
Rinse-Off	*42*	*12*	6.7	*1-6*				
Diluted for (Bath) Use	*NR*	*2*	NR	*NR*				
Exposure Type								
Eye Area	138	11	3-15.3	0.5-30				
Incidental Ingestion	32	9	7.4-12.9	15				
Incidental Inhalation-Spray	14; 122[a]; 115[b]	3; 13[a]; 17[b]	1.3-22.9; 2.5[a]; 26[b]	2; 1-5[a]; 0.5-10[b]				
Incidental Inhalation-Powder	71;115[b]; 1[c]	55; 17[b]	15-30; 26[b]	1-15; 0.5-10[b];				
Dermal Contact	699	159	0.087-30	0.5-30				
Deodorant (underarm)	4[a]	NR	NR	4[a]				
Hair - Non-Coloring	35	2	1.3-11.4	NR				
Hair-Coloring	13	NR	6.7	NR				
Nail	NR	NR	NR	NR				
Mucous Membrane	34	18	7.4-12.9	15				
Baby Products	1	NR	NR	NR				

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

** -a concentration of use survey is currently being conducted; these data will be added once they are received

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays..

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders

NR – no reported use

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 21, 2018

SUBJECT: Concentration of Use by FDA Product Category: Aluminum Starch Octenylsuccinate and Related Ingredients

Concentration of Use by FDA Product Category – Aluminum Starch Octenylsuccinate and Related Ingredients*

Aluminum Starch Octenylsuccinate
Acacia Seyal Gum Octenylsuccinate
Hydrolyzed Hydroxypropyl Starch Octenylsuccinate
Quinoa Starch Octenylsuccinate
Sodium Glycogen Octenylsuccinate
Sodium Hydroxypropyl Cyclodextrin Octenylsuccinate
Sodium Trehalose Octenylsuccinate

Ingredient	Product Category	Maximum Concentration of Use
Aluminum Starch Octenylsuccinate	Eyebrow pencils	5.2%
Aluminum Starch Octenylsuccinate	Eyeliners	3-9.6%
Aluminum Starch Octenylsuccinate	Eye shadows	8-15.3%
Aluminum Starch Octenylsuccinate	Eye lotions	1.8%
Aluminum Starch Octenylsuccinate	Mascaras	0.9%
Aluminum Starch Octenylsuccinate	Other eye makeup preparations	3%
Aluminum Starch Octenylsuccinate	Colognes and toilet waters	1.8%
Aluminum Starch Octenylsuccinate	Powders (dusting and talcum)	17.2%
Aluminum Starch Octenylsuccinate	Other fragrance preparations	22.9%
Aluminum Starch Octenylsuccinate	Hair sprays Aerosols	 7.5-11.4%
Aluminum Starch Octenylsuccinate	Tonics, dressings and other hair grooming aids	1.3-6.1%
Aluminum Starch Octenylsuccinate	Other hair preparations (noncoloring)	1.1%
Aluminum Starch Octenylsuccinate	Hair rinses (coloring)	6.7%
Aluminum Starch Octenylsuccinate	Hair color sprays	0.1%
Aluminum Starch Octenylsuccinate	Blushers	3-10%
Aluminum Starch Octenylsuccinate	Face powders	15-30%
Aluminum Starch Octenylsuccinate	Foundations	2.5-16.7%
Aluminum Starch Octenylsuccinate	Lipstick	7.4-12.9%
Aluminum Starch Octenylsuccinate	Makeup bases	6.6-21.3%
Aluminum Starch Octenylsuccinate	Rouges	12%
Aluminum Starch Octenylsuccinate	Makeup fixatives	13%
Aluminum Starch Octenylsuccinate	Other makeup preparations	4%
Aluminum Starch Octenylsuccinate	Deodorants Not spray Aerosol	 0.99-1.3% 0.1-3.5%
Aluminum Starch Octenylsuccinate	Aftershave lotions	0.087-4.3%
Aluminum Starch Octenylsuccinate	Shaving cream	0.86%
Aluminum Starch Octenylsuccinate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	5%
Aluminum Starch Octenylsuccinate	Face and neck products Not spray	 1-15.9%
Aluminum Starch Octenylsuccinate	Body and hand products Not spray	 0.86-6.9%
Aluminum Starch Octenylsuccinate	Foot powders and sprays	26%

Aluminum Starch Octenylsuccinate	Moisturizing products Not spray	 1.8-2%
Aluminum Starch Octenylsuccinate	Night products Not spray	 0.86-3%
Aluminum Starch Octenylsuccinate	Paste masks and mud packs	0.86%
Aluminum Starch Octenylsuccinate	Other skin care preparations	1%
Aluminum Starch Octenylsuccinate	Suntan products Not spray	 1-6%
Aluminum Starch Octenylsuccinate	Indoor tanning preparations	2.5%
Aluminum Starch Octenylsuccinate	Other suntan preparations	2%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2018

Table prepared May 21, 2018

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Priya Cherian
Scientific Analyst/Writer
Date: May 23, 2018
Subject: Wave 2 Data on Dialkyl Dimer Dilinoleates

The concentration of use data on Diisopropyl Dimer Dilinoleate and Diisostearyl Dimer Dilinoleate (*dilin062018wave2_data*) were received from the Council and are being submitted as an attachment to this memorandum. An updated use table with 2018 concentration of use data is included (*dilin062018wave2_usetable*).

An increase in the frequency of use and a decrease in the concentration of use of Diisopropyl Dimer Dilinoleate are reported from the years of 1998/1999 to 2018. The highest frequency of use was reported to be lipstick formulations, with 12 and 87 reported uses in 1998 and 2018, respectively. The highest reported Diisopropyl Dimer Dilinoleate lipstick formulation concentration was 53% in 1999, and 29% in 2018.

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Current and historical frequency and concentration of use of Dialkyl Dimer Dilinoleates according to duration and exposure

	Diisopropyl Dimer Dilinoleate				Diisostearyl Dimer Dilinoleate			
	2018[4]	**1998[1]**	**2018**	**1999[1]**	**2018[4]**	**1998[1]**	**2018**	**1999[1]**
Totals*	**145**	**35**	**1-29**	**0.1-53**	**20**	**20**	**5-16**	**1-12**
Duration of Use								
Leave-On	*142*	*30*	*1-29*	*0.05-53*	*19*	*20*	*5-16*	*1-12*
Rinse-Off	*3*	*5*	*NR*	*0.1-5*	*1*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	12	2	NR	0.1-3	7	11	6	5-11
Incidental Ingestion	87	12	10.8-29	4-53	5	2	16	7-12
Incidental Inhalation-Spray	13[a]	4[a]; 4[b]	1.5-5.3[a]	9.25; 2-10[a]; 3-5[b]	4[a]	NR	NR	NR
Incidental Inhalation-Powder	NR	4[b]	NR	3-5[b]; 30	NR	3	NR	1-7
Dermal Contact	53	22	1-5	0.05-30	15	18	5-6	1-11
Deodorant (underarm)	NR	NR	NR	20[a]	NR	NR	NR	NR
Hair - Non-Coloring	5	1	1.5-5.3	9.25-10	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	88	12	10.8-29	4-53	6	2	16	7-12
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a] Includes products that can be sprays, but it is not known whether the reported uses are sprays

[b] Not specified whether this product is a spray or a powder or neither, but it is possible it may be a spray or a powder, so this information is captured for both categories of incidental inhalation

NR – no reported use

Concentration of Use by FDA Product Category – Dimer Dilinoleate Ingredients*

Dicetearyl Dimer Dilinoleate
Diethylhexyl Dimer Dilinoleate
Diisopropyl Dimer Dilinoleate
Diisostearyl Dimer Dilinoleate
Dioctyldodecyl Dimer Dilinoleate
Ditridecyl Dimer Dilinoleate
Di-C16-18 Alkyl Dimer Dilinoleate
Di-C20-40 Alkyl Dimer Dilinoleate

Ingredient	Product Category	Maximum Concentration of Use
Diisopropyl Dimer Dilinoleate	Tonics, dressings and other hair grooming aids	1.5-5.3%
Diisopropyl Dimer Dilinoleate	Blushers	1-2.8%
Diisopropyl Dimer Dilinoleate	Foundations	1-2.8%
Diisopropyl Dimer Dilinoleate	Lipstick	10.8-29%
Diisopropyl Dimer Dilinoleate	Rouges	5%
Diisostearyl Dimer Dilinoleate	Eye shadows	6%
Diisostearyl Dimer Dilinoleate	Foundations	5%
Diisostearyl Dimer Dilinoleate	Lipstick	16%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2018
Table prepared May 21, 2018

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: May 23, 2018
Subject: Wave 2 Data on Organo-Titanium Ingredients

Chemistry and safety test data, received from the Council and listed below, on a product containing Isopropyl Titanium Triisostearate or a Isopropyl Titanium Triisostearate trade name mixture, are being submitted as an attachment to this memorandum. A data summary document (*organo062018wave2studysummaries*) is also attached for the Panel's review. The data that were received include:

organo062018data5:

- HRIPT on an eye powder containing 1.4% Isopropyl Titanium Triisostearate

organo062018data6 (data on Isopropyl Titanium Triisostearate trade name mixture (> 98%)):

- Definition and chemical/physical properties
- Method of manufacture
- Impurities analysis of titanium isopropoxide (used in manufacture of Isopropyl Titanium Triisostearate)
- Acute oral toxicity (rats)
- Acute dermal toxicity (rabbits)
- Genotoxicity (*in vitro*)
- Skin irritation (rabbits)
- Ocular irritation (rabbits)

These data will be added to the safety assessment after the Panel meeting.

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Wave 2 Data on Organo-Titanium Ingredients

CHEMISTRY

Definition and General Characterization

Isopropyl Titanium Triisostearate

A chemical supplier has reported that an Isopropyl Titanium Triisostearate trade name mixture consists of > 98% Isopropyl Titanium Triisostearate and < 2% isopropyl alcohol.

Chemical and Physical Properties

Isopropyl Titanium Triisostearate

Chemical and physical properties of an Isopropyl Titanium Triisostearate trade name mixture are presented in Table 1.[1]

Method of Manufacture

Isopropyl Titanium Triisostearate

An Isopropyl Titanium Triisostearate trade name material (> 98% Isopropyl Titanium Triisostearate and < 2% isopropyl alcohol) is produced by reacting titanium isopropoxide with 3 equivalents of isostearic acid.[1] The product is predominantly titanium substituted with 1 isopropoxy and 3 isostearate ligands. However, it will also contain the tetra-isostearate and the di-isopropoxy and di-isostearate titanates as well.

Impurities

Isopropyl Titanium Triisostearate

The results of an impurities analysis of titanium isopropoxide (a starting material used in the manufacture of Isopropyl Titanium Triisostearate) indicated the presence of calcium (3 ppm) and titanium (16.99%).[2] The following other metals were not detected.

Silver	Mercury	Ruthenium
Aluminum	Indium	Antimony
Arsenic	Potassium	Scandium
Gold	Lithium	Selenium
Boron	Magnesium	Silicon
Barium	Manganese	Tin
Beryllium	Molybdenum	Strontium
Bismuth	Sodium	Tantalum
Cadmium	Niobium	Tellurium
Cobalt	Neodymium	Thallium
Chromium	Nickel	Vanadium
Copper	Phosphorus	Tungsten
Dysprosium	Lead	Yttrium
Iron	Palladium	Zinc
Gallium	Platinum	Zirconium
Germanium	Rhodium	Lanthanum

Polychlorinated biphenyls and the following halogens were also undetectable: Fluorine, Chlorine, Bromine, and Iodine.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Isopropyl Titanium Triisostearate

The dermal toxicity of an Isopropyl Titanium Triisostearate trade name mixture (> 98% Isopropyl Titanium Triisostearate and < 2% isopropyl alcohol) was evaluated using New Zealand White rabbits (number not stated).[3] The test material was administered under a semi-occlusive wrap for 4 h. There were no signs of gross toxicity or remarkable pathology.

Oral

Isopropyl Titanium Triisostearate

The acute oral toxicity of an Isopropyl Titanium Triisostearate trade name mixture (> 98% Isopropyl Titanium Triisostearate and < 2% isopropyl alcohol) was evaluated using male and female Sprague-Dawley rats (number not stated).[3] An LD_{50} of > 30,000 mg/kg was reported for males only, females only, and males and females together. There were no signs of gross toxicity or remarkable pathology. A target organ was not identified in this study.

GENOTOXICITY STUDIES

In Vitro

Isopropyl Titanium Triisostearate

The genotoxicity of an Isopropyl Titanium Triisostearate trade name mixture (> 98% Isopropyl Titanium Triisostearate and < 2% isopropyl alcohol) was studied using the following *Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538.[3] The trade name material was tested at doses ranging from 0.2 µg to 500 µg per plate with and without metabolic activation. No increase in the number of revertants per plate was observed with or without metabolic activation.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Isopropyl Titanium Triisostearate

The skin irritation potential of an Isopropyl Titanium Triisostearate trade name mixture (> 98% Isopropyl Titanium Triisostearate and < 2% isopropyl alcohol) was evaluated using New Zealand White rabbits (number not stated).[3] The test material (dose per cm^2 not stated) was administered under a semi-occlusive wrap for 4 h. Scores for erythema and edema were recorded at 4 h, 24 h, 48 h, and 72 h after removal of the wrap. The following primary dermal irritation scores were reported: erythema (0.3), edema (0), and overall score (0.3). The test material was not corrosive.

Sensitization

Human

Isopropyl Titanium Triisostearate

An HRIPT on an eye powder containing 1.4% Isopropyl Titanium Triisostearate was performed using 101 subjects.[4] Specifically, the test was identified as an 8 mm Finn chamber occlusive patch test. During induction, the product (20 mg; dose per cm^2 not stated) was applied to one side of the infrascapular area of the back on Mondays, Wednesdays, and Fridays for 3 consecutive weeks (9 applications total). The challenge phase was initiated after a 2-week non-treatment period. Challenge patches were applied for 48 h to the induction site and to a new site. Challenge reactions were scored at 48 h and 96 h after patch application. The authors concluded that the product was non-irritating and non-sensitizing.

OCULAR IRRITATION STUDIES

Isopropyl Titanium Triisostearate

The ocular irritation potential of an Isopropyl Titanium Triisostearate trade name mixture (> 98% Isopropyl Titanium Triisostearate and < 2% isopropyl alcohol) was evaluated using New Zealand White rabbits (number not stated).[3] The test material (0.1 ml) was instilled into the conjunctival sac, and scores for corneal opacity, iritis, and conjunctivitis were recorded at 1 h, 24 h, 48 h, and 72 h post-instillation. The following primary ocular irritation scores were reported: 10 (at 1 h), 0.7 (at 24 h), 0 (at 48 h), and 0 (at 72 h). There were no signs of gross toxicity or remarkable pathology. The test material was not corrosive.

TABLES

Table 1. Chemical and Physical Properties of a Isopropyl Titanium Triisostearate tradename mixture (> 98% Isopropyl Titanium Triisostearate and < 2% isopropyl alcohol).[1]

Physical State	Liquid
Color	Reddish
Odor	Waxy fatty acid
Boiling point °C	149
Flash point °C	> 93
Evaporation rate (relative to n-butyl acetate)	Slower
Relative density @ 60°F	0.95
Solubility (H_2O)	Insoluble
Solubility to other solvents	< 5% xylene
Viscosity @ 77°F	125 cps

References

1. Kenrich Petrochemicals, Inc. Summary information. Isopropyl Titanium Triisostearate. Unpublished data submitted by the Personal Care Products Council on 5-15-2018. 2018. pp.1

2. SGS. Test report (metal analysis of TIPT (titanium compound used in the manufacture of isopropyl titanium triisostearate)). Unpublished data submitted by the Personal Care Products Council on 5-15-2018. 2016. pp.1-10.

3. Richard Costlow Consulting, LLC. Ken-React® KR® TTS (Isopropyl Titanium Triisostearate): Summary of toxicology data for regulatory compliance. Unpublished data submitted by the Personal Care Products Council on 5-15-2018. 2018. pp.1-5.

4. TKL Research, Inc. Human repeated patch test with challenge (eye powder containing 1.4% isopropyl titanium triisostearate). Unpublished data submited by the Personal Care Products Council on 5-8-2018. 2012. pp.1-19.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 8, 2018

SUBJECT: Isopropyl Titanium Triisostearate

TKL Research, Inc. 2012. Human repeated insult patch test with challenge (eye powder containing 1.4% Isopropyl Titanium Triisostearate).

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TKL RESEARCH

[redacted]

TKL Study No. [redacted]

TKL Study Report [redacted]

Human Repeated Insult Patch Test with Challenge

(FINN-CHAMBERS 48 H)

Sponsor:	[redacted]
Document type:	Clinical Study Report
Investigational Product:	[redacted]
Batch No.:	[redacted]
Product Type:	EYE POWDER contains 1.4%
Study Monitor:	[redacted] Isopropyl Titanium
Investigator:	[redacted] Triisostearate
Investigating Site:	TKL Research, Inc 1 Palmer Terrace Carlstadt, NJ 07072
Investigating Laboratory:	TKL Research, Inc 365 W Passaic Street Rochelle Park, NJ 07662
Number of Pages:	41
Document Version:	Final Date: November 16, 2012

[redacted]

Prepared by TKL Research, Inc
365 W Passaic Street
Rochelle Park, NJ 07662 USA

Version 1.0

SIGNATURES

This study was conducted in compliance with the requirements of the protocol and TKL's Standard Operating Procedures (SOPs), and in the spirit of GCP ICH Topic E6 [1]. The report accurately reflects the raw data for this study.

[redacted], MD
Principal Investigator
Dermatologist

11/16/12
Date

[redacted]
Director, Dermatologic Safety Testing

11/16/12
Date

Michelle Medina
Manager, Dermatologic Safety Testing

11/16/12
Date

[1] ICH Topic E6 "Note for guidance on GCP (CPMP/ICH/135/95)" – ICH Harmonised Tripartite Guideline for GCP having reached Step 5 of the ICH Process at the ICH Steering Committee meeting on 1 May 1996.

STATEMENT OF TKL RESEARCH, INC QUALITY CONTROL

All data and supporting documentation for this study have been reviewed by the TKL Quality Control Department and found to be accurate, complete and in compliance with the requirements of the protocol and TKL's SOPs, and this report has been reviewed and accurately reflects the raw data for this study.

The Quality Control Department has conducted the following inspections for this study. Written status reports of inspections and findings are submitted to Management according to TKL SOPs.

Date of Inspection	Type of Inspections	Date Reported to Management
09/06/2012	Initial Binder Review Prior to Study Start	09/06/2012
09/14/2012	Screeners/Consents 100% Review	09/14/2012
10/26/2012	After Study Binder Review	10/26/2012
10/26/2012	Tables and Data Listings	10/26/2012
11/16/2012	Final Study Report	11/16/2012

Quality Control

11/16/12
Date

Study Title: Human Repeated Insult Patch Test with Challenge

Sponsor: [redacted]

Protocol #: RCL-SEC-01/E

Contract Research Organization:
TKL Research, Inc
365 W Passaic Street
Rochelle Park, NJ 07662 USA

TKL Study Report #: [redacted]

Investigating Site:
TKL Research, Inc
1 Palmer Terrace
Carlstadt, NJ 07072

Dates of Study: September 10, 2012 – October 19, 2012

STUDY PERSONNEL

Principal Investigator — [redacted] MD
Dermatologist

Director, Dermatologic Safety Testing — [redacted]

Manager, Dermatologic Safety Testing — [redacted]

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SUMMARY

One investigational product, Formula No. [redacted] was evaluated as supplied to determine if the application of the investigational product, Formula No. [redacted] did not cause a delayed contact allergic response in volunteer subjects with normal skin using an 8 mm Finn Chamber occlusive human repeat insult patch test. One hundred one (101) subjects completed the study.

Under the conditions employed in this study, Formula No. [redacted] was non-sensitizing and non-irritating.

1 INTRODUCTION

The test consists in the repeated dermal application of the investigational product to human volunteer subjects under conditions which exaggerate the normal conditions of product use.

2 STUDY OBJECTIVE

The main objective of this study was to confirm that the application of a cosmetic product to volunteer subjects under maximized conditions according to the "modified Marzulli and Maibach" method did not cause a delayed contact allergic response.

Secondarily, skin compatibility of certain products may have been evaluated during the Induction Phase.

3 STUDY DESIGN

3.1 OVERALL STUDY DESIGN

This was a single center, within-subject comparison study of the investigational product. All subjects had sites designated for the investigational product on the infrascapular area of the back for the purpose of determining sensitization potential.

During the Induction Phase of the study, the study products were applied to one side of the infrascapular area of the back. Evaluation of dermal reactions at the application sites was assessed clinically using a visual scale that rated the degree of erythema, edema, and other signs of cutaneous irritation. A total of 9 applications were made during the Induction Phase.

Following Induction, subjects had a 2-week rest phase, after which they entered the Challenge Phase that consisted of one 48-hour patch application to the original site and a naive site on the opposite side of the back. Observations at the naive site during Challenge and the patterns of reactivity during the Induction Period provided a basis for an interpretation of contact allergic response.

If a cutaneous response observed in the Challenge Phase indicated possible sensitization, or at the discretion of the Dermatologist Investigator, a Rechallenge was performed. In such cases, a narrative description of reactions in the Challenge and Rechallenge Phases were reported together with the opinion of the Dermatologist Investigator as to whether such reactions were felt to be indicative of contact allergic response.

A total of 10 patch applications were made over a period of 6 weeks.

3.2 DISCUSSION OF DESIGN

This study design is based on the Modified Draize procedure (Marzulli & Maibach 1974), and is accepted standard methodology used for assessment of skin sensitization [2, 3].

Substances that come into contact with human skin need to be evaluated for their propensity to irritate and/or sensitize. Once an appropriate pre-clinical safety evaluation has been performed, a reproducible, standardized, quantitative patch evaluation procedure must be used to demonstrate that a particular investigational product can be applied safely to human skin without significant risk of adverse reactions [4].

Repeated insult patch test (RIPT) evaluation is a predictive patch study that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Irritant reactions may also be detected using this evaluation method, although this is not the primary purpose of this procedure.

3.3 STUDY PROCEDURES

3.3.1 Screening / Day 1

At Screening, the subjects were informed of the study procedures and the informed consent (IC) of each volunteer was obtained. Background information, including the date of birth, gender, and race, and a medical history for each subject was reviewed and recorded at Screening. Eligibility was determined by review of the inclusion/non-inclusion criteria. If the subject fulfilled all the inclusion and none of the non-inclusion criteria, he/she was allowed to participate in the study, and received a unique enrollment number in order to preserve the subject's confidentiality. Qualified subjects were given oral and written instructions as follows:

- When bathing, avoid getting the patches and the application areas wet by taking a low tub bath or shower the front of your body only.
- No swimming is permitted during the study.
- You must notify staff if patches come off.
- Do not engage in activities (especially sports) that cause excessive sweating.
- Throughout the entire study, and for 2 weeks after study completion, avoid exposure to the sun or tanning beds.
- Avoid excessive scrubbing around patch area, which may cause irritation and may remove patch site markings.
- Do not apply any products in or around the patch area (including sunscreens). You must notify the staff if you do.
- Inform the staff of any vaccinations and/or use of medications during the study.
- Notify the staff if anything unusual occurs at any time during the study or within 2 weeks of completing the study. Please bear in mind that if TKL discontinues your participation in this study due to an adverse event (AE) or severe reaction, you will be paid for your participation.
- Please inform us if you experience any discomfort beyond mild itching. Contact us as soon as possible at (201) 587-0505.
- During the entire study, including rest week, we ask that you do not participate in any other patch or photopatch study with any research company.
- Do not participate in a similar study within 3 months of completing this study.

3.3.2 Induction

The Induction Phase consisted of a series of 9 applications of the investigational product and subsequent evaluations of the application sites. Patches were to be applied on Mondays, Wednesdays, and Fridays for 3 consecutive weeks. The subjects returned to the facility at 48-hour intervals to have the patches removed. Using a tissue, the Dermatologist Investigator-trained evaluator removed any remaining excess investigational product to avoid transference of products between sites. The sites were evaluated 15 to 30 minutes after patch removal by a Dermatologist

Investigator-trained evaluator using the scoring system detailed in Table 3.1 in Appendix I. Scores were entered into the data sheets by the evaluator. Identical patches were then applied to the same sites. Patches applied on a Friday remained in place for 72 hours until Monday.

3.3.3 Rest Period

During the 2-week Rest Period, subjects did not receive any application of the investigational product.

3.3.4 Challenge

At Challenge, subjects who completed the Induction Phase and the Rest Period had identical patches applied to the original and to naive sites. Patches remained in place for 48 hours. The sites were graded at least 30 minutes as well as 48 hours following patch removal (ie, 48 and 96 hours after patch application) using the procedures described above for the Induction Phase.

3.3.5 Rechallenge

At the discretion of the Dermatologist Investigator and after discussion with the Sponsor, a subject may have been rechallenged to the investigational product in the event of a doubtful reaction during the Challenge Phase. Rechallenge patches would be applied as soon as challenge reactions had resolved. The investigational product would be applied to naive sites on the back for 48 hours and graded at 48, 72, and 96 hours after application and if necessary, every day until resolution.

A similar or more severe response observed at Rechallenge would have been considered indicative of a sensitization reaction. At the Dermatologist Investigator's discretion, further follow-up or retesting may have been necessary to confirm an interpretation of the finding.

3.3.6 Study Flow Chart

Week 1
1 Obtained informed consent, reviewed completed medical screening form, applied patches
3 Staff removed patches, graded, applied patches
5 Staff removed patches, graded, applied patches

Week 2
1 Staff removed patches, graded, applied patches
3 Staff removed patches, graded, applied patches
5 Staff removed patches, graded, applied patches

Week 3
1-7 Same as Week 2

Week 4
1 Staff removed patches, graded
2-7 Began Rest Period

Week 5
1-7 Rest Period

Week 6

1 Staff applied patches
3 Staff removed patches, graded
5 Staff graded

3.4 SELECTION OF SUBJECTS

A sufficient number of subjects were enrolled in order to provide 100 completed subjects evaluable for analysis; an individual subject was allowed to participate in the study one time only.

To be considered a **completed case**, a subject must have had 9 applications of the investigational product and 9 subsequent readings during Induction and one application followed by 2 subsequent readings at Challenge. Only completed cases were used to assess sensitization.

3.4.1 Inclusion Criteria

Subjects included in the study were those who:

1. Were healthy males or females, 18 to 65 years of age (no more than 10% ages 60-65), with a permanent address,

2. Were able to give written consent,

3. Were informed of the test procedures, were capable of reading the documents presented to them, and were capable of understanding them in the language used,

4. Were subjects who benefited from social security or medical insurance (according to the legislation in force in the country where the test takes place),

5. Were subjects selected according to the procedures established by the Investigating Laboratory. These criteria were evaluated using the questionnaires recorded in the Investigator's CRF.

3.4.2 Non-inclusion Criteria

Subjects excluded from the study were those who:

1. Refused to undertake to refrain from participating simultaneously in other bio-medical studies,

2. Did not comply with the non-inclusion period stipulated at the time of their participation in the previous test,

3. Had been deprived of their freedom by a legal or administrative decision, or people undergoing an emergency medical treatment[redacted]

4. Were minors or subjects protected by law, as well as those admitted into a health, social, or mental institution[redacted]

5. Refused to give their agreement by not signing the informed consent declaration,

6. Had an organ removed (kidney, lung, spleen, hepatic lobe, etc), a transplant, or suffered from a cranial trauma with after-effects,

7. Were pregnant or nursing women, or those who have not taken contraceptive precautions,

8. Presented a condition which is considered unacceptable for the study: such as skin marks at the test site that may interfere with the evaluation of the skin reactions (pigmentation problems, scarring, excessive hair growth, excessive numbers of freckles and moles, sunburn, etc), an immune deficiency, a previous history of contact allergies, immediate allergic reactions currently under treatment (asthma, periodic spasmodic rhinitis, conjunctivitis, etc), a fever lasting for more than 24 hours, in the 8 days preceding the product application,

9. Had undergone long-term treatment or who were currently undergoing long-term treatment involving insulin, antihistamines, corticoids, beta-blockers (including eye drops), antibiotics, immunosuppressive drugs (cyclosporine), and/or in a period of de-sensitization,

10. Had treatment with vitamin A or its derivatives less than 3 months before the beginning of the study,

11. Had been vaccinated in the 3 weeks prior to the study or intend to be vaccinated during the study,

12. Had been presenting cutaneous hyperactivity or skin disorder,

13. Had strong reactions to sticking plaster of patches,

14. Had been exposed to natural sunshine or UV lamp on the test area, during the month preceding the study,

15. Showed a disorder due to excessive alcohol or drug use.

3.4.3 Informed Consent

A properly executed IC document in compliance with FDA regulations (21 CFR Part 50) and the Helsinki Declaration (1964) and subsequent amendments [5] was obtained from each subject prior to entering the study. Each subject dated and signed an IC document, which was witnessed and dated and signed by the Dermatologist Investigator's designee. The signed IC document is maintained in the study file. In addition, the subject was provided with a copy of the IC document (see Appendix III).

3.4.4 Interruption or Discontinuation of Treatment

In accordance with legal requirements and ICH-GCP guidelines, every subject or his/her legal representative had the right to refuse further participation in the study at any time and without providing reasons. A subject's participation was terminated immediately upon his/her request. The Dermatologist Investigator or designee was to seek to obtain and record the reason.

The termination of an individual's participation was to be considered in the case of a serious adverse event (SAE). If the subject, during the course of the study, developed a condition(s) which would have prevented his/her entry into the study according to the safety-related medical non-inclusion criteria, he/she was to be withdrawn immediately.

The subject may have been withdrawn from the study at any time at the discretion of the Dermatologist Investigator for medical reasons and/or due to non-adherence to the treatment scheme and other duties stipulated in the study protocol. The reasons were to be fully documented on the CRF.

An erythema score of 2 or more to a study product (see Table 3.1 in Appendix I for interpretation of scores) observed at the first or second reading of the Induction Phase would have indicated the subject was most likely presensitized and the Sponsor was to be immediately notified. Application of the product in question would have been discontinued at the original site and the treatment moved to an adjacent site. The grading will continue on the first site until the effects are reversed and on the 2nd site until the end of the Induction Phase. The site may only be changed once. In the case of a suspected allergic reaction, the product would not be applied again and the decision to reapply would be discussed with the Sponsor.

Withdrawals

The following medical and other reasons justified a premature termination (by subject or Dermatologist Investigator) of any of the study products:

- Withdrawal of informed consent,
- Serious adverse event,
- Allergic reactions to the investigational products,
- Subject's request,
- Occurrence of one of the safety criteria for non-inclusion after treatment had been instituted,
- The patches became dislodged or were misplaced such that continuous contact with the skin had been interrupted,
- Subject was lost to follow-up, and/or
- Dermatologist Investigator's judgment.

If a subject withdrew from the study, all efforts were made to complete a final evaluation, if possible. Subjects discontinued for having experienced an adverse event (AE) were followed until the AE was resolved, a reasonable explanation was provided for the event, or the subject was referred to his/her own primary medical doctor (PMD). The specific AE in question was recorded on the appropriate CRF.

3.5 INVESTIGATIONAL PRODUCT (IP)

3.5.1 Investigational Product Specifications

IP Category	:	EYE POWDER
Formula No.	:	[redacted]
Batch No.	:	[redacted]
Description	:	BEIGE POWDER
Amount Applied	:	Sufficient to cover patch
Patch Type	:	Occlusive
Evaporation	:	No
Dilution	:	No
Storage Conditions	:	Room Temperature
Special Instructions	:	Switched to semi-occlusive patch if reactions $\geq$ 2 occurred.

3.5.2 Description of Patch Conditions

Products evaluated under occlusive patch conditions are applied under a Finn Chamber. This chamber, formed of an 8 mm aluminum cup affixed to Scanpor tape, provides an isolation chamber in which the investigational product is placed. An amount of investigational product sufficient to fill the chamber (20 µL or mg) is placed within the Finn Chamber such that it does not extend onto the adhesive tape surfaces. Liquid investigational product is soaked into a small filter disk placed within the Finn Chamber. For gels and ointments, an amount sufficient to fill the chamber is applied. The chamber is maintained in place by a hypoallergenic adhesive strip (Micropore) and serves to limit the investigational product to the designated skin contact site. Liquids are applied to the patch using an Eppendorf single channel adjustable pipette set at the appropriate amount to be applied to the patch, usually 20 µL. Creams, semi-solids, and solids are weighed by applying product to a patch that has been pre-weighed on a pre-calibrated weight balance. The product and patch are then weighed on the pre-calibrated weight balance to determine the appropriate amount of product, usually 20 mg. The weighed patch is used as a visual guide to prepare patches. Distilled water (20µL) was added to the Finn Chamber, and a sufficient amount of product to cover the patch was applied. A spatula was used to press the product into the chamber.

3.5.3 Storage, Handling, and Documentation of the Investigational Product

Receipt of the investigational product used in this study was documented in a general log book which serves as a permanent record of the receipt, storage, and disposition of all investigational products received by TKL Research, Inc. On the basis of information provided by the Sponsor, the investigational product was considered reasonably safe for evaluation on human subjects. The investigational product was kept locked in product storage rooms at the TKL clinical site at 4 Forest Avenue, Paramus, NJ 07652 prior to the start of the study and at the completion of the study. During the study the product was kept at the TKL clinical test site at 1 Palmer Terrace, Carlstadt, NJ 07072. The product was only accessible to TKL clinical staff members. The investigational product was destroyed upon acceptance of the final report and a sample was retained for a period of 6 months.

3.5.4 Treatment Compliance

All patches were applied and removed by clinical study staff. Whereas bathing was allowed (low tub bath/frontal showers), the patched area was not to be soaked and was to be kept as dry as possible,

per the instructions given to each subject (see Section 3.3.1). A Dermatologist Investigator-trained, experienced evaluator assessed study compliance. Records of patch applications and visit schedule compliance were recorded on the subjects' CRFs.

3.6 SAFETY EVALUATIONS

3.6.1 Local Tolerability Assessments

Assessment of the patch sites was performed 9 times during the Induction Phase, 2 times following Challenge and, if applicable, 3 times following Rechallenge. The examination of the treated sites was carried out under an artificial 60 watt blue light. The scores outlined in Table 3.1, Appendix I were used to express the response observed at the time of examination. Allergy was evaluated according to the International Contact Dermatitis Research Group [6].

3.6.2 Adverse Events

An AE is defined as an occurrence of a new symptom(s) of a medical nature during use of the investigational product whether or not considered related to the investigational product, eg, headache, influenza, broken bones, fever, nausea. A SAE is defined as death, a life threatening adverse experience, inpatient hospitalization, a persistent or significant disability/incapability, or a congenital anomaly/birth defect. Serious adverse events were to be reported to the Sponsor within 24 hours of the investigative personnel's knowledge of the event. All AEs, whether observed by the clinical staff or by the subject, and whether or not thought to be study-related, were to be recorded on an Adverse Event Form. Assessment of severity and causality will be based on definitions found on the Adverse Event Form. Pregnancy, although not itself an AE, was also to be reported on an Adverse Event Form.

Expected Adverse Events

Any observed response that was denoted using the irritation criteria summarized in Table 3.1 was not considered an AE. Likewise, any tape-related irritation was not noted as an AE.

3.7 QUALITY CONTROL

The Quality Control (QC) Unit of the Dermatological Safety Department conducted a 100% review of all study-related documents. The protocol was reviewed prior to the start of the study, the medical screening forms and IC documents were reviewed in-process of the study, and the regulatory binder was reviewed post-study.

4 DATA MANAGEMENT

4.1 DOCUMENTATION

Case report forms were designed by TKL Research, Inc to identify each subject by subject entry number and, where appropriate, the subject's initials. Originals or copies of all CRFs, source documents, correspondence, and study reports, etc will be kept on hard-copy file by TKL Research, Inc for a minimum of 10 years from completion of the study. The hard-copy file will be maintained at the study site, TKL Research, Inc at 4 Forest Avenue, Paramus, NJ 07652, in a secured room accessible only to TKL employees for a period of one year, after which it will be sent to the off-site

archive location, Allstate Business Archives, 80 Beckwith Avenue, Paterson, NJ 07503, that provides a secure environment with burglar/fire alarm systems, camera detection, and controlled temperature and humidity, for a period of 9 years. Archive destruction will be done only by a formal signed written agreement from the Sponsor. The Dermatologist Investigator/institution permit study-related monitoring, audits, IRB/IEC review, and regulatory inspection, and provide direct access to source data/documents on the premises of TKL Research, or at a secure location off-site.

4.2 Database Management and Quality Control

Data were double-keyed and validated using ClinPlus (DZS Software Solutions), which directly generated SAS® data sets. After resolution of double-key discrepancies and a combination of manual and automated data review procedures, the final data sets were subject to a QC audit. SAS® programs for data analysis and presentation were applied to secure validated data sets.

5 INTERPRETATION OF THE RESULTS

5.1 Sample Size

With a sample size of 100, in the absence of any sensitization reactions, a 95% upper confidence bound on the population rate of sensitization would be 3.5% [7].

5.2 Populations

All subjects who were treated were evaluable for AEs. The evaluation of sensitization was based on all subjects who completed the Challenge Phase of the study.

5.3 Criteria of Evaluation of Skin Compatibility

Skin compatibility was evaluated from the skin reactions observed (number, intensity, frequency) and compared with that established for the chosen investigational product as a reference with the untreated control site. The analysis of skin compatibility includes all subjects in the test, however many times they were evaluated during the Induction Phase.

5.4 Dermal Sensitization Potential

The determination of dermal sensitization potential was based on specific scoring criteria derived from observations in the Challenge Phase of the study, and confirmed in the Rechallenge Phase, if necessary.

The recurrence of a cutaneous response at Rechallenge equivalent to or more severe than that observed at Challenge was considered indicative of a sensitization reaction. The observation of such a response in even a single subject suggested that the study product may have the potential to cause hypersensitivity.

For all subjects who entered Rechallenge, a narrative description of reactions in the Challenge and Rechallenge Phases was to be provided together with the opinion of the Dermatologist Investigator as to whether such reactions were felt to be indicative of contact allergic response.

6 RESULTS

Summary data tables are provided in Appendix I of this report. Supportive listings are provided in Appendix II.

6.1 SUBJECTS EVALUATED

6.1.1 Subject Disposition

Subject disposition is shown in Table 1 and summarized in Text Table 6-1; these data are supported by Data Listing 1.

Text Table 6-1 Subject Disposition

Number of subjects enrolled	120
Number of subjects treated	120
Number of subjects discontinued	19
Lost to follow-up	16
Voluntary withdrawal	3
Number of subjects completed	101

Source: Table 1, Appendix I

6.1.2 Protocol Deviations

Eleven point seven (11.7) percent of the study population was between the ages of 60 and 65. This is a deviation from the protocol-specified no more than 10% of the subjects between the ages of 60 and 65.

TKL Research, Inc used an artificial 60 watt blue light during subject evaluations. This light is not consistent with the D65 North daylight Illuminator specified in the protocol. This deviation did not affect the validity of the study.

The in-phase inspection was conducted bi-yearly in accordance with TKL's SOPs. An audit was not conducted for this study as specified in the protocol. This deviation did not affect the validity of the study.

[redacted] This is in agreement with the Sponsor and the deviation does not affect the validity of the study.

6.1.3 Protocol Amendments

No amendments to the protocol were issued for this study.

6.1.4 Baseline Demographic and Background Characteristics

All subjects met the inclusion criteria. Demographic information is summarized in Table 2, Appendix I; these data are supported by Data Listing 2, Appendix II. The study population contained 89 (74.2%) females and 31 (25.8%) males, of whom 69 (57.5%) were Caucasian, 50 (41.7%) were Hispanic, and 1 (0.8%) was American Indian. Subject ages ranged from 18 to 65 years; the mean was 44 years.

6.2 SAFETY RESULTS

6.2.1 Induction and Challenge Responses

One hundred two (102) subjects completed the Induction Phase and were included in determining the presence of significant irritation. One hundred one (101) subjects completed the Challenge Phase of the study and were included in the sensitization analysis. There was no requirement for a Rechallenge Phase to be conducted. A summary of the repeated insult patch test responses during the Induction and Challenge Phases of the study is provided in Table 3 of Appendix I, a by-subject listing of the sensitization response data is provided in Data Listing 3, Appendix II.

6.2.2 Overall Experience of Adverse Events

There were no AEs reported.

7 CONCLUSIONS

Under the conditions employed in this study, Formula No. [redacted] was non-sensitizing and non-irritating.

8 REFERENCES

1. ICH Topic E6 "Note for guidance on Good Clinical Practices (CPMP/ICH/135/95)" – ICH Harmonised tripartite Guideline for Good Clinical Practices having reached Step 5 of the ICH Process at the ICH Steering Committee meeting on 1 May 1996.
2. Jordan, WP. 24-, 48-, and 48/48-hour Patch Tests. *Contact Dermatitis* 1980. 6: 151-152.
3. Marzulli F. N.; Maibach H. I. Contact Allergy: Predictive Test in Man. *Contact Dermatitis* 1976. 2:1-17.
4. Lanman, BM, EB Elvers, and CJ Howard. "The Role of Human Patch Testing in a Product Development Program." Joint Conference on Cosmetic Goods Association, Washington DC, April 21-23, 1968.
5. Declaration of Helsinki adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, and amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975; 35th World Medical Assembly, Venice, Italy, October 1983; 41st World Medical Assembly, Hong Kong, September 1989; 48th World Medical Assembly, Somerset West, Republic of South Africa, October 1996; 52nd World Medical Assembly, Edinburgh, Scotland, October 2000; 53rd World Medical Assembly, Washington, USA, 2002; 55th World Medical Assembly, Tokyo, Japan, 2004; 59th General Assembly, Seoul, Korea, October 2008.
6. CDRG = The International Contact Dermatitis Research Group, Fregert S. Manual of Contact Dermatitis, 2nd Edition.
7. Mood AM, Graybill FA, Boes DC. Introduction to the Theory of Statistics. 3rd ed. New York: McGraw-Hill Higher Ed; 1974:Chapter 7.

[redacted]

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 15, 2018

SUBJECT: Isopropyl Titanium Triisostearate

Kenrich Petrochemicals, Inc. 2018. Summary information Isopropyl Titanium Triisostearate.

Richard Costlow Consulting, LLC. 2018. Ken-React® KR® TTS (Isopropyl Titanium Triisostearate): Summary of toxicology data for regulatory compliance.

SGS. 2016. Test report (metal analysis of TIPT (titanium compound used in the manufacture of Isopropyl Titanium Triisostearate)).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

May 15, 2018

Summary Information Isopropyl Titanium Triisostearate

Kenrich Petrochemicals, Inc. reports that their Isopropyl Titanium Triisostearate trademarked Ken-React® KR® TTS is sold to the cosmetics industry as >98% Isopropyl Titanium Triisostearate and <2% Isopropyl Alcohol. The product is made by reacting tetra-isopropyl titanate with three equivalents of iso-stearic acid. The product is predominantly the titanate compound with one iso-propyl and three iso-stearic acid ligands, as noted in the report for the CIR. However it will also contain the tetra-isostearate titanate and the di-isopropyl and di-isostearate titanate as well. This has been confirmed by NMR and FTIR analysis. The metal content of the starting titanium compound has been determined (attached).

The Isopropyl Titanium Triisostearate described above has been tested for acute oral toxicity, dermal and ocular irritation and genotoxicity. Summaries of these studies are provided.

Physical and Chemical Properties of Isopropyl Titanium Triisostearate

Property	Values/Data
Physical state	Liquid
Color	Reddish
Odor	Waxy fatty acid
pH	5-6
Boiling point	149 °C (300 °F)
Flash point	>93 °C (>200 °F)
Evaporation rate (relative to n-butyl acetate)	Slower
Relative density @ 60 °F	0.95
Solubility (H_2O)	Insoluble
Solubility to other solvents	<5% xylene
Viscosity @ 77 °F	125 cps

RICHARD COSTLOW CONSULTING, LLC

Regulatory Toxicology and Risk Assessment for the Chemical Industry
Suite 2 East, 2080 Parkview Drive
Lansdale, PA 19446-5023

Title:

Ken-React® KR® TTS: Summary of Toxicology Data for Regulatory Compliance

Isopropyl Titanium Triisostearate

Page 1 of 5 Pages

Toxicology Summary for Ken-React KR TTS

Acute toxicity: oral

Summary of relevant acute toxicity studies

Method	Results	Remarks	Reference
Rat (SD) male/female Oral: administered by gavage in two equally divided doses two hours apart Method: similar to US EPA Guideline (Acute Oral Toxicity)	LD50: >30,000 mg/kg bw (male/female) LD50: >30,000 mg/kg bw (female) LD50: >30,000 mg/kg bw (male) No signs of gross toxicity or remarkable pathology	Klimisch 2 (reliable with restrictions) Key study Test material: **Ken-React KRTTS** K-16558, Lot 2597 (purity not specified)	Shapiro (1976) Report T-126

Human information

No data are available.

Summary and discussion of acute toxicity

Data are conclusive and support no classification for this endpoint under REACH.

Specific target organ toxicity – single exposure (STOT SE)

The acute oral studies did not identify a target organ in animals treated with KR TTS.

Irritation: Dermal

Summary of relevant toxicity studies

Method	Results	Remarks	Reference
Rabbit (NZW) Dermal: administered under a semi-occluded wrap for 4 hours. Scores for erythema and edema were recorded at 4, 24, 48, and 72 hours after removal of the covering. Method: similar to US EPA Guideline (Acute Dermal Irritation)	Primary Dermal Irritation Scores Erythema: 0.3 Edema: 0.0 Overall 0.3 Classification: Not classified. No signs of gross toxicity or remarkable pathology	Klimisch 1 (reliable with no restrictions) Key study Test material: **Ken-React KR TTS** Lot R-1082S (purity not specified)	Prince, HN (1986) Report GBL 27535, Gibraltar Biological Laboratories Inc.

Human information

No data are available.

Summary and discussion of skin irritation

Data are conclusive and support no classification for this endpoint under REACH.

Human information

No data are available.

Corrosivity

The substance was not corrosive in the dermal irritation test. No data specific to corrosivity

Irritation: Ocular

Summary of relevant toxicity studies

Method	Results	Remarks	Reference
Rabbit (NZW) Ocular: 0.1 ml administered into the conjunctival sac. Scores for corneal opacity, iritis and conjunctivitis were recorded at 1, 24, 48, and 72 hours after instillation. Method: US EPA Guideline (Acute Ocular Irritation)	Primary Ocular Irritation Scores At 1 hour: 10 At 24 hours: 0.7 At 48 hours: 0 At 72 hours: 0 Classification: Not classified. No signs of gross toxicity or remarkable pathology	Klimisch 1 (reliable with no restrictions) Key study Test material: **Ken-React KR TTS** Lot #22059 (purity not specified)	Wnorowski, G (2003) Report13319, Product Safety Labs

Human information

No data are available.

Summary and discussion of ocular irritation

Data are conclusive and support no classification for this endpoint under REACH.

Corrosivity

The substance was not corrosive in the ocular irritation test. No data specific to corrosivity.

Mutagenicity: Bacterial

Summary of relevant toxicity studies

Method	Results	Remarks	Reference
Salmonella typhimuirium In vitro: administered to 5 strains of bacteria [TA98, TA100, TA1535, TA1537 and TA1538] at doses of 0.2 to 500 micrograms per plate in the presence and absence of metabolic activation. Scores for revertants per plate were recorded. Method: similar to US EPA Guideline (Ames test)	Revertants per plate For TA98, TA100, TA1535, TA1537 and TA1538 were all recorded as (-); no increase in revertants with or without metabolic activation Classification: Not classified.	Klimisch 2 (reliable with restriction) Test material: **Ken-React KR TTS** Lot 279 (purity not specified)	Combines legible data pages from 2 files of Anonymous (1980) Report 367353, Stillwell and Gladding, Inc., bacterial mutagenicity [5 strains; +/- activation)

Summary and discussion of bacterial mutagenicity

Data are conclusive and support no classification for this endpoint under REACH.

SGS

Test Report

No. : CM/2010/B0073 Date : 2010/11/16 Page : 1 of 4

BORICA CO., LTD.
6F, NO. 158, XIN SHENG S. RD., SEC. 1, 10061 TAIPEI, TAIWAN

The following sample(s) was/were submitted and identified by/on behalf of the client as:

Sample Description	:	TIPT
Other Info.	:	BATCH NO.:309980y
Sample Receiving Date	:	2010/11/05
Testing Period	:	2010/11/05 TO 2010/11/16

==========

Test Result(s) : Please refer to next page(s).

SGS TAIWAN LTD SGS TAIWAN

James Lu

James Lu / Supervisor
Signed for and on behalf of
SGS TAIWAN LTD.
Chemical Laboratory – Taipei

SGS

Test Report

BORICA CO., LTD.
6F, NO. 158, XIN SHENG S. RD., SEC. 1, 10061 TAIPEI, TAIWAN

Test Result(s)

PART NAME No.1 : TRANSPARENT LIQUID

Test Item (s):	Unit	Method	MDL	Result
				No.1
Silver (Ag)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Aluminum (Al)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Arsenic (As)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Gold (Au)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Boron (B)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Barium (Ba)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Beryllium (Be)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Bismuth (Bi)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Calcium (Ca)	mg/kg	ICP-AES after as per acid digestion.	2	3
Cadmium (Cd)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Cobalt (Co)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Chromium (Cr)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Copper (Cu)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Dysprosium (Dy)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Iron (Fe)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Gallium (Ga)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Germanium (Ge)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Mercury (Hg)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Indium (In)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Potassium (K)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Lithium (Li)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Magnesium (Mg)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Manganese (Mn)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Molybdenum (Mo)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Sodium (Na)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Niobium (Nb)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Neodymium (Nd)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Nickel (Ni)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Phosphorus (P)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Lead (Pb)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Palladium (Pd)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Platinum (Pt)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Rhodium (Rh)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.

SGS

Test Report

BORICA CO., LTD.
6F, NO. 158, XIN SHENG S. RD., SEC. 1, 10061 TAIPEI, TAIWAN

Test Item (s):	Unit	Method	MDL	Result No.1
Ruthenium (Ru)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Antimony (Sb)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Scandium (Sc)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Selenium (Se)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Silicon (Si)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Tin (Sn)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Strontium (Sr)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Tantalum (Ta)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Tellurium (Te)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Titanium (Ti)	%	ICP-AES after as per acid digestion.	0.0002	16.99
Thallium (Tl)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Vanadium (V)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Tungsten (W)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Yttrium (Y)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Zinc (Zn)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Zirconium (Zr)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Lanthanum (La)	mg/kg	ICP-AES after as per acid digestion.	2	n.d.
Polychlorinated Biphenyls (PCBs) (CAS No.: 001336-36-3)	mg/kg	With reference to US EPA 3540C method. Analysis was performed by GC/MS.	0.5	n.d.
Halogen				
Halogen-Fluorine (F) (CAS No.: 014762-94-8)	mg/kg	With reference to BS EN 14582:2007. Analysis was performed by IC.	50	n.d.
Halogen-Chlorine (Cl) (CAS No.: 022537-15-1)			50	n.d.
Halogen-Bromine (Br) (CAS No.: 010097-32-2)			50	n.d.
Halogen-Iodine (I) (CAS No.: 014362-44-8)			50	n.d.

Note :

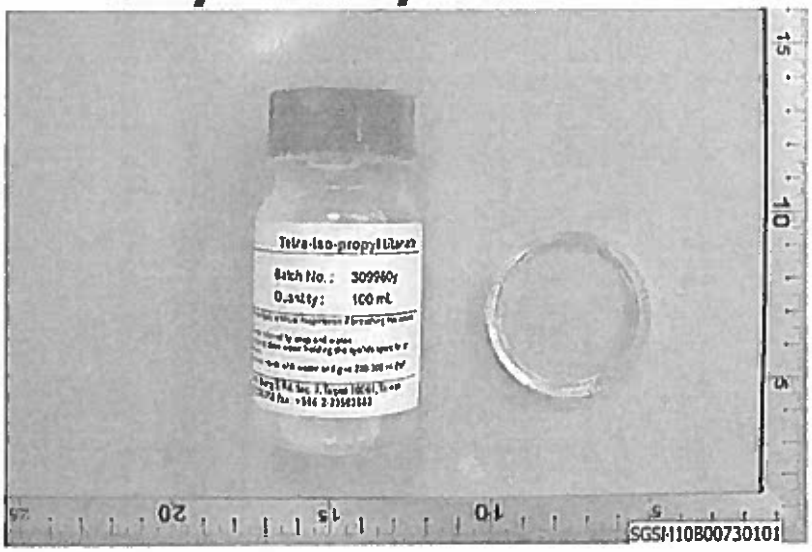
1. mg/kg = ppm ; 0.1wt% = 1000ppm
2. n.d. = Not Detected
3. MDL = Method Detection Limit

SGS

Test Report

BORICA CO., LTD.
6F, NO. 158, XIN SHENG S. RD., SEC. 1, 10061 TAIPEI, TAIWAN

CM/2010/B0073



** End of Report **

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: May 23, 2018
Subject: Wave 2 Data on Polyfluorinated Polymers

A human repeated insult patch test (HRIPT) on a cosmetic product containing 9% PTFE (in *fluoro062018data3*) and the method of manufacture of PTFE (in *fluoro062018data4*) were received from the Council, and are being submitted as attachments to this memorandum. A data summary document (*fluoro062018wave2studysummaries*) is also attached for the Panel's review. These data will be added to the safety assessment after the Panel meeting.

Please note that the method of manufacture provided begins with high molecular weight PTFE that, perhaps, was provided by another company. With this in mind, the Panel may want to determine whether or not the method of manufacture of the starting material (high molecular weight PTFE) is needed.

1620 L Street, N.W., Suite1200, Washington, DC 20036
(Main) 202-331-0651 (Fax) 202-331-0088
(Email) cirinfo@cir-safety.org (Website) www.cir-safety.org

Wave 2 Data on Polyfluorinated Polymers

CHEMISTRY

Method of Manufacture

PTFE

The method of manufacture of PTFE (from a supplier) is summarized below.[1] Virgin grade high molecular weight PTFE is the starting material. The molecular weight of the starting material needs to be reduced in order for PTFE to be micronized into a fine powder. Electron beam irradiation is used to lower the molecular weight, typically into the 15,000 to 50,000 Daltons (Da) range. This process is controlled via melting point reduction monitoring (reduced from ~ 341ºC to ~ 330ºC). The irradiated PTFE is then post-baked to remove volatiles (including any trace perfluorooctanoic acid (PFOA) to below 25 ppb and tetrafluoroethylene (monomer in PTFE) is not detected (detection limit: 75 ppb)). The resultant material is jet mill micronized to the particle size specification.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Sensitization

Human

PTFE

An HRIPT on a cosmetic product containing 9% PTFE (undiluted) was performed using 206 subjects.[2] During induction, the subjects received 9 consecutive 24-h patch (2 cm x 2 cm semi-occlusive patch) applications of the product (volume and dose per cm^2 not stated) on the infrascapular area of the back. Induction sties were evaluated at 48-h intervals. Patches removed on Friday were removed on the following Monday, i.e., 72 h after patch application. The challenge phase was initiated (during week 6) after a 10- to 15-day non-treatment period. Challenge patches were applied for 24 h to new test sites, and reactions were scored at 48 h and 72 h post-application. There was no evidence of skin sensitization in any of the subjects tested.

References

1. Anonymous. PTFE method of manufacture. Unpublished data submitted by the Personal Care Products Council on 5-17-2018. 2018. pp.1

2. TKL Research, Inc. Repeated insult patch study of a cosmetic product containing 9% PTFE. Unpublished data submitted by the Personal Care Products Council on 5-8-2018. 2001. pp.1-15.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 8, 2018

SUBJECT: PTFE

TKL Research, Inc. 2001. Repeated insult patch study of a cosmetic product containing 9% PTFE.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

REPEATED INSULT PATCH STUDY

TKL STUDY [redacted]

Product containing 9% PTFE

[redacted]

[redacted]

DATE OF REPORT:

December 4, 2001

TKL Research, Inc., 4 Forest Avenue, Paramus, NJ 07652 Tel (201) 587-0500 Fax (201) 587-0878 Email: info@tklresearch.com

[redacted]

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APPENDICES

I SUMMARY TABLES
II DATA LISTINGS
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IV INFORMED CONSENT DOCUMENT

TITLE OF STUDY

Repeated Insult Patch Study

SPONSOR

[redacted]

STUDY MATERIAL

[redacted]

DATE STUDY INITIATED

August 27, 2001

DATE STUDY COMPLETED

October 18, 2001

DATE OF REPORT

December 4, 2001

INVESTIGATIVE PERSONNEL

[redacted]

[redacted]

CLINICAL SITES

TKL RESEARCH, INC.
4 Forest Avenue
Paramus, NJ 07652

TKL RESEARCH, INC.
1099 Wall Street West
Lyndhurst, NJ 07071

STATEMENT OF QUALITY ASSURANCE

This report has been reviewed by the TKL Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Clinical research studies are performed by TKL Research, Inc. in accordance with federal regulations and proposed guidelines for good clinical practices which include:

21 CFR Part 312,	Investigational New Drug Application
21 CFR Part 50,	Protection of Human Subjects
21 CFR Part 56,	Institutional Review Boards

Quality Assurance

5 December 2001
Date

SUMMARY

One product, Sample No. [redacted] was evaluated neat using a semi-occlusive repeated insult patch study to determine its ability to sensitize the skin of normal volunteer subjects. Two hundred six subjects completed the study.

Under the conditions employed in this study, there was no evidence of sensitization to Sample No. [redacted].

1.0 OBJECTIVE

The objective of this study was to determine the ability of the study material to cause sensitization by repeated topical applications to the skin of humans under controlled patch study conditions.

2.0 RATIONALE

Substances that come into contact with human skin need to be evaluated for their propensity to irritate and/or sensitize. Once an appropriate pre-clinical safety evaluation has been performed, a reproducible, standardized, quantitative patch evaluation procedure must be used to demonstrate that a particular material can be applied safely to human skin without significant risk of adverse reactions. The method herein employed is generally accepted for such a purpose.

Repeated insult patch evaluation is a modified predictive patch study that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Irritant reactions may also be detected using this evaluation method, although this is not the primary purpose of this procedure. Results are interpreted according to interpretive criteria based upon published works, as well as the clinical experience of TKL Research, Inc. These interpretive criteria are periodically reviewed and amended as new information becomes available.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of volunteer subjects were screened and enrolled to provide 200 completed subjects.

3.1.1 Inclusion Criteria

Individuals were eligible for inclusion in the study if they:

1. were males or females, 18 years of age or older, in general good health;

2. were free of any systemic or dermatologic disorder which, in the opinion of the investigative personnel, would have interfered with the study results or increased the risk of adverse events;

3. were of any skin type or race providing the skin pigmentation would allow discernment of erythema;

4. had completed a patch study Medical Screening form as well as a Medical/Personal History form; and

5. had read, understood and signed an informed consent agreement.

3.1.2 Exclusion Criteria

Individuals were excluded from participation in the study if they:

1. had any visible skin disease at the study site which, in the opinion of the investigative personnel, would have interfered with the evaluation;

2. were receiving systemic or topical drugs or medication which, in the opinion of the investigative personnel, would have interfered with the study results;

3. had psoriasis and/or active atopic dermatitis/eczema;

4. were females who were pregnant, planning to become pregnant during the study, or breast-feeding; and/or

5. had a known sensitivity to cosmetics, skin care products, or topical drugs as related to the material being evaluated.

3.1.3 Informed Consent

A properly executed informed consent document in compliance with FDA regulations (21 CFR 50) was obtained from each subject prior to entering the study. The signed informed consent document is maintained in the study file. In addition, the subject was provided with a copy of the informed consent document. A sample is included as Appendix IV.

3.2 Description of Study

3.2.1 Outline of Study Procedures

Each study enrollment group participated over a 6-week period involving 3 phases: (1) Induction, (2) Rest, and (3) Challenge. Prior to study entry, the subjects were screened to assure that they met the inclusion/exclusion criteria. Informed consent was obtained. Each subject was provided with a schedule of the study activities. All subjects were told to avoid wetting the patches and were asked not to engage in activities that caused excessive perspiration. They were instructed to notify the staff if they experienced any discomfort beyond mild itching or observed any adverse changes at the evaluation sites while on the study or within 2 weeks of completing the study.

The Induction Phase consisted of 9 consecutive applications of the study material and subsequent evaluations of the patch sites. Prior to application of the patches, the sites were outlined with a skin marker, e.g., gentian violet. The subjects were required to remove the patches approximately 24 hours after application. They returned to the

facility at 48-hour intervals to have the sites evaluated and identical patches applied to the same sites. Patches applied on Friday were removed by subjects after 24 hours and sites were evaluated on the following Monday, i.e., 72 hours after patch application.*

Following the ninth evaluation, the subjects were dismissed for a rest period of approximately 10-15 days.

Subjects who were absent once during the 3-week, 9-patch induction phase received a make-up (MU) patch at the last induction visit. The MU applications were graded 48 hours later at the MU visit or were recorded as N9G (no ninth grading).

The Challenge Phase was initiated during the sixth week of the study. Identical patches were applied to sites previously unexposed to the study material. The patches were removed by subjects after 24 hours and the sites graded after additional 24-hour and 48-hour periods (i.e., 48 and 72 hours after application). Rechallenge was conducted as required.

To be considered a completed case, a subject must have had 9 applications and no fewer than 8 subsequent readings during induction and 1 application and 2 readings during challenge. Only completed cases were used to assess sensitization.

3.2.2 Definitions Used for Grading Responses

The symbols found in the data listings accompanying this report were used to express the response observed at the time of examination:

-	=	No reaction
?	=	Minimal or doubtful response, slightly different from surrounding normal skin
+	=	Definite erythema No edema
++	=	Definite erythema Definite edema
+++	=	Definite erythema Definite edema and vesiculation

SPECIAL NOTATIONS

E	=	Marked/severe erythema
S	=	Spreading of reaction beyond patch site (i.e., reaction where study material was not in contact with the skin).

*A Monday or Friday holiday may result in evaluation at 96 hours after patch application.

p	=	Papular response > 50%
pv	=	Papulovesicular response > 50%
D	=	Damage to epidermis: oozing, crusting and/or superficial erosions
I	=	Itching
X	=	Subject absent
PD	=	Patch dislodged
NA	=	Not applied
NP	=	Not patched (due to reaction achieved)
N9G	=	No ninth grading

3.2.3 Evaluation of Responses

All responses were graded by a trained dermatologic evaluator meeting TKL's strict certification requirements to standardize the assignment of response grades.

4.0 STUDY MATERIAL

4.1 Storage, Handling, and Documentation of Study Material

Receipt of the material used in this study was documented in a general logbook, which serves as a permanent record of the receipt, storage, and disposition of all study material received by TKL Research, Inc. On the basis of information provided by the Sponsor, the study material was considered reasonably safe for evaluation on human subjects. A sample of the study material was reserved and will be stored for a period of 6 months. At the conclusion of the clinical study, the remaining study material was discarded and the disposition documented in the logbook. All information regarding the receipt, storage and disposition of the study material was also recorded on a Clinical Material Record form (see Appendix III) which is incorporated in this study report. All study material is kept in a locked product storage room accessible to clinical staff members only.

4.2 Nature of Study Material

Identification	:	[redacted]
Description	:	clear colorless liquid
Quantity Provided	:	1 x 16 oz
Amount Applied	:	0.2 mL
Special Instructions	:	Volatilized for at least 30 minutes prior to patch application.

4.3 APPLICATION OF STUDY MATERIAL

Study material was applied to patch as instructed and patch was applied to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm.

4.4 DESCRIPTION OF PATCH CONDITIONS

Material evaluated under semi-occlusive patch conditions will be applied to a 2-cm x 2-cm Webril pad, which will be affixed to the skin with hypoallergenic tape (Micropore).

5.0 INTERPRETATION

Sensitization is characterized by an acute allergic contact dermatitis. Typical sensitization reactions begin with an immunologic response in the dermis resulting in erythema, edema formation, and secondary epidermal damage (vesiculation), sometimes extending beyond the patch site and often accompanied by itching. Sensitization reactions tend to be delayed. The reaction typically becomes evident between 24 and 48 hours, peaks at 48-72 hours and subsequently subsides. The reaction is often greater at 72 hours than at 48 hours. The severity of the reaction is generally greater during the challenge phase of an RIPT than that seen during induction.

Irritant reactions are characterized as a non-immunologic, localized, superficial, exudative, inflammatory response of the skin due to an externally applied material. The typical initial reaction does not develop much edema or vesiculation but results in scaling, drying, cracking, oozing, crusting and erosions. The reaction is usually sharply delineated, not spreading beyond the patch site. Irritant reactions are typically evident by 24 hours and diminish over the next 48-72 hours. Removal of the offending agent results in gradual improvement of the epidermal damage. The reaction seen at 72 hours is, therefore, less severe than that seen at 48 hours. Finally, the severity of the reaction experienced in the challenge phase is generally similar to that seen during induction.
If the results of the study indicate the likelihood of sensitization, the recommended practice is to rechallenge the subjects who have demonstrated sensitization-like reactions to confirm that these reactions are, indeed, associated with the product. Our preferred rechallenge procedure involves the application of the product to naïve sites, under both occlusive and semi-occlusive patch conditions. Use of the semi-occlusive patch condition helps to differentiate irritant and sensitization reactions.

Generally speaking, if a product is a sensitizer it will produce a similar reaction under both occlusion and semi-occlusion. Whereas, if the product has caused an irritant reaction, the reactions will be less pronounced under the semi-occlusive condition.

6.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms were designed to identify each subject by subject number and subject's initials, the study material evaluated and the reactions observed. Originals or copies of all case report forms, source documents, IRB documents (if required), correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 5 years from completion of the study. Storage is maintained either at a TKL Research, Inc. facility in a secured room accessible only to TKL employees, or at an offsite location which provides a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the Sponsor's review on the premises of TKL Research, Inc.

7.0 RESULTS & DISCUSSION

Two hundred twenty-three subjects between the ages of 20 and 72 were enrolled and 206 subjects, completed the study. Three subjects discontinued due to protocol violations; 1 was taking an exclusion medication, 1 scratched in the patch area, and 1 was over age 70. See Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II.

The following table summarizes subject enrollment and disposition.

Number enrolled:		223
Number discontinued:		17
Lost to follow-up:	8	
Voluntarily withdrew:	6	
Protocol violation:	3	
Number completed:		206

Source: Table 1, Appendix I

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

8.0 CONCLUSION

Under the conditions employed in this study, there was no evidence of sensitization to Sample No. [redacted].

6.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms were designed to identify each subject by subject number and subject's initials, the study material evaluated and the reactions observed. Originals or copies of all case report forms, source documents, IRB documents (if required), correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 5 years from completion of the study. Storage is maintained either at a TKL Research, Inc. facility in a secured room accessible only to TKL employees, or at an offsite location which provides a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the Sponsor's review on the premises of TKL Research, Inc.

7.0 RESULTS & DISCUSSION

Two hundred twenty-three subjects between the ages of 20 and 72 were enrolled and 206 subjects, none over the age of 70, completed the study. See Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II.

The following table summarizes subject enrollment and disposition.

Number enrolled:		223
Number discontinued:		17
Lost to follow-up:	8	
Voluntarily withdrew:	6	
Protocol violation:	3	
Number completed:		206

Source: Table 1, Appendix I

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

8.0 CONCLUSION

Under the conditions employed in this study, there was no evidence of sensitization to Sample No. [redacted]

9.0 REFERENCES

Kligman AM. The identification of contact allergens by human assay II. A critique of standard methods. J Invest Dermatol 1966; 47:369.

Kligman AM. The identification of contact allergens by human assay II. Factors influencing the induction and measurement of allergic contact dermatitis. J Invest Dermatol 1966; 47:375.

Hardy J. Allergy hypersensitivity in cosmetics. J Soc Cosmet Chem 1973; 24:423.

Marzulli FN, Maibach HI. Contact allergy: predictive testing in man. Contact Dermatitis 1976; 2:1.

Marzulli FN, Maibach HI. Effects of vehicles and elicitation concentration in contact dermatitis testing I: experimental contact sensitization in humans. Contact Dermatitis 1976; 2:325.

Marzulli FN, Maibach HI. Dermatotoxicology. 4th ed. New York:Hemisphere, 1991.

Fisher AA. 3rd ed. Contact Dermatitis. Philadelphia:Lea & Feiberger, 1986.

Shelanski HA, Shelanski MV. A new technique of human patch tests. Proc Sci Sect Toilet Goods Assoc 1953; 204:107-110.

Jordan WP, King SF. Related hypersensitivity in families. Contact Dermatitis 1977; 3:19-26.

Kligman AM, Epstein W. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1975; 1:231-239.

Stotts, J. Planning, conduct and interpretation of human predictive sensitization patch tests. In: Drill VA, Lazar P, eds. Current Concepts In Cutaneous Toxicity. New York:Academic Press, 1980:41-53.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 2, 2018

SUBJECT: PTFE

Anonymous. 2018. PTFE Method of manufacture.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

May 2018

PTFE Method of Manufacture

A company supplying PTFE to the cosmetics industry provided the following information:

1. We begin with virgin grade high molecular weight PTFE.

2. In order to be able to micronize the PTFE into a fine powder, molecular weight needs to be reduced.

3. Electron beam irradiation is used to lower molecular weight into a range typically 15,000-50,000 daltons.

    a. This process is controlled via melt point reduction (starting around 341 °C and ending around 330 °C).

4. The irradiated PTFE is post-baked to remove volatiles (including any trace PFOA to below 25 ppb)

5. The irradiated and baked material is jet mill micronized to the particle size specification. A typical particle size curve is attached.

Trace tetrafluoroethylene monomer in PTFE is not detected (DL 75 ppb).

MALVERN MASTERSIZER

Result: Histogram Report

Sample Details

Sample ID: PTFE
Sample File: M2775
Sample Path: C:\SIZERS\DATA\
Sample Notes:

Run Number: 9
Record Number: 5

Measured: Sat Feb 10 2018 8:40AM
Analysed: Sat Feb 10 2018 8:40AM
Result Source: Analysed

System Details

Range Lens: 300RF mm
Presentation: 3NHD
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm

Sampler: None

Obscuration: 17.3 %
Residual: 1.342 %

Result Statistics

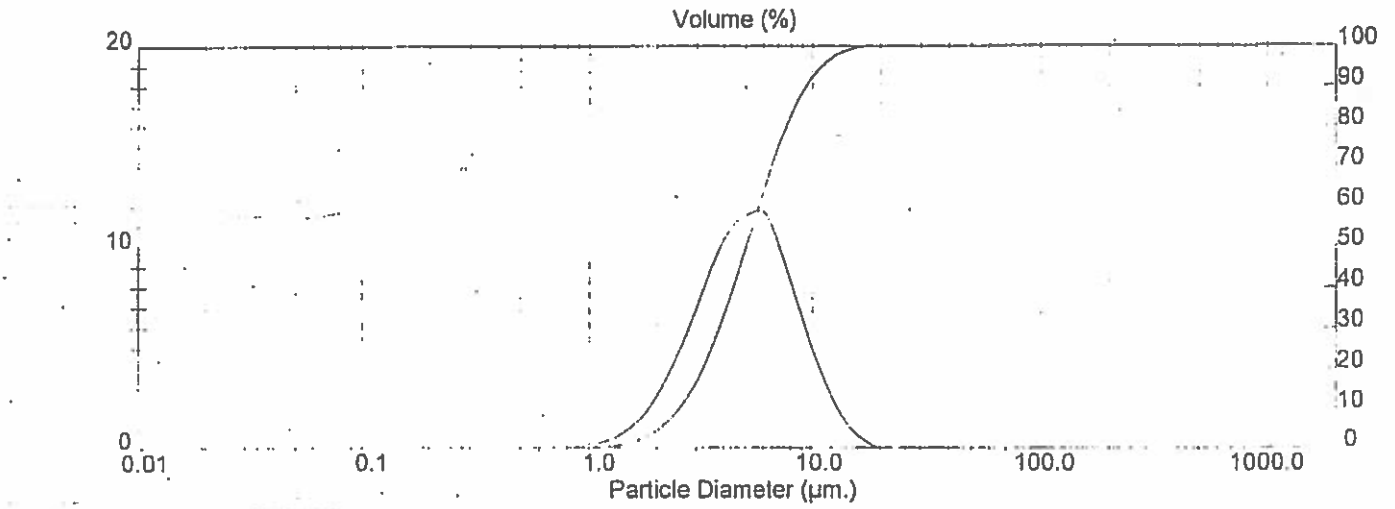
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 5.58 um

Concentration = 0.0108 %Vol
D (v, 0.1) = 2.52 um
D [3, 2] = 4.31 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 5.04 um
Span = 1.359E+00

Specific S.A. = 1.3915 sq. m / g
D (v, 0.9) = 9.38 um
Uniformity = 4.257E-01

Size (um)	Volume Under %	Size (um)	Volume Under %	Size (um)	Volume Under %	Size (um)	Volume Under %
0.055	0.00	0.635	0.00	7.31	77.38	84.15	100.00
0.061	0.00	0.700	0.00	8.06	83.04	92.79	100.00
0.067	0.00	0.772	0.00	8.89	87.79	102.3	100.00
0.074	0.00	0.851	0.00	9.80	91.61	112.8	100.00
0.082	0.00	0.938	0.02	10.81	94.54	124.4	100.00
0.090	0.00	1.03	0.15	11.91	96.67	137.2	100.00
0.099	0.00	1.14	0.31	13.14	98.14	151.3	100.00
0.109	0.00	1.26	0.54	14.49	99.08	166.8	100.00
0.121	0.00	1.39	0.91	15.97	99.62	183.9	100.00
0.133	0.00	1.53	1.47	17.62	99.91	202.8	100.00
0.147	0.00	1.69	2.27	19.42	100.00	223.6	100.00
0.162	0.00	1.86	3.38	21.42	100.00	246.6	100.00
0.178	0.00	2.05	4.92	23.62	100.00	271.9	100.00
0.196	0.00	2.26	6.99	26.04	100.00	299.8	100.00
0.217	0.00	2.49	9.68	28.72	100.00	330.6	100.00
0.239	0.00	2.75	13.07	31.66	100.00	364.6	100.00
0.263	0.00	3.03	17.22	34.92	100.00	402.0	100.00
0.290	0.00	3.34	22.17	38.50	100.00	443.3	100.00
0.320	0.00	3.69	27.89	42.45	100.00	488.8	100.00
0.353	0.00	4.07	34.30	46.81	100.00	539.0	100.00
0.389	0.00	4.48	41.23	51.62	100.00	594.3	100.00
0.429	0.00	4.94	48.48	56.92	100.00	655.4	100.00
0.473	0.00	5.45	55.89	62.76	100.00	722.7	100.00
0.522	0.00	6.01	63.45	69.21	100.00	796.9	100.00
0.576	0.00	6.63	70.81	76.32	100.00	878.7	100.00



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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr., Senior Scientific Analyst
Date: May 23, 2018
Subject: Wave 2 Data on Polyol Phosphates

The data listed below (in *phytic062018data4 and phytic062018data5*) on Phytic Acid and Sodium Phytate were received from the Council and are being submitted as an attachment to this memorandum. A data summary document (*phytic062018wave2studysummaries*) is also attached for the Panel's review. The data that were received include:

phytic062018data4:

- HRIPT on a cosmetic product containing 1% Phytic Acid
- HRIPT on another cosmetic product containing 1% Phytic Acid

phytic062018data5:

- HRIPT on a rouge containing 0.19% Sodium Phytate data

phytic062018data6:

- Bovine corneal opacity and permeability assay on 3% Sodium Mannose Phosphate
- Skin irritation test on 3% Sodium Mannose Phosphate using the Epiderm™ skin model
- Genotoxicity data (Ames test) on Sodium Mannose Phosphate
- KeratiinoSens™ assay for evaluating the sensitization potential of Sodium Mannose Phosphate

These data will be added to the safety assessment after the Panel meeting.

Wave 2 Data on Polyol Phosphates

GENOTOXICITY STUDIES

In Vitro

Sodium Mannose Phosphate

The genotoxicity of Sodium Mannose Phosphate was evaluated in the Ames test using the following bacterial strains: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A.[1] Sodium Mannose Phosphate was tested at doses up to 5000 µg/plate, with and without metabolic activation. The test material was not genotoxic in any of the bacterial strains tested, with or without metabolic activation.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

Sodium Mannose Phosphate

The skin irritation potential of 3% Sodium Mannose Phosphate was evaluated using the Epiderm™ skin model (reconstructed human epidermis).[2] Epiderm™ tissues were treated in triplicate with the test material for 60 ± 1 min and then transferred to well plates. A 1 mg/ml solution of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added to each well to assess the ability of the test material to directly reduce MTT during a 3 ± 0.1 h incubation period (i.e., MTT cytotoxicity assay). The negative control was CMF-DPBS (acronym not defined) and the positive control was sodium dodecyl sulfate. Relative cell viability was calculated as % of the mean of the negative control tissues. Skin irritation is predicted if the remaining relative cell viability is below 50%. The test material was not observed to directly reduce MTT in the absence of viable cells. Mean viability in the presence of the test material was 101.1%. Mean viability in the presence of the positive control was 3.34%.

Sensitization

In Vitro

Sodium Mannose Phosphate

The sensitization potential of Sodium Mannose Phosphate was evaluated using the KeratinoSens™ assay.[3] This assay is a cell-based assay with a reporter cell line for the detection of potential skin sensitizers by their ability to induce the Nrf2-response. The KeratinoSens™ cell line is derived from the human keratinocyte culture HaCaT. Sodium Mannose Phosphate (in dimethyl sulfoxide (DMSO)) was tested at 12 concentrations ranging from 0.49 to 1000 ppm. Cinnamic aldehyde served as the positive control. The following 2 endpoints were measured: 1) luciferase induction after a 48-h treatment with the test material and 2) cytotoxicity, as determined with the MTT assay. For Luciferase induction, the maximal fold-induction over solvent control (I_{max}) and the concentration needed to reach a 1.5-, 2-, and 3-fold induction (EC1.5, EC2, and EC3) were calculated. For cytotoxicity, the IC_{50} value was extrapolated. Sodium Mannose phosphate did not induce the luciferase reporter above the threshold of 1.5 at any concentration in 2 of 3 repetitions, whereas a weak induction at the highest concentration was noted in the third repetition. According to the prediction model of the KeratinoSens™ assay, Sodium Mannose Phosphate was classified as a non-sensitizer.

Human

Phytic Acid

The skin irritation and sensitization potential of a cosmetic product containing 1% Phytic Acid was evaluated in an HRIPT involving 104 male and female subjects.[4] The product (~ 0.2 ml on a 2 cm x 2cm semiocclusive patch) was applied for 24 h to the back (between the scapulae), which means that ~ 0.05 mL/cm^2 was applied. This procedure was repeated on Mondays, Wednesdays, and Fridays, for a total of 9 induction applications. Patch removals on Tuesdays and Thursday were followed by a 24-h non-treatment period. Patch removals on Saturdays were followed by a 48-h non-treatment period. Removal of the last induction patch was followed by a 2-week non-treatment period. A challenge patch was applied to a new test site on each subject and reactions were scored at 24 h and 72 h after patch application. Reactions were not observed during the induction phase. A challenge reaction (+

reaction (barely perceptible erythema) at 72-h reading) was observed in one subject, and was classified as not being indicative of sensitization. It was concluded that application of the product was not associated with clinically significant skin irritation or allergic contact dermatitis.

The skin sensitization potential of another cosmetic product containing 1% Phytic Acid was evaluated in an HRIPT involving 98 male and female subjects.[5] The test procedure summarized in the preceding paragraph was used. Again, the product (~ 0.2 ml on a 2 cm x 2cm semiocclusive patch) was applied to the back. Skin reactions were not observed at any time during the study. It was concluded that application of the product was not associated with clinically significant skin irritation or allergic contact dermatitis.

Sodium Phytate

An HRIPT on a rouge containing 0.19% Sodium Phytate (undiluted) was performed using 106 male and female subjects (Fitzpatrick skin types II to IV).[6] The product (20 µl) was applied to the upper back (dose per cm^2 not stated), under an occlusive patch, and this procedure was repeated for a total of 9 induction patch applications over a period of 3 consecutive weeks. Induction applications (application period undefined) were followed by a 2-week non-treatment period, after which the challenge phase was initiated. Challenge patches were applied (application period undefined) to the induction site and to a new test site. An occlusive patch application of distilled water served as the control. Repeated applications of the product did not cause significant skin irritation and the product was said to have had very good skin compatibility. Also there was no evidence of an allergic reaction after challenge application of the product.

OCULAR IRRITATION STUDIES

In Vitro

Sodium Mannose Phosphate

The ocular irritation potential of 3% Sodium Mannose Phosphate was evaluated in the bovine opacity and permeability assay using excised corneas.[7] An aliquot (750 µl) of the test material was introduced into the anterior chamber of 5 corneas, and the corneas were incubated for 10 min. Three positive control corneas were incubated with ethanol and 3 negative control corneas were incubated with deionized water. The change in opacity for each cornea was calculated by subtracting the initial opacity reading from the final opacity reading. These values were then corrected by subtracting, from each, the average change in opacity observed for the negative control corneas. For permeability measurements, corneas were incubated for 90 min and medium was removed from the posterior chamber. The optical density (OD) of the medium at 490 nm was determined. The final corrected OD_{490} values of the test material and positive control were calculated by subtracting the average corrected OD_{490} value of the negative control corneas from the corrected OD_{490} value of each treated cornea. The opacity value was -0.1 and the OD_{490} value was 0.004. The in vitro ocular irritation score for Sodium Mannose Phosphate was 0 (compared with 55 for a positive control). According to the in vitro ocular irritation scoring system, a score of ≤ 25 is classified as mildly irritating.

References

1. Charles River Laboratories Skokie, LLC. *Salmonella-E. coli*/Mammalian microsome reverse mutation assay (Sodium Mannose Phosphate). Unpublished data submitted by the Personal Care Products Council on 5-21-2018. pp.1-49.

2. Institute for In Vitro Sciences, Inc. Skin irritation test (SIT) using the Epiderm™ skin model (3% sodium mannose phosphate). Unpublished data submitted by the Personal Care Products Council on 5-21-2018. 2016. pp.1-14.

3. Anonymous. KeratinoSens™ assay: Test report on D-mannose-6-phosphate (INCI: Sodium Mannose Phosphate). Unpublished data submitted by the Personal Care Products Council on 5-21-2018. 2016. pp.1-17.

4. Essex Testing Clinic, Inc. Clincial safety evaluation repeated insult patch test (product containied 1% phytic acid). Unpublished data submitted by the Personal Care Products Council on 5-7-2018. 2012. pp.1-13.

5. Essex Testing Clinic, Inc. Clinical safety evaluation repeated insult patch test (product contained 1% phytic acid). Unpublished data submitted by the Personal Care Products Council on 5-7-2018. 2011. pp.14-25.

6. Anonymous. Human repeated insult patch test with challenge (rouge containing 0.19% sodium phytate). Unpublished data submitted by the Personal Care Products Council on 5-8-2018. 2012. pp.1-7.

7. Institute for In Vitro Sciences, Inc. Bovine corneal opacity and permeability assay (3% sodium mannose phosphate). Unpublished data submitted by the Personal Care Products Council on 5-21-2018. 2017. pp.1-13.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 7, 2018

SUBJECT: Studies on Products Containing Phytic Acid

Essex Testing Clinic, Inc. 2012. Clinical safety evaluation repeated insult pat test (product contained 1% Phytic Acid).

Essex Testing Clinic, Inc. 2011. Clinical safety evaluation repeated insult pat test (product contained 1% Phytic Acid).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Essex Testing Clinic, Inc.

ETC

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

TRA/P# 12-028

Contains 1% Phytic Acid

Sponsor

[redacted]

Sponsor Representative

[redacted]

Clinical Testing Facility

Essex Testing Clinic, Inc.
799 Bloomfield Avenue
Verona, NJ 07044

Sponsor Code: T28
ETC Panel Nos.: 12115/12124
ETC Entry No.: 21615
TRA Project No.: 99001-398

Date of Final Report

5-9-12

ETC Panel Nos.: 12115/12124
ETC Entry No.: 21615

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

TRA/P# 12-028

AE Hollenback — 3 MAY 2012

Annemarie E. Hollenback, BA
Laboratory Manager
Study Director

Date

5/3/12

Toni F. Miller, PhD, DABT, BCFE
Scientific Director
Principal Investigator

Date

5/3/12

John A. Erianne, MD
Board-Certified Dermatologist
Medical Investigator

Date

Essex Testing Clinic, Inc.

QUALITY ASSURANCE STATEMENT

This study (ETC Panel Nos.: 12115/12124; ETC Entry No.: 21615) was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in 21 CFR Part 50 (Protection of Human Subjects – Informed Consent) and the Standard Operating Procedures of Essex Testing Clinic, Inc.

For purposes of this clinical study:

_X_	Informed Consent was obtained.
____	Informed Consent was not obtained.
_X_	An IRB review was not required.
____	An IRB review was conducted and approval to conduct the proposed clinical research was granted.

To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the applicable study records and report. This report is considered a true and accurate reflection of the testing methods and source data.

Sherri L. Sayles, MS
Manager, Quality Assurance

8 May 2012
Date

ETC Panel Nos.: 12115/12124
ETC Entry No.: 21615

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TABLE 1 - INDIVIDUAL SCORES

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

TRA/P# 12-028

1.0 OBJECTIVE

The objective of this study was to determine the irritation and/or sensitization potential of the test article after repeated application under semi-occlusive patch test conditions to the skin of human subjects (non-exclusive panel).

2.0 SPONSOR

[redacted]

2.1 Sponsor Representative

[redacted]

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

Essex Testing Clinic, Inc.
799 Bloomfield Avenue
Verona, NJ 07044

4.0 CLINICAL INVESTIGATORS

Study Director:	Annemarie E. Hollenback, BA
Principal Investigator:	Toni F. Miller, PhD, DABT, BCFE
Medical Investigator:	John A. Erianne, MD, Board-Certified Dermatologist

5.0 STUDY DATES

Study initiation:	March 7, 2012 (Panel No. 12115) March 14, 2012 (Panel No. 12124)
Final evaluation:	April 13, 2012 (Panel No. 12115) April 21, 2012 (Panel No. 12124)

6.0 ETHICS

6.1 Ethical Conduct of the Study

This study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in Title 21 of the U.S. Code of Federal Regulations (CFR), the Declaration of Helsinki and/or Essex Testing Clinic (ETC) Standard Operating Procedures.

6.2 Subject Information and Consent

This study was conducted in compliance with CFR Title 21, Part 50 (Informed Consent of Human Subjects). Informed Consent was obtained from each subject in the study and documented in writing before participation in the study. A copy of the Informed Consent was provided to each subject.

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on February 27, 2012 and identified as follows:

ETC Entry No.	Test Article I.D.	Description
21615	TRA/P# 12-028	Transparent Colorless Liquid

8.0 TEST SUBJECTS

At least 100 male and female subjects ranging in age from 18 to 79 years were to be empanelled for this test.

The subjects chosen were to be dependable and able to read and understand instructions. The subjects were not to exhibit any physical or dermatologic condition that would have precluded application of the test article or determination of potential effects of the test article.

9.0 TEST PROCEDURE

The 9 Repeated Insult (semi-occlusive) Patch Test (9-RIPT)[1] was conducted as follows:

9.1 Induction Phase

A sufficient amount of the test article (approximately 0.2 mL) was placed onto a 2 cm x 2 cm square of Webril® cotton fabric (approximately 0.05 mL/cm^2 of test material) affixed to Scanpor (Allerderm) semi-occlusive surgical tape. The patch was then applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

The subjects were instructed to remove the patch 24 hours after application. Twenty-four hour rest periods followed the Tuesday and Thursday removals and 48-hour rest periods followed each Saturday removal. Subjects returned to the Testing Facility and the site was scored by a trained examiner just prior to the next patch application.

If a subject developed a positive reaction of a level 2 erythema or greater during the Induction phase or if, at the discretion of the Study Director, the skin response warranted a change in site, the patch was applied to a previously unpatched, adjacent site for the next application. If a level 2 reaction or greater occurred at the new site, no further applications were made. However, any reactive subjects were subsequently Challenge patch tested.

9.2 Challenge Phase

After a rest period of approximately 2 weeks (no applications of the test article), the Challenge patch was applied to a previously unpatched (virgin) test site. The site was scored 24 and 72 hours after application. All subjects were instructed to report any delayed skin reactivity that occurred after the final Challenge patch reading. When warranted, selected test subjects were called back to the Clinic for additional examinations and scoring to determine possible increases or decreases in Challenge patch reactivity.

Dermal responses for both the Induction and Challenge phases of the study were scored according to the following 6-point scale:

0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

All other observed dermal sequelae (eg, edema, dryness, hypo- or hyperpigmentation) were appropriately recorded on the data sheet and described as mild, moderate or severe.

[1] Marzulli FN, Maibach HI. (1976) Contact allergy: predictive testing in man. *Contact Dermatitis.* 2, 1-17.

9.0 TEST PROCEDURE (CONT'D)

9.3 Data Interpretation

Edema, vesicles, papules and/or erythema that persist or increase in intensity either during the Induction and/or Challenge phase may be indicative of allergic contact dermatitis. Allergic responses normally do not resolve or improve markedly at 72-96 hours.

Exceptions to typical skin reactions may occur. These may include, but not be limited to, symptoms of allergic contact sensitivity early in the Induction period to one or more test products. When this occurs in one subject, such a reaction usually suggests either an idiosyncratic response or that the subject had a pre-exposure/sensitization to the test material or component(s) of the test material or a cross-reactivity with a similar product/component. Data for such reactions will be included in the study report but will not be included in the final study analysis/conclusion of sensitization.

10.0 RESULTS AND DISCUSSION

(See Table 1 for Individual Scores)

A total of 110 subjects (30 males and 80 females ranging in age from 20 to 76 years) were empanelled for the test procedure. One hundred four (104/110) subjects satisfactorily completed the test procedure on Test Article: TRA/P# 12-028. Six (6/110) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued subject data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

Induction Phase Summary

Test Article	Induction Scores (Number of Responses)						Evidence of Irritation
	0.5	1	2	3	4	Other	
TRA/P# 12-028	0	0	0	0	0	0	No

Challenge Phase Summary

Test Article	Challenge Scores (Number of Responses)						Evidence of Sensitization
	0.5	1	2	3	4	Other	
TRA/P# 12-028	1	0	0	0	0	0	No

There was no skin reactivity observed during the Induction phase.

The response seen on one subject during the Challenge phase was low in severity and was not indicative of sensitization.

11.0 CONCLUSIONS

Under the conditions of a repeated insult (semi-occlusive) patch test procedure conducted in 104 subjects, Test Article: TRA/P# 12022 was "Dermatologist-Tested" and was not associated with clinically significant skin irritation or allergic contact dermatitis in human subjects.

ETC Panel No.: 12115
ETC Entry No.: 21615

TABLE 1

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - SEMI-OCCLUSIVE

Test Article: TRA/P# 12-028

Subj.	Induction Evaluation Number									Challenge Virgin Site	
No.	1	2	3	4	5	6	7	8	9	24hr	72hr
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

Essex Testing Clinic, Inc._____

ETC Panel No.: 12115
ETC Entry No.: 21615

TABLE 1 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - SEMI-OCCLUSIVE

Test Article: TRA/P# 12-028

Subj.	Induction Evaluation Number									Challenge Virgin Site	
No.	1	2	3	4	5	6	7	8	9	24hr	72hr
31	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	Discontinued					
38	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	Discontinued					
43	0	0	0	0	0	Discontinued					
44	0	0	0	0	0	Discontinued					
45	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

ETC Panel No.: 12124
ETC Entry No.: 21615

TABLE 1 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - SEMI-OCCLUSIVE

Test Article: TRA/P# 12-028

Subj.	Induction Evaluation Number									Challenge Virgin Site	
No.	1	2	3	4	5	6	7	8	9	24hr	72hr
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	Discontinued	
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	+
30	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

ETC Panel No.: 12124
ETC Entry No.: 21615

TABLE 1 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - SEMI-OCCLUSIVE

Test Article: TRA/P# 12-028

Subj.	Induction Evaluation Number									Challenge Virgin Site	
No.	1	2	3	4	5	6	7	8	9	24hr	72hr
31	0	0	0	0	0	0	0	0	0	0	0
32	0	Discontinued									
33	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

Essex Testing Clinic, Inc.

ETC

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

TRA/P #11-194

Contains 1% phytic Acid

Sponsor

[redacted]

Sponsor Representative

[redacted]

Clinical Testing Facility

Essex Testing Clinic, Inc.
799 Bloomfield Avenue
Verona, NJ 07044

Sponsor Code: T28
ETC Panel Nos.: 11261/11279
ETC Entry No.: 20532
TRA Project No.: 99001-356

Date of Final Report

10-7-11

799 Bloomfield Avenue • Verona, NJ 07044 • (973) 857-9541 • Fax # (973) 857-9662

ETC Panel Nos.: 11261/112
ETC Entry No.: 20532

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

TRA/P #11-194

AEHollenback

Annemarie E. Hollenback, BA
Laboratory Manager
Study Director

6 Oct 2011
Date

Toni F. Miller, PhD, DABT, BCFE
Scientific Director
Principal Investigator

6 Oct 2011
Date

John A. Erianne, MD
Board-Certified Dermatologist
Medical Investigator

10/7/11
Date

Essex Testing Clinic, Inc.

QUALITY ASSURANCE STATEMENT

This study (ETC Panel Nos.: 11261/11279; ETC Entry No.: 20532) was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in 21 CFR Part 50 (Protection of Human Subjects – Informed Consent) and the Standard Operating Procedures of Essex Testing Clinic, Inc.

For purposes of this clinical study:

_X_	Informed Consent was obtained.
____	Informed Consent was not obtained.
_X_	An IRB review was not required.
____	An IRB review was conducted and approval to conduct the proposed clinical research was granted.

To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the applicable study records and report. This report is considered a true and accurate reflection of the testing methods and source data.

Sherri L Sayles 7 Oct 2011

Sherri L. Sayles, MS
Manager, Quality Assurance

Date

Essex Testing Clinic, Inc.

ETC Panel Nos.: 11261/112
ETC Entry No.: 20532

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CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

TRA/P #11-194

1.0 OBJECTIVE

The objective of this study was to determine the irritation and/or sensitization potential of the test article after repeated application under semi-occlusive patch test conditions to the skin of human subjects (non-exclusive panel).

2.0 SPONSOR

[redacted]

2.1 Sponsor Representative

[redacted]

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

Essex Testing Clinic, Inc.
799 Bloomfield Avenue
Verona, NJ 07044

4.0 CLINICAL INVESTIGATORS

Study Director:	Annemarie E. Hollenback, BA
Principal Investigator:	Toni F. Miller, PhD, DABT, BCFE
Medical Investigator:	John A. Erianne, MD, Board-Certified Dermatologist

5.0 STUDY DATES

Study Initiation:	August 3, 2011 (Panel No. 11261) August 17, 2011 (Panel No. 11279)
Final evaluation:	September 9, 2011 (Panel No. 11261) September 23, 2011 (Panel No. 11279)

6.0 ETHICS

6.1 Ethical Conduct of the Study

This study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in Title 21 of the U.S. Code of Federal Regulations (CFR), the Declaration of Helsinki and/or Essex Testing Clinic (ETC) Standard Operating Procedures.

6.2 Subject Information and Consent

This study was conducted in compliance with CFR Title 21, Part 50 (Informed Consent of Human Subjects). Informed Consent was obtained from each subject in the study and documented in writing before participation in the study. A copy of the Informed Consent was provided to each subject.

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on July 26, 2011 and identified as follows:

ETC Entry No.	Test Article I.D.	Description
20532	TRA/P #11-194	Transparent Pale Yellow Liquid

8.0 TEST SUBJECTS

At least 100 male and female subjects ranging in age from 18 to 79 years were to be empanelled for this test.

The subjects chosen were to be dependable and able to read and understand instructions. The subjects were not to exhibit any physical or dermatologic condition that would have precluded application of the test article or determination of potential effects of the test article.

9.0 TEST PROCEDURE

The 9 Repeated Insult (semi-occlusive) Patch Test (9-RIPT)[1] was conducted as follows:

9.1 Induction Phase

A sufficient amount of the test article (approximately 0.2 mL) was placed onto a 2 cm x 2 cm square of Webril® cotton fabric (approximately 0.05 mL/cm^2 of test material) affixed to Scanpor (Allerderm) semi-occlusive surgical tape. The patch was then applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

The subjects were instructed to remove the patch 24 hours after application. Twenty-four hour rest periods followed the Tuesday and Thursday removals and 48-hour rest periods followed each Saturday removal. Subjects returned to the Testing Facility and the site was scored by a trained examiner just prior to the next patch application.

If a subject developed a positive reaction of a level 2 erythema or greater during the Induction phase or if, at the discretion of the Study Director, the skin response warranted a change in site, the patch was applied to a previously unpatched, adjacent site for the next application. If a level 2 reaction or greater occurred at the new site, no further applications were made. However, any reactive subjects were subsequently Challenge patch tested.

9.2 Challenge Phase

After a rest period of approximately 2 weeks (no applications of the test article), the Challenge patch was applied to a previously unpatched (virgin) test site. The site was scored 24 and 72 hours after application. All subjects were instructed to report any delayed skin reactivity that occurred after the final Challenge patch reading. When warranted, selected test subjects were called back to the Clinic for additional examinations and scoring to determine possible increases or decreases in Challenge patch reactivity.

Dermal responses for both the Induction and Challenge phases of the study were scored according to the following 6-point scale:

0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

All other observed dermal sequelae (eg, edema, dryness, hypo- or hyperpigmentation) were appropriately recorded on the data sheet and described as mild, moderate or severe.

---

[1] Marzulli FN, Maibach HI (1976) Contact allergy: predictive testing in man. *Contact Dermatitis* 2, 1-17.

9.0 TEST PROCEDURE (CONT'D)

9.3 Data Interpretation

Edema, vesicles, papules and/or erythema that persist or increase in intensity either during the Induction and/or Challenge phase may be indicative of allergic contact dermatitis. Allergic responses normally do not resolve or improve markedly at 72-96 hours.

Exceptions to typical skin reactions may occur. These may include, but not be limited to, symptoms of allergic contact sensitivity early in the Induction period to one or more test products. When this occurs in one subject, such a reaction usually suggests either an idiosyncratic response or that the subject had a pre-exposure/sensitization to the test material or component(s) of the test material or a cross-reactivity with a similar product/component. Data for such reactions will be included in the study report but will not be included in the final study analysis/conclusion of sensitization.

10.0 RESULTS AND DISCUSSION

(See Table 1 for Individual Scores)

A total of 110 subjects (22 males and 88 females ranging in age from 18 to 79 years) were empanelled for the test procedure. Ninety-eight (98/110) subjects satisfactorily completed the test procedure on Test Article: TRA/P #11-194. Twelve (12/110) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued subject data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

Induction Phase Summary

Test Article	Induction Scores (Number of Responses)						Evidence of Irritation
	0.5	1	2	3	4	Other	
TRA/P #11-194	0	0	0	0	0	0	No

Challenge Phase Summary

Test Article	Challenge Scores (Number of Responses)						Evidence of Sensitization
	0.5	1	2	3	4	Other	
TRA/P #11-194	0	0	0	0	0	0	No

There was no skin reactivity observed at any time during the course of the study.

11.0 CONCLUSIONS

Under the conditions of a repeated insult (semi-occlusive) patch test procedure conducted in 98 subjects, Test Article: TRA/P #11-194 was "Dermatologist-Tested" and was not associated with skin irritation or allergic contact dermatitis in human subjects.

ETC Panel No.: 11261
ETC Entry No.: 20532

TABLE 1

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - SEMI-OCCLUSIVE

Test Article: TRA/P #11-194

Subj. No.	Induction Evaluation Number									Challenge Virgin Site	
	1	2	3	4	5	6	7	8	9	24hr	72hr
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	Discontinued										
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	Discontinued				
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	Discontinued				
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

Essex Testing Clinic, Inc.

ETC Panel No.: 11261
ETC Entry No.: 20532

TABLE 1 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - SEMI-OCCLUSIVE

Test Article: TRA/P #11-194

Subj. No.	Induction Evaluation Number									Challenge Virgin Site	
	1	2	3	4	5	6	7	8	9	24hr	72hr
31	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0
42	0	Discontinued								0	0
43	0	0	Discontinued								
44	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0
46	0	Discontinued									
47	0	0	0	Discontinued							
48	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

ETC Panel No.: 11279
ETC Entry No.: 20532

TABLE 1 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - SEMI-OCCLUSIVE

Test Article: TRA/P #11-194

Subj. No.	Induction Evaluation Number									Challenge Virgin Site	
	1	2	3	4	5	6	7	8	9	24hr	72hr
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	Discontinued				
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	Discontinued						0	0
30	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

ETC Panel No.: 11279
ETC Entry No.: 20532

TABLE 1 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - SEMI-OCCLUSIVE

Test Article: TRA/P #11-194

Subj. No.	Induction Evaluation Number									Challenge Virgin Site	
	1	2	3	4	5	6	7	8	9	24hr	72hr
31	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0
41	Discontinued										
42	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0
44	Discontinued										
45	Discontinued										
46	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
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4 = Severe (Deep red erythema with/without vesiculation or weeping)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heidreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 8, 2018

SUBJECT: Sodium Phytate

Anonymous. 2012. Human repeated insult patch test with challenge (rouge containing 0.19% Sodium Phytate).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

[redacted]- 07/04/11

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

Study report – version n° 1 (28/02/2012)

STUDY REFERENCES [redacted]

INVESTIGATIONAL PRODUCT

Denomination [redacted] Rouge containing

Formula number [redacted] 0.19% Sodium

Batch number [redacted] phytate

SPONSOR	[redacted]
STUDY MONITOR	[redacted]
COORDINATING CENTRE	[redacted]
INVESTIGATING CENTRE	[redacted]
MAIN INVESTIGATOR	[redacted]

Initiation date of study performance	**09/01/2012**
Completion date of study performance	**17/02/2012**

Date of the study report: 28/02/2012

[redacted]

[redacted]

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

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[redacted] [redacted]

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

[redacted] synopsis

STUDY OBJECTIVES	Mainly, to confirm, in a panel of healthy human adult subjects, that the application of the investigational product, under maximalising conditions of exposure, did not induce delayed contact sensitisation Secondarily, to assess the skin compatibility of the investigational product during the induction phase of the study
SPONSOR	[redacted]
COORDINATING CENTRE	[redacted]
INVESTIGATING CENTRE	[redacted]
MAIN INVESTIGATOR	[redacted]
TYPE OF THE STUDY	Monocentric randomised study performed in simple blind
DATES OF STUDY PERFORMANCE	From January 09th to February 17th 2012
INVESTIGATIONAL PRODUCT	**ROUGE** [redacted] **Modalities of use in the study:** As it is - 20 µl under occlusive patch (Finn Chamber standard ®) Switch to semi-occlusive patch if reactions ≥ 2 occur

[redacted]

[redacted]

[redacted]synopsis (continuation)

STUDY POPULATION	**Number of valid cases: 106** **Population characteristics: test subjects** • suitable to participate in the study (after the clinical examination and questioning) and corresponding to the quality of "healthy subject" • declaring to have a health coverage • signing an "informed consent form" for this study • certifying not to take part in another clinical study in another investigating centre • certifying the truth of the personal information declared to the investigator • capable of following directions and reliable to respect the constraints of the protocol • free to ensure the visits to the investigating centre • aged from 18 to 62 • female and/or male • with a phototype (Fitzpatrick): II - IV • declaring not to have exposed themselves to a risk of pregnancy for at least 3 months before the beginning of the study and committing themselves to use effective contraceptive method throughout the study (for the women of childbearing potential) • with all types of skin on body **Non inclusion criteria: test subjects** • being in exclusion period • deprived of freedom by administrative or legal decision or under guardianship • who cannot be contacted in case of emergency • admitted in a residential care • planning an hospitalisation during the study • belonging to the staff of the investigating centre • being of age but protected by law • having received vaccination within the 3 weeks prior to the study or intending to be vaccinated during the course of the study • with personal history of adverse reactions to the same type of product as the investigational product • with personal history of adverse reaction to colophony, rubber, nickel, aluminium, patch materials, adhesive plaster, • with documented history of contact allergy • with orthoergic skin reactivity on body • with family or personal history of atopy • exhibiting skin marks and/or moles and/or freckles in too great quantity, hyperpilosity on the experimental area able to interfere with the assessment of the possible skin reactions • with still visible eczematous reaction, scar or pigmentary after-effects of previous tests on the experimental area • under treatment, prior to the study, able to interfere with the interpretation of the study results • foreseeing, during the study, a treatment able to interfere with the interpretation of the study results • having had a fever lasting more than 24 hours, within the 8 days prior to the study • lactating or pregnant or planning a pregnancy during the study (for the women of childbearing potential) • having started or changed oestrogen-progesterone contraception or hormonal treatment, within the 3 months prior to the study or foreseeing it for the duration of the study • having had any invasive aesthetic cares on chest and back (peeling, laser...) by a dermatologist within the 2 months prior to the study or foreseeing it for the duration of the study • having had any non invasive aesthetic cares on chest and back (scrub, skin cleansing...) by an aesthetician within the month prior to the study or foreseeing it for the duration of the study

[redacted]

Synopsis (continuation)

STUDY POPULATION	• having received excessive or intensive exposure to sunlight (natural or artificial) within the month prior to the study or foreseeing UV exposures for the duration of the study • under treatment with PUVA or UVB within the month prior to the study • having participated in a human repeated insult patch test with challenge without sun exposure or in a cumulative irritability test within the 3 months prior to the study or in a single patch test within the month prior to the study • having already participated in 5 clinical studies involving patch test, including 3 human repeated insult patch tests with challenge or cumulative irritability test, within the year prior to the study • foreseeing bath (in bathtub, sea or swimming-pool), sauna or Turkish bath during the study period • regularly practicing intensive sport causing sweating and requiring frequent showers
METHODOLOGY	Application of the investigational product, in healthy human subjects, by a technician, at the investigating centre, to a skin site on the upper back, under maximalising conditions of exposure (under occlusive patch) for a defined time Repeated applications 9 times to the same site (induction site) over a period of 3 consecutive weeks, period necessary to induce a possible allergy (induction phase) After a minimal 2-week rest period, with no product application, single application of the investigational product, under patch, to the induction site and to a virgin site and for a defined time, enabling to reveal a possible induced allergy (challenge) Application in parallel of distilled water under occlusive patch at the same defined times as the investigational product = control site Skin examination of the application site, before the 1st product application of the induction phase and the application of the challenge and after each patch removal by the same investigator / technician, supervised by the investigator Reporting of the sensations of discomfort directly by the test subjects to the investigator / technician, during the study Assessment of the allergic potential - checking of the skin compatibility: • Accurate description of the skin reactions observed • Evaluation of the allergic reaction according to the ICDRG scale: ?+, (+), (++), (+++) • Calculation of the percentage of reactive test subjects during the challenge and the induction phase

[redacted]

[redacted]

[redacted] synopsis (continuation)

RESULTS

Checking of the skin compatibility

No reaction was noted on the control site

For the investigational product:

Induction phase			
Type of reaction	**Description of the reaction on the induction site**	**Number and percentage of reactive test subjects**	**Total number and percentage of reactive test subjects**
E: Erythema	None	0 / 0%	0 / 0%
A: ICDRG scale	None	0 / 0%	
M: Complementary mention	None	0 / 0%	

Challenge			
Type of reaction	**Description of the reactions on the induction site and the virgin site**	**Number and percentage of reactive test subjects**	**Total number and percentage of reactive test subjects**
E: Erythema	None	0 / 0%	0 / 0%
A: ICDRG scale	None	0 / 0%	
M: Complementary mention	None	0 / 0%	

OVERALL CONCLUSION

Under the experimental conditions adopted, the repeated applications of the product, **ROUGE** [redacted] under occlusive patch on a panel of 106 test subjects, with all types of skin on body, induced no significant reaction of irritation and the product has a very good skin compatibility.

Moreover, the repeated applications induced no allergic reaction.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 21, 2018

SUBJECT: Sodium Mannose Phosphate

Institute for In Vitro Sciences, Inc. 2017. Bovine corneal opacity and permeability assay (3% Sodium Mannose Phosphate).

Institute for In Vitro Sciences, Inc. 2016. Skin irritation test (SIT) using the Epiderm™ skin model (3% Sodium Mannose Phosphate).

Charles River Laboratories Skokie, LLC. 2017. *Salmonella-E. coli*/Mammalian microsome reverse mutation assay (Sodium Mannose Phosphate).

Anonymous. 2016. KeratinoSens™ assay: Test report on D-mannose 6-phosphate (INCI: Sodium Mannose Phosphate).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

FINAL REPORT

Study Title

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

Test Article 3% Sodium Mannose Phosphate

[redacted]

Authors

Greg Mu, B.A.
Megan Lamm, B.A.

Study Completion Date

15 February 2017

Performing Laboratory

Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Study Number

[redacted]

Laboratory Project Number

[redacted]

Sponsor Study Number

[redacted]

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STATEMENT OF COMPLIANCE

The Bovine Corneal Opacity and Permeability Assay performed on the test article, [redacted] was conducted in compliance with the U.S. EPA GLP Standards 40 CFR 792 and the principles presented in the OECD series on Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test article and controls have not been determined by the testing facility. Certificates of analysis for the test article and controls are in Appendix C.

The stability of the test article and the controls under the storage conditions at the testing facility and under the actual test conditions has not been determined by the testing facility and is not included in the final report.

Greg Mun, B.A.
Study Director

15 February 2017
Date

QUALITY ASSURANCE STATEMENT

Study Title: Bovine Corneal Opacity and Permeability Assay

Study Number: [redacted]

Study Director: Greg Mun, B.A.

This study was divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitored each of these phases over a series of studies. Procedures, documentation, equipment records, etc., were examined in order to assure that the study was performed in accordance with the U.S. EPA GLP Standards (40 CFR 792) and the OECD Principles of Good Laboratory Practice and to assure that the study was conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director and Management
Protocol and Initial Paperwork	20-Sept-16	20-Sept-16
Permeability Measurement	29-Sept-16	29-Sept-16
Draft Report and Data	1-Feb-17	2-Feb-17
Final Report	14-Feb-17	15-Feb-17

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Nicholas Minster, B.S.
Quality Assurance

15-Feb-2017
Date

SIGNATURE PAGE

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

Initiation Date:	20 September 2016
Completion Date:	15 February 2017
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility:	Institute for In Vitro Sciences, Inc. 30 W. Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
Archive Location:	Institute for In Vitro Sciences, Inc. Gaithersburg, MD 20878

Study Director: _____ 15 February 2017

Greg Mun, B.A. Date

TEST ARTICLE RECEIPT

IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Condltions*
[redacted]	[redacted]	clear colorless non-viscous liquid	8 September 2016	2-8°C

* - Protected from exposure to light

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

INTRODUCTION

The Bovine Corneal Opacity and Permeability Assay (BCOP) was used to assess the potential ocular irritancy of the test article to isolated bovine corneas. Bovine corneas, obtained as a by-product from freshly slaughtered animals, were mounted in special holders and exposed to the test article. An *in vitro* score was determined for the test article based on the induction of opacity and permeability (to fluorescein) in the isolated bovine corneas.

The purpose of this study was to evaluate the potential ocular irritancy of the test article, [redacted] supplied by [redacted] as measured by changes in opacity and permeability (to fluorescein) in isolated bovine corneas. The laboratory phase of this study was conducted from 22 September 2016 to 29 September 2016 at the Institute for In Vitro Sciences, Inc.

The results of the first definitive assay (Trial 1, performed on 22 September 2016) were considered invalid because the positive control results did not fall within the acceptable range. An additional definitive assay (Trial 2) was performed on 29 September 2016. Only the results from Trial 2, with acceptable control results, were considered valid and are presented in this report. In Trial 2, five corneas were treated with the test article. Based on changes in corneal opacity and permeability (relative to the control corneas), an *in vitro* score was determined.

MATERIALS AND METHODS

Bovine Eyes

Bovine eyes were obtained from a local abattoir as a by-product from freshly slaughtered animals (J.W. TREUTH & SONS, Inc., Baltimore, MD). The eyes were excised and then placed in Hanks' Balanced Salt Solution, containing Penicillin/Streptomycin (HBSS), and transported to the laboratory on ice packs. Immediately upon receipt of the eyes into the laboratory, preparation of the corneas was initiated.

Preparation of Corneas

The eyes were grossly examined for damage and those exhibiting defects were discarded. The tissue surrounding the eyeball was carefully pulled away and the cornea was excised such that a 2 to 3 mm rim of sclera was present around the cornea. The isolated corneas were then stored in a petri dish containing HBSS until they were mounted in a corneal holder. The corneas were mounted in the holders with the endothelial side against the O-ring of the posterior chamber. The anterior chamber was then positioned on top of the cornea and the screws were tightened. Starting with the posterior chamber, the two chambers were then filled with Minimum Essential Medium (EMEM) without phenol red, containing 1% fetal bovine serum and 2 mM L-glutamine (Complete MEM (without phenol red)). Each corneal holder was uniquely identified with a number written in permanent marker, on both the anterior and posterior chambers. The corneal holders were incubated at 32 ± 1°C for a minimum of 1 hour.

Controls

The positive control used in this study was ethanol (Pharmco). The negative control used in this study was sterile, deionized water (Quality Biological).

Test Article Preparation

As instructed by the Sponsor, the test article was administered to the test system without dilution.

Test Article pH Determination

The pH of the test article was determined using pH paper (EMD Millipore Corporation). Initially, the test article was added to 0-14 pH paper with 1.0 pH unit increments to approximate a narrow pH range. Next, the test article was added to 5-10 pH paper with 0.5 pH unit increments to obtain a more accurate pH value. The pH value obtained from the narrower range pH paper is presented in Table 1.

Bovine Corneal Opacity and Permeability Assay

After a minimum of 1 hour of incubation, the corneas were removed from the incubator. The medium was removed from both chambers and replaced with fresh Complete MEM (without phenol red). The initial opacity was determined for each cornea using an Electro Design OP-KIT opacitometer. Any cornea whose initial opacity was greater than 7 was not used in the assay. The treatment of each cornea was identified with the test article number written in permanent

marker on colored tape, affixed to each holder. The medium was then removed from the anterior chamber and replaced with the test article, positive control, or negative control.

Method for Testing Liquid or Surfactant Materials

The liquid test article, [redacted], was tested neat. An aliquot of 750 µL of the test article, positive control, or negative control was introduced into the anterior chamber while slightly rotating the holder to ensure uniform distribution over the comea. Three corneas were incubated in the presence of the positive control at 32 ± 1°C for 10 minutes. Three corneas were incubated in the presence of the negative control at 32 ± 1°C for 10 minutes. Five corneas were incubated in the presence of the test article at 32 ± 1°C for 10 minutes. After the 10-minute exposure time, the control or test article treatments were removed. The epithelial side of the corneas was washed at least three times with Complete MEM (containing phenol red) to ensure total removal of the control or test articles. The corneas were then given a final rinse with Complete MEM (without phenol red). The anterior chambers were refilled with fresh Complete MEM (without phenol red) and an opacity measurement was performed. The corneas were returned to the incubator for approximately 2 hours after which a final measure of opacity was obtained.

After the final opacity measurement was performed, the medium was removed from both chambers of the holder. The posterior chamber was filled with fresh Complete MEM (without phenol red) and 1 mL of a 4 mg/mL fluorescein solution was added to the anterior chamber. The corneas were then incubated in a horizontal position (anterior side up) for approximately 90 minutes at 32 ± 1°C. At the end of the 90-minute incubation period, the medium was removed from the posterior chambers and placed into tubes numbered with corresponding chamber numbers. Aliquots of 360 µL from the numbered tubes were placed into their designated wells on a 96-well plate. The optical density at 490 nm (OD_{490}) was determined using a Molecular Devices *V*max kinetic microplate reader. If the OD_{490} value of a control or test article sample was greater than 1.500, a 1:5 dilution of the sample was prepared in Complete MEM (without phenol red) (to bring the OD_{490} value within the linear range of the platereader). A 360 µL sample of each 1:5 dilution was transferred to its specified well on the 96-well plate. The plate was read again and the final reading was saved to a designated print file.

Presentation of Data

Opacity Measurement: The change in opacity for each cornea (including the negative control corneas) was calculated by subtracting the initial opacity reading from the final opacity reading. These values were then corrected by subtracting from each the average change in opacity observed for the negative control corneas. The mean opacity value of each treatment group was calculated by averaging the corrected opacity values of each cornea for that treatment condition.

Permeability Measurement: The mean OD_{490} value for the blank wells was calculated. The mean blank OD_{490} value was then subtracted from the raw OD_{490} value of each well (corrected OD_{490}). Any dilutions that were made to bring the OD_{490} readings into the linear range of the platereader (OD_{490} should be less than 1.500), had each diluted OD_{490} reading multiplied by the dilution factor, after the subtraction of the mean blank OD_{490}. The final corrected OD_{490} values of the test article and the positive control were then calculated by subtracting the average corrected OD_{490} value of the negative control corneas from the corrected OD_{490} value of each treated comea:

Final Corrected OD_{490} = (raw OD_{490} – mean blank OD_{490}) – average corrected negative control OD_{490}

The mean OD_{490} value of each treatment group was calculated by averaging the final corrected OD_{490} values of the treated corneas for that treatment condition.

The following formula was used to determine the *in vitro* score:

In Vitro Score = Mean Opacity Value + (15 x Mean OD_{490} Value)

Criteria for Determination of a Valid Test

The BCOP assay was accepted when the positive control (ethanol) caused an *in vitro* score that fell within two standard deviations of the historical mean.

RESULTS AND DISCUSSION

Bovine Corneal Opacity and Permeability Assays

The test article was tested in two definitive assays. The results of the first definitive assay (Trial 1, performed on 22 September 2016) were considered invalid because the positive control results did not fall within the acceptable range. An additional definitive assay (Trial 2) was performed on 29 September 2016. Only the results from Trial 2, with acceptable control results, were considered valid and are presented in this report.

Table 1 summarizes the opacity, permeability, and *in vitro* score for the test article and the positive control. Since the results of the positive control fell within two standard deviations of the historical mean (within a range of 39.1 to 63.6), the assay was considered valid. The opacity and permeability data for the individual corneas may be found in Appendix B.

For regulatory purposes, the *In Vitro* Score (IVIS) cut-off values for identifying test chemicals as inducing serious eye damage (UN GHS Category 1) and test chemicals not requiring classification for eye irritation or serious eye damage (UN GHS No Category) are found in the table below (OECD 437, adopted 26 July 2013). This guidance on categorization applies only to test article evaluated using the appropriate standard protocols as described in OECD 437[1].

IVIS	UN GHS
≤3	No Category
>3; ≤55	No prediction can be made
>55	Category 1

For non-regulatory purposes, the following classification system was established by Sina et al.[2] based on studies with a wide range of test materials. While this classification system provides a good initial guide to interpretation of these *in vitro* data, these specific ranges may not be applicable to all classes of materials or other exposure times. Whenever possible, results should be compared to "benchmark" materials tested under similar exposure conditions.

In Vitro Score:

≤ 25	= mild irritant
from 25.1 to 55	= moderate irritant
from 55.1 and above	= severe irritant

[1] OECD (2013),*Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing. doi: 10.1787/9789264203846-en

[2] Sina, J.F., Galer, D.M., Sussman, R.G., Gautheron, P.D., Sargent, E.V., Leong, B., Shah, P.V., Curren, R.D., and Miller, K. (1995) A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. **Fundamental and Applied Toxicology** 26:20-31.

Table 1
BCOP Results of the Test Article and the Positive Control

Assay Date	IIVS Test Article Number	Sponsor's Designation	Conc.	Exposure Time	Opacity Value	OD_{490} Value	*In Vitro* Score	pH
29 September 2016	[redacted]	[redacted]	Neat	10 minutes	-0.1	0.004	0.0	5.5
	Positive Control	Ethanol	NA	10 minutes	37.3	1.183	55.1	NA

NA – Not Applicable

Performed on 29-Sep-2016 Study No. [redacted]

IN VITRO SCORE

In Vitro Score = Mean Opacity Value + (15 x Mean OD490)

Test Article	Concentration	Exposure Period	Mean Opacity	Mean OD490	In vitro Score
[redacted]	Neat	10 minutes	-0.1	0.004	0.0
Ethanol	Neat	10minutes	37.3	1.183	55.1

FINAL REPORT

Study Title

SKIN IRRITATION TEST (SIT) USING THE EPIDERM™ SKIN MODEL

Test Substance 3% Sodium Mannose Phosphate

[redacted]

Authors

Gertrude-Emilia Costin, Ph.D., M.B.A.
Rebecca Pham, B.S.

Study Completion Date

6 December 2016

Performing Laboratory

Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Study Number

[redacted]

Laboratory Project Number

[redacted]

Sponsor Study Number

[redacted]

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APPENDIX C (CERTIFICATES OF ANALYSIS)....C1-C3

STATEMENT OF COMPLIANCE

The Skin Irritation Test (SIT) using the EpiDerm™ Skin Model of the test substance, W1601, was conducted in compliance with the U.S. EPA GLP Standards 40 CFR 792, and the principles presented in the OECD series on Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test substance and assay controls has not been determined by the testing facility. A Certificate of Analysis (regarding the non-GLP analysis of the test substance) was provided by the Sponsor (test substance) and was included in Appendix C along with Certificates of Analysis for the assay controls provided by their respective manufacturers.

The stability of the test substance and assay controls under the storage conditions at the testing facility and under the actual test conditions has not been determined by the testing facility and is not included in the final report.

Gertrude-Emilia Costin, Ph.D., M.B.A.
Study Director

6 December 2016
Date

QUALITY ASSURANCE STATEMENT

Study Title: Skin Irritation Test (SIT) Using the EpiDerm™ Skin Model

Study Number: [redacted]

Study Director: Gertrude-Emilia Costin, Ph.D., M.B.A.

This study was divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitored each of these phases over a series of studies. Procedures, documentation, equipment records, etc., were examined in order to assure that the study was performed in accordance with the U.S. EPA TSCA GLP Standards (40 CFR 792) and the OECD Principles of Good Laboratory Practice and to assure that the study was conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director and Management
Protocol and Initial Paperwork	26-Sept-16	26-Sept-16
Weighing of solid test article	28-Sept-16	28-Sept-16
Draft Report and Data	21 & 23-Nov-16	28-Nov-16
Final Report	29-Nov-16	29-Nov-16

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Nicholas Minster, B.S.
Quality Assurance

6-Dec-2016
Date

SIGNATURE PAGE

SKIN IRRITATION TEST (SIT)
USING THE EPIDERM™ SKIN MODEL

Initiation Date:	21 September 2016
Completion Date:	6 December 2016
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility:	Institute for In Vitro Sciences, Inc. 30 W. Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
Archive Location:	Institute for In Vitro Sciences, Inc. Gaithersburg, MD 20878
Study Director:	Gertrude-Emilia Costin, Ph.D., M.B.A. — 6 December 2016 (Date)
Director of Laboratory Services:	Greg Mun, B.A.

TEST SUBSTANCE RECEIPT

IIVS Test Substance Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
[redacted]	[redacted]	clear colorless non-viscous liquid	08 September 2016	2°C - 8°C

* - Protected from exposure to light

SKIN IRRITATION TEST (SIT)
USING THE EPIDERM™ SKIN MODEL

INTRODUCTION

The purpose of this study was to evaluate the skin irritation potential of the test substance, supplied by [redacted] in the context of identification and classification of skin irritation hazard according to the UN GHS and EU classification system (Category 2 or Category 1 and No Category). The skin irritation potential was evaluated based upon measuring the relative conversion of MTT (3-[4,5 - dimethylthiazol-2-yl] - 2,5 - diphenyltetrazolium bromide)[1] in the test substance-treated tissues after exposure to the test substance for a 60-minute exposure period, followed by a 42-hour post-exposure expression period. Skin irritation potential of the test substance was predicted if the relative viability was less than or equal to 50%. The protocol was based upon the EpiDerm™ SOP, Version 7.0 (Revised March 2009), Protocol for: In vitro EpiDerm™ skin irritation test (EPI-200-SIT), for use with MatTek Corporation's reconstructed human epidermal model EpiDerm (EPI-200)[2]. The protocol met the requirements of the OECD guideline, "*In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method" (TG **439**)[3].

The test substance was tested in one valid definitive assay to determine the identification and classification of skin irritation hazard according to the UN GHS and EU classification system (Category 2 or Category 1 and No Category). The laboratory phase of the study was conducted from 26 September 2016 to 30 September 2016 at the Institute for In Vitro Sciences, Inc.

[1] Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. Biochemica 4:14-19.
[2] EpiDerm™ SOP, Version 7.0 (Revised March 2009), Protocol for: In vitro EpiDerm™ skin irritation test (EPI-200-SIT). For use with MatTek Corporation's reconstructed human epidermal model EpiDerm (EPI-200).
[3] OECD Guidelines for the Testing of Chemicals. *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (OECD TG 439), adopted 28 July 2015.

MATERIALS AND METHODS

Receipt of the EpiDerm™ Skin Model

Upon receipt of the EpiDerm™ Skin Kit (MatTek Corporation), the solutions were stored as indicated by the manufacturer. The EpiDerm™ tissues were stored at 2-8°C until use. On the day prior to testing, EpiDerm™ Maintenance Medium was set to room temperature prior to use. Nine-tenths mL of Maintenance Medium were aliquotted into the appropriate wells of 6-well plates. Each 6-well plate was labeled with the test substance, positive control, or negative control. Each EpiDerm™ tissue was inspected for air bubbles between the agarose gel and cell culture insert prior to opening the sealed package. Tissue inserts with air bubbles covering greater than 50% of the cell culture insert area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiDerm™ tissues were transferred aseptically into the 6-well plates. The EpiDerm™ tissues were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air (standard culture conditions) for 60±5 minutes. After 60±5 minutes, the EpiDerm™ tissues were transferred to appropriate wells containing 0.9 mL of fresh warmed (to 37°C) Maintenance Medium. The plates were returned to the incubator for 18±3 hours to acclimate the tissues.

Test Substance Preparation

As instructed by the Sponsor, the test substance was administered to the test system without dilution. For ease of handling the test substance and per Study Director's instructions, an aliquot of the test substance was placed into a 1.5 mL Eppendorf tube, protected from the light, and allowed to reach room temperature before administering to the test system.

Assessment of Test Substance/Nylon Mesh Compatibility

Prior to performing the assay, the compatibility of the test substance with the nylon mesh was evaluated. Nylon meshes (ELKO) were placed on a slide and 30 µL of the test substance was applied. A negative control, 30 µL of sterile, Calcium and Magnesium Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS) (Gibco), was tested concurrently. The slides holding the treated meshes were placed into a covered petri dish and incubated at standard culture conditions for 60±1 minutes. Using a microscope, each mesh was checked after 60±1 minutes of exposure to assess any interaction between the test substance and the mesh.

The test substance was not observed to interact with the nylon mesh, and therefore a nylon mesh was used to aid in the spreading of the test substance after dosing the EpiDerm™ tissues.

Assessment of Direct Test Substance Reduction of MTT

The test substance was added to a 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) containing 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. Approximately 30 µL of the test substance were added to 1 mL of the MTT solution and the mixture was incubated in the dark at standard culture

conditions for one to three hours. A negative control, 30 µL of sterile, Calcium and Magnesium Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS), was tested concurrently. If the MTT solution color turned blue/purple, the test substance was presumed to have reduced the MTT.

The test substance was not observed to directly reduce MTT in the absence of viable cells.

Assessment of Colored or Staining Materials

The ability of the test substance to interfere with the photometric MTT measurement was assessed (*i.e.*, its ability to absorb light significantly at the wavelength used for the MTT determination.). Approximately 30 µL of the test substance were added to 2.0 mL isopropanol in a 6-well plate and placed on an orbital shaker for 2-3 hours at room temperature. The test substance-isopropanol mixture was transferred in duplicate (at 200 µL) to a 96-well plate. Two blank samples of the isopropanol were transferred to the 96-well plate and the optical density (OD) was measured using a plate reader at a wavelength of 570 nm.

The corrected absorbance of the test substance sample was determined by subtracting the mean isopropanol blank OD value from the OD value of the test substance sample. The OD of the test substance-mixture was <0.08, therefore the test substance was not considered to have potential interference with the MTT measurement.

pH Determination

The pH of the test substance was measured using pH paper (EMD Millipore Corporation). Initially, the test substance was added to pH paper with a 0-14 pH range in 1.0 pH unit increments to approximate a narrow pH range. Next, the test substance was added to pH paper with a narrower range of 5.0-10.0 pH units with 0.5 pH unit increments, to obtain a more accurate pH value. The pH value obtained from the narrower range pH paper is presented in Table 1.

Controls

The definitive assay included a negative control and a positive control. The negative control was 30 µL of sterile, CMF-DPBS and the positive control was 30 µL of 5% Sodium Dodecyl Sulfate (SDS). Both the positive and negative controls were tested in triplicate, and at the same exposure time as the test substances (60±1 minutes).

Skin Irritation Test (SIT) Definitive Assay

The test substance was tested in one valid definitive trial.

After the overnight incubation for 18±3 hours, the 6-well plates containing the EpiDerm™ tissues were removed from the incubator and placed at room temperature for at least 5 minutes prior to dosing.

The EpiDerm™ tissues were treated in triplicate with the test substance for 60±1 minutes. Thirty microliters of the test substance was applied to each of three tissues at 1 minute intervals per tissue. A nylon mesh was placed gently over the dose to spread the test substance. If necessary, the mesh was gently pressed down to assure even spreading. The EpiDerm™ tissues were tested in triplicate with the positive or negative control for 60±1 minutes. Thirty microliters of each control were applied to each of three tissues at 1 minute intervals per tissue. Immediately after control administration onto the tissue, a nylon mesh was placed gently over the dose to spread the negative and positive controls. The plates with dosed tissues were kept in the laminar flow hood until the last tissue was dosed. After the last tissue was dosed, all of plates were transferred to the incubator for 35±1 minutes at standard culture conditions. After 35±1 minutes, all of the plates were removed from the incubator, placed into the laminar flow hood and kept at room temperature until the exposure period was completed for the first dosed tissue.

After 60±1 minutes of test or control substance exposure, the tissues were rinsed with sterile, CMF-DPBS by filling and emptying the tissue insert 15 times. A stream of CMF-DPBS was directed onto the tissue surface. For the test and control substances where a mesh was used, the mesh was carefully removed with forceps (if necessary) after the 5th rinse. After the removal of the mesh, the rinsing procedure of the tissue continued for 10 times. After the 15th rinse, each of the 3 inserts per treatment group (test substance, positive and negative control) was completely submerged, gently swirled, and rinse media dumped in a beaker containing approximately 150 mL of CMF-DPBS and specifically assigned for each treatment group; this procedure was repeated three times for each insert of each treatment group. Finally, the tissues were rinsed once more on the inside and outside of the tissue insert with sterile CMF-DPBS from the wash bottle, and the excess CMF-DPBS was decanted. The bottoms of the tissue inserts were blotted on sterile paper towels and the inserts were transferred to new 6-well plates containing 0.9 mL of fresh warmed (to 37°C) Maintenance Medium. The tissue surface was carefully blotted with sterile cotton-tipped applicators to remove any excess moisture, and the tissue surface was visually observed for residual test substance using a dissecting scope. The tissues were then placed into the incubator at standard culture conditions for a post-treatment expression incubation of 42±2 hours. After an initial 24±1 hours of incubation, the 6-well plates were removed from the incubator and the tissues were transferred into new 6-well plates pre-filled with 0.9 mL fresh Maintenance Medium warmed to approximately 37°C. The tissues were placed back into the incubator at standard culture conditions for an additional 18±1 hours for the remainder of the 42±2 hour post-treatment expression incubation.

MTT Preparation

A 10X stock of MTT prepared in PBS (filtered at time of batch preparation) was thawed and diluted in warm MTT Addition Medium to produce a 1.0 mg/mL solution no more than two hours before use. Three hundred microliters of the MTT solution were added to each designated well of a pre-labeled 24-well plate.

After the total 42 ± 2 hours post-exposure expression incubation, the 6-well plates were removed from the incubator. Each tissue was blotted on a sterile paper towel and transferred to

an appropriate well containing 0.3 mL of MTT solution. The 24-well MTT plates were incubated at standard culture conditions for 3±0.1 hours.

After the 3±0.1 hours incubation, the EpiDerm™ tissues were submerged, gently swirled, and rinse media decanted in a beaker containing approximately 150 mL of CMF-DPBS three times. The tissue was then blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabelled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plate was covered with parafilm and shaken for at least 2 hours at room temperature to extract the MTT. At the end of the extraction period, the insert was gently agitated up and down in its extractant well. The tissues were pierced with forceps to allow the extract to flow back into the well from which the insert was removed, and the cell culture inserts were discarded. The extract solution was mixed (homogenized by pipetting up and down three times) and two x 200 µL aliquots were transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol were added to the wells designated as blanks. The absorbance at 570 nm (OD_{570}) of each well was measured with a Molecular Devices Vmax plate reader with the AUTOMIX function selected.

Presentation of Data

The mean OD_{570} value of the blank wells was calculated. Individual blank-corrected OD_{570} values for each of the duplicate aliquots of each test substance or control tissue was determined by subtracting the mean OD_{570} value of the blank wells from their individual OD_{570} values. All calculations were performed using an Excel spreadsheet.

$$\text{Corrected Individual } OD_{570} = \text{Individual } OD_{570} - \text{mean Blank } OD_{570}$$

Mean corrected OD_{570} values were calculated for each individual test substance and control tissue from the duplicate aliquots. The group mean of the corrected OD_{570} values for the negative controls were calculated.

The following % of Control viability calculations were made for each individual tissue:

$$\% \text{ Viability} = \frac{\text{Mean Corrected } OD_{570} \text{ of Aliquots of Individual Test Substance or Control Tissue}}{\text{Corrected Group Mean } OD_{570} \text{ of Negative Control}} \times 100$$

The individual % of Control viability values were tabulated for each individual tissue. Mean (and standard deviation) viability values were calculated for the test substance and control treatment groups. Finally, the mean viability values were plotted on a bar graph (with 1 standard deviation error bar) for the test substance and positive control.

Evaluation of Test Results

The following Prediction Model has been endorsed by the European Centre for the Validation of Alternative Methods (ECVAM) Scientific Advisory Committee (ESAC) for the prediction of skin irritation. A test substance was predicted to be an irritant (GHS Category 1 or 2) if the mean relative viability of the three treated tissues is less than or equal to 50% of the mean viability of the negative control.

In Vitro Result	*In Vivo* Prediction	GHS Category
mean tissue viability ≤ 50%	Irritant (I)	Category 1 or 2*
mean tissue viability > 50%	Non-irritant (NI)	No Category

*- *Additional testing (e.g., reconstructed human epidermis (RhE) OECD TG 431) in a tiered testing approach would be warranted to discriminate between GHS Category 1 and GHS Category 2.*

Criteria for a Valid Test

The assay was accepted when the following criteria were met: 1) the positive control resulted in a mean tissue viability ≤ 20%, 2) the mean OD_{570} value of the negative control tissues was ≥ 0.8 and < 2.8, and 3) the standard deviations of the positive and negative control calculated from individual percent tissue viabilities of the three identically treated replicates were < 18%.

RESULTS AND DISCUSSION

The test substance was tested using the EpiDerm™ Skin Model for the Skin Irritation Test (SIT). Table 1 summarizes the results of the Skin Irritation Test (SIT) for the test substance and the positive control. The raw and analyzed data for the test substance and the negative and positive controls are included in Appendix B. The mean OD_{570} of the negative control, CMF-DPBS, was 1.683. The mean viability of the positive control, 5% SDS, was 3.34%. The standard deviation calculated from individual percent tissue viabilities of the 3 identically treated replicates was <18% for the positive control and negative control. Since the acceptance criteria were met, the assay was considered valid.

The test substance was not observed to directly reduce MTT in the absence of viable cells.

The test substance was not considered to have potential interference with the MTT measurement (was not considered a colorant).

SIT Results Using the EpiDerm™ Skin Model
Table 1

Assay Date	IIVS Test Substance Number	Sponsor's Designation	Conc.	Mean Viability (%)	pH
28 September 2016	[redacted]	[redacted]	Neat	101.1	5.5
	Positive Control	SDS	5% (w/v)	3.34	NA

NA – Not Applicable

APPENDIX B

EPIDERM™ SIT COLORANT CONTROLS ASSESSMENT

Study No. [redacted]
Plate: 1
Read Date: 09/26/18 Read By: RP
Raw Data: Optical Density (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			0.068	0.068								
C												
D												
E												
F												
G												0.036
H												0.035

Plate Map:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			T1OC1	T1OC1								
C												
D												
E												
F												
G												BLNK
H												BLNK

Average Blank OD: 0.037

	Trial	Map Prefix	Average OD_{550} Values	Corrected OD_{550} (Avg. TA - Avg. Blank OD)	Possible MTT Interference? (Corrected OD550>0.08)
Test Article [redacted]	B1	T1OC1	0.067	0.031	No

Negative control (NC)	Negative Control (NC)
Positive control (PC)	Positive Control (PC)
Test Chemical	[redacted]

Dosing Date:	28-Sep-16

96 WELL PLATE MAP

PLATE 1

	1	2	3	4	5	6	7	8	9	10	11	12	
A	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK							
B	NC	PC		[redacted]									Tissue1
C	NC	PC											
D	NC	PC		[redacted]									Tissue2
E	NC	PC											
F	NC	PC		[redacted]									Tissue3
G	NC	PC											
H													

RAW DATA

PLATE 1

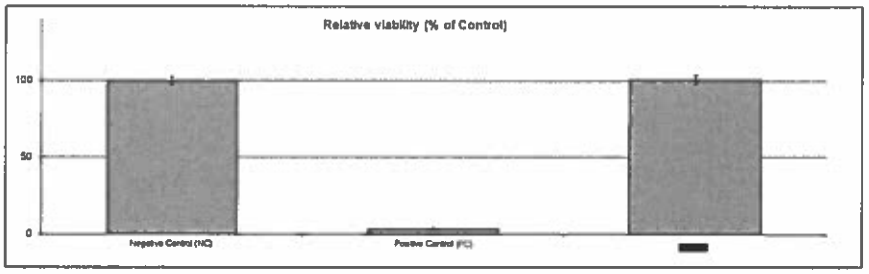
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.036	0.033	0.034	0.036	0.035	0.036							
B	1.749	0.094		1.810									Tissue1
C	1.778	0.090		1.748									
D	1.682	0.099		1.649									Tissue2
E	1.667	0.093		1.760									
F	1.735	0.081		1.739									Tissue3
G	1.695	0.090		1.707									
H													

COMMENTS

RESULTS

Dosing Date	26-Sep-18

Blank	0.036
	0.033
	0.034
	0.036
	0.035
	0.036
Mean	0.035



Chemical Code	Tissue n	OD Alq. 1	OD Alq. 2	Blank corrected OD data Alq. 1	Blank corrected OD data Alq. 2	mean of OD (aliquots)	% of viability
Negative Control (NC)	1	1.749	1.778	1.714	1.743	1.729	102.7
	2	1.682	1.667	1.647	1.632	1.640	97.4
	3	1.735	1.695	1.700	1.660	1.680	99.8
Positive Control (PC)	1	0.094	0.090	0.059	0.055	0.057	3.39
	2	0.088	0.093	0.054	0.059	0.061	3.63
	3	0.091	0.090	0.048	0.048	0.051	3.00
[redacted]	1	1.810	1.748	1.775	1.713	1.744	103.6
	2	1.648	1.780	1.614	1.725	1.670	99.2
	3	1.739	1.707	1.704	1.672	1.688	100.3

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
Negative Control (NC)	1.693	0.045	100.6	2.65	2.65
Positive Control (PC)	0.056	0.005	3.34	0.31	9.44
[redacted]	1.701	0.039	101.1	2.31	2.28

Classification

Negative Control (NC)	Nil
Positive Control (PC)	I
[redacted]	NE

Negative control (NC)	Negative Control (NC)
Positive control (PC)	Positive Control (PC)
Test Chemical	[redacted]

charles river

FINAL REPORT

Laboratory Project ID [redacted]

[redacted]

Salmonella-E. Coli/Mammalian Microsome Reverse Mutation Assay

Sodium Mannose Phosphate

Test Guideline: OECD 471

Author: Sara B. Hurtado, PhD

SPONSOR:

[redacted]

PERFORMING LABORATORY:
Charles River Laboratories Skokie, LLC
8025 Lamon Avenue
Skokie, IL 60077
United States

22 February 2017

Total Number of Pages: 49

COMPLIANCE STATEMENT

This study, designated [redacted] was conducted in compliance with U.S. Environmental Protection Agency's Toxic Substances Control Act (EPA TSCA) Good Laboratory Practice (GLP) standards; CFR Title 40, Part 792; the Charles River SOPs; and the protocol as approved by the Sponsor with the following exceptions. A Certificate of Analysis for the test substance was provided by the Sponsor (presented in Appendix 2); the characterization analyses were not compliant with GLP standards, but with written laboratory standards. As the analyses were conducted to a specified laboratory standard, this deviation has no impact on the validity or integrity of the study. Also, no analysis of the formulated test substance was conducted concurrently for this study with respect to either test substance concentration or stability. As precipitates and cytotoxicity were not observed during the course of this study, the impact of this exception is unknown. However, to limit the impact, the dosing formulations were prepared by a documented serial dilution procedure from a single stock solution prepared by a single weighing using a balance which was function verified prior to use. Additionally, the samples were prepared fresh on each assay date.

This study was in general accordance with the OECD Guideline No. 471. There were no known circumstances or errors that affected the quality or integrity of the study. The Study Director certifies that the evaluation of the data for this test substance represents an appropriate conclusion and is an accurate representation of the raw data.

Study Director: Sara B. Hurtado Date: 22Feb2017
Signature

Typed Name of Signer: Sara B Hurtado

Typed Name of Laboratory: Charles River Laboratories

Sponsor: _____ Date: _____
Signature

Typed Name of Signer: _____

Typed Name of Laboratory: _____

Submitter: _____ Date: _____
Signature

Typed Name of Signer: _____

Typed Name of Company: _____

[redacted]

QUALITY ASSURANCE STATEMENT

Date(s) of Inspection	Critical Phase Inspected (Study-Based)	Date Observations Reported to:	
		Charles River Skokie Study Director	Charles River Skokie Management
20-Sep-2016	Dosing of initial assay	21-Sep-2016	21-Sep-2016
28-Sep-2016	Final protocol	30-Sep-2016	30-Sep-2016
29-Sep-2016	Final protocol amendment 1	30-Sep-2016	30-Sep-2016
01-Nov-2016	Final protocol amendment 2	01-Nov-2016	01-Nov-2016
01-Dec-2016	Raw data	02-Dec-2016	02-Dec-2016
01-Dec-2016	Report	02-Dec-2016	02-Dec-2016

This study was inspected in accordance with the applicable GLP Regulations, the Charles River SOPs, and the protocol as approved by the Sponsor, with the following exceptions. The data located in Appendix 2 (Test Substance Information) were the responsibility of the Sponsor and/or supplier. Quality Assurance observations derived from the inspections during the conduct of the study and from the inspections of the protocol, raw data, and report are documented and have been reported to the Study Director. A biannual internal facility inspection is conducted by the Charles River Quality Assurance Unit. Reports of all Quality Assurance inspections are submitted to management.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol and the Charles River SOPs.

Lucia Podbielski Date: 22 Feb 2017

Lucia Podbielski, BS, RQAP-GLP
Senior Manager, Regulatory Compliance

[redacted]

REPORT APPROVAL

Report Authored and Approved by:

S. B Hurtado Date: 22 Feb 2017

Sara B. Hurtado, PhD
Research Scientist, Genetic and In Vitro Toxicology
Study Director

[redacted]

LIST OF ABBREVIATIONS

2AA	2-Aminoanthracene
2NF	2-Nitrofluorene
CFR	Code of Federal Regulations
DMSO	dimethylsulfoxide
EPA	U.S. Environmental Protection Agency
GLP	Good Laboratory Practice
ICR	ICR-191 Acridine
KCl	potassium chloride
$MgCl_2$	magnesium chloride
NADP	nicotinamide adenine dinucleotide phosphate
NQNO	4-Nitroquinoline-N-Oxide
OECD	Organisation for Economic Co-operation and Development
S9	9000 × g liver supernatant fraction
SA	sodium azide
SD	Standard deviation
SOP	standard operating procedure
TSCA	Toxic Substances Control Act

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Table 4.2 Confirmatory Assay: Individual Data of the Preincubation Experiment for [redacted] in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay 49

1. ABSTRACT

[redacted] (lot no. [redacted]) was evaluated for mutagenic activity in the in vitro *Salmonella-E. coli*/mammalian microsome reverse mutation assay. Four tester strains of *Salmonella typhimurium* (TA1537, TA98, TA100, and TA1535) and 1 *Escherichia coli* strain (WP2 *uvrA*) were used for mutagenicity testing. [redacted] was prepared as a stock formulation in sterile water at concentrations of 50 mg/mL for each assay. Mutagenicity testing was performed in triplicate at each concentration with and without an Aroclor™ 1254-induced rat liver S9 metabolic activation system.

In the initial assay, [redacted] was tested at 25, 50, 100, 250, 500, 1000, 2500, and 5000 µg/plate using the plate incorporation method. Little or no colony growth was observed in the initial assay; therefore, the assay was repeated as outlined in the initial assay. In the repeat initial assay, precipitates were not observed in any strain either with or without metabolic activation. Cytotoxicity (i.e., reduction in the background lawn and/or > 50% reduction in the mean number of revertant colonies compared to the vehicle control) was not observed in any strain with or without metabolic activation. In the repeat initial assay, strain TA1537 contained contamination and was repeated in a 2nd repeat initial assay. In the 2nd repeat initial assay, no precipitates or cytotoxicity were observed.

Based on the results of the repeat initial assay, [redacted] was tested at 100, 250, 500, 1000, 2500, and 5000 µg/plate using the preincubation method in the confirmatory assay. Precipitates were not observed in any strain either with or without metabolic activation. Cytotoxicity (i.e., reduction in the background lawn and/or > 50% reduction in the mean number of revertant colonies compared to the vehicle control) was not observed in any strain with or without metabolic activation.

In all acceptable assays, criteria for a negative response were met for all tester strains with and without metabolic activation. The data from the vehicle and positive control substances demonstrated the validity and sensitivity of this test system for detecting chemical mutagens with and without metabolic activation.

These data support the conclusion that [redacted] is negative for mutagenic activity in the *Salmonella* strains TA1537, TA98, TA100, and TA1535 and in the *E. coli* strain WP2 *uvrA*, with and without metabolic activation, under the conditions of this assay.

2. INTRODUCTION

[redacted] (lot no. [redacted] was tested for potential mutagenic activity in the in vitro *Salmonella-E. coli*/mammalian microsome reverse mutation assay using the plate incorporation and preincubation methods.[1,2] This assay has been proven to be effective for detecting potential mammalian mutagens and carcinogens.[3,4]

[redacted] sterile water, and the appropriate positive control substances were tested in triplicate using each of 5 tester strains (TA1537, TA98, TA100, TA1535, and WP2 *uvrA*) for mutagenicity testing. The tests were performed both in the presence and absence of an Aroclor™ 1254-induced rat liver S9 homogenate which allows detection of potentially indirect-acting and direct-acting mutagens, respectively.

2.1. Key Study Dates

Date(s)	Event(s)
15 Sep 2016	Study initiation
20 Sep 2016	Dosing of initial assay
05 Oct 2016	Dosing of repeat initial assay
02 Nov 2016	Dosing of 2nd repeat initial assay
02 Nov 2016	Dosing of confirmatory assay
04 Nov 2016	Last date of plate scoring

The study protocol is presented in Appendix 1. No deviations from the protocol were known to have occurred during the conduct of this study.

2.2. Key Study Personnel

Sara B. Hurtado, PhD	Study Director
Jose Martinez, BS	Study Scientist
Maxwell Pedre, BS	Study Scientist
Erica Pinkus, BS	Study Scientist
Lucia Podbielski, BS, RQAP-GLP	Quality Assurance
Christopher Farabaugh, PhD	Management
[redacted]	Sponsor Representative

2.3. Charles River Computer Systems

The major computer systems used on this study include, but are not limited to, the following systems. All computerized systems used for data collection during the conduct of this study have been validated (with the exception of Microsoft Office®); when a particular system has not satisfied all requirements, appropriate administration and procedural controls were implemented to assure the quality and integrity of the data.

Text Table 1
Critical Computerized Systems

Program/System	Description
Archive Management System (AMS)	In-house developed application for storage, maintenance, and retrieval of information for archived materials (e.g., lab books, study data, etc.).
InSight® Publisher	Electronic publishing system (output is Adobe Acrobat, PDF).
Master Schedule	Used to maintain the master schedule at the Test Facility
MD5 Checksum Tool, version 3.1	Used to generate and verify MD5 checksums during the final report generation process to create a significant, permanent link between the electronic study report and the signature page.
Microsoft Office® 2013	Used to generate raw data tables, calculate mean and standard deviation, and/or maintain the master schedule at the Test Facility.
ProtoCol 3 Colony Counter Software, version 1.0.2.0	Automated bacterial colony counting software at the Test Facility.
Tempurity	Temperature monitoring system, used as applicable, at the Test Facility.

3. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

3.1. Test Substance

[redacted] lot no. [redacted], supplied by [redacted] was received at ambient conditions and room temperature (19°C to 25°C). The test substance Certificate of Analysis appears in Appendix 2. Following finalization of the report, the Sponsor will be contacted regarding the disposition of the remaining neat test substance.

3.1.1. Test Substance in Vehicle

A stock solution of [redacted] was prepared in sterile water at a concentration of 50 mg/mL on the days of the initial and confirmatory assays. The lower concentrations were prepared by serial dilution with sterile water to attain the appropriate concentration for testing. A correction factor of 7.11 was used in the preparation of all dose formulations to correct for purity. The dosing volume for all assays was 100 µL per plate.

3.2. Vehicle Control Substance

The vehicle control substance for all assays was sterile water, MediaTech, lot no. 04616009.

3.3. Positive Control Substance

For tests without metabolic activation, ICR-191 acridine (0.5 µg/plate) was used as the positive control substance with strain TA1537; 2-nitrofluorene (2.5 µg/plate) was used with strain TA98; sodium azide (1.0 µg/plate) was used with strains TA100 and TA1535; and 4-nitroquinoline-N-oxide (2.0 µg/plate) was used for WP2 *uvrA*. For testing with metabolic

activation, 2-aminoanthracene was used as the positive control substance for all strains: TA1537, TA98, TA100, TA1535 (2.5 µg/plate), and WP2 *uvrA* (10 µg/plate).

The source and lots of the positive control substances are as follows:

2-Aminoanthracene, Sigma-Aldrich, Inc. lot #STBD3302V
ICR-191 acridine, Sigma-Aldrich, Inc. lot # SLBH1792V
2-Nitrofluorene, Sigma-Aldrich, Inc. lot #S43858V
Sodium Azide, Sigma-Aldrich, Inc. lot #MKBX7529V
4-Nitroquinoline-N-oxide, Supelco lot # LC15235V

All positive control substance articles were dissolved in DMSO at the appropriate concentrations and stored at -60°C to -80°C for up to 6 months.

3.4. Indicator Organisms

The *Salmonella* strains TA1537, TA98, TA100, and TA1535 and the *E. coli* strain WP2 *uvrA* were originally purchased from Molecular Toxicology, Inc. (Boone, NC).

Strains TA1537 and TA98 detect frameshift mutations, whereas strains TA100, TA1535, and WP2 *uvrA* detect base pair substitutions. WP2 *uvrA* contains the ochre mutation which enables this strain to detect a variety of oxidative mutagens, some of which are not detected or are detected poorly by the other tester strains.[5] In addition to having mutations in the histidine operon, the *Salmonella* tester strains have a deep rough mutation (*rfa*-) that causes the cell wall to be defective in the lipopolysaccharide coat resulting in increased permeability to large molecules. These strains also have a DNA deletion that affects genes involved in biotin synthesis (*bio*-). All tester strains have a DNA deletion that affects repair of ultraviolet light damage (*uvrB*- for the *Salmonella typhimurium* strains and *uvrA*- for the *Escherichia coli* strain), which greatly increases sensitivity in detecting mutagens. Strains TA98 and TA100 contain the R-factor plasmid, pKM101, which confers ampicillin resistance and increased sensitivity to some mutagens over that of other bacterial strains.

The clones used for this assay were stored at -60°C to -80°C until use. Prior to each test day, an inoculum from a frozen clone of each strain was incubated overnight for approximately 8 to 10 hours in Oxoid nutrient broth at 36°C to 38°C with shaking. Optical density readings were performed at 650 nm on all strains to check the level of growth. All cultures gave acceptable absorbance readings (in the range of 0.2 - 0.5) prior to each assay. Viable cell titers were also performed in each assay, and for all strains between 10^7 and 10^9 cells per mL of culture were used.

3.4.1. Test System Identification

The test system identification methods are described in the protocol in Appendix 1.
Study number, tester strain, treatment group, concentration, and the presence or absence of metabolic activation were identified on each test plate.

3.5. Metabolic Activation System

For detection of potential indirect-acting mutagens, the liver metabolic activation system was prepared and used as previously described.[3,6] The Aroclor™ 1254-induced rat liver S9 fraction (lot nos. 3605 and 3649) was purchased from Molecular Toxicology, Inc. (Boone, NC).

The metabolic activation mixture was prepared fresh on each day of testing and maintained on ice throughout the assay.

The S9 mixture (7.5% v/v) for metabolic activation consists of the following in a total volume of 10 mL:

0.75 mL of S9 fraction
0.20 mL of $MgCl_2$ (0.4M) - KCl (1.65M)
0.05 mL of glucose-6-phosphate (1M)
0.40 mL of nicotinamide adenine dinucleotide phosphate (NADP) (0.1M)
5.00 mL of phosphate buffered saline
3.60 mL of sterile distilled deionized water

3.6. Mutagenicity Assay

All 4 *Salmonella typhimurium* strains used were histidine auxotrophs. The *Escherichia coli* strain used was a tryptophan auxotroph. When these cells are grown on minimal medium plates containing a trace amount of histidine or tryptophan, only those cells that have reverted to histidine prototrophy (*his*+) or tryptophan prototrophy (*trp*+) are able to grow and form colonies.[4] Because DNA replication is usually required for mutagenesis to occur, the small amount of histidine or tryptophan allows all the bacteria on the plate to undergo a few cycles of cell division. The bacteria that revert to (*his*+) or (*trp*+) are easily seen as colonies against the slight background growth. The spontaneous mutation (or reversion) frequency of each strain is relatively constant, but, when a known mutagen (positive control substance) is added to the test system, the mutation frequency is typically increased from 2 to over 100 times.

3.6.1. Plate Incorporation Procedure

Sterile 12 × 75 mm test tubes were placed in heating blocks at approximately 46°C and the following items were added in a stepwise manner for each concentration of test or control substance:

1) 2.00 mL of top agar, supplemented with 10% of a 0.5mM histidine/biotin/tryptophan solution
2) 0.10 mL of indicator organisms (overnight culture)
3) 0.10 mL of vehicle control substance or test substance, or 0.05 mL of positive control substance agent
4) 0.50 mL of metabolic activation mixture or phosphate-buffered saline, for tests with or without S9, respectively

The tube contents were mixed gently and then poured onto minimal glucose plates. The top agar was allowed to set and the plates were incubated at 36°C to 38°C for 2 days.

3.6.2. Preincubation Procedure

To sterile tubes, the following items were added in a step-wise manner and incubated with shaking (100-150 rpm) at 36°C to 38°C for 20 minutes:

1) 0.10 mL vehicle control substance or test substance, or 0.050 mL of positive control substance
2) 0.50 mL of metabolic activation mixture or phosphate-buffered saline, for tests with or without S9, respectively
3) 0.10 mL of indicator organisms (overnight culture)

After the pre-incubation period, the tubes were removed from the shaker incubator. Two mL of top agar, supplemented with 10% of a 0.5mM histidine/biotin/tryptophan solution, was then added and the mixture was vortexed gently and then poured onto minimal glucose agar plates. After the agar set, the plates were incubated at 36°C to 38°C for 2 days.

3.7. Statistical Procedures

For each concentration level and for each condition, the mean revertant count and standard deviation (SD) were determined.

Mean = $\bar{x} = \frac{\sum x_N}{N}$ Where x = the individual values and N = the number of values.

Standard Deviation = SD = $\sqrt{\frac{\sum (x - \bar{x})^2}{N - 1}}$

3.8. Data Acquisition

All equipment used in this study were documented in the raw data and used in accordance with applicable Charles River SOPs. A list of computer systems used in this study can be found in Section 2.3 (Charles River Computer Systems).

4. INTERPRETATION OF RESULTS

4.1. Criteria for Acceptability and Interpretation of Assay

The following criteria were used as guidelines in evaluating the activity of a test substance in this system. These criteria are not absolute. The Study Director, based on sound scientific judgment, may take additional factors into consideration when determining the final test results.

4.1.1. Assay Acceptance Criteria

The vehicle and positive control substance plates for the current study were compared against revertant count ranges that are found in historical data ranges. Historical data are listed in Appendix 3.

4.2. Criteria for a Positive Response

The test substance was considered positive for mutagenicity if it induced an increase of revertants per plate with increasing concentration. The increases should be at least 2 times the vehicle control substance background frequency for strains with high spontaneous levels (i.e., TA100) and 3 times for those with low spontaneous levels (TA1537, TA98, TA1535, and WP2 *uvrA*). These increases should be seen in at least 2 or more successive concentrations or the response should be repeatable at a single concentration.

4.3. Criteria for a Negative Response

The test substance was considered to be negative for inducing mutagenicity if it did not induce a response which fulfills the criteria for a positive response.

4.4. Criteria for an Equivocal Response

Cases which did not clearly fit into the positive or negative criteria may be judged equivocal. In these cases the Study Director, based on sound scientific judgment, may take additional factors into consideration in evaluating the test results.

5. RESULTS AND DISCUSSION

The data presented in Section 9. (Summary Tables) represent the mean values and standard deviations (SD) for the results of the initial and confirmatory assays. The total number of revertant colonies for each individual plate can be found in Appendix 4.

For each assay, [redacted] was prepared as a solution in sterile water at a stock concentration of 50 mg/mL on the day of the assay. Testing was performed with and without a rat liver homogenate metabolic activation system using the plate incorporation and preincubation methods. In each acceptable assay, the means of the vehicle control substance data were comparable to the historical data. The means of all positive control substance data were at least 3-fold greater than the means of the vehicle control substance data and comparable to the historical data with the following exception. The positive control response for TA98 without metabolic activation in the confirmatory assay was greater than the historical range. As this represents a more robust response, there is no impact to the quality or integrity of the data.

5.1. Ames Assay Results

5.1.1. Initial Assay

The results of the repeat and 2nd repeat initial plate incorporation assay are shown in Section 9. (Summary Tables), Table 1 and Table 4.1 (Individual Data).

The concentrations tested in the initial assay were 25, 50, 100, 250, 500, 1000, 2500, and 5000 µg/plate. No colony growth was observed in the initial assay; therefore, the assay was repeated as outlined in the initial assay. In the repeat initial assay, precipitates were not observed in any strain either with or without metabolic activation. Cytotoxicity (i.e., reduction in the background lawn and/or > 50% reduction in the mean number of revertant colonies compared to the vehicle control) was not observed in any strain with or without metabolic activation. In the repeat initial assay strain TA1537 was contaminated and was therefore not evaluated for mutagenicity. Strain TA1537 was repeated in a 2nd repeat initial assay both with and without

metabolic activation. In the 2nd repeat initial assay, no precipitates or cytotoxicity were observed with or without metabolic activation. Criteria for a negative response were met for all tester strains with and without metabolic activation.

5.1.2. Confirmatory Assay

The results of the confirmatory preincubation assay are shown in Section 9. (Summary Tables), Table 2 and Table 4.2 (Individual Data).

The concentrations tested in the confirmatory assay were 100, 250, 500, 1000, 2500, and 5000 µg/plate using the preincubation method. Precipitates were not observed in any strain either with or without metabolic activation. Cytotoxicity (i.e., reduction in the background lawn and/or > 50% reduction in the mean number of revertant colonies compared to the vehicle control) was not observed in any strain with or without metabolic activation. Criteria for a negative response were met for all tester strains with and without metabolic activation.

6. CONCLUSIONS

The data from the vehicle and positive control substances demonstrated the validity and sensitivity of this test system for detecting chemical mutagens with and without metabolic activation.

Mean increases in the number of revertant colonies indicative of a positive response were not observed with [redacted] (lot no. [redacted]) in the *Salmonella* strains TA1537, TA98, TA100, and TA1535 and in the *E. coli* strain WP2 *uvrA* with and without metabolic activation under the conditions of this assay. Therefore, [redacted] is considered to be negative for inducing mutagenicity in this assay.

7. REFERENCES

1 Kier LD, Brusick DJ, Auletta AE, et al. The Salmonella typhimurium/mammalian microsomal assay. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat Res.* 1986;168:69-240. Erratum in *Mutat Res.* 1986;185:320.

2 Gatehouse D, Haworth S, Cebula T, et al. Recommendations for the performance of bacterial mutation assays. Mutat Res. 1994;312:217-233

3 Ames BN, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutat Res.* 1975;31(6):347-364.

4 Green MHL, Muriel WJ. Mutagen testing using trp+ reversion in Escherichia coli. *Mutat Res.* 1976;38:3-32.

5 Brusick DJ, Simmon VF, Rosenkranz HS, Ray VA, Stafford RS. An evaluation of the Escherichia coli WP2 and WP2 uvrA reverse mutation assay. *Mutat Res.* 1980;76:169-190.

6 Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. *Mutat Res.* 1983;113:173-215.

8. DATA RETENTION

Data and reports from this study are the property of the Sponsor. Unless otherwise requested by the sponsor, following completion of the final study report, all raw data and reports will be maintained at the originating facility in accordance with applicable SOPs. All raw data and reports generated by Charles River Skokie will be maintained for a period of at least 6 months. The Sponsor will be notified at the end of the storage period regarding the disposition of the records.

9. SUMMARY TABLES

Table 1
Repeat and 2nd Repeat Initial Assay: Summary Results of Plate Incorporation Experiment for [redacted] in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD) [a]

Treatment Group	µg/plate	TA1537[b]		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
Sterile Water	100 µL	3	(3)	15	(7)	142	(22)	8	(3)	40	(8)
ICR	0.5	138	(19)								
2NF	2.5			1187	(167)						
SA	1.0					615	(28)	432	(19)		
NQNO	2.0									907	(29)
[redacted]	25	5	(3)	10	(2)	139	(20)	10	(2)	40	(2)
	50	4	(2)	21	(6)	142	(11)	9	(7)	43	(2)
	100	4	(2)	13	(1)	124	(13)	9	(2)	43	(4)
	250	7	(2)	13	(2)	124	(14)	11	(3)	40	(5)
	500	5	(2)	21	(5)	125	(18)	10	(2)	45	(7)
	1000	5	(2)	22	(6)	127	(6)	11	(3)	35	(16)
	2500	5	(4)	18	(9)	130	(5)	10	(2)	48	(17)
	5000	5	(2)	14	(4)	136	(5)	11	(4)	61	(10)
WITH ACTIVATION											
Sterile Water	100 µL	4	(2)	26	(6)	139	(8)	8	(1)	60	(2)
2AA	2.5	99	(18)	2107	(77)	1151	(121)	157	(17)		
2AA	10.0									242	(49)
[redacted]	25	4	(3)	22	(5)	124	(9)	5	(4)	49	(6)
	50	2	(0)	21	(2)	175	(26)	5	(1)	57	(13)
	100	6	(4)	26	(2)	131	(19)	13	(5)	53	(7)
	250	7	(2)	22	(3)	154	(37)	11	(2)	53	(11)
	500	7	(2)	21	(8)	132	(22)	7	(3)	70	(18)
	1000	4	(2)	24	(7)	155	(6)	12	(4)	56	(26)
	2500	7	(0)	26	(3)	151	(7)	9	(2)	59	(20)
	5000	3	(3)	19	(1)	131	(9)	12	(4)	41	(13)

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
Note: All plates had a confluent background lawn
[a] Calculated from triplicate plates
[b] Data presented from the 2nd Repeat Initial Assay

Table 2
Confirmatory Assay: Summary Results of Preincubation Experiment for [redacted] in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD) [a]

Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
Sterile Water	100 µL	14	(3)	18	(6)	66	(13)	8	(1)	57	(17)
ICR	0.5	423	(46)								
2NF	2.5			1284	(180)						
SA	1.0					325	(35)	477	(70)		
NQNO	2.0									1896	(64)
[redacted]	100	11	(4)	14	(6)	56	(11)	8	(1)	56	(7)
	250	7	(8)	11	(3)	60	(9)	7	(4)	60	(6)
	500	7	(2)	13	(2)	68	(5)	9	(1)	61	(13)
	1000	14	(7)	8	(4)	65	(10)	8	(2)	57	(8)
	2500	9	(1)	8	(1)	69	(10)	12	(7)	58	(3)
	5000	13	(4)	13	(1)	82	(13)	8	(2)	62	(1)
WITH ACTIVATION											
Sterile Water	100 µL	13	(3)	15	(3)	71	(9)	12	(3)	48	(9)
2AA	2.5	136	(29)	2407	(379)	1443	(78)	191	(15)		
2AA	10.0									330	(4)
[redacted]	100	10	(3)	17	(3)	59	(7)	9	(2)	63	(14)
	250	7	(2)	16	(5)	77	(4)	8	(4)	50	(15)
	500	13	(2)	16	(6)	63	(6)	13	(4)	53	(4)
	1000	9	(3)	24	(3)	68	(9)	9	(3)	51	(8)
	2500	11	(7)	19	(3)	83	(11)	8	(4)	59	(15)
	5000	12	(4)	20	(2)	65	(11)	11	(3)	67	(5)

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
Note: All plates had a confluent background lawn
[a] Calculated from triplicate plates

Study Number: [redacted]

FINAL REPORT

Study Title

KeratinoSens™ assay: Test report on D-MANNOSE 6-PHOSPHATE

INCI: Sodium Mannose Phosphate

Author(s): [redacted]

Test facility [redacted]

Study Number: [redacted]

Sponsor [redacted]

Report Issued: 9.11.2016

Study Number: [redacted]

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Figure 4. Dose response curves for D-MANNOSE 6-PHOSPHATE. Average and standard deviation of al three repetitions.

Figure 5. Dose response curve for the positive control cinnamic aldehyde.

Figure 6. Historical results for luciferase induction by the positive control in the test laboratory.

Study Number: [redacted]

Approval Section

KeratinoSens™ test report on D-MANNOSE 6-PHOSPHATE

[redacted] attests to the content of the report and personally supervised the experimental proceedings of the study.

The study was conducted in the [redacted] between 29.8.2016 and 8.9.2016.

The study described in this report has been correctly reported and was conducted in compliance with: ECVAM. (2014), DB-ALM protocol 155: KeratinoSens™ protocol [1] and OECD test guideline 442d..

The sponsor is responsible for all test substance information.

Analysis of stability, homogeneity and concentration of the test substance under test conditions was not performed as part of this study.

Study Director: [redacted]

Signature: _____ Date: 15.11.2016

[redacted]

Sponsor:

Signature: [redacted] Date: 15.11.2016

Study Number: [redacted]

Responsible Personnel

Study Management:

[redacted]

Contributing scientist:

[redacted]

Archive statement

All primary data are recorded and permanently archived within the [redacted]. The data of this study are stored under experiment number [redacted].

Study Number: [redacted]

Summary

Introduction

The KeratinoSens™ assay is a cell-based assay with a reporter cell line to detect potential skin sensitizers by their ability to induce the Nrf2-response.

This assay has been validated for a broad range of low-molecular weight chemicals and it was found to respond to skin sensitizers from a broad range of so called applicability domains, i.e. chemicals reacting with proteins by different mechanisms. It was validated by ECVAM and proposed to be used as part of an integrated approach for testing and assessment (IATA).

Experimental

The test substance D-MANNOSE 6-PHOSPHATE was dissolved in cell culture medium containing DMSO and tested according to the standard operating procedure of the KeratinoSens™ assay at 12 concentrations in three repetitions, each time in three replicates. After 48 h incubation time, luciferase induction and cellular viability at each of the concentrations were determined.

Results

D-MANNOSE 6-PHOSPHATE was non-toxic to the KeratinoSens™ cells. In two of three repetitions, it did not induce the luciferase gene above a threshold of 1.5, while a weak induction at top dose was observed in one repetition. It is therefore considered a non-sensitizer according to the prediction model of the KeratinoSens™ assay.

1. Introduction

The Cosmetic Directive is phasing out of animal testing for new products. This mandate has led to the development of potential alternative assays to screen for sensitizing potential. These new assays were tested against a large list of reference chemicals in order to prove their applicability domain. The KeratinoSens™ assay is a cell-based assay with a reporter cell line to detect potential skin sensitizers by their ability to induce the Nrf2-response [2].

This assay has been validated for a broad range of low-molecular weight chemicals [2-8] and it was found to respond to skin sensitizers from a broad range of so called applicability domains, i.e. chemicals reacting with proteins by different mechanisms.

The assay underwent validation at the European Centre for the Validation of Alternative Methods (ECVAM) [9] and an OECD test guideline was adopted (OECD TG 442d). The protocol was published as DB-ALM protocol 155 [1]. The assay was proposed by ECVAM [9] to be to be used as part of an integrated approach for testing and assessment (IATA).

2. Test and Reference Substances

2.1. Test substance specification and solubility in vehicles

Table 1 gives specifications for the test substance, while Table 2 and 3 summarize specifications of positive control and negative (solvent) control.

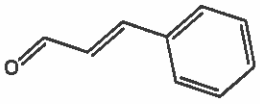
The test substance was freely soluble in cell culture medium containing 4 mM DMSO. This solvent was thus used for the preparation of a stock solution with 0.4% of the test item.

Table 1. Test Substance specification.

Trivial name	:	D-Mannose 6-Phosphate
Chemical name	:	D-Mannose 6-Phosphate
Molecular weight	:	Mixture
Molecular formula	:	Mixture
Purity	:	INCI Name: Aqua, Sodium Mannose Phosphate, Mannose Composition/information on ingredients : Water (>80%) D-Mannose-6-phosphate sodium salt (CAS 70442-25-0): 13.5 - 14.5% D-Mannose (CAS 3458-28-4): 5.0 -6.0%
Supplier	:	[redacted]
Product code	:	[redacted]
CAS number	:	CAS 70442-25-0 and CAS 3458-28-4
EC number	:	n.a.
Expiration date	:	21.7.2017
Batch number	:	[redacted]
Physical form	:	Liquid
Concentrations tested	:	12 binary dilutions from 1000 ppm to 0.49 ppm
Storage conditions	:	4°C

2.2. Positive reference compound

Table 2. Positive control specification.

Trivial name	:	Cinnamic aldehyde
Chemical structure	:	
Chemical name	:	Trans-Cinnamaldehyde
Molecular weight	:	132.16
Molecular formula	:	C_9H_8O
Purity	:	>99%
Supplier	:	Aldrich
Product code	:	239968
CAS number	:	104-55-2 / 14371-01-9
EC number	:	203-213-9
Batch number	:	STBB9109V / 101121271
Physical form	:	Liquid
DMSO solubility	:	Freely soluble at 200 mM
Water solubility	:	1.1 g/L (20 °C)
Treatment prior to testing	:	None
Concentrations tested	:	64 µM, 32 µM, 16 µM, 8 µM, 4 µM
Storage conditions	:	4°C

2.3. Negative (vehicle) control

Table 3. Negative (vehicle) control specification.

Trivial name	:	Dimethylsulfoxide
Chemical structure	:	
Molecular weight	:	78.13
Molecular formula	:	C_2H_6OS
Purity	:	>99.5%
Supplier	:	SIGMA - Aldrich
Product code	:	D5879
CAS number	:	67-68-5
Batch number	:	BCBB8306
EC number	:	200-664-3
Physical form	:	liquid
Concentrations tested	:	1%
Storage conditions	:	Ambient Temperature

2.4 . Test reagents

All test reagents were sourced as indicated in the standard operating procedure. Luciferin was sourced from Promega.

The Luciferase substrate was prepared according to the following recipe: 20 mM Tricine; 2.67 mM $MgSO_4$; 0.1 mM EDTA; 33.3 mM DTT; 270 µM Coenzyme A; 470 µM Luciferin; 530 µM ATP; pH 7.8

3. Experimental Procedures

DMSO (i.e. the standard solvent of the SOP) could be used for preparation of test solutions. The test was run according to the final validated SOP published by ECVAM (Dbalm protocol 155) [1].

BASIS OF THE METHOD

The only feature all skin sensitizers have in common is their intrinsic electrophilicity or their potential to be metabolically transformed to electrophilic chemicals. The signaling pathway with the repressor protein Keap1(Kelch-like ECH-associated protein 1) and the transcription factor Nrf2 (nuclear factor (erythroid-derived 2)-like 2), which binds to the antioxidant / electrophile response element (ARE / EpRE), is known to respond to electrophilic chemicals and it was found to be a valuable cellular endpoint to detect skin sensitizers *in vitro* [10]. This result was confirmed by independent laboratories [11-16].

EXPERIMENTAL DESCRIPTION

Test System(s):

The KeratinoSens™ cell line is derived from the human keratinocyte culture HaCaT. It contains a stable insertion of a Luciferase gene under the control of the ARE-element of the gene AKR1C2 [2].

The KeratinoSens™ cell line was developed by the testing lab and stored on liquid nitrogen. It was grown in 10 cm petri dishes as described in the SOP to 80% confluency prior to testing for 3 – 4 days.

Cells were counted using a counting chamber and adjusted to the desired density. During seeding into 96-well plates, the cell suspension was gently stirred and cell sedimentation was avoided by repeatedly pipetting up and down to ensure homogeneous distribution of cells.

Basic Procedure:

Cells are grown for 24 h in 96-well plates. The medium is then replaced with medium containing a final level of 1% of the solvent DMSO containing the test substance. The test compound was tested at 12 concentrations in the range from 0.49 to 1000 ppm. **Note:** Routinely chemicals are tested up to 1000 µM or 200 ppm in case there is no defined molecular weight, e.g. in case of mixtures. In this case a maximal concentration of 1000 ppm was selected since the test preparation contains 80% water, thus a final level of 200 ppm active compound was effectively tested, as required by SOP. The ingredient was dissolved at 0.4% in cell culture medium containing 1% FCS and 4% DMSO, and this mixture was further diluted in order to obtain final concentration of DMSO of 1% as required by SOP.

Each test plate contains six wells with the solvent control, 1 well with no cells for background value and 5 wells with a dose response of the positive control cinnamic aldehyde. In each repetition, three parallel replicate plates are run with this same set-up, and a forth parallel plate is prepared for cytotoxicity determination.

Positive control

In each test Cinnamic aldehyde is included as positive control. It is tested in each test plate at five concentrations from 4 – 64 µM.

Endpoint & Endpoint Detection:

Two endpoints are measured: (i) Luciferase induction after a 48 h treatment with test substances and (ii) cytotoxicity as determined with the MTT assay recorded in a parallel plate with the same cell batch and made up with the same dilutions of the test substances.

Luminescence was read in a Promega Glomax Luminometer programmed to

i. add 50 µl of the luciferase substrate to each well,

ii. to then wait for 1 second and

iii. then to integrate the luciferase activity for 2 seconds.

Endpoint Value:

For Luciferase induction the maximal fold-induction over solvent control (I_{max}) and the concentration needed to reach an 1.5-, 2- and 3- fold induction (EC1.5, EC2 and EC3) are calculated. For cytotoxicity the IC50 value is extrapolated.

Data Processing

Data evaluation is automatically performed by a standardized Excel template which forms part of the SOP. The test plates are read by a plate reader, and the generated raw data are directly pasted into this template, and all data processing is performed automatically by this Excel sheet.

For both the MTT and the luciferase data, first the background value recorded in an empty well without added cells is subtracted.

For the MTT data the % viability is then calculated for each well in the test plate in relation to average of the six solvent control wells.

For the luciferase data the average value of the six solvent control wells is set to 1, and for each well in the test plate the fold induction is calculated in relation to this value.

The following parameters are then calculated from these processed raw data:

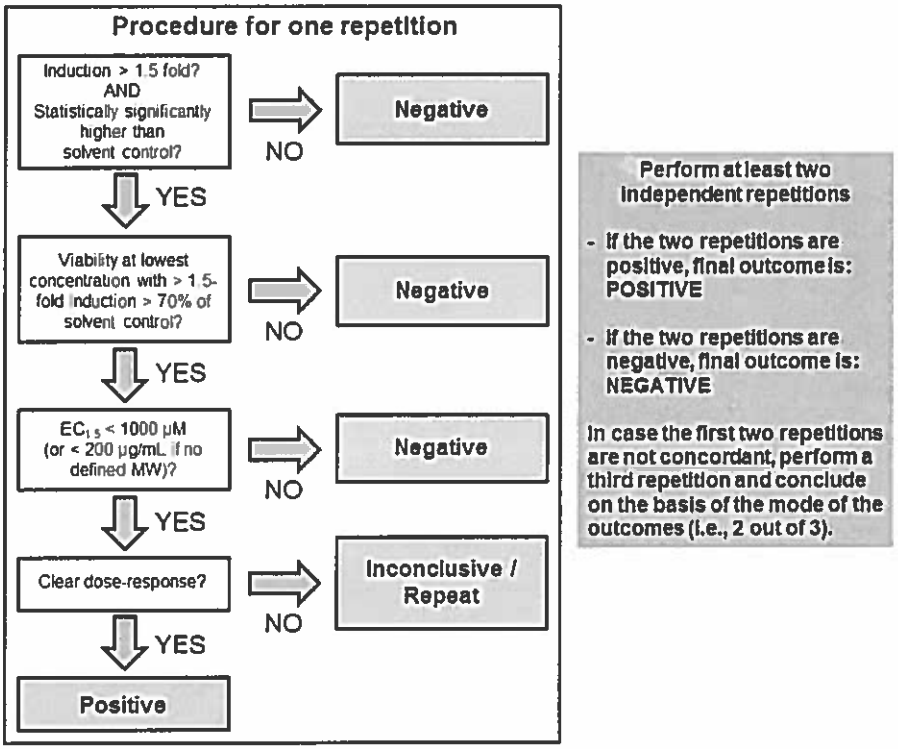
- I_{max} Maximal fold-gene induction of the luciferase gene over the full dose-response up to 1000 ppm
- EC 1.5 Concentration in ppm for 1.5-fold gene induction
- EC 2 Concentration in ppm for 2-fold gene induction
- EC 3 Concentration in ppm for 3-fold gene induction
- Pos / Neg Rating of substance according to prediction model
- reps. Positive number of independent repetitions positive / number of repetitions done
- IC50 Concentration in ppm for 50% reduction of cell viability

Study Number: [redacted]

Prediction Model

Substances are rated positive if the following conditions are met (see also Scheme 1):

- The I_{max} indicates > 1.5-fold gene induction, and this induction is statistically significant above the solvent control in a particular repetition as determined by students T-test. The EC1.5 value is below 1000 ppm in all three repetitions or in at least 2 repetitions. (If the I_{max} is exactly equal to 1.5, the substance is still rated negative and no EC1.5 value is calculated by the evaluation sheet.)
- At the lowest concentration with a gene induction above 1.5-fold (i.e. at the EC 1.5 determining value), the cellular viability is above 70%.
- There is an apparent overall dose-response for luciferase induction, which is similar between the repetitions.



Scheme 1: Prediction model of the KeratinoSens assay

Testing of Proficiency chemicals and historical positive control data in the test facility

[redacted]

Results for repeated testing of the positive control are shown in Figure 6.

These validations show a very good reproducibility of the assay over time.

4. Results

4.1 Cytotoxic activity of the test substance

Table 4 lists the IC50 values for the test substance. The full dose response curve is in the Figures section in Appendix A (Figures 1 – 4). D-MANNOSE 6-PHOSPHATE was not cytotoxic in the tested concentration range.

Table 4. Cytotoxicity determinations. Given is the IC50 value as the concentration in ppm reducing the viability by 50%.

	Test substance	Rep 1 IC50 (ppm) (*)	Rep 2 IC50 (ppm)	Rep 3 IC50 (ppm)	Geometric Mean IC 50 (ppm)	Standard deviation IC 50 (ppm)
1	D-MANNOSE 6-PHOSPHATE	>1000	>1000	>1000	>1000	n.a.

(*) The three values given come from three independent experiments

n.a. not applicable

4.2 Luciferase induction by the test substances

Table 5 lists the results of luciferase determinations as expressed as the I_{max} values indicating maximal induction over the tested range up to a concentration of 1000 ppm. Table 6 lists the EC1.5, EC2 and EC3 value for the test substance. Table 7 lists the overall rating of the test substance according to the prediction mode. The full dose response curve is in the Figures section in Appendix A (Figures 1 – 4).

D-MANNOSE 6-PHOSPHATE did not induce the luciferase gene above the threshold of 1.5 at any concentration in 2 of 3 repetitions, while a weak induction at top dose was noted in the third repetition (Figure 3). It is thus rated negative in the KeratinoSens™ assay.

Table 5. Luciferase determinations. Given is the I_{max} values indicating maximal fold-induction up to a concentration of 1000 ppm.

	Test substance	Rep 1 i_{MAX} (fold induction)	Rep 2 i_{MAX} (fold induction)	Rep 3 I_{MAX} (fold induction)	Average i_{MAX} (fold induction)	Standard deviation I_{MAX} (fold induction)
1	D-MANNOSE 6-PHOSPHATE	1.28	1.18	1.79	1.42	0.33

Table 6. Luciferase determinations. Given is the EC1.5, EC2 and EC3 value as the concentration in ppm inducing the luciferase activity 1.5-fold, 2-fold and 3-fold up to a concentration of 1000 ppm.

	Test substance	Extrapolated value	Rep 1 (ppm)	Rep 2 (ppm)	Rep 3 (ppm)	Geometric Mean (ppm)	Standard deviation (ppm)
1	D-MANNOSE 6-PHOSPHATE	EC 1.5	n.i.	n.i.	693.9	n.i.	n.a.
1	D-MANNOSE 6-PHOSPHATE	EC 2	n.i.	n.i.	n.i.	n.i.	n.a.
1	D-MANNOSE 6-PHOSPHATE	EC 3	n.i.	n.i.	n.i.	n.i.	n.a.

n.i. no induction above a given threshold; n. a., not applicable

Table 7. Overall rating of the test substance according to the prediction model and the number of positive repetitions.

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		Reps pos.	Overall rating
1	D-MANNOSE 6-PHOSPHATE	1 of 3	NEGATIVE

4.3. Positive and negative control

Cinnamic aldehyde was run in all three repetitions. Here the detailed results for this positive control are reported in Table 8 and Figure 5. Cinnamic aldehyde needs to be positive for a run to be accepted (i.e. induction > 1.5 fold). This was the case in all three repetitions. The induction at 64 µM and the EC 1.5 for cinnamic aldehyde were also calculated. The targets are: (i) Average induction in the three replicates for cinnamic aldehyde at 64 µM should be between 2 and 8, and (ii) the EC 1.5 value should be between 7 µM and 30 µM. At least one of these two numerical criteria must be met in order to accept a repetition. In the experiments performed here both criteria were fulfilled in all three repetitions. Thus all three repetitions were valid for the positive control.

Table 8. Numerical results for the positive control cinnamic aldehyde.

Quality control: Induction values Reference							Criteria	
cinnamic aldehyde	*4 µM*	*8 µM*	*16 µM*	*32 µM*	*64 µM*	EC 1.5	EC 1.5	Ind. 64 µM
rep1	1.27	1.40	1.50	1.85	3.40	15.77	TRUE	TRUE
rep2	1.23	1.29	1.45	1.81	3.41	18.31	TRUE	TRUE
rep3	1.15	1.30	1.34	1.86	2.70	20.87	TRUE	TRUE
Average	**1.22**	**1.33**	**1.43**	**1.84**	**3.17**	**18.32**		

As second performance criterion, the variability of the solvent control should be below 20%. Table 9 lists the results of the three repetitions. Repetitions 2 and 3 were valid for the solvent control, while repetition 1 was slightly above the threshold. However, since the positive control was highly reproducible (see Figure 5 and Table 8) in all three repetitions and also a stable dose response curve was observed in repetition 1 (see Figure 1), this is considered acceptable.

Table 9. The variability of the solvent controls.

	% variability blanks	
rep1	21.3	Borderline
rep2	12.1	ACCEPTED
rep3	11.4	ACCEPTED

5. Discussion and Conclusions

The result of the KeratinoSens™ assay should be used as part of an integrated approach for testing and assessment (IATA)[9]. A parallel test in the DPRA may indicate whether congruent results are obtained by both test methods. According to a detailed analysis on large set of substances, two congruent results in these two tests give a good prediction of the sensitizer hazard [5, 7, 18], in particular when comparing against human data, while an additional test in a dendritic cell line assessing expression of surface markers may be needed in case of discordant results.

D-MANNOSE 6-PHOSPHATE did not induce the luciferase gene above the threshold of 1.5 at any concentration in 2 of 3 repetitions, while a weak induction at top dose was noted in the third repetition (Figure 3). According to the prediction model of the KeratinoSens™ assay, the test substance is rated as non-sensitizer. This conclusion is also clearly supported by the analysis of the

dose-response curve in Figure 4 with overall no induction of the luciferase reporter gene to be observed.

6. References

1. ECVAM, *KeratinoSens™ protocol.* DB-ALM database, 2014. **Protocol 155**: p. http://ecvam-dbalm.jrc.ec.europa.eu/index.cfm?idmm=6, Last accessed 3.2.2014.
2. Emter, R., G. Ellis, and A. Natsch, *Performance of a novel keratinocyte-based reporter cell line to screen skin sensitizers in vitro.* Toxicol. Appl. Pharmacol., 2010. **245**: p. 281-290.
3. Natsch, A., et al., *The intra- and inter-laboratory reproducibility and predictivity of the KeratinoSens assay to predict skin sensitizers in vitro: results of a ring-study in five laboratories.* Toxicol. In Vitro, 2011. **25**(3): p. 733-44.
4. Delaine, T., et al., *Structure-Activity Relationship between the in Vivo Skin Sensitizing Potency of Analogues of Phenyl Glycidyl Ether and the Induction of Nrf2-Dependent Luciferase Activity in the KeratinoSens in Vitro Assay.* Chem. Res. Toxicol., 2011. **24**(8): p. 1312-8.
5. Natsch, A., et al., *A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation.* J. Appl. Toxicol., 2013. **33**(11.): p. 1337-52.
6. Natsch, A., et al., *Predicting Skin Sensitizer Potency Based on In Vitro Data from KeratinoSens and Kinetic Peptide Binding: Global Versus Domain-Based Assessment.* Toxicol. Sci., 2015. **143**(2): p. 319-32.
7. Urbisch, D., et al., *Assessing skin sensitization hazard in mice and men using non-animal test methods.* Regul. Toxicol. Pharmacol., 2015. **71**(2): p. 337-51.
8. Ball, N., et al., *Evaluating the sensitization potential of surfactants: Integrating data from the local lymph node assay, guinea pig maximization test, and in vitro methods in a weight-of-evidence approach.* Regul. Toxicol. Pharmacol., 2011. **60**: p. 389-400.
9. ECVAM, *EURL ECVAM Recommendation on the KeratinoSens™ assay for skin sensitisation testing* http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/recommendation-keratinosens-skin-sensitisation, last accessed 26.3.2014, 2014.
10. Natsch, A., *The Nrf2-Keap1-ARE toxicity pathway as a cellular sensor for skin sensitizers--functional relevance and a hypothesis on innate reactions to skin sensitizers.* Toxicol. Sci., 2010. **113**(2): p. 284-92.
11. Ade, N., et al., *HMOX1 and NQO1 genes are upregulated in response to contact sensitizers in dendritic cells and THP-1 cell line: Role of the Keap1/Nrf2 pathway.* Toxicol. Sci., 2009. **107**(2): p. 451-460.
12. van der Veen, J.W., et al., *Applicability of a keratinocyte gene signature to predict skin sensitizing potential.* Toxicol. In Vitro, 2013. **27**(1): p. 314-22.
13. Vandebriel, R.J., et al., *Keratinocyte gene expression profiles discriminate sensitizing and irritating compounds.* Toxicol. Sci., 2010. **117**(1): p. 81-9.
14. Johansson, H., et al., *A genomic biomarker signature can predict skin sensitizers using a cell-based in vitro alternative to animal tests.* BMC Genomics, 2011. **12**(1): p. 399.
15. Miyazawa, M. and A. Takashima, *Development and validation of a new in vitro assay designed to measure contact allergen-triggered oxidative stress in dendritic cells.* J. Dermatol. Sci., 2012. **68**(2): p. 73-81.
16. Ramirez, T., et al., *LuSens: A keratinocyte based ARE reporter gene assay for use in integrated testing strategies for skin sensitization hazard identification.* Toxicol In Vitro, 2014.
17. [redacted]
18. Bauch, C., et al., *Putting the parts together: Combining in vitro methods to test for skin sensitizing potentials.* Regul Toxicol Pharmacol, 2012. **63**(3): p. 489-504.
19. Natsch, A. and R. Emter, *Nrf2 activation as a key event triggered by skin sensitisers: The development of the stable KeratinoSens reporter gene assay.* Altern Lab Anim, 2016. **44**(5): p. 443-451.

Study Number: [redacted]

Appendix A: Figures

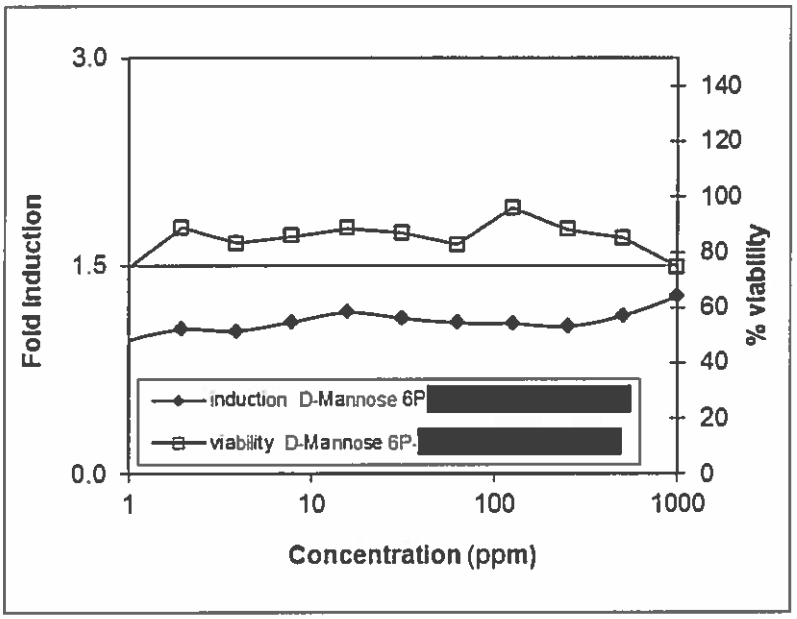

Figure 1. Dose response curves for D-MANNOSE 6-PHOSPHATE. Given is the fold induction of the luciferase gene over solvent control (filled diamonds) and the % viability as determined with the MTT assay (open squares). Results of Repetition 1.

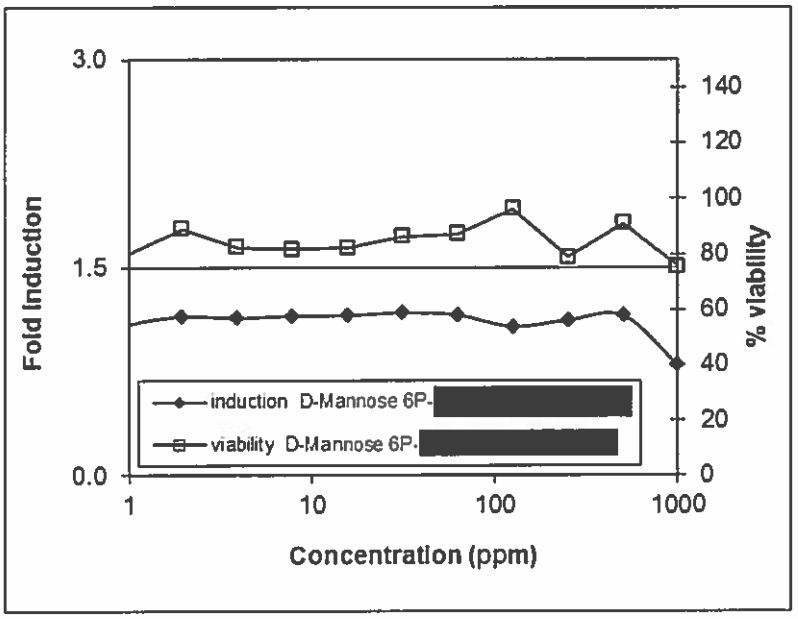

Figure 2. Dose response curves for D-MANNOSE 6-PHOSPHATE. Given is the fold induction of the luciferase gene over solvent control (filled diamonds) and the % viability as determined with the MTT assay (open squares). Results of Repetition 2.

Study Number: [redacted]

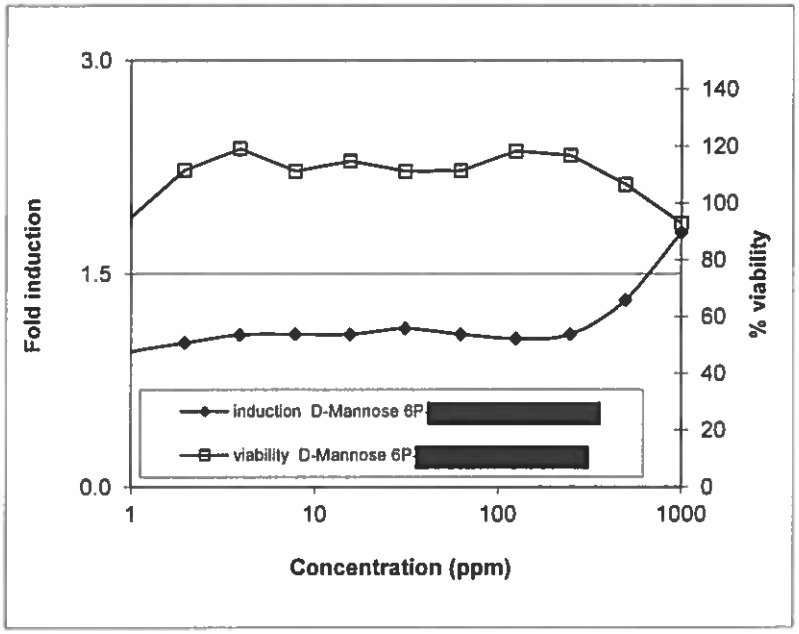

Figure 3. Dose response curves for D-MANNOSE 6-PHOSPHATE. Given is the fold induction of the luciferase gene over solvent control (filled diamonds) and the % viability as determined with the MTT assay (open squares). Results of Repetition 3.

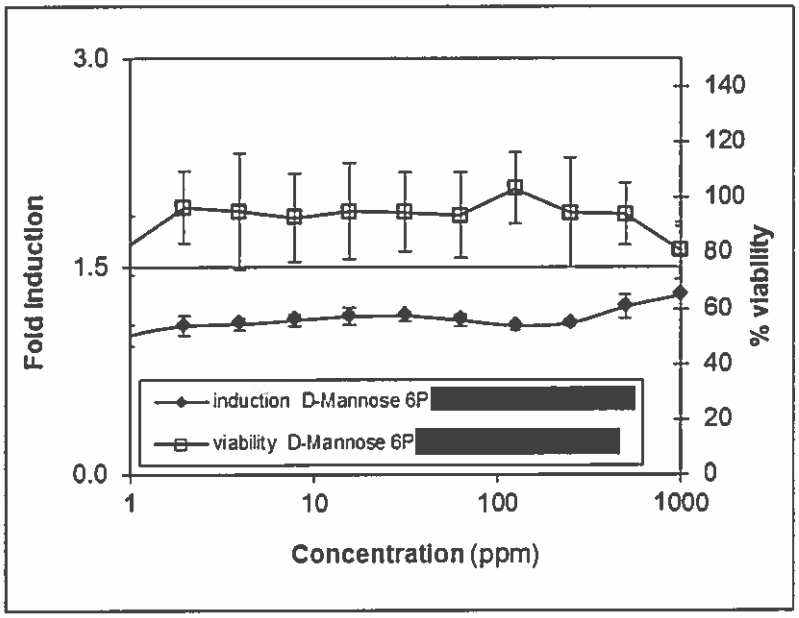

Figure 4. Dose response curves for D-MANNOSE 6-PHOSPHATE. Given is the fold induction of the luciferase gene over solvent control (filled diamonds) and the % viability as determined with the MTT assay (open squares). Average and standard deviation of all three repetitions.

Study Number: [redacted]

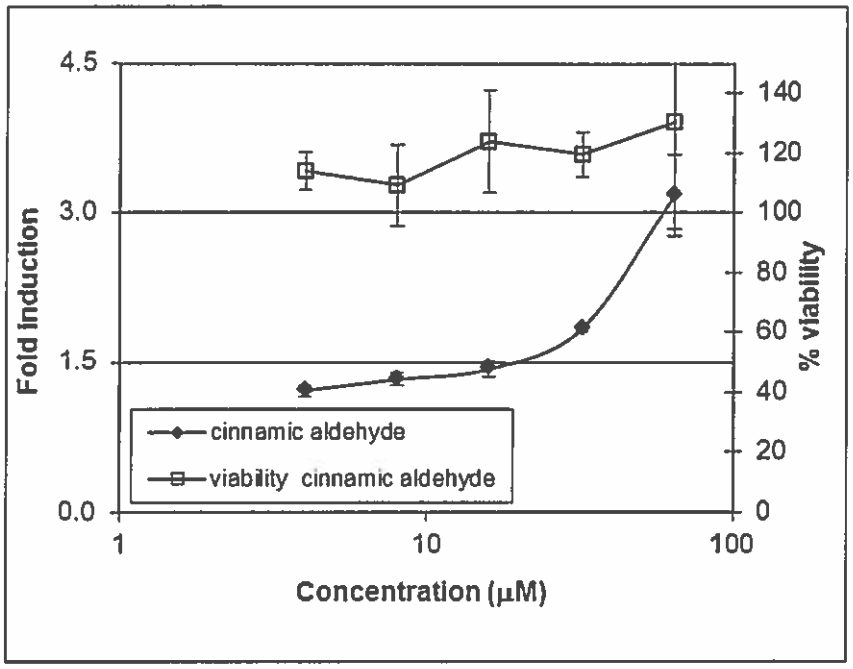

Figure 5. Dose response curve for the positive control cinnamic aldehyde. Given is the fold induction of the luciferase gene over solvent control (filled diamonds) and the % viability as determined with the MTT assay (filled squares)

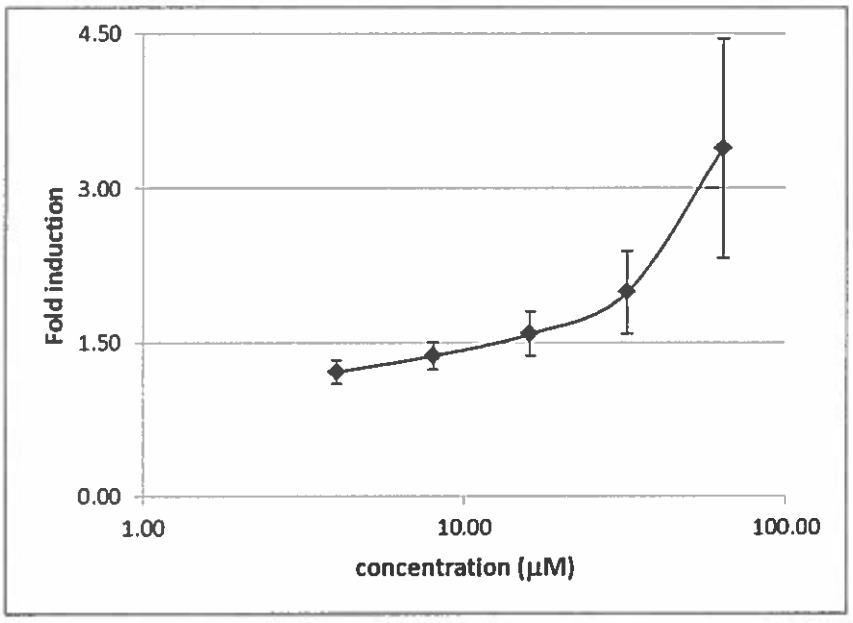

Figure 6. Historical results for luciferase induction by the positive control in the test laboratory: Shown are average and standard deviations from 242 valid runs conducted in 2014 – 2016 [19].