Safety Assessment of Caprylhydroxamic Acid as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	May 10, 2019
Panel Meeting Date:	June 6-7, 2019

The 2019 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Monice M. Fiume, Senior Director.

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Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Monice M. Fiume MMF
Senior Director
Date: May 10, 2019
Subject: Safety Assessment of Caprylhydroxamic Acid as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Caprylhydroxamic Acid as Used in Cosmetics. (It is identified in this report package as *caphyd062019rep*.) This is the first time the Panel is seeing the safety assessment on this ingredient.

The following unpublished data were received either from the Council or as a direct submission to CIR, and are included in the report:

1. Inolex. 2019. Method of manufacture for Caprylhydroxamic Acid. (*caphyd062019data_1)*
2. PCPC. 2018. Council concentration of use survey: Caprylhydroxamic Acid. (*caphyd062019data_2)*
3. Nelson Laboratories Inc. 2007. The *Salmonella typhimurium* reverse mutation assay (Ames test), liquids or soluble chemicals, with caprylohydroxamic acid. (*caphyd062019data_3)*
4. BioReliance. 2013. *In vitro* mammalian cell micronucleus assay in human peripheral blood lymphocytes (HPBL) with Caprylhydroxamic Acid. (*caphyd062019data_4)*
5. MatTek Corporation. 2018. Evaluation of the skin irritation potential of diheptyl succinate and Caprylhydroxamic Acid using the EpiDerm skin irritation test OECD TG 439. (*caphyd062019data_5)*
6. Consumer Product Testing Company. 2014. Repeated insult patch test of an eyeliner containing 0.105% Caprylhydroxamic Acid. (*caphyd062019data_6)*
7. Consumer Product Testing Company. 2018. Repeated insult patch test of a lotion containing 0.15% Caprylhydroxamic Acid, tested undiluted. (*caphyd062019data_7)*
8. Consumer Product Testing Company. 2018. Repeated insult patch test of W/O thick balm containing 0.15% Caprylhydroxamic Acid, tested undiluted. (*caphyd062019data_8)*
9. Consumer Product Testing Company. 2018. Repeated insult patch test of a wipe juice containing 0.15% Caprylhydroxamic Acid, tested undiluted. (*caphyd062019data_9)*
10. Anonymous. 2019. Summary of an HRIPT of a facial cream containing 0.15% Caprylhydroxamic Acid. (*caphyd062019data_10)*
11. Anonymous. 2019. Summary of an HRIPT on a brow thickening powder containing 0.195% Caprylhydroxamic Acid. (*caphyd062019data_11)*
12. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #3 containing 5% Caprylhydroxamic Acid, tested as a 6% dilution. (*caphyd062019data_12)*
13. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #5 containing 7.5% Caprylhydroxamic Acid, tested as a 4% dilution. (*caphyd062019data_13)*
14. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #2 containing 10% Caprylhydroxamic Acid, tested as a 3% dilution. (*caphyd062019data_14)*
15. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #1 containing 15% Caprylhydroxamic Acid, tested as a 2% dilution. (*caphyd062019data_15)*

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16. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #4 containing 15% Caprylhydroxamic Acid, tested as a 2% dilution. (*caphyd062019data_16)*
17. Clinical Research Laboratories Inc. 2008. Repeated insult patch test of undiluted caprylohydroxamic acid. (*caphyd062019data_17)*
18. MB Research Laboratories. 2011. Bovine Corneal Opacity and Permeability Test (BCOP) with a 20% solution of Caprylhydroxamic Acid. (*caphyd062019data_18)*
19. MB Research Laboratories. 2010. MatTek EpiOcular™ MTT Viability Assay with CHA (Caprylhydroxamic Acid). (*caphyd062019data_19)*

The Panel should be aware that in the NICNAS dossier, the following statements were made. "Based on the low molecular weight, potential surface activity and irritancy potential, it is likely that [Caprylhydroxamic Acid] will be able to be absorbed into the skin. Hydroxamic acids are known to inhibit certain enzymes such as urease … and therefore have been shown to have protein reactivity, an important factor in skin sensitisation potential. The skin sensitisation potential of [Caprylhydroxamic Acid] cannot be ruled out." Please comment on whether these statements should be included in the report.

Comments on the SLR that were received from the Council were addressed, and are included (*caphyd062019PCPC*). The following are also included as a part of this report package:

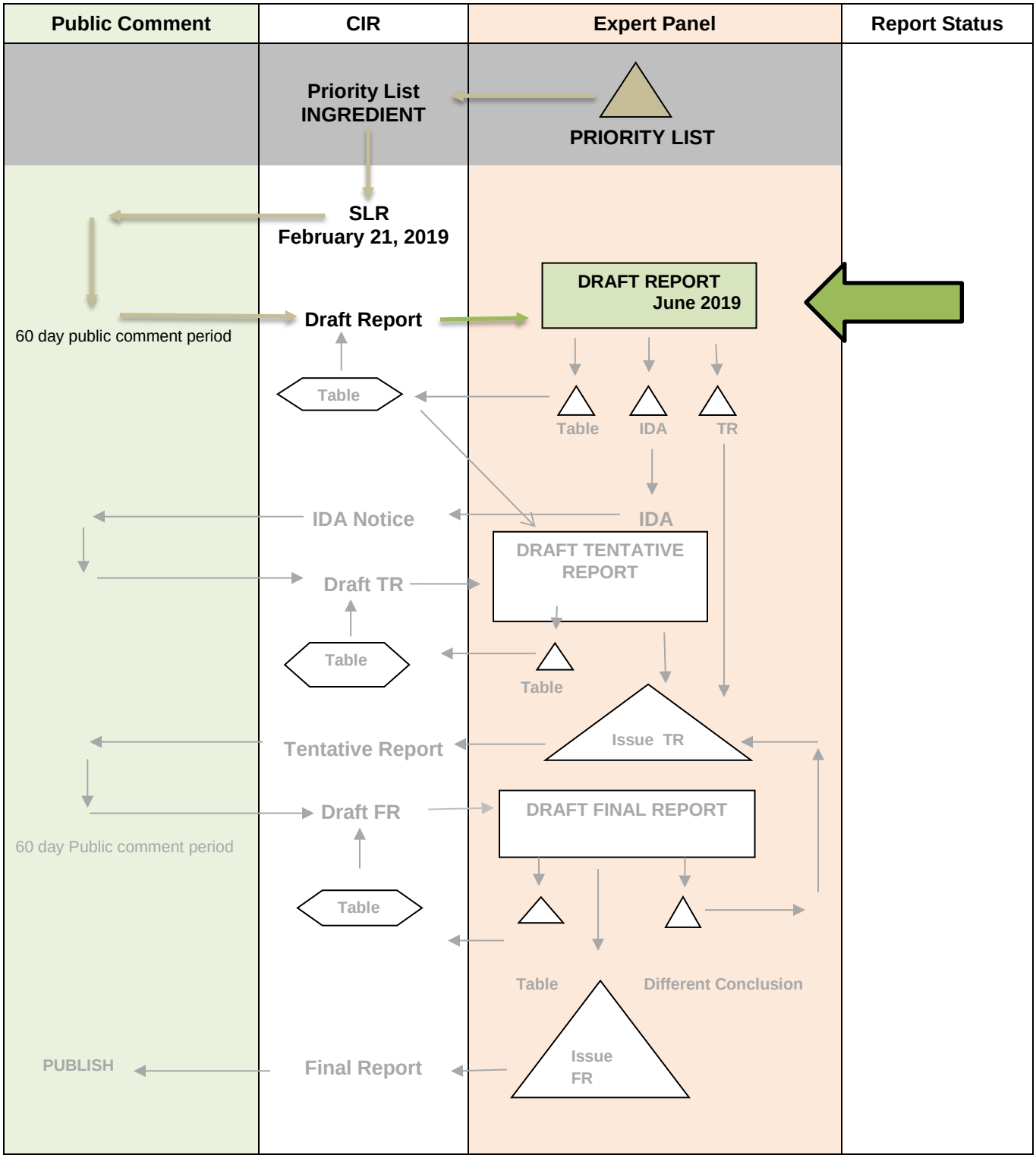
caphyd062019flow: report flowchart
caphyd062019hist: report history
caphyd062019prof: data profile
caphyd062019strat: search strategy
caphyd062019FDA: 2019 VCRP data

If the data included in this report adequately address the safety of Caprylhydroxamic Acid, the Panel should be prepared to formulate a tentative conclusion, provide the rationale to be described in the Discussion, and issue a Tentative Report for public comment. If the data are not sufficient for making a determination of safety, then an Insufficient Data Announcement (IDA) should be issued that provides a listing of the additional data that are needed.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Caprylhydroxamic Acid

MEETING June 2019



CIR Report History: Caprylhydroxamic Acid

SLR: February 21, 2019
The following data were received prior to announcing the SLR:"

1. PCPC. 2018. Council concentration of use survey: Caprylhydroxamic Acid.

Draft Report: June 6-7, 2019
The following unpublished data were received either from the Council or as a direct submission to CIR prior to review of the Draft Report:

1. Inolex. 2019. Method of manufacture for Caprylhydroxamic Acid.
2. Nelson Laboratories Inc. 2007. The *Salmonella typhimurium* reverse mutation assay (Ames test), liquids or soluble chemicals, with caprylohydroxamic acid.
3. BioReliance. 2013. *In vitro* mammalian cell micronucleus assay in human peripheral blood lymphocytes (HPBL) with Caprylhydroxamic Acid.
4. MatTek Corporation. 2018. Evaluation of the skin irritation potential of diheptyl succinate and Caprylhydroxamic Acid using the EpiDerm skin irritation test OECD TG 439.
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6. Consumer Product Testing Company. 2018. Repeated insult patch test of a lotion containing 0.15% Caprylhydroxamic Acid, tested undiluted.
7. Consumer Product Testing Company. 2018. Repeated insult patch test of W/O thick balm containing 0.15% Caprylhydroxamic Acid, tested undiluted.
8. Consumer Product Testing Company. 2018. Repeated insult patch test of a wipe juice containing 0.15% Caprylhydroxamic Acid, tested undiluted.
9. Anonymous. 2019. Summary of an HRIPT of a facial cream containing 0.15% Caprylhydroxamic Acid
10. Anonymous. 2019. Summary of an HRIPT on a brow thickening powder containing 0.195% Caprylhydroxamic Acid.*)*
11. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #3 containing 5% Caprylhydroxamic Acid, tested as a 6% dilution.
12. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #5 containing 7.5% Caprylhydroxamic Acid, tested as a 4% dilution.
13. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #2 containing 10% Caprylhydroxamic Acid, tested as a 3% dilution.
14. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #1 containing 15% Caprylhydroxamic Acid, tested as a 2% dilution.
15. Consumer Product Testing Company. 2018. Repeated insult patch test of CHA blend #4 containing 15% Caprylhydroxamic Acid, tested as a 2% dilution.
16. Clinical Research Laboratories Inc. 2008. Repeated insult patch test of undiluted caprylohydroxamic acid.
17. MB Research Laboratories. 2011. Bovine Corneal Opacity and Permeability Test (BCOP) with a 20% solution of Caprylhydroxamic Acid.
18. MB Research Laboratories. 2010. MatTek EpiOcular™ MTT Viability Assay with CHA (Caprylhydroxamic Acid).

Caprylhydroxamic Acid* – June 6-7, 2019 – Writer, Monice Fiume

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies		
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Provocative Testing	Case Reports
Caprylhydroxamic Acid	yes	X	X	X		X		X			X			X	X				X		X			X		X			X	X

* "X" indicates that data were available in a category for the ingredient

Caprylhydroxamic Acid – 2/7/19

Ingredient	CAS #	SciFin	PubMed	FDA	EU	ECHA	ECETOC	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Caprylhydroxamic Acid	7377-03-9	5/160	2/7	no	X	X	no	X	no	no	no	no	no	no	no

Search Strategy

PubMed (2/7/19; updates received weekly): ((((Caprylhydroxamic Acid) OR 7377-03-9[EC/RN Number]) OR Octanamide, N-Hydroxy-) OR N-hydroxyoctanamide) OR Octanohydroxamic Acid – 7 hits/2 useful
SciFinder (2/7/19; updates received weekly): searched by CAS No; refined by document type – 160 hits/5 useful

Google searches

Caprylhydroxamic Acid sensitization
Adverse event reporting caprylhydroxamic acid
Adverse event reporting phenostat
Sensitization to Phenostat
Allergic contact dermatitis caused by cosmetic products.
Allergic contact dermatitis caused by preservatives in cosmetic products.
Contact dermatitis caused by preservatives.
Chemistry of hydroxamic acids
hydroxamic acids and the effect of straight versus cyclic chains

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)
- Scifinder (https://scifinder.cas.org/scifinder)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org

- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- EAFUS: http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- OTC ingredient list: https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- ChemPortal: https://www.echemportal.org/echemportal/index.action
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/

- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://www.oecd.org/env/ehs/risk-assessment/publishedassessments.htm
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/

- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

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INTRODUCTION

This assessment reviews the safety of Caprylhydroxamic Acid as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), this ingredient is reported to function as a chelating agent in cosmetics.[1]

Included in this safety assessment are relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some of the data included in this safety assessment was found on Australia's National Industrial Chemicals Notification and Assessment Scheme (NICNAS)[2] and the European Chemicals Agency (ECHA)[3] websites. Please note that these websites provide summaries of information from other sources, and it is those summary data that are reported in this safety assessment when NICNAS or ECHA is cited.

CHEMISTRY

Definition and Structure

According to the *Dictionary*, Caprylhydroxamic Acid (CAS No. 7377-03-9) is the organic compound that conforms to the keto form depicted in Figure 1.[1] However, hydroxamic acids may exist in both keto and enol tautomeric forms.[4] The keto form is likely to predominate in acidic formulation, while the enol may dominate under alkaline conditions.

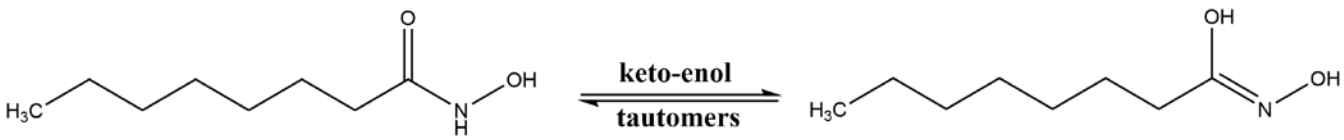

Figure 1. Caprylhydroxamic Acid

The hydroxamic acid functional group makes Caprylhydroxamic Acid an excellent chelating agent. It is known that some bacteria synthesize and use hydroxamic acids as siderophores (iron scavengers/chelators).[4] Additionally, Caprylhydroxamic Acid forms strong complexes with oxidized transition metals almost instantaneously, and it may react with oxidizers and acids.[2] In general, hydroxamic acids are capable of the inhibition of a variety of enzymes, including ureases, peroxidases, and matrix metalloproteinases.[5] (Data concerning the effects of Caprylhydroxamic Acid on enzyme activity were not found in the published literature.)

Caprylhydroxamic Acid is stable under normal environmental and usage conditions.[2] However, at very high or low pH, it may be hydrolyzed to caprylic acid and hydroxylamine. Decomposition products at high temperature are ammonia and oxides of carbon and nitrogen.

Physical and Chemical Properties

Caprylhydroxamic Acid is a white to tan crystalline solid,[2,3] with a molecular weight of 159.23 Da.[6] Additional physical and chemical properties are described in Table 1.

Method of Manufacture

A supplier reports that as a cosmetic ingredient, Caprylhydroxamic Acid is most frequently synthesized via the transamidation of either methyl caprylate or ethyl caprylate with hydroxylamine to yield Caprylhydroxamic Acid; methanol or ethanol, respectively, is a byproduct of the process.[7] Depending on which caprylate ester is used, the reaction is conducted in either methanol or ethanol under refluxing conditions. Caprylhydroxamic Acid is then isolated and purified via recrystallization from ethyl acetate, followed by washing, filtering, and drying to obtain Caprylhydroxamic Acid (> 99% pure). Figure 2 depicts an example of the synthesis route for the commercial production of Caprylhydroxamic Acid.

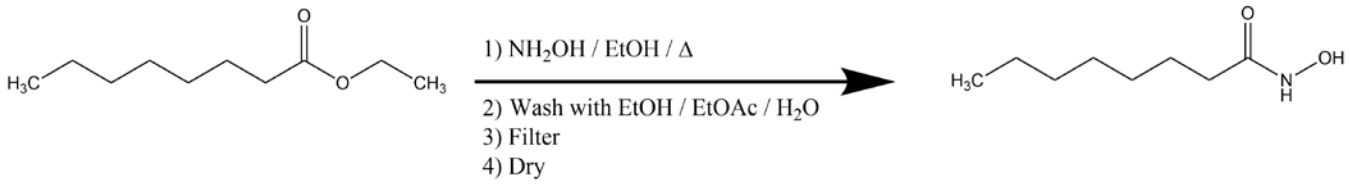

Figure 2. Example of the synthesis route for the commercial production of Caprylhydroxamic Acid, using ethyl caprylate

Impurities

Caprylhydroxamic Acid is reported to be > 99% pure, and it does not contain any "non-hazardous" (> 1% by weight) or "hazardous" impurities.[2] According to NICNAS, formulators should consider monitoring products for formation of hydroxylamine if formulated at pH < 5 or pH > 8, or if formulation intermediates are substantially acidic or basic.

Nitrosation

Nitrosamides are chemicals containing the R-C(O)-N=NO functional group. Due to the presences of a reactive *N*-hydrogen substituent (i.e., identity as a secondary amide), the theoretical potential for the formation of nitrosamides exists with hydroxamic acid derivatives. Of concern in cosmetics, is the conversion of secondary amides into nitrosamides that may be carcinogenic. In a group of *N*-nitroso compounds that have been tested, 79 of the 86 nitrosamides have been shown to produce cancer in laboratory animals.[8] Nitrosation can occur under physiologic conditions.[9] Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. However, nitrosation occurs most commonly under acidic conditions. Atmospheric NO_2 may also participate in nitrosation in aqueous solution.[10]

However, while indirect test methods have supported the likelihood of formation, such *N*-nitrosated hydroxamic acid derivatives have yet to be isolated (likely due either to rapid decomposition or facile molecular rearrangement).[11] Also, no carcinogenicity studies specific to *N*-nitrosated hydroxamic acid derivatives were found in the publicly available literature.

Enzymatic Activity

In general, hydroxamic acids are capable of the inhibition of a variety of enzymes, including ureases, peroxidases, and matrix metalloproteinases.[5] Data concerning the effects of Caprylhydroxamic Acid on enzyme activity were not found in the published literature.

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2019 VCRP survey data and the results of the concentration of use survey conducted by the Council in 2018, Caprylhydroxamic Acid is reported to be used in 227 formulations[12] at maximum leave-on and rinse-off concentrations of 0.25% in body and hand products and 0.3% in bath soaps and detergents, respectively.[13] (Table 2) Caprylhydroxamic Acid is used in products applied near the eye at up to 0.2% (in eyebrow pencils and in "other" eye makeup preparations), in formulations that come into contact with mucous membranes at up to 0.3% (in bath soaps and detergents), and in baby lotions, oils, and creams at up to 0.15%. Although there are uses reported to the VCRP that could result in incidental ingestion (i.e., lipsticks), concentration of use data were not reported for these uses.

Additionally, Caprylhydroxamic Acid is used in cosmetic sprays and could possibly be inhaled. It is reported to be used at 0.075% in both aerosol and pump hair spray formulations. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles < 10 µm compared with pump sprays.[14,15] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[16,17]

Caprylhydroxamic Acid is not restricted from use in any way under the rules governing cosmetic products in the European Union.[18]

Exposure Assessment

NICNAS estimated the total systemic exposure dose (SED) to Caprylhydroxamic Acid from cosmetic applications.[2] For the assessment, it was assumed that the user is a 60 kg body weight (bw) female, and that dermal absorption is 100% (worst-case scenario). Additionally, it was assumed that Caprylhydroxamic Acid is always used at 0.5% in cosmetic formulations, that it is not used in oral care products, and that there is daily exposure to 6 make-up products, 5 leave-on skin and hair care products (including body lotion), and 4 rinse-off skin and hair cleansing products containing this ingredient, for a total exposure of 15.1 g/day (234 mg/kg bw/day) to products containing Caprylhydroxamic Acid. Based on these parameters, the total SED to Caprylhydroxamic Acid through the use of cosmetics was calculated as 1.17 mg/kg bw/day.

The margin of exposure (MOE) was then calculated using the total SED of 1.17 mg/kg bw/day and a no-observable-adverse-effect-level (NOAEL) of 50 mg/kg bw/day (that was derived in a subchronic oral toxicity study in rats, described later in this report). Using these values, the MOE was calculated to be 43. Because an MOE greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences, which was not achieved with a use concentration of 0.5%, a concentration of 0.3% was considered in the calculations. Using 0.3% as the maximum concentration of use, the MOE was calculated to be 71. NICNAS stated that even though this MOE is still below 100, given that the exposure estimate is based on the conservative assumption of 100% dermal absorption of the amount left on the skin following application and the simultaneous use of various products containing the maximum concentration of Caprylhydroxamic Acid, the risk to the public is not considered unreasonable if products contain a maximum of 0.3%.

Non-Cosmetic

Use of Caprylhydroxamic Acid as a growth-promoting feed additive was reported.[19] (No details were provided.)

Very little information specific to the non-cosmetic use of Caprylhydroxamic Acid was found in the published literature. However, hydroxamic acids have use in numerous applications, including biomedical use as therapeutic agents; agriculturally as insecticides, antimicrobials, and plant growth regulators; and industrially as antioxidants, corrosion inhibitors, for the extraction of toxic elements, as a means of flotation of minerals, and as redox switches for electronic devices.[5]

TOXICOKINETICS STUDIES

Dermal Penetration

Based on the physicochemical properties of Caprylhydroxamic Acid, including low molecular weight (159.23 Da) and the octanol/water partition coefficient (1.66, estimated), it is likely this ingredient will absorb through the skin.[2]

Absorption, Distribution, Metabolism, and Excretion

Given the low molecular weight of Caprylhydroxamic Acid, absorption across the gastrointestinal (GI) tract is possible by passive diffusion through the aqueous pores or micellar solubilization.[2]

In Vitro

Caprylhydroxamic Acid was rapidly hydrolyzed to caprylic acid and hydroxylamine by rat liver homogenates.[20] (Only an English abstract was available, therefore additional details are not presented.)

Animal

Oral

Following oral administration of 1-[^{14}C]-Caprylhydroxamic Acid (1.27 mg/kg) to rats, hydroxamic acid was not detected in any tissues (except in the GI tract) 2 h after administration.[20] "Considerable amounts" of radioactivity were found in the liver and the heart, but most was excreted as expired $^{14}CO_2$; approximately 25% of the total radioactivity was excreted as $^{14}CO_2$ at 2 h. Within 24 h, 6.9% and 0.6% was excreted in the urine and the feces, respectively. (Only an English abstract was available, therefore additional details are not presented.)

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

The oral LD_{50} of Caprylhydroxamic Acid is reported to be > 8820 mg/kg in rats.[2] Another source reported that the oral LD_{50} in rats is > 10,700 mg/kg.[21] (Details were not available.)

Subchronic Toxicity Studies

Oral

Groups of 10 male and 10 female Wistar rats were dosed for 13 wks with 0, 100, 500, or 2500 mg/kg bw/day 10% Caprylhydroxamic Acid in lactose (corresponding to 0, 10, 50, and 250 mg/kg bw Caprylhydroxamic Acid, respectively) by

gavage.[2,22] The vehicle was 5% aqueous (aq.) gum arabic. There was no mortality attributed to the test article; however, 2 female animals of the mid-dose group died due to dosing errors. Signs of toxicity were observed only in the high dose group, and all the following observations were reported for this group. Clinical observations included "slowness in activity." There were significant decreases in alanine amino transferase, glucose and potassium levels in males, and there was a significant increase in leukocyte count and significant decreases in erythrocyte, hematocrit, and hemoglobin counts in males and females. Spleen weights (absolute and relative to bw) were increased in males and females, and adrenal weights were significantly decreased in males. Slight atrophy in the epithelial cells of the renal glomeruli and hemosiderin deposits in the spleen were reported upon microscopic examination. The NOAEL of the test article (10% Caprylhydroxamic Acid in lactose) was determined to be 500 mg/kg bw/day; accordingly, the NOAEL of undiluted Caprylhydroxamic Acid is expected to be 50 mg/kg bw/day.[2]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Groups of 18 mated female Wistar rats were dosed with 0, 50, 250, and 500 mg/kg bw/day 10% Caprylhydroxamic Acid (corresponding to 0, 5, 25, and 50 mg/kg bw Caprylhydroxamic Acid, respectively) by gavage on days 9 through 14 of gestation.[2,23] The vehicle was 5% gum arabic solution. Twelve dams of the 0, 50, and 250 mg/kg bw/day groups, and all of the dams of the 500 mg/kg bw/day group, were killed on day 20 of gestation. The remaining dams were allowed to litter naturally. There was no mortality during the study, and there were no clinical signs of maternal toxicity. Body weight gains and feed consumption of the 250 and 500 mg/kg bw/day groups were "a little lower" than those of the controls; fetal weights in these groups were also lower than those in the control group, subsequently resulting in delayed ossification. Neonatal body weights from dams of the 250 mg/kg bw/day dose group were significantly lower at birth and at weaning. Decreased growth that was observed for fetuses and neonates of the higher dose groups were considered to be a result of the slight suppression of maternal body weight gains and feed consumption. Caprylhydroxamic Acid tested at 10% and at doses up to 500 mg/kg bw (corresponding to up to 50 mg/kg bw Caprylhydroxamic Acid) was not teratogenic under the conditions of this study.

GENOTOXICITY

In Vitro

In an Ames test using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, and *Escherichia coli* WP2 *hcr trp*, with and without metabolic activation, Caprylhydroxamic Acid in dimethyl sulfoxide (DMSO; 0 - 2000 µg/plate) showed weak but clear dose-dependent mutagenic activity towards *E. coli* at concentrations up to 1000 µg/plate, but was not mutagenic to *S. typhimurium*.[19] In another Ames test (performed in accord with Organisation for Economic Co-operation (OECD) test guideline (TG) 471), Caprylhydroxamic Acid in DMSO, tested at concentrations of 16 - 5000 µg/plate using *S. typhimurium* TA1535, TA98, TA100, TA102, and TA97a with and without metabolic activation, was not mutagenic.[24] Solvent and positive controls gave expected results.

Caprylhydroxamic Acid was not genotoxic in a recombination–repair (rec) assay using *Bacillus subtilis* H17 Rec^+ and M45 Rec^-.[19] (No other details were provided.)

The genotoxic potential of Caprylhydroxamic Acid (98.09% pure) was also evaluated in an in vitro mammalian cell micronucleus test using human peripheral blood lymphocytes, with and without metabolic activation, in accord with OECD TG 487.[25] The dose levels tested were 25 – 450 µg/ml with and without activation for 4 h, and 7.5 – 50 µg/ml without activation for 24 h. DMSO served as the vehicle. No increase in micronucleated binucleated cells was observed following the 4 h exposure, with or without activation. With 24 h exposure (without activation), a statistically significant increase in the percentage of micronucleated binucleated cells was observed with 15 and 30 µg/ml Caprylhydroxamic Acid (0.4% and 0.7% increase, respectively) as compared to the vehicle control; however, these values were within the historical solvent control range (0.01 – 1.0%). Caprylhydroxamic Acid was not considered genotoxic in this study. Vehicle and positive controls gave appropriate results.

In Vivo

In vivo genotoxicity studies were not found in the published literature, and unpublished data were not submitted.

CARCINOGENICITY STUDIES

Carcinogenicity studies were not found in the published literature, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION

The dermal irritation and sensitization studies summarized below are detailed in Table 3.

Caprylhydroxamic Acid, tested neat using reconstructed human epidermis tissue containing keratinocytes in an EpiDerm™ skin irritation test (OECD TG 439), was classified as non-irritant; tissue viability was 102.6%.[21]

In human repeated insult patch tests (HRIPTs), formulations containing 0.105% Caprylhydroxamic Acid (54 subjects; 24-h semi-occlusive patches),[26] 0.15% Caprylhydroxamic Acid (104 subjects, 24-h occlusive patches;[27-29] 109 subjects, 48-h occlusive patches[30]), and 0.195% Caprylhydroxamic Acid (52 subjects; 24-h semi-occlusive patches[31]) were not irritants or sensitizers. In several other HRIPTs (104 subjects; 24-h occlusive patches) in which formulations containing 5% - 15% Caprylhydroxamic Acid were tested as dilutions in distilled water, with a resulting test concentration of 0.3% Caprylhydroxamic Acid, no clinically significant potential for dermal irritation or allergic contact sensitization was observed.[32-36] However, it should be noted that in all of the studies with 104 subjects (all formulations were tested at the same time in the same subjects), scattered, transient, barely perceptible to mild or moderate erythema with occasional edema were noted throughout the test; the researchers stated that neither the number of responses nor the peak level of the responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. Undiluted Caprylhydroxamic Acid was not an irritant or a sensitizer in an HRIPT (52 subjects; 24-h semi-occlusive patches).[37]

OCULAR IRRITATION STUDIES

In Vitro

The ocular irritation potential of a 20% solution of Caprylhydroxamic Acid was evaluated in a bovine corneal opacity and permeability (BCOP) test performed in accord with OECD TG 437.[38] A 4-h exposure period was followed by a 3-h incubation period. The vehicle (minimal essential media) served as the negative control; a positive control was not used. The corrected mean opacity score was 10.5, and the corrected mean optical density (permeability) score was 0.108. The resulting in vitro irritancy score of 12.12 corresponds to a classification of mild irritant; a 20% solution of Caprylhydroxamic Acid was not considered a corrosive or severe ocular irritant under the conditions of the test.

A MatTek EpiOcular™ methyl thiazole tetrazolium (MTT) viability assay was also performed to evaluate the ocular irritation potential of Caprylhydroxamic Acid.[39] The chemical was tested neat (100 mg), the test samples were treated in duplicate, and the exposure periods were 16, 64, and 256 min. Appropriate negative and positive controls were used. The ET_{50} (i.e., the time at which the EpiOcular™ tissue viability was reduced 50% compared to control tissues) was 130.8 min, and the ocular irritancy classification for undiluted Caprylhydroxamic Acid was "non-irritating, minimal."

CLINICAL STUDIES

Provocative Testing

Patch testing was performed according to the European Society of Contact Dermatitis test guidelines in 39 patients with compromised skin that were suspected of developing contact allergy.[40] Symptoms, which appeared as acute, itchy, often sharply demarcated erythematous eczema, were thought to be due to the use of a moisturizer in Finland that had recently been reformulated; in early 2014, the moisturizer was reformulated to remove parabens. The new moisturizer formulation contained 0.75% of a preservative mixture that consisted of 65 – 75% phenoxyethanol, 10 – 20% Caprylhydroxamic Acid, and 5 – 10% methylpropanediol, resulting in an actual concentration of 0.075 – 0.15% Caprylhydroxamic Acid in the new formulation.

The test group was patch-tested with the old paraben-containing formulation (as a cream and oily cream); the new formulation containing the preservative mixture (as a cream, oily cream, and lotion); another test formulation that contained phenoxyethanol only; a preservative-free oily cream; the preservative mixture itself diluted in petrolatum (pet.; test concentrations, 0.05% - 1.5%); and Caprylhydroxamic Acid (or its potassium salt) diluted in pet. (test concentrations, 0.001% - 3.2%). Occlusive patches were applied for 2 days, and the test sites were scored upon patch removal and on days 4 and 5. A control group of 20 eczema patients, who had not used the new moisturizer formulation that contained the preservative mixture, was patched-tested with the preservative mixture and with Caprylhydroxamic Acid. A second control group of 13 subjects, all with uncompromised skin, was patch-tested with all the test materials.

Patch test results for the test group are presented in Table 4. In the test group of patients with compromised skin that developed contact allergy, positive reactions were seen with the new moisturizer formulation (that contained the preservative mixture), Caprylhydroxamic Acid, and the preservative mixture itself; however, reactions were not reported with the old moisturizer formulation (which was preserved with parabens), the formulation with phenoxyethanol only, or the preservative-free cream. For Caprylhydroxamic Acid, +++ reactions were reported with test concentrations ≥ 0.1%, ++ reactions with concentrations ≥ 0.032%, and + reactions with concentrations ≥ 0.01%. Negative results were reported in both the eczema-patient control group and the normal control subjects. The study authors did not elaborate on the lack of reaction by the 33 control subjects to the preservative mixture or Caprylhydroxamic Acid.

As a follow-up, 1% Caprylhydroxamic Acid (pet.) was added to the 2017 epicutaneous preservative series at Helsinki University Central Hospital in an effort to determine if there were any new cases of contact allergy to Caprylhydroxamic Acid in patients with no previous use of the moisturizer series described above; it is not clear if the researchers were referring

only to use of the "new" formulation that contained Caprylhydroxamic Acid.[41] A total of 16 patients with a positive patch test reaction were identified, three with a (++)-reaction and the remainder with a (+)-reaction. Twelve of the 16 patients that presented with atopic dermatitis, hand eczema, or psoriasis had previously used the moisturizer. Of the remaining 4 patients (2 of which had a ++ reaction), 3 presented with eczema of the face or eyelids, and 1 was a hairdresser with hand eczema. The use of products containing Caprylhydroxamic Acid could not be identified, but make up or hair products were suspected. The researchers stated that simultaneous contact allergy to other allergens may facilitate the sensitization, and also that further follow-up is needed to clarify the significance of Caprylhydroxamic Acid as a contact allergen.

Case Reports

In Finland, two case reports of contact allergy were attributed to use of a moisturizer that contained Caprylhydroxamic Acid.[42] Although the moisturizer had been reformulated to no longer include a preservative that contained Caprylhydroxamic Acid (it was only included in formulations produced 2014 – 2016), the patients had used products that had been obtained prior to reformulation. Patch tests were not performed, but the contact allergy was attributed to the Caprylhydroxamic Acid-containing moisturizer based on medical history, use of the old formulation, outbreaks, and clinical presentation.

SUMMARY

Caprylhydroxamic Acid is reported to function in cosmetics as a chelating agent. Hydroxamic acids, such as Caprylhydroxamic Acid, may exist in both keto and enol tautomeric forms; the keto form is likely to predominate in acidic formulation, while the enol may dominate under alkaline conditions. Hydroxamic acids are capable of the inhibition of a variety of enzymes, including ureases, peroxidases, and matrix metalloproteinases. At very high or low pH, Caprylhydroxamic Acid may be hydrolyzed to caprylic acid and hydroxylamine.

Caprylhydroxamic Acid is most frequently synthesized via the transamidation of either methyl or ethyl caprylate with hydroxylamine to yield Caprylhydroxamic Acid. Methanol or ethanol, respectively, is a byproduct of the process. Caprylhydroxamic Acid is reported to be > 99% pure.

According to 2019 FDA VCRP data and Council survey results, Caprylhydroxamic Acid is reported to be used in 227 formulations at maximum leave-on and rinse-off concentrations of 0.25% in body and hand products and 0.3% in bath soaps and detergents, respectively. It is used in products applied near the eye at up to 0.2%, in lipsticks (concentration of use data not reported), in formulations that come into contact with mucous membranes at up to 0.3%, and in baby lotions, oils, and creams at up to 0.15%. It is also reported to be used in products that could possibly be inhaled; a maximum concentration of use of 0.075% was reported for both aerosol and pump hair spray formulations.

NICNAS estimated the total SED to Caprylhydroxamic Acid from cosmetic applications. Assuming that the user is a 60 kg female, that dermal absorption is 100%, that Caprylhydroxamic Acid is always used at 0.5% in cosmetic formulations, and that there is daily exposure to 15 leave-on and rinse-off skin and hair formulations containing this ingredient, the total SED to Caprylhydroxamic Acid through the use of cosmetics was calculated as 1.17 mg/kg bw/day. Using this SED and an NOAEL of 50 mg/kg bw/day (that was derived in a subchronic oral toxicity study in rats), an MOE of 43 was calculated. Because this is not an acceptable MOE, the calculations were again performed with a maximum use concentration of 0.3% in formulations. With this concentration, the MOE was calculated to be 71. Even though this MOE is still below the generally acceptable value of 100, NICNAS stated, given that the exposure estimate is based on the conservative assumption of 100% dermal absorption, and the simultaneous use of various products containing the maximum concentration of Caprylhydroxamic Acid, the risk to the public is not considered unreasonable if products contain a maximum of 0.3%.

Based on the physicochemical properties of Caprylhydroxamic Acid, such as low molecular weight, both percutaneous absorption and absorption across the GI tract are considered likely. Caprylhydroxamic Acid was rapidly hydrolyzed by rat liver homogenates to caprylic acid and hydroxylamine. In rats orally administered 1-[^{14}C]-Caprylhydroxamic Acid, approximately 25% of the radioactivity was excreted as $^{14}CO_2$ after 2 h, and by 24 h, 6.9% and 0.6% was excreted in the urine and the feces, respectively.

The oral LD_{50} of Caprylhydroxamic Acid is reported to be > 8820 mg/kg in rats. In a 13-wk study in which groups of 20 rats were dosed by gavage with up to 2500 mg/kg bw/day 10% Caprylhydroxamic Acid in lactose, with 5% aq. gum arabic as the vehicle, the NOAEL of the test article was determined to be 500 mg/kg bw/day; accordingly, the NOAEL of undiluted Caprylhydroxamic Acid is expected to be 50 mg/kg bw/day. Changes in some clinical chemistry parameters and organ weights (specifically an increase in absolute and relative spleen weight) were observed in the high dose group.

Caprylhydroxamic Acid (10% in 5% gum arabic solution) was administered to groups of 18 mated rats, at doses up to 500 mg/kg bw/day, on days 9 – 14 of gestation. The majority of the dams were killed on day 20 of gestation; some were allowed to litter naturally. There was no mortality during the study, and there were no clinical signs of maternal toxicity. Caprylhydroxamic Acid (tested at 10% and at doses up to 500 mg/kg bw, corresponding to up to 50 mg/kg bw Caprylhydroxamic Acid) was not teratogenic.

In the Ames test, Caprylhydroxamic Acid in DMSO (at up to 5000 µg/plate) was not mutagenic to *S. typhimurium*, with or without metabolic activation, but there was weak but clear dose-dependent mutagenic activity towards *E. coli* at

concentrations up to 1000 µg/plate. Caprylhydroxamic Acid was not genotoxic in a rec assay using *Bacillus subtilis*, and it was not genotoxic in an in vitro mammalian cell micronucleus test (at doses up to 450 µg/ml) using human peripheral blood lymphocytes, with or without metabolic activation.

Caprylhydroxamic Acid was not irritating or sensitizing in numerous studies. Tested neat, it was classified as non-irritant in an EpiDerm™ skin irritation test reconstructed human epidermis tissue containing keratinocytes. Additionally, formulations containing 0.105% Caprylhydroxamic Acid (54 subjects; 24 h semi-occlusive patches), 0.15% Caprylhydroxamic Acid (104 subjects, 24-h occlusive patches; 109 subjects, 48-h occlusive patches), and 0.195% Caprylhydroxamic Acid (52 subjects; 24-h semi-occlusive patches) were not irritants or sensitizers in the HRIPT. In several other HRIPTs (104 subjects; 24-h occlusive patches) in which formulations containing 5% - 15% Caprylhydroxamic Acid were tested as dilutions in distilled water, with a resulting test concentration of 0.3% Caprylhydroxamic Acid, no clinically significant potential for dermal irritation or allergic contact sensitization was observed. However, in all of the studies that used 104 subjects (all formulations were tested at the same time in the same subjects), scattered, transient, barely perceptible to mild or moderate erythema with occasional edema were noted throughout the test; the researchers stated that neither the number of responses nor the peak level of the responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. Undiluted Caprylhydroxamic Acid was not an irritant or a sensitizer in an HRIPT (52 subjects; 24-h semi-occlusive patches).

According to the results of in vitro ocular irritation studies, Caprylhydroxamic Acid is not expected to be an ocular irritant. In a BCOP test, it was concluded that 20% Caprylhydroxamic Acid was not considered an ocular corrosive or severe eye irritant under the conditions of the test. Additionally, in a MatTek EpiOcular™ MTT viability assay, the undiluted test article was classified as non-irritating to the eye.

In provocative testing, a patch test was conducted using 39 patients with compromised skin that had suspected allergenicity to a specific moisturizer formulation that contained 0.075 – 0.15% Caprylhydroxamic Acid. In this test group, positive results were reported to the new moisturizer containing the preservative mixture, to the preservative mixture, and to Caprylhydroxamic Acid itself. A '+' reaction was observed with concentrations ≥ 0.01%, '++' reactions with ≥ 0.032%, and '+++' reactions with ≥ 0.1% Caprylhydroxamic Acid. However, when the same patients were tested with an "old" version of the moisturizer that was preserved with parabens, negative results were reported with the old formulation. Additionally, in 33 control subjects (20 with eczema who had not used this specific moisturizer product that contained the preservative mixture, and 13 with uncompromised skin barrier function), negative results were reported to the preservative mixture and to Caprylhydroxamic Acid alone.

TABLES

Table 1. Physical and chemical properties

Property	Value	Reference
Physical Form	crystalline solid	2,3
Color	white white to tan	3 2
Odor	mild, characteristic	3
Molecular Weight (Da)	159.23	6
Density (g/mL @ 25ºC)	0.3413 (sample not compressed) 0.4789 (sample tamped down)	2,3
Vapor pressure (mm Hg @ 25 ºC)	2.50×10^{-6} (estimated)	2
Melting Point (ºC)	≥ 78 to ≤ 81 81 79 - 81	3 2 21
Boiling Point (°C)	343.32	21
Water Solubility (g/L @ 23ºC)	1.55	2,3
log K_{ow} (@ 25°C)	1.66 (estimated) 2.827 ± 0.191 (estimated)	2,3 6
Disassociation constants (pKa; (@ 25°C)	9.56 ± 0.20 (estimated)	6

Table 2. Frequency (2019) and concentration (2018) of use of Caprylhydroxamic Acid

	# of Uses[12]	Max Conc of Use (%)[13]
Totals*	**227**	**0.075 – 0.3**
Duration of Use		
Leave-On	*162*	*0.075 – 0.25*
Rinse-Off	*65*	*0.12 – 0.3*
Diluted for (Bath) Use	*NR*	*NR*
Exposure Type		
Eye Area	14	0.11 – 0.2
Incidental Ingestion	2	NR
Incidental Inhalation-Spray	1; 43[a]; 68[b]	0.075 (aerosol and pump) 0.075 - 0.23[a]
Incidental Inhalation-Powder	3; 68[b]; 4[c]	0.12[c]
Dermal Contact	206	0.11 – 0.3
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	18	0.075 – 0.23
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	6	0.13 – 0.3
Baby Products	6	0.15

*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] 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

Table 3. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population/System	Procedure	Results	Reference
IN VITRO					
Irritation					
Caprylhydroxamic Acid	undiluted	reconstructed human epidermis tissue containing keratinocytes	EpiDerm™ skin irritation test, in accord with OECD TG 439; tissue viability was determined with the MTT assay	classified as non-irritant; tissue viability was 102.6%	21
HUMAN					
Irritation and Sensitization					
eyeliner formulation containing 0.105% Caprylhydroxamic Acid	applied neat; 0.2 ml	54 subjects	HRIPT induction: 24-h semi-occlusive patch (1 sq in) applied to the upper back 3 x/wk for 3 wks, for a total of 9 applications; test sites were evaluated 24 or 48 h after patch removal challenge: after a 2-wk non-treatment period, a 24-h patch was applied to a previously untreated test site on the back; test sites were evaluated upon patch removal and at 48 and 72 h	not an irritant or sensitizer	26
lotion containing 0.15% Caprylhydroxamic Acid (also, 72.35% water; 5% caprylic/ capric triglyceride; 5% isopropyl myristate; 4.5% arachidyl alcohol (and) behenyl alcohol (and) arachidyl glucoside; 4% petrolatum; 3% cetyl alcohol; 3% stearyl alcohol; 3% glycerin)	applied neat; 0.2 ml	104 subjects	HRIPT induction: 24-h occlusive patch (¾ in x ¾ in) applied to the upper back 3 x/wk for 3 wks, for a total of 9 applications; test sites were evaluated 24 or 48 h after patch removal challenge: after a 2-wk non-treatment period, a 24-h patch was applied to a previously untreated test site on the back; test sites were evaluated upon patch removal and at 48 or 72 h	"no clinically significant potential for dermal irritation or allergic contact sensitization" - one subject (#10) exhibited barely perceptible to moderate erythema and edema after induction patches 2-5, resulting in the discontinuation of subsequent patch applications; the researchers stated this pattern of skin reactivity was indicative of a pre-existing hyper-sensitivity to one or more ingredients in the formulation - in the remaining subjects, scattered, transient barely perceptible to mild erythema with occasional edema was noted throughout the test; specifically, 1 subject (#42) that had barely perceptible erythema following induction patches 8 and 9 exhibited barely perceptible erythema upon challenge patch removal and mild erythema and edema 48 h after challenge; the researchers stated that neither the number of responses or the peak level of these responses were inconsistent with similar diluted formula-tions evaluated under repetitive, occlusive patch conditions	27
water-in-oil (W/O) thick balm containing 0.15% Capryl-hydroxamic Acid (also, 66.35% water; 10% sunflower seed oil; 10% isopropyl palmitate; 5% petrolatum; 3.5% octyldodecanol (and) octyldodecyl xyloside (and PEG-30 dipolyhydroxy-stearate; 3% glycerin; 2% beeswax) [concentrations stated as provided]	applied neat; 0.2 ml	104 subjects	HRIPT – same protocol as above	"did not indicate a clinically significant potential for dermal irritation or allergic contact sensitization" - one subject (#10) exhibited mild to moderate erythema and edema after induction patches 4 and 5, resulting in the discontinuation of subsequent patch applications; same comment by the researchers as given above - in the remaining subjects, scattered, transient barely perceptible to mild erythema with occasional edema was noted throughout the test; specifically, 1 subject (#42) that had barely perceptible erythema following induction patches 5-9 exhibited mild erythema and edema 48 h after challenge; same statement applies as given above by the researchers	28

Table 3. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population/System	Procedure	Results	Reference
"wipe juice" containing 0.15% Caprylhydroxamic Acid containing 0.15% Capryl-hydroxamic Acid (also, 94.85% water; 3% propane-diol; 2% polysorbate 20)	applied neat; 0.2 ml	104 subjects	HRIPT – same protocol as above	"no clinically significant potential for dermal irritation or allergic contact sensitization" - scattered, transient barely perceptible (0.5) to mild (1) erythema with occasional edema was noted throughout the test; specifically, 1 subject (#42) that had barely perceptible erythema following induction patches 6 and 8 exhibited mild erythema and edema 48 h after challenge; same statement applies as given above by the researchers	29
facial cream containing 0.15% Caprylhydroxamic Acid	applied neat; 0.02 ml	109 subjects	HRIPT induction: 48-h occlusive patch applied 3x/wk for 3 wks challenge: after a 2-wk non-treatment period, patches were applied to inducted and previously untreated test sites; test sites were evaluated at 30 min, 48 h and 72 h after patch removal	not a sensitizer 1 subject had "low level reaction" (score of 0 or 1) during challenge; no reactions during induction	30
brow thickening powder containing 0.195% Caprylhydroxamic Acid	applied neat; 200 mg product (0.39 mg Caprylhydroxamic Acid) dose/unit area: 0.06 mg/cm^2	52 subjects	HRIPT induction: 24-h semi-occlusive patch (application area 6.45 cm^2) moistened to ensure adherence of the test article applied to the back 3 x/wk for 3 wks, for a total of 9 applications; test sites were evaluated 24 or 48 h after patch removal challenge: after a 2-wk non-treatment period, a 24-h patch was applied to previously untreated test site on the back; test sites were evaluated upon patch removal and 48 h later	not an irritant or sensitizer	31
formulation containing 5% Caprylhydroxamic Acid (and 30% hexanediol; 65% propanediol)	tested as a 6% dilution with distilled water (resultant test concentration – 0.3% Caprylhydroxamic Acid); 0.2 ml	104 subjects	HRIPT induction: 24-h occlusive patch (¾ in x ¾ in) applied to the upper back 3 x/wk for 3 wks, for a total of 9 applications; test sites were evaluated 24 or 48 h after patch removal challenge: after a 2-wk non-treatment period, a 24-h patch was applied to a previously untreated test site on the back; test sites were evaluated upon patch removal and at 48 or 72 h	"no clinically significant potential for dermal irritation or allergic contact sensitization" - scattered, transient barely perceptible to moderate erythema with occasional edema was noted throughout the test; specifically, 1 subject (#42) that had barely perceptible erythema following induction patches 4 and 8 and mild erythema following induction patch 9 exhibited barely perceptible erythema at challenge patch removal and mild erythema and edema 48 h after challenge; the researchers stated that neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions	32
formulation containing 7.5% Caprylhydroxamic Acid (and 92.5% propanediol)	tested as a 4% dilution with distilled water (resultant test concentration – 0.3% Caprylhydroxamic Acid); 0.2 ml	104 subjects	HRIPT – same protocol as above	"no clinically significant potential for dermal irritation or allergic contact sensitization" - scattered, transient barely perceptible to mild erythema with occasional edema was noted throughout the test; specifically, 1 subject (#42) that had barely perceptible erythema following induction patches 4 - 8 exhibited mild erythema and edema 48 h after challenge; same statement applies as given above by the researchers	33

Table 3. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population/System	Procedure	Results	Reference
formulation containing 10% Caprylhydroxamic Acid (and 75% glyceryl caprylate and 15% glycerin) [concentrations stated as provided]	tested as a 3% dilution with distilled water (resultant test concentration – 0.45% Caprylhydroxamic Acid); 0.2 ml	104 subjects	HRIPT – same protocol as above	"no clinically significant potential for dermal irritation or allergic contact sensitization" - scattered, transient barely perceptible to mild erythema with occasional edema was noted throughout the test; specifically, 1 subject (#42) that had barely perceptible erythema following induction patches 5, 6, and 8 and mild erythema following induction patch 9 exhibited barely perceptible erythema at challenge patch removal and mild erythema and edema 48 h after challenge; same statement applies as given above by the researchers	34
formulation containing 15% Caprylhydroxamic Acid (and 70% phenoxyethanol; 7.5% methylpropanediol; 7.5% water)	tested as a 2% dilution with distilled water (resultant test concentration – 0.3% Caprylhydroxamic Acid); 0.2 ml	104 subjects	HRIPT – same protocol as above	"no clinically significant potential for dermal irritation or allergic contact sensitization" - scattered, transient barely perceptible to moderate erythema with occasional edema was noted throughout the test; specifically, 1 subject (#42) that had barely perceptible erythema following induction patches 5, 6, and 8 and mild erythema following induction patch 9 exhibited barely perceptible erythema at challenge patch removal and mild erythema and edema 48 h after challenge; same statement applies as given above by the researchers	35
formulation containing 15% Caprylhydroxamic Acid (and 71% caprylyl glycol and 14% glycerin)	tested as a 2% dilution with distilled water (resultant test concentration – 0.3% Caprylhydroxamic Acid); 0.2 ml	104 subjects	HRIPT – same protocol as above	"no clinically significant potential for dermal irritation or allergic contact sensitization" - scattered, transient barely perceptible to moderate erythema with occasional edema was noted throughout the test; specifically, 1 subject (#42) that had barely perceptible erythema following induction patches 5- 8 exhibited barely perceptible erythema 48 h after chal-lenge; same statement applies as given above by the researchers	36
Caprylhydroxamic Acid	undiluted; no vehicle indicated	52 subjects	HRIPT induction: 24-h semi-occlusive patch (1 in^2) applied to the upper back 3 x/wk for 3 wks, for a total of 9 applications; test sites were evaluated 24 or 48 h after patch removal challenge: after a 2-wk non-treatment period, a 24-h patch was applied to a previously untreated test site on the back; test sites were evaluated upon patch removal and at 48 and 72 h	not an irritant or sensitizer	37

Abbreviations: HRIPT - human repeated insult patch test; MTT - 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide ; OECD - Organisation for Economic Co-operation; TG - test guideline

Table 4. Patch test results in patients with compromised skin that had suspected contact allergy to a new moisturizer formulation[40]

	New Moisturizer Formulation							
	cream	**oily cream**	**lotion**					
+++	6	7	4					
++	13	11	10					
+	13	15	12					
?+	2	1	2					
negative	0	2	1					
irritant reaction	0	0	0					
no. tested	***34***	***36***	***29***					
	Caprylhydroxamic Acid (or its potassium salt)							
	0.001%	**0.0032%**	**0.01%**	**0.032%**	**0.10%**	**0.32%**	**1.0%**	**3.2%**
+++	0	0	0	0	1	4	10	9
++	0	0	0	3	6	15	21	6
+	0	0	1	14	18	17	7	0
?+	0	1	3	6	10	2	1	1
negative	7	6	8	16	4	1	0	0
irritant reaction	0	0	0	0	0	0	0	0
no. tested	***7***	***7***	***12***	***39***	***39***	***39***	***39***	***16***
	Preservative Mixture							
	0.05%	**0.15%**	**0.5%**	**1.5%**				
+++	0	0	2	5				
++	2	3	6	10				
+	7	8	10	16				
?+	0	8	10	4				
negative	30	18	10	3				
irritant reaction	0	2	1	1				
no. tested	***39***	***39***	***39***	***39***				

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Caprylhydroxamic Acid – 2019 VCRP data

CAPRYLHYDROXAMIC ACID	01A - Baby Shampoos	1
CAPRYLHYDROXAMIC ACID	01B - Baby Lotions, Oils, Powders, and Creams	4
CAPRYLHYDROXAMIC ACID	01C - Other Baby Products	1
CAPRYLHYDROXAMIC ACID	03A - Eyebrow Pencil	5
CAPRYLHYDROXAMIC ACID	03D - Eye Lotion	5
CAPRYLHYDROXAMIC ACID	03F - Mascara	1
CAPRYLHYDROXAMIC ACID	03G - Other Eye Makeup Preparations	3
CAPRYLHYDROXAMIC ACID	04A - Cologne and Toilet waters	1
CAPRYLHYDROXAMIC ACID	05A - Hair Conditioner	3
CAPRYLHYDROXAMIC ACID	05F - Shampoos (non-coloring)	8
CAPRYLHYDROXAMIC ACID	05G - Tonics, Dressings, and Other Hair Grooming Aids	1
CAPRYLHYDROXAMIC ACID	05I - Other Hair Preparations	5
CAPRYLHYDROXAMIC ACID	07B - Face Powders	3
CAPRYLHYDROXAMIC ACID	07C - Foundations	2
CAPRYLHYDROXAMIC ACID	07E - Lipstick	2
CAPRYLHYDROXAMIC ACID	07I - Other Makeup Preparations	1
CAPRYLHYDROXAMIC ACID	10A - Bath Soaps and Detergents	2
CAPRYLHYDROXAMIC ACID	10E - Other Personal Cleanliness Products	2
CAPRYLHYDROXAMIC ACID	11E - Shaving Cream	1
CAPRYLHYDROXAMIC ACID	12A - Cleansing	15
CAPRYLHYDROXAMIC ACID	12C - Face and Neck (exc shave)	53
CAPRYLHYDROXAMIC ACID	12D - Body and Hand (exc shave)	15
CAPRYLHYDROXAMIC ACID	12F - Moisturizing	36
CAPRYLHYDROXAMIC ACID	12G - Night	6
CAPRYLHYDROXAMIC ACID	12H - Paste Masks (mud packs)	33
CAPRYLHYDROXAMIC ACID	12J - Other Skin Care Preps	18

INOLEX

20 March 2019

Cosmetic Ingredient Review
1620 L St, NW, Suite 1200
Washington, DC 20036-4702
Attn: Dr. Bart Heldreth, Executive Director

Subject: Method of Manufacture for Caprylhydroxamic Acid

Dear Dr. Heldreth:

I am writing to provide CIR with information relating to the commercial manufacturing methods employed for the production of caprylhydroxamic acid (CHA) for use as a cosmetic ingredient.

Contrary to the methods reported in the Scientific Literature Review for Public Comment dated 21 February 2019, commercial CHA production does not involve the use of caprylaldehyde, nor capryloyl chloride, as starting materials.

CHA is most frequently synthesized via the transamidation of either methyl caprylate or ethyl caprylate with hydroxylamine to yield CHA with either methanol or ethanol as a byproduct, respectively. The reaction is conducted in either methanol or ethanol (depending on which caprylate ester is used as the starting material) under refluxing conditions. CHA is isolated and purified via recrystallization from ethyl acetate, followed by washing and drying of the crystalline CHA to obtain the ingredient at purities > 99%. The entire method of manufacture for CHA is summarized in the process flow diagram below (Figure 1).

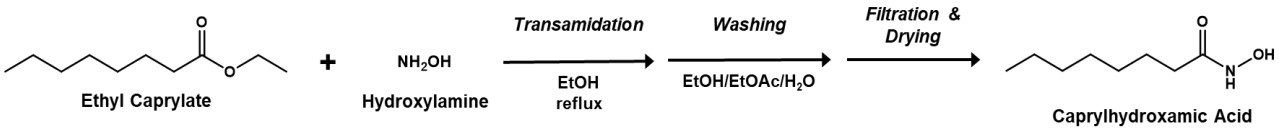

Figure 1. Example process flow diagram for commercial scale production of CHA.

Many variations of this synthesis route are reported in the open literature and patents. See for example U.S. Patent 6,739,454 and the references cited therein.

The SLR for CHA should be amended to indicate this synthesis route as the method of manufacture for CHA. To the best of my company's knowledge, this route and minor variations on it are the only methods practiced for the commercial production of CHA used as a cosmetic ingredient.

Please feel free to contact me with any questions or concerns regarding this matter.

INOLEX

Sincerely,

Michael J. Fevola, Ph.D.
Vice President, Research & Development
INOLEX, Inc.
2101 S. Swanson St.
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FINAL REPORT

THE *SALMONELLA TYPHIMURIUM* REVERSE MUTATION ASSAY (AMES TEST), LIQUIDS OR SOLUBLE CHEMICALS

PROCEDURE NO. STP0098 REV 01
PROTOCOL DETAIL SHEET NO. 200701143 REV 01

LABORATORY NO. 373535

PREPARED FOR:

[redacted]

SUBMITTED BY:

NELSON LABORATORIES, INC.
6280 S. REDWOOD RD.
SALT LAKE CITY, UT 84123-6600
801-290-7500

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NELSON LABORATORIES, INC.

QAU AUDIT STATEMENT

[X] USFDA (21 CFR PART 58) [ ] USEPA (40 CFR PART 160)

THE *SALMONELLA TYPHIMURIUM* REVERSE MUTATION ASSAY (AMES TEST), LIQUIDS OR SOLUBLE CHEMICALS

Study Director: Final Report Dated:

Chad Summers, A.S. 04 June 2007

1. The test was conducted in accordance with the USFDA or USEPA Regulations as noted above. All laboratory results pertaining to this study are recorded in Nelson Laboratories' Data File Number 373535.

2. In accordance with the Good Laboratory Practice Regulations, the Sample Preparation phase(s) of this study was inspected by the Quality Assurance Unit on: 01 May 2007. The findings of the inspection(s) were reported to Management and to the Study Director on: 30 May 2007.

3. The Quality Assurance Unit has reviewed this report and has determined that the methods and standard operating procedures are accurately described, and that the reported results accurately reflect the raw data.

4. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study:

Michelle Lee
Chad Summers
Heidi Waldron
Dr. Jerry Nelson
Jeff Hills

QUALITY ASSURANCE: April Wannstrom DATE: 04 Jun 2007

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THE *SALMONELLA TYPHIMURIUM* REVERSE MUTATION ASSAY (AMES TEST), LIQUIDS OR SOLUBLE CHEMICALS

LABORATORY NUMBER:	373535
PROCEDURE NUMBER:	STP0098 REV 01
PROTOCOL DETAIL SHEET NUMBER:	200701143 REV 01
SAMPLE SOURCE:	[redacted]
SAMPLE IDENTIFICATION:	Caprylohydroxamic Acid P.O. #JP0407A
DEVIATIONS:	None
PROTOCOL APPROVAL DATE:	30 Apr 2007
SAMPLE RECEIVED DATE:	20 Apr 2007
LAB PHASE START DATE:	30 Apr 2007
LAB PHASE COMPLETION DATE:	31 May 2007
REPORT ISSUE DATE:	04 June 2007

INTRODUCTION:

The *Salmonella typhimurium* reverse mutation assay (Ames test) is used to determine the potential mutagenic activity of the test sample. The assay is based on exposing a large number of the test organisms to the test sample in agar plates. The agar plates are monitored for growth of revertants (organisms mutating to the wild type) which are counted and used to estimate the mutagenic potential of the test article.

The Ames test employs several histidine dependant (His+) strains of *S. typhimurium* which require the amino acid histidine for growth. The test detects mutations which cause the bacterial strains to revert to histidine independent (His-) bacteria which are capable of synthesizing histidine and can grow in the absence of histidine. The assay used tester strains TA97a, TA98 TA100, TA102 and TA1535 which were selected to detect various types of mutagens. The test is performed both with and without metabolic activation using an S-9 activation system. The S-9 activation system is designed to simulate mammalian liver enzyme systems and is used to detect substances which undergo metabolic activation from non-mutagenic forms.

PROCEDURE:

Broth Culture Preparation: Commercial culture discs were used to inoculate nutrient broth for testing. The cultures were incubated at 37 ± 2°C for 10-14 hours on an orbital shaker until when measured spectrophotometrically at 660 nm, an absorbance reading of approximately 1.0 to 2.0 was obtained. Validation data of the cultures showed absorbance readings in the above range resulted in concentrations of approximately 10^9 CFU/mL.

Strain Genotype Verification: The culture disc lot numbers used were checked for presence of appropriate strain genotype characteristics. These tests included verification of the following:

- Presence of uvrB mutation
- Presence or absence of R-factor plasmid
- Presence of rfa mutation
- Requirement for histidine

The uvrB mutation was verified by demonstrating UV sensitivity (lack of repair system). The R-factor was checked by determining sensitivity or resistance to ampicillin (0.08% in 0.02 NaOH). The presence of the rfa mutation was verified by demonstrating sensitivity to crystal violet (0.1% in water) on nutrient agar plates. The histidine requirement was assured by plating onto minimal glucose agar plates spread with 0.1 mL of 0.5 mM biotin and both with and without 0.1 mL of 0.1 M histidine.

Sample Preparation: The sample was dissolved and diluted in Dimethyl Sulfoxide (DMSO) and tested at the following concentrations per plate: 5mg, 1.6mg, 0.5mg, 0.16mg, 0.05mg and 0.016mg/plate. The concentrations tested were based on the OECD 471 recommended concentrations. An aliquot of the DMSO used was tested as the negative solvent control.

Note: The test sample was analyzed on two separate test dates in all strains except TA97a. In strains TA98, TA100, TA102 and TA1535 an additional concentration (0.016mg/plate) was tested in order to meet the OECD requirement of 4 analyzable doses below the toxic level. All six concentrations were tested on the same test day in strain TA97a.

Metabolic Activation System: The S-9 activation system was used to screen for the presence of mutagens from byproducts of the test sample. Rat liver S-9 homogenate was obtained from Molecular Toxicology, Inc. The homogenate was kept frozen at ≤-60°C upon receipt. Plates requiring activation contained approximately 20 µL rat liver S-9 per plate. When working with soft agar the plates did not exceed 47°C.

Top Agar Preparation: Aliquots of top agar were melted and maintained at 45 ± 2°C. Each 100 mL aliquot of top agar was fortified with 5-10 mL of 0.5 mM biotin and 0.5 mM histidine prior to use.

Plate Incorporation Tests: The test sample, solvent control and chemical controls were tested both with and without S-9 activation. Sterile 13 x100 mm test tubes were transferred to a waterbath held at 45 ± 2°C. Two mL aliquots of top agar were transferred to each test tube. Three replicates for each of the materials were prepared and the test organism and materials were added as follows:

Three replicates for the solvent control with 100 µL test organism plus 100 µL solvent control
Three replicates for the each sample concentration with 100 µL test organism plus 100 µL sample.

Three test tubes for each chemical control with 100 µL test organism plus 10 µL chemical control.

Each replicate requiring S-9 activation had 0.5 mL of the prepared S-9 mix added.

The replicates were vortexed, poured onto MGPA plates, swirled to form an even layer and allowed to solidify. The plates were incubated for growth 37 ± 2°C for 48-72 hours.

Spot Tests: The sample was also analyzed using the spot method on plates with and without the S-9 activation system. Two mL aliquots of the top agar mixture and 0.1 mL of the appropriate test organism was added to minimal glucose agar plates. The plates were allowed to harden then 10 µL of the sample was added as a spot on the surface of the plate. The plates were incubated for growth of the organisms at 37 ± 2°C for 48-72 hours. Only the highest sample concentration was tested using the spot method.

Chemical Control Materials: The following chemical controls were used: Sodium Azide, Mitomycin-C, 4-nitro-0-phenylene-diamine (NPD), and 2 aminofluorene (2AF). The chemical controls were tested using the plate incorporation method only.

Acceptance Criteria: The criteria for acceptance of the test and criteria for determination of a mutagen are listed below.

1) Tested strains for genotype verification and achieved the appropriate responses.
2) All chemical controls included in the test gave the appropriate responses.
3) The reversion rates for each tester strains was within the historical ranges as outlined in the protocol.

Criteria for a Mutagen: A two-fold increase in the number of revertants when compared to the solvent control. (percent of control >200%). A clear dose related response when multiple concentrations were tested.

Criteria for a Non-Mutagen: A less than two-fold increase in the number of revertants when compared to the solvent control. (percent of control <200%). No clear dose related response when multiple concentrations were tested.

RESULTS/CONCLUSION:

The test results are summarized in Tables 1-11. The results are calculated using a validated computer program. Manual calculations may differ slightly due to rounding. Tables 1-9 contain the results for the plate incorporation tests. All results greater than 300 colony forming units (CFU) are considered estimates. Table 10 contains the results for the spot tests recorded as + (positive) or - (negative). A positive result indicates that the material showed a zone of

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increased reversion at the inoculation site. A negative result indicates that the material did not show a zone of increased reversion at the inoculation site. Table 11 contains the results for the genotype verification. All five tester strain cultures showed the appropriate results in the genotype verification assay.

SUMMARY:

The test sample did not produce a two-fold increase in the number of revertants or a clear dose related response in any of the five tester strains. The spot plates showed a clear zone at the inoculation site indicating that the sample was toxic to the bacteria but was not surrounded by a ring of increased reversion which indicates that the sample was not mutagenic. In summary, the sample concentrations tested did not meet the criteria for a potential mutagen

DATA DISPOSITION:

The raw data and final report from this study are archived at NLI or an approved off-site location.

STATEMENT OF UNCERTAINTY:

If applicable, the statement of uncertainty is available to sponsors upon request.

Technical Reviewer

Chad Summers, A.S.
Study Director

04 Jan 2007
Study Completion Date

CJS/ad

TABLE 1. TA97a Results
5mg, 1.6mg, 0.5mg, 0.16mg, 0.05mg and 0.016/plate.
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	135	172	131	146	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	105	113	115	111	76
Sample 0.16mg/plate	110	124	144	126	86
Sample 0.05mg/plate	161	125	134	140	96
Sample 0.016mg/plate	158	181	169	169	116
Sodium Azide:	145	140	138	141	97 (-)
NPD:	529	700	549	593	406 (+)
2AF:	180	155	124	153	105 (-)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	235	250	252	246	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	207	222	182	204	83
Sample 0.16mg/plate	205	225	227	219	89
Sample 0.05mg/plate	232	234	200	222	90
Sample 0.016mg/plate	209	168	220	199	81
Sodium Azide:	200	184	182	189	77 (-)
NPD:	567	524	692	594	242 (+)
2AF:	1127	1154	1402	1228	500 (+)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( ).

UFA: Unsuitable For Analysis. No growth on the plates due to toxicity to the bacterial cells.

TABLE 2. TA98 Results
5mg, 1.6mg, 0.5mg, 0.16mg, and 0.05mg/plate.
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	22	17	16	18	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	25	15	13	18	96
Sample 0.16mg/plate	18	13	15	15	84
Sample 0.05mg/plate	17	25	15	19	104
Sodium Azide:	25	27	15	22	122 (-)
NPD:	352	318	725	465	2536 (+)
2AF:	29	21	38	29	160 (-)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	31	34	27	31	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	22	26	26	25	80
Sample 0.16mg/plate	23	27	18	23	74
Sample 0.05mg/plate	26	26	29	27	88
Sodium Azide:	18	23	21	21	67 (-)
NPD:	996	963	1050	1003	3271 (+)
2AF:	3059	2800	2922	2927	9545 (+)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( )

TABLE 3. TA98 Results
0.016mg/plate.
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	20	21	18	20	
Sample 0.016mg/plate	19	16	23	19	98
Sodium Azide:	23	25	21	23	117 (-)
NPD:	448	462	479	463	2354 (+)
2AF:	19	21	29	23	117 (-)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	35	24	32	30	
Sample 0.016mg/plate	26	22	22	23	77
Sodium Azide:	28	36	28	31	101 (-)
NPD:	1926	1366	1947	1746	5757 (+)
2AF:	3379	3627	3680	3562	11743 (+)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( ).

TABLE 4. TA100 Results
5mg, 1.6mg, 0.5mg, 0.16mg, and 0.05mg/plate.
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	140	145	148	144	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	128	103	144	125	87
Sample 0.16mg/plate	176	161	180	172	119
Sample 0.05mg/plate	151	134	167	151	104
Sodium Azide:	973	867	914	918	636 (+)
NPD:	464	499	532	498	345 (+)
2AF:	177	163	172	171	118 (-)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	155	170	156	160	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	144	150	130	141	88
Sample 0.16mg/plate	158	153	175	162	101
Sample 0.05mg/plate	144	167	160	157	98
Sodium Azide:	466	343	690	500	312 (+)
NPD:	476	468	535	493	307 (+)
2AF:	2264	2106	2312	2227	1389 (+)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( ).

UFA: Unsuitable For Analysis. No growth on the plates due to toxicity to the bacterial cells.

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[redacted]
Lab Number 373535

TABLE 5. TA100 Results
0.016mg/plate
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	179	213	166	186	
Sample 0.016mg/plate	205	188	169	187	101
Sodium Azide:	849	1787	715	1117	601 (+)
NPD:	613	533	594	580	312 (+)
2AF:	204	210	211	208	112 (-)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	181	173	176	177	
Sample 0.016mg/plate	199	184	199	194	110
Sodium Azide:	1546	1517	1395	1486	841 (+)
NPD:	521	547	512	527	298 (+)
2AF:	2344	2529	2468	2447	1385 (+)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( ).

TABLE 6. TA102 Results
5mg, 1.6mg, 0.5mg, 0.16mg, and 0.05mg/plate
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	296	360	343	333	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	276	345	239	287	86
Sample 0.16mg/plate	352	288	334	325	97
Sample 0.05mg/plate	333	336	346	338	102
Sodium Azide:	325	319	321	322	97 (-)
NPD:	319	329	355	334	100 (-)
MITOMYCIN-C:	1615	1477	1686	1593	478 (+)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	412	417	401	410	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	599	555	520	558	136
Sample 0.16mg/plate	492	465	581	513	125
Sample 0.05mg/plate	453	496	465	471	115
Sodium Azide:	414	404	441	420	102 (-)
NPD:	443	427	419	430	105 (-)
MITOMYCIN-C:	1339	1525	1738	1534	374 (+)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( ).

UFA: Unsuitable For Analysis. No growth on the plates due to toxicity to the bacterial cells.

TABLE 7. TA102 Results
0.016mg/plate.
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	329	342	312	328	
Sample 0.016mg/plate	327	324	369	340	104
Sodium Azide:	321	367	361	350	107 (-)
NPD:	410	301	303	338	103 (-)
MITOMYCIN-C:	1690	1552	1547	1596	487 (+)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	422	448	411	427	
Sample 0.016mg/plate	494	452	414	453	106
Sodium Azide:	484	425	358	422	99 (-)
NPD:	405	437	429	424	99 (-)
MITOMYCIN-C:	1502	1984	1910	1799	421 (+)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( ).

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TABLE 8. TA1535 Results
5mg, 1.6mg, 0.5mg, 0.16mg, and 0.05mg/plate.
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	14	21	17	17	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	21	5	9	12	67
Sample 0.16mg/plate	15	24	18	19	110
Sample 0.05mg/plate	21	20	16	19	110
Sodium Azide:	1033	1076	1040	1050	6056 (+)
NPD:	29	23	24	25	146 (-)
2AF:	20	17	29	22	127 (-)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE.	PERCENT OF CONTROL:
DMSO Negative Control	11	16	10	12	
Sample 5mg/plate	UFA	UFA	UFA	NA	NA
Sample 1.6mg/plate	UFA	UFA	UFA	NA	NA
Sample 0.5mg/plate	4	4	2	3	27
Sample 0.16mg/plate	11	9	12	11	86
Sample 0.05mg/plate	9	12	10	10	84
Sodium Azide:	484	526	495	502	4068 (+)
NPD:	13	17	18	16	130 (-)
2AF:	17	17	19	18	143 (-)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( ).

UFA: Unsuitable For Analysis. No growth on the plates due to toxicity to the bacterial cells.

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TABLE 9. TA1535 Results
0.016mg/plate.
(Number of Revertants)

RESULTS WITHOUT ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	20	18	24	21	
Sample 0.016mg/plate	15	23	15	18	85
Sodium Azide:	1048	1420	948	1139	5510 (+)
NPD:	30	33	23	29	139 (-)
2AF:	18	14	19	17	82 (-)
RESULTS WITH S-9 ACTIVATION:					
IDENTIFICATION:	PLATE COUNT RESULTS:			AVERAGE:	PERCENT OF CONTROL:
DMSO Negative Control	25	23	21	23	
Sample 0.016mg/plate	17	23	24	21	93
Sodium Azide:	920	1431	1016	1122	4880 (+)
NPD:	22	23	30	25	109 (-)
2AF:	16	20	23	20	86 (-)

Note: Percent of control results greater than 200 qualify as positive according to the test acceptance criteria. The expected result for the chemical controls is included as ± in the parentheses ( ).

TABLE 10. Spot Test Results

RESULTS WITHOUT ACTIVATION:					
Identification:	TA97A	TA98	TA100	TA102	TA1535
Test Sample 5mg:	-	-	-	-	-
RESULTS WITH S-9 ACTIVATION:					
Identification:	TA97A	TA98	TA100	TA102	TA1535
Test Sample 5mg:	-	-	-	-	-

Note: Results are reported as ±. The spot plates showed a clear zone at the inoculation site indicating that the sample was toxic to the bacteria but was not surrounded by a ring of increased reversion which indicates that the sample was not mutagenic.

TABLE 11. Strain Verification Results

PARAMETER	STRAINS				
	TA97A	TA98	TA100	TA102	TA1535
uvrB	+	+	+	-	+
R-factor	+	+	+	+	-
rfa	+	+	+	+	+
Histidine Requirement	+	+	+	+	+

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FINAL REPORT

Study Title

***In Vitro* Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL)**

Test Article

Caprylhydroxamic Acid

Authors

Shambhu Roy, Ph.D.
Meena Jois, B.S.

Study Completion Date
03 April 2013

Testing Facility

BioReliance
9630 Medical Center Drive
Rockville, Maryland 20850

BioReliance Study Number

AD64VD.348REACH.BTL

Sponsor

[redacted]

STATEMENT OF COMPLIANCE

Study No. AD64VD.348REACH.BTL was conducted in compliance with the US FDA Good Laboratory Practice Regulations as published in 21 CFR 58 and the OECD Principles of Good Laboratory Practices C(97)186/Final in all material aspects with the following exceptions:

1. The identity, strength, purity and composition or other characteristics to define the test article and the stability of the test article were determined by the Sponsor. However, the Certificate of Analysis does not indicate the regulations under which the analyses were conducted.

   Study Director Impact Statement: Since the test article was characterized and released for use in this study, the Study Director has concluded that this had no adverse impact on the integrity of the data or the validity of the study conclusion.

2. Analyses to determine the uniformity, concentration and/or stability of the test article dosing formulations were not performed by the testing facility or the Sponsor.

   Study Director Impact Statement: Due to the lack of dose formulation analysis, the interpretation of the study data was based on the nominal dose levels as documented in the study records and not on the actual formulated test article concentrations as confirmed by analytical results. Nevertheless, toxicity in the assay demonstrated that the test system was dosed up to the regulatory-required level.

Shambhu Roy, Ph.D.
Study Director

03 April 2013
Date

BioReliance by SAIC

Quality Assurance Statement

Study Information

Number: AD64VD.348REACH.BTL

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practices 21CFR 58
OECD Principles of Good Laboratory Practices (C(97)186/Final)

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
01-Mar-2013	01-Mar-2013	Observation of Test System	01-Mar-2013	01-Mar-2013
19-Mar-2013	19-Mar-2013	Data and Draft Reporting	19-Mar-2013	19-Mar-2013
03-Apr-2013	03-Apr-2013	Final Reporting	03-Apr-2013	03-Apr-2013

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

For a multisite study, test site QA Statements are located in the corresponding contributing scientist report.

E-signature

Quality Assurance: Carlton Hall 03-Apr-2013 7:38 pm GMT

Reason for signature: QA Approval

Printed by:Carlton Hall
Printed on:3-Apr-13

In Vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL)

STUDY INFORMATION

Sponsor:	[redacted]
Sponsor's Authorized Representative:	[redacted]
Testing Facility:	**BioReliance** **9630 Medical Center Drive** **Rockville, Maryland 20850**
Testing Facility Management:	**Timothy E. Lawlor, MA** **Director, Study Management** **Genetic Toxicology**
Study Director:	**Shambhu Roy, Ph.D.**
BioReliance Study No.:	**AD64VD.348REACH.BTL**
Test Article I.D.:	**Caprylhydroxamic Acid**
Test Article Lot No.:	**AM4744**
Test Article CAS No.:	**7377-03-9**
Test Article Purity:	**98.09% (per Certificate of Analysis)**
Test Article Molecular Weight:	**159.23 (provided by Sponsor)**
Test Article Description:	**White powder**
Storage Conditions:	**Room temperature, stored protected from light**
Test Article Receipt/Login Date:	**03 Dec 2012**
Study Initiation:	**18 Dec 2012**
Experimental Starting Date (first day of data collection):	**07 Jan 2013**
Experimental Start Date (first day test article administered to test system):	**09 Jan 2013**
Experimental Completion Date:	**04 Mar 2013**
Laboratory Supervisor:	**Shyam Kumar, B.S.**

TABLE OF CONTENTS

SUMMARY

The test article, Caprylhydroxamic Acid, was tested in the *in vitro* mammalian cell micronucleus test using human peripheral blood lymphocytes (HPBL) in both the absence and presence of an Aroclor-induced S9 activation system. A preliminary toxicity was performed to establish the dose range for testing in the micronucleus test. The micronucleus assay was used to evaluate the aneugenic and clastogenic potential of the test article.

Dimethyl sulfoxide (DMSO) was used as the vehicle based on the solubility of the test article and compatibility with the target cells. In a solubility test conducted at BioReliance, the test article formed a soluble and clear solution in DMSO at a concentration of approximately 500 mg/mL, the maximum concentration tested for solubility.

In the preliminary toxicity assay, the doses tested ranged from 0.159 to 1590 µg/mL (10 mM). HPBL cells were treated for 4 and 24 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 24 hours after treatment initiation. Substantial cytotoxicity [50 to 60% cytokinesis-blocked proliferation index (CBPI) relative to the vehicle control] was observed at dose levels ≥ 447 µg/mL in the non-activated and S9-activated 4-hour exposure groups, and at dose levels ≥ 47.7 µg/mL in the non-activated 24-hour exposure group. Based on these findings, the doses chosen for the micronucleus assay ranged from 25 to 450 µg/mL for the non-activated and S9-activated 4-hour exposure groups, and from 7.5 to 50 µg/mL for the non-activated 24-hour exposure group.

In the micronucleus assay, the cells were treated for 4 and 24 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 24 hours after treatment initiation. The highest dose analyzed under each treatment condition produced 50 to 60% reduction in CBPI which met the dose limit as recommended by testing guidelines for this assay. A minimum of 1000 binucleated cells from each culture were examined and scored for the presence of micronuclei.

The percentage of cells with micronucleated binucleated cells in the non-activated and S9-activated 4-hour exposure groups was not significantly increased relative to vehicle control at any dose level ($p > 0.05$, Fisher's Exact test).

The percentage of cells with micronucleated binucleated cells in the non-activated 24-hour exposure group was statistically increased relative to vehicle control at dose levels 15 and 30 µg/mL ($p \leq 0.05$ or $p \leq 0.01$, Fisher's Exact test). However, percentage of cells with micronucleated binucleated cells at 15 and 30 µg/mL (0.4% and 0.7%, respectively) was within the historical solvent control range of 0.0% to 1.0%. Therefore, the statistically significant increase was not considered to be biologically relevant.

The results for the positive and negative controls indicate that all criteria for a valid assay were met. Based on the findings of this study, Caprylhydroxamic Acid was concluded to be negative for the induction of micronuclei in both non-activated and S9-activated test systems in the *in vitro* mammalian cell micronucleus test using human peripheral blood lymphocytes.

PURPOSE

The purpose of this study was to evaluate the potential of a test article and/or its metabolites to induce micronuclei in HPBL using cytokinesis-block methodology in the presence and absence of an exogenous metabolic activation system. A copy of the study protocol and amendment is included in Appendix I.

The study was conducted in compliance with the OECD testing guideline 487 (OECD 2010).

CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, Caprylhydroxamic Acid, was received by BioReliance on 03 Dec 2012 and was assigned the code number AD64VD. Upon receipt, the test article was described as a white powder and was stored at room temperature, protected from light.

The Sponsor has determined the identity, strength, purity and composition or other characteristics to define the test article and the stability of the test article. A copy of the Certificate of Analysis for the test article is included in Appendix II. Based on the re-evaluation date in the Certificate of Analysis, the test article was considered stable through 12 Jun 2013.

The vehicle used to deliver Caprylhydroxamic Acid to the test system was DMSO (CAS No. 67-68-5, Lot No. 51283202, Exp. Date Aug 2015) obtained from EMD Chemicals. Test article dilutions were prepared immediately before use and delivered to the test system at room temperature under yellow light.

Vinblastine (VB, CAS 143-67-9, Lot No. BCBG4454V, Exp. Date 30 Sep 2013) was obtained from Sigma Chemical Company, and was dissolved in sterile distilled water (Gibco; CAS No. 7732-18-5, Lot No. 1119693, Exp. Date Feb 2014) to stock concentration of 0.0005 and 0.001 mg/mL (final concentrations of 5 and 10 ng/mL) as the positive control in the non-activated test system for aneugenicity. Cyclophosphamide (CP; CAS No. 6055-19-2, Lot No. 120M1253V, Exp. Date 31 Dec 2013) was obtained from Sigma-Aldrich, and was dissolved and diluted in sterile distilled water to stock concentrations of 0.25, 0.5, and 0.75 mg/mL (final concentrations of 2.5, 5, and 7.5 µg/mL) for use as the positive control article in the S9-activated test system. For CP, only two concentrations (5, and 7.5 µg/mL) were used in the study. For each positive control article, one dose level exhibiting a sufficient number of scorable metaphase cells was selected for analysis. The vehicle for the test article was used as the vehicle control for each treatment group.

Cytochalasin B (cyto B) (CAS No.14930-96-2, Lot No. 101M4043V, Exp. 18 September 2014) was obtained from Sigma-Aldrich. It was dissolved in DMSO (CAS 67-68-5, Lot No. 51283202, Exp. Date Aug 2015 obtained from EMD Chemicals) to a stock concentration of 2 mg/mL. It was used at 6 µg/mL concentration to block cytokinesis.

The vehicle and positive controls have been characterized as per the Certificates of Analysis on file with the Testing Facility. The stability of the vehicle and positive controls and their mixtures was demonstrated by acceptable results that met the criteria for a valid test.

MATERIALS AND METHODS

Test System

Peripheral blood lymphocytes were obtained from a healthy non-smoking 26-year-old adult male on 07 Jan 2013 for the preliminary toxicity assay and from a healthy non-smoking 25-year-old adult male on 22 Jan 2013 for the definitive assay. The donors had no recent history of radiotherapy, viral infection or the administration of drugs. This system has been demonstrated to be sensitive to the clastogenic activity of a variety of chemicals (Preston et al., 1981).

Identification of Test System

Using computer generated labels, the treatment tubes were identified by the BioReliance study number, dose level, test phase, treatment condition, activation system and/or replicate design.

Activation System

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 (Lot No. 2877) was obtained from Molecular Toxicology Inc. (Boone, NC). Each bulk preparation of S9 was assayed for sterility and its ability to metabolize at least two pro-mutagens to forms mutagenic to *Salmonella typhimurium* TA100.

Immediately prior to use, the S9 was thawed and mixed with a cofactor pool to contain 2 mM magnesium chloride, 6 mM potassium chloride, 1 mM glucose-6-phosphate, 1 mM nicotinamide adenine dinucleotide phosphate (NADP) and 20 µL S9 per milliliter medium (RPMI 1640 serum-free medium supplemented with 100 units penicillin/mL and 100 µg streptomycin/mL and 2 mM L-glutamine).

Solubility Test

A solubility test was conducted to determine the maximum soluble concentration or workable suspension using water and DMSO. The vehicle was selected in the order of preference that permitted preparation of the highest soluble stock concentration, up to 50 mg/mL in water and up to 500 mg/mL in DMSO.

Experimental Design

The *in vitro* mammalian cell micronucleus assay was conducted using standard procedures (Kirsch-Volders et al. 2000; Parry and Sors 1993; Fenech and Morley, 1985; Fenech 1993) by exposing HPBL to appropriate concentrations of the test article as well as the concurrent

positive and vehicle controls, in the presence and absence of an exogenous metabolic activation system.

Preparation of Cells and Cells Culture Condition

Approximately 0.5 mL heparinized blood was inoculated into centrifuge tubes containing 5 mL RPMI-1640 complete medium (RPMI-1640 containing 15% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units penicillin/mL, 100 µg streptomycin/mL) supplemented with 2% phytohemagglutinin (PHA). The cultures were incubated at standard conditions (37±1°C in a humidified atmosphere of 5±1% CO2 in air) for approximately 44-48 hours.

Preliminary Toxicity Test for Selection of Dose Levels

HPBL were exposed to vehicle alone and to nine concentrations of test article with half-log dose spacing using single cultures. The precipitation in the treatment medium was determined using unaided eye at the beginning and conclusion of treatment. The osmolality of the solvent, the highest dose level, and the highest soluble dose level in treatment medium was measured. Dose levels for the micronucleus assay were based upon post-treatment toxicity [cytokinesis-blocked proliferation index (CBPI) relative to the vehicle control].

Micronucleus Assay

Eight dose levels were tested using duplicate cultures at appropriate dose intervals based on the toxicity profile of the test article. The precipitation in the treatment medium was determined using unaided eye at the beginning and conclusion of treatment. The highest dose level evaluated for the micronucleus was based on approximately 50 to 60% cytotoxicity (CBPI relative to the vehicle control). At least two additional dose levels, demonstrating moderate to minimal or no toxicity, were evaluated in the micronucleus assay.

Treatment of Target Cells (Preliminary Toxicity Test and Definitive Assay)

Test article dosing solutions were prepared immediately prior to use. Treatment was carried out by re-feeding the cultures with 5 mL complete medium for the non-activated exposure or 5 mL S9 mix (4 mL culture medium + 1 mL of S9 cofactor pool) for the S9-activated exposure, to which was added 50 µL of dosing solution of vehicle or test article. In the definitive assays, positive control cultures were resuspended in either 5 mL of complete medium for the non-activated studies, or 5 mL of the S9 reaction mixture (4 mL serum free medium + 1 mL of S9 cofactor pool), to which was added 50 µL of positive control in solvent.

After the 4 hour treatment in the non-activated and the S9-activated studies, the cells were centrifuged, the treatment medium was aspirated, the cells were washed with calcium and magnesium free phosphate buffered saline (CMF-PBS), refed with complete medium containing cyto B at 6.0 µg/mL and returned to the incubator under standard conditions. For the 24 hour treatment in the non-activated study, cyto B (6.0 µg/mL) was added at the beginning of the treatment.

Collection of Cells (Preliminary Toxicity Test and Definitive Assay)

Cells were collected after being exposed to cyto B for 24 hours (± 30 minutes), 1.5 to 2 normal cell cycles, to ensure identification and selective analysis of micronucleus frequency in cells that have completed one mitosis evidenced by binucleated cells (Fenech and Morley, 1986). The cyto B exposure time for the 4 hour treatment in the non-activated and the S9-activated studies was 20 hours (± 30 minutes).

Cells were collected by centrifugation, swollen with 0.075M KCl, washed with fixative (methanol: glacial acetic acid, 25:1 v/v), capped and may be stored overnight or longer at 2-8°C. To prepare slides, the cells were collected by centrifugation and if necessary, the cells were resuspended in fresh fixative. The suspension of fixed cells was applied to glass microscope slides and air-dried. The slides were stained with acridine orange and identified by the BioReliance study number and a code system to designate at least the treatment condition, dose level, and test phase.

Cell Cycle Kinetics Scoring (Preliminary Toxicity Test and Definitive Assay)

For the preliminary toxicity test, at least 500 cells were evaluated to determine the CBPI at each dose level and the control. For the micronucleus assay, at least 1000 cells (500 cells per culture) were evaluated to determine the CBPI at each dose level and the control. The CBPI was determined using the following formula:

$$\text{CBPI} = \frac{\text{1X Mononucleated cells} + 2 \times \text{Binucleated cells} + 3 \times \text{Multinucleated cells}}{\text{Total number of cells scored}}$$

% Cytostasis (cytotoxicity) = 100 -100 {(CBPIt-1) /(CBPIc-1)}

T = test article treatment culture
C = vehicle control culture

Micronucleus Scoring (Definitive Assay)

The slides from at least three test article treatment groups were coded using random numbers by an individual not involved with the scoring process and scored for the presence of micronuclei based on cytotoxicity. Whenever possible, a minimum of 2000 binucleated cells from each concentration (1000 binucleated cells from each culture) were examined and scored for the presence of micronuclei.

Micronuclei in a binucleated cell (MN-BN) were recorded if they meet the following criteria:

- the micronucleus should have the same staining characteristics as the main nucleus.
- the micronuclei should be separate from the main nuclei or just touching (no cytoplasmic bridges).

- the micronuclei should be of regular shape and approximately 1/3 or less than the diameter of the main nucleus.

Criteria for Determination of a Valid Test

The frequency of cells with micronucleus induction in the vehicle control must be within the historical control range. The percentage of cells with micronucleus induction must be statistically increased ($p \leq 0.05$, Fisher's exact test) in the positive control condition relative to the vehicle control. The Historical Control Data is included in Appendix III.

Evaluation of Test Results

Toxicity induced by treatment was based upon CBPI and was reported for the cytotoxicity and micronucleus portions of the study. The percent frequency of micronucleated binucleated (MN-BN) cells was determined out of at least 2000 total binucleated cells per dose levels, when possible, and reported for each treatment group.

Statistical analysis of the percentage of micronucleated cells was performed using the Fisher's exact test. The Fisher's test was used to compare pairwise the percent micronucleated cells of each treatment group with that of the vehicle control. The Cochran-Armitage test was used to measure dose-responsiveness.

The test article was considered positive response if it induced a statistically significant and dose-dependent increase the frequency of MN-BN cells ($p \leq 0.05$). If only one criterion was met (statistically significant OR dose-dependent increase), the result was considered equivocal. If neither criterion was met, the results were considered to be negative.

Other criteria also may be used in reaching a conclusion about the study results (e.g., comparison to historical control values, biological significance, etc.). In such cases, the Study Director used sound scientific judgment and clearly report and describe any such considerations.

Electronic Data Collection Systems

The primary computer or electronic systems used for the collection or analysis of data included, but were not limited to, the following:

BRIQS (BioReliance), LIMS System (BioReliance), Excel 2007 (Microsoft Corporation) and Kaye Lab Watch Monitoring System (Kaye GE).

Deviations

No significant deviations from the study protocol or assay method SOPs occurred during the conduct of this study. No unforeseen circumstances were observed during the conduct of the study.

Archives

All raw data, the protocol, pertinent study email correspondence, slides and/or specimens (as applicable), and all reports for procedures performed at BioReliance will be maintained in the archives at BioReliance, Rockville, MD for at least five years. At that time, the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials will first be copied and the copy will be retained at the BioReliance archives for a minimum of 10 years. The raw data, reports, and other documents generated at locations other than BioReliance will be archived by the test site. All unused test article was disposed prior to report finalization.

RESULTS AND DISCUSSION

Solubility Test

DMSO was used as the vehicle based on the solubility of the test article and compatibility with the target cells. In a solubility test conducted at BioReliance, the test article formed a soluble and clear solution in DMSO at a concentration of approximately 500 mg/mL, the maximum concentration tested for solubility.

Preliminary Toxicity Assay

A preliminary toxicity assay was conducted to observe the cytotoxicity profile of the test article and to select suitable dose levels for the definitive micronucleus assay. HPBL cells were first exposed to nine concentrations of Caprylhydroxamic Acid ranging from 0.159 to 1590 µg/mL, as well as vehicle controls, in both the absence and presence of an Aroclor-induced S9 activation system for 4 hours, or continuously for 24 hours in the absence of S9 activation. The test article was soluble in DMSO at all concentrations tested. Visible precipitate was observed in treatment medium at 1590 µg/mL, while dose levels ≤ 477 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. At the conclusion of the treatment period, hemolysis was observed at 1590 µg/mL in all three treatment conditions.

The osmolality in treatment medium of the highest concentration tested, 1590 µg/mL, was 409 mmol/kg. The osmolality in treatment medium of the highest soluble concentration, 477 µg/mL, was 416 mmol/kg. The osmolality of the vehicle (DMSO) in the treatment medium was 420 mmol/kg. The osmolality of the test article dose levels in treatment medium is acceptable because it did not exceed the osmolality of the vehicle by more than 20%. The pH of the highest concentration of test article in treatment medium was 7.5.

The results of the evaluation of CBPI and % cytotoxicity are presented in Tables 1, 2 and 3. Substantial cytotoxicity [50 to 60% cytokinesis-blocked proliferation index (CBPI) relative to the vehicle control] was observed at dose levels ≥ 447 µg/mL in the non-activated and S9-activated 4-hour exposure groups, and at dose levels ≥ 47.7 µg/mL in the non-activated 24-hour exposure group. Based on the results of the preliminary toxicity assay, the dose levels selected for testing in the micronucleus assay were as follows:

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	20 hr	25, 50, 88, 175, 350, 400, 425, 450
	24 hr	0 hr	7.5, 15, 20, 25, 30, 35, 40, 50
S9-activated	4 hr	20 hr	25, 50, 88, 175, 350, 400, 425, 450

Micronucleus Assay

In the micronucleus assay, the test article was soluble in DMSO and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period. The pH of the highest concentration of test article in treatment medium was 7.5.

Results of the micronucleus analysis in the non-activated 4-hour exposure group are presented in Table 7. The dose levels selected for analysis of micronucleus were 50, 88, and 175 µg/mL. At the highest test concentration, 175 µg/mL, cytotoxicity was 53% relative to the vehicle control (Table 4). The percentage of cells with micronuclei in the test article-treated group was not significantly increased relative to vehicle control at any dose level ($p > 0.05$, Fisher's Exact test). The percentage of micronucleated cells in the VB (positive control) group (1.2%) was statistically significant ($p \leq 0.01$, Fisher's Exact test).

Results of the micronucleus analysis in the S9-activated 4-hour exposure group are presented in Table 8. The dose levels selected for analysis of micronucleus were 50, 88, and 175 µg/mL. At the highest test concentration, 175 µg/mL, cytotoxicity was 50% relative to the vehicle control (Table 5). The percentage of cells with micronuclei in the test article-treated group was not significantly increased relative to vehicle control at any dose level ($p > 0.05$, Fisher's Exact test). The percentage of micronucleated cells in the CP (positive control) group (1.4%) was statistically significant ($p \leq 0.01$, Fisher's Exact test).

Results of the micronucleus analysis in the non-activated 24-hour exposure group are presented in Table 9. The dose levels selected for analysis of micronucleus were 7.5, 15, and 30 µg/mL. At the highest test concentration, 30 µg/mL, cytotoxicity was 52% relative to the vehicle control (Table 6). The percentage of cells with micronucleated binucleated cells in the non-activated 24-hour exposure group was statistically increased relative to vehicle control at dose levels 15 and 30 µg/mL ($p \leq 0.05$ or $p \leq 0.01$, Fisher's Exact test). The Cochran-Armitage test was also positive for a dose response ($p \leq 0.05$). However, the percentage of cells with micronucleated binucleated cells at 15 and 30 µg/mL (0.4% and 0.7%, respectively) was within the historical solvent control range of 0.0% to 1.0%. Therefore, the statistically significant increase was not considered to be biologically relevant. The percentage of micronucleated cells in the VB (positive control) group (1.2%) was statistically significant ($p \leq 0.01$, Fisher's Exact test).

The results for the positive and vehicle controls indicate that all criteria for a valid assay were met. Based on these criteria, the negative result is justified and does not require a repeat of any portions of the study.

The Common Technical Document (CTD) Summary Table is included in Appendix IV.

Dose Formulation Analysis

The dosing formulation analysis for concentration and stability was not conducted. Due to the lack of dose formulation analysis, the interpretation of the study data was based on the nominal dose levels as documented in the study records and not on the actual formulated test article concentrations as confirmed by analytical results. Nevertheless, toxicity in the assay demonstrated that the test system was dosed up to the regulatory required level.

CONCLUSION

The positive and vehicle controls fulfilled the requirements for a valid test.

Under the conditions of the assay described in this report, Caprylhydroxamic Acid was concluded to be negative for the induction of micronuclei in the non-activated and S9-activated test systems in the *in vitro* mammalian micronucleus test using human peripheral blood lymphocytes.

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TABLE 1
PRELIMINARY TOXICITY ASSAY USING Caprylhydroxamic Acid
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
4-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition µg/mL	Total # of Cells Counted	Count per total cells Cells with # of nuclei 1	2	>2	CBPI[1]	Cytotoxicity[2]
DMSO	500	346	149	5	1.318	
Caprylhydroxamic Acid						
0.159	500	323	169	8	1.370	-16%
0.477	500	312	181	7	1.390	-23%
1.59	500	311	185	4	1.386	-21%
4.47	500	331	167	2	1.342	-8%
15.9	500	311	180	9	1.396	-25%
47.7	500	363	134	3	1.280	12%
159	500	392	105	3	1.222	30%
447	500	454	43	3	1.098	69%
1590 P	0	0	0	0	-	-

[1]CBPI = Cytokinesis-Block Proliferation Index

[2]Relative to vehicle control

p: Visible precipitate was observed in the treatment medium at the conclusion of the treatment period.

TABLE 2
PRELIMINARY TOXICITY ASSAY USING Caprylhydroxamic Acid
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
4-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition µg/mL	Total # of Cells Counted	Count per total cells Cells with # of nuclei 1	2	>2	CBPI[1]	Cytotoxicity[2]
DMSO	500	295	196	9	1.428	
Caprylhydroxamic Acid						
0.159	500	318	179	3	1.370	14%
0.477	500	320	175	5	1.370	14%
1.59	500	314	182	4	1.380	11%
4.47	500	345	152	3	1.316	26%
15.9	500	325	169	6	1.362	15%
47.7	500	331	166	3	1.344	20%
159	500	374	122	4	1.260	39%
447	500	452	47	1	1.098	77%
1590 p	0	0	0	0	-	-

[1]CBPI = Cytokinesis-Block Proliferation Index

[2]Relative to vehicle control

p: Visible precipitate was observed in the treatment medium at the conclusion of the treatment period.

TABLE 3
PRELIMINARY TOXICITY ASSAY USING Caprylhydroxamic Acid
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
24-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition µg/mL	Total # of Cells Counted	Count per total cells Cells with # of nuclei 1	2	>2	CBPI[1]	Cytotoxicity[2]
DMSO	500	257	222	21	1.528	
Caprylhydroxamic Acid						
0.159	500	290	192	18	1.456	14%
0.477	500	276	209	15	1.478	9%
1.59	500	278	194	28	1.500	5%
4.47	500	325	167	8	1.366	31%
15.9	500	321	172	7	1.372	30%
47.7	500	446	52	2	1.112	79%
159	500	451	48	1	1.100	81%
447	500	483	17	0	1.034	94%
1590 p	0	0	0	0	-	-

[1]CBPI = Cytokinesis-Block Proliferation Index

[2]Relative to vehicle control

p: Visible precipitate was observed in the treatment medium at the conclusion of the treatment period.

TABLE 4
CONCURRENT CYTOTOXICITY TEST USING Caprylhydroxamic Acid
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
4-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition	Replicate Culture	Total # of Cells Counted	Count per total cells Cells with # of nuclei			CBPI[1]	Cytotoxicity[2]
			1	2	>2		
DMSO	A	500	276	213	11	1.478	
	B	500	264	229	7		
Caprylhydroxamic Acid 25 µg/mL	A	500	266	229	5	1.496	-4%
	B	500	251	241	8		
50 µg/mL	A	500	268	225	7	1.471	2%
	B	501	272	226	3		
88 µg/mL	A	500	306	191	3	1.382	20%
	B	500	318	179	3		
175 µg/mL	A	500	387	109	4	1.226	53%
	B	500	396	99	5		
350 µg/mL	A	500	447	51	2	1.103	78%
	B	500	455	42	3		
400 µg/mL	A	500	447	44	9	1.119	75%
	B	500	447	49	4		
425 µg/mL	A	500	430	68	2	1.115	76%
	B	500	460	37	3		
450 µg/mL	A	500	457	41	2	1.087	82%
	B	500	458	42	0		
VB, 5 ng/mL	A	500	286	207	7	1.433	9%
	B	500	294	200	6		
VB, 10 ng/mL	A	500	310	182	8	1.377	21%
	B	500	327	167	6		

[1]CBPI = Cytokinesis-Block Proliferation Index

[2]Relative to vehicle control

TABLE 5
CONCURRENT CYTOTOXICITY TEST USING Caprylhydroxamic Acid
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
4-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition µg/mL	Replicate Culture	Total # of Cells Counted	Count per total cells Cells with # of nuclei 1	2	>2	CBPI[1]	Cytotoxicity[2]
DMSO	A	500	302	193	5	1.404	
	B	500	306	187	7		
Caprylhydroxamic Acid							
25	A	500	304	190	6	1.367	9%
	B	500	337	161	2		
50	A	500	302	196	2	1.377	7%
	B	500	324	175	1		
88	A	500	330	170	0	1.356	12%
	B	500	318	178	4		
175	A	500	405	95	0	1.201	50%
	B	500	396	102	2		
350	A	500	439	59	2	1.127	69%
	B	500	437	62	1		
400	A	498	424	72	2	1.138	66%
	B	500	440	58	2		
425	A	500	454	45	1	1.129	68%
	B	500	419	80	1		
450	A	500	432	68	0	1.107	74%
	B	500	462	37	1		
CP, 5	A	500	363	136	1	1.255	37%
	B	500	383	117	0		
CP, 7.5	A	500	384	116	0	1.224	45%
	B	500	393	106	1		

[1]CBPI = Cytokinesis-Block Proliferation Index

[2]Relative to vehicle control

TABLE 6
CONCURRENT CYTOTOXICITY TEST USING Caprylhydroxamic Acid
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
24-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition	Replicate Culture	Total # of Cells Counted	Count per total cells Cells with # of nuclei			CBPI[1]	Cytotoxicity[2]
			1	2	>2		
DMSO	A	500	254	226	20	1.509	
	B	500	276	205	19		
Caprylhydroxamic Acid 7.5 µg/mL	A	500	304	188	8	1.407	20%
	B	500	312	173	15		
15 µg/mL	A	500	343	148	9	1.328	36%
	B	500	342	154	4		
20 µg/mL	A	500	358	138	4	1.290	43%
	B	500	360	136	4		
25 µg/mL	A	500	391	109	0	1.243	52%
	B	500	368	130	2		
30 µg/mL	A	500	385	113	2	1.242	52%
	B	500	377	121	2		
35 µg/mL	A	500	421	77	2	1.163	68%
	B	500	420	78	2		
40 µg/mL	A	500	449	50	1	1.113	78%
	B	500	439	61	0		
50 µg/mL	A	500	447	52	1	1.099	81%
	B	500	456	43	1		
VB, 5 ng/mL	A	500	343	151	6	1.331	35%
	B	500	340	152	8		
VB, 10 ng/mL	A	501	471	28	2	1.074	85%
	B	500	461	36	3		

[1]CBPI = Cytokinesis-Block Proliferation Index

[2]Relative to vehicle control

TABLE 7

MICRONUCLEUS ANALYSIS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES TREATED WITH Caprylhydroxamic Acid IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION DEFINITIVE ASSAY: 4-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition	CBPI[1]	Cytotoxicity[2]	Percentage of MNBN[3] Cells per Total BN[4] Cells Counted
DMSO	1.478		0.2%
Caprylhydroxamic Acid			
50 µg/mL	1.471	2%	0.1%
88 µg/mL	1.382	20%	0.2%
175 µg/mL	1.226	53%	0.1%
VB, 10 ng/mL	1.377	21%	1.2%**

[1]CBPI = Cytokinesis-Block Proliferation Index
[2]Relative to vehicle control.
[3]MNBN = micronucleated binucleated
[4]BN = binucleated
* $p \leq 0.05$; ** $p \leq 0.01$, Fisher's exact test, relative to the solvent control.

TABLE 8

MICRONUCLEUS ANALYSIS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES TREATED WITH Caprylhydroxamic Acid IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

DEFINITIVE ASSAY: 4-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition µg/mL	CBPI[1]	Cytotoxicity[2]	Percentage of MNBN[3] Cells per Total BN[4] Cells Counted
DMSO	1.404		0.1%
Caprylhydroxamic Acid			
50	1.377	7%	0.2%
88	1.356	12%	0.2%
175	1.201	50%	0.1%
CP, 7.5	1.224	45%	1.4%**

[1]CBPI = Cytokinesis-Block Proliferation Index
[2]Relative to vehicle control.
[3]MNBN = micronucleated binucleated
[4]BN = binucleated
* $p \leq 0.05$; ** $p \leq 0.01$, Fisher's exact test, relative to the solvent control.

TABLE 9

MICRONUCLEUS ANALYSIS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES TREATED WITH Caprylhydroxamic Acid IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

DEFINITIVE ASSAY: 24-HOUR TREATMENT, 24-HOUR HARVEST

Treatment Condition	CBPI[1]	Cytotoxicity[2]	Percentage of MNBN[3] Cells per Total BN[4] Cells Counted
DMSO	1.509		0.1%
Caprylhydroxamic Acid			
7.5 µg/mL	1.407	20%	0.2%
15 µg/mL	1.328	36%	0.4%*
30 µg/mL	1.242	52%	0.7%**
VB, 5 ng/mL	1.331	35%	1.2%**

[1]CBPI = Cytokinesis-Block Proliferation Index
[2]Relative to vehicle control.
[3]MNBN = micronucleated binucleated
[4]BN = binucleated
* $p \leq 0.05$; ** $p \leq 0.01$, Fisher's exact test, relative to the solvent control.

APPENDIX I

Study Protocol and Amendment

PROTOCOL AMENDMENT 1

Sponsor: [redacted]

BioReliance Study No.: AD64VD.348REACH.BTL

Title: *In Vitro* Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL)

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1. Page 4, Section 8, EXPERIMENTAL DESIGN AND METHODOLOGY
   Preparation of Target Cells

Replace: Peripheral blood lymphocytes will be cultured in complete medium (RPMI-1640 containing 15% fetal bovine serum, 2mM L-glutamine, 100 units penicillin and 100 µg/mL streptomycin) by adding 0.6 mL heparinized blood to a centrifuge tube containing 9.4 mL of complete medium with 1% phytohemagglutinin.

With: Peripheral blood lymphocytes will be cultured in complete medium (RPMI-1640 containing 15% fetal bovine serum, 2mM L-glutamine, 100 units penicillin and 100 µg/mL streptomycin) by adding 0.5 mL heparinized blood to a centrifuge tube containing 5 mL of complete medium with 2% phytohemagglutinin.

Reason: To correct the size of culture volume and use of phytohemagglutinin.

Approvals:

Shambhu Roy, PhD
Study Director

07 Jan 2013
Date

[redacted]

08-Jan-2013
Date

BioReliance
by SAFC

Protocol

Study Title	***In Vitro* Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL)**
Study Director	Shambhu Roy, PhD
Testing Facility	BioReliance Corporation 9630 Medical Center Drive Rockville, MD 20850
Sponsor	[redacted]
Sponsor's Authorized Representative	[redacted]
BioReliance Study Number	AD64VD.348REACH.BTL

1. KEY PERSONNEL

Study Director	Shambhu Roy, PhD BioReliance Corporation Phone: 301-610-2956 Email: shambhu.roy@bioreliance.com
BioReliance Quality Assurance Representative	Karen Westray, RQAP-GLP BioReliance Corporation Phone: 301-610-2856 Email: karen.westray@bioreliance.com
Sponsor's Authorized Representative	[redacted]

2. TEST SCHEDULE

Proposed Experimental Initiation Date	10 January 2013
Proposed Experimental Completion Date	07 March 2013
Proposed Report Date	21 March 2013

3. REGULATORY REQUIREMENTS

This study will be performed in compliance with the following Good Laboratory Practices (GLP) regulations.

- US FDA Good Laboratory Practices 21 CFR Part 58
- OECD Principles of Good Laboratory Practice (C(97)186/Final)

At a minimum, all work performed at US test site(s) will comply with the US GLP regulations stated above. Non-US sites must follow the GLP regulations governing their site. The regulations that were followed will be indicated on the compliance statement in the final contributing report.

4. QUALITY ASSURANCE

The protocol, any amendments, at least one in-lab phase, the raw data, draft report(s), and final report(s) will be audited by BioReliance Quality Assurance (QA) and a signed QA Statement will be included in the final report.

Test Site Quality Assurance (where applicable)

Test Site QA is responsible for performing an in-lab phase inspection, auditing raw data and final report(s), and providing the inspection results to the Principal Investigator, Study Director, and their respective management. A signed QA Statement documenting the type of audit performed, the dates it was performed,

and the dates in which the audit results were reported to the Study Director, Principal Investigator and their respective management must be submitted by the test site QA.

5. PURPOSE

The purpose of this study is to evaluate the potential of a test article and/or its metabolites to induce micronuclei in HPBL using cytokinesis-block methodology in the presence and absence of an exogenous metabolic activation system. The assay design is based on the OECD Guideline 487, updated and adopted 22 July 2010.

6. TEST ARTICLE INFORMATION

Identification	Caprylhydroxamic Acid
Storage Conditions	Room Temperature
Purity	98.09 % (no correction factor will be used for dose formulations)
Molecular Weight	159.23

Characterization of Test Article
Characterization of the Test Article is the responsibility of the Sponsor.

Test Article Reserve Sample
A reserve sample of the Test Article is the responsibility of the Sponsor.

Characterization of Dose Formulations
Dose formulations will not be analyzed.

Stability of Test Article in Vehicle
Stability of Test Article in Vehicle, under the conditions of use, is the responsibility of the Sponsor.

Disposition of Test Article and Dose Formulations
All unused test article will be disposed prior to report finalization unless the test article is used on another study. Residual dose formulations will be discarded after use.

7. TEST SYSTEM

Peripheral blood lymphocytes will be obtained from healthy adults, 18-35 years of age, non-smokers, without a recent history of radiotherapy, viral infections or the administration of drugs. This system has been demonstrated to be sensitive to the clastogenic activity of a variety of chemicals (Preston et al., 1981).

8. EXPERIMENTAL DESIGN AND METHODOLOGY

The *in vitro* mammalian cell micronucleus assay will be conducted by exposing HPBL to appropriate concentrations of the test article as well as the concurrent positive and vehicle controls, in the presence and absence of an exogenous metabolic activation system.

Solubility Determination

The Sponsor has indicated that the test article, Caprylhydroxamic Acid, is soluble in water at 1.5 g/L.

As needed, a solubility determination will be conducted to determine the maximum soluble concentration or workable suspension as indicated below. Vehicles compatible with this test system, in order of preference, include but are not limited to deionized water (CAS 7732-18-5), dimethyl sulfoxide (CAS 67-68-5), ethanol (CAS 64-17-5) and acetone (CAS 67-64-1). The vehicle of choice, selected in order of preference, will be that which permits preparation of the highest workable or soluble stock concentration, up to 50 mg/mL for aqueous vehicles and up to 500 mg/mL for organic vehicles. Based on the molecular weight of the test article, the solvents to be tested and the dose to be achieved in the assay, alternate stock concentrations may be tested, as needed.

Preparation of Target Cells

Peripheral blood lymphocytes will be cultured in complete medium (RPMI-1640 containing 15% fetal bovine serum, 2mM L-glutamine, 100 units penicillin and 100 µg/mL streptomycin) by adding 0.6 mL heparinized blood to a centrifuge tube containing 9.4 mL of complete medium with 1% phytohemagglutinin. Alternate volumes of blood and media may be used if necessary. The cultures will be incubated under standard conditions (37 ± 1°C in a humidified atmosphere of 5 ± 1% CO_2 in air) for 44-48 hours.

Identification of Test System

The cultures will be identified by the BioReliance study number and a code system to designate at least the treatment condition, dose level, and test phase.

Exogenous Metabolic Activation System

Liver Homogenate

Liver homogenate (S9) will be purchased commercially (MolTox; Boone, NC). It is prepared from male Sprague-Dawley rats that have been injected intraperitonealy with Aroclor™ 1254 (200 mg/mL in corn oil), at a dose of 500 mg/kg, 5 days before sacrifice.

S9 Mix

S9 mix will be prepared on the day of use and added to the test system at 20% (v/v). The final concentrations of the components in the test system are as indicated below.

Component	Final Concentration in Cultures
NADP (sodium salt)	1 mM
Glucose-6-phosphate	1 mM
Potassium chloride	6 mM
Magnesium chloride	2 mM
S9 homogenate	20 µL

Controls

No analyses will be performed on the positive control articles or the positive control dose formulations. The neat positive control articles and the vehicles used to prepare the test article and positive control formulations will be characterized by the Certificates of Analysis provided by the Supplier(s). Copies of the Certificates of Analysis will be kept on file at BioReliance.

Vehicle Control

The vehicle for the test article will be used as the vehicle control for each treatment group. For vehicles with no historical control data, an untreated control will be included.

Positive Controls

Results obtained from these articles will be used to assure responsiveness of the test system but not to provide a standard for comparison with the test article. One dose level of each positive control will be evaluated microscopically for micronucleus induction.

Positive Control	CAS #	S9	Concentrations*
Cyclophosphamide (CP)	6055-19-2	+	5 and 7.5 µg/mL
Vinblastine (VB)	143-67-9	–	5 and 10 ng/mL

*Prepared in water

Frequency and Route of Administration

Target cells will be treated for 4 hours in the absence and presence of S9, and for 24 hours in the absence of S9, by incorporation of the test article vehicle mixture into the treatment medium.

Preliminary Toxicity Test for Selection of Dose Levels

HPBL will be exposed to vehicle alone and to nine concentrations of test article with half-log dose spacing using single cultures. Unless limited by solubility, the test article will be evaluated at a maximum concentration of 5000 µg/mL (or 10 mM, whichever is lower). If limited by solubility in the vehicle, the test article will be evaluated at the highest concentration able to be prepared and administered as a workable suspension. The osmolality of the highest dose level, lowest precipitating dose level (where applicable) and the highest soluble dose level (where applicable) in treatment medium will be measured. If the osmolality of the dose levels in the treatment medium is considered excessive (>20% of vehicle), the Sponsor will be consulted. Dose levels for the micronucleus assay will be based upon post-treatment toxicity (cytokinesis-blocked

proliferation index (CBPI) relative to the vehicle control) and will be documented in the raw data and report.

Micronucleus Assay
At least four dose levels will be tested using duplicate cultures at appropriate dose intervals based on the toxicity profile of the test article. Whenever possible, the highest dose level evaluated for the micronucleus will be selected to give 50 to 60% cytotoxicity (CBPI relative to the vehicle control), irrespective of solubility. At least two additional dose levels, demonstrating moderate to minimal or no toxicity, will be evaluated in the micronucleus assay. For poorly soluble test article, the highest dose to be evaluated for microneclus induction will be the concentration resulting in minimum precipitation in test medium, provided that there is no interference with scoring. The precipitation will be determined with the unaided eye at the beginning and conclusion of treatment. The maximum concentration to be evaluated in the definitive assay will be the limit dose for this assay (5000 µg/mL or 10mM), or be expected to induce 50 to 60% cytotoxicity (CBPI relative to the vehicle control), or be minimally insoluble (whichever is lowest).

Treatment of Target Cells (Preliminary Toxicity Test and Definitive Assay)
Test article dosing solutions will be prepared immediately prior to use. The pH will be measured at the highest test article concentration prior to dosing and will be adjusted, if necessary, in order to maintain a neutral pH in the treatment medium. The lower concentrations may be measured and adjusted to neutral pH as needed. All test article dosing will be at room temperature under yellow light. Treatment will be carried out by refeeding the cultures with 5 mL complete medium for the non-activated exposure or 5 mL S9 mix (4 mL culture medium + 1 mL of S9 cofactor pool) for the S9-activated exposure, to which will be added 50 µl of dosing solution of vehicle, test, and/or control article. Larger volumes of dosing solution may be used as appropriate based on the compatibility to the test system. If larger volumes of dosing solutions are used, media volume will be adjusted accordingly for a total volume of 5 mL.

After the 4 hour treatment in the non-activated and the S9-activated studies, the cells will be centrifuged, the treatment medium will be aspirated, the cells will be washed with calcium and magnesium free phosphate buffered saline (CMF-PBS), refed with complete medium containing Cytochalasin B (cytoB) at 6.0 µg/mL and returned to the incubator under standard conditions. For the 24 hour treatment in the non-activated study, cyto B (6.0 µg/mL) will be added at the beginning of the treatment.

Collection of Cells (Preliminary Toxicity Test and Definitive Assay)
Cells will be collected after being exposed to cyto B for 24 hours (± 30 minutes), 1.5 to 2 normal cell cycles, to ensure identification and selective analysis of micronucleus frequency in cells that have completed one mitosis evidenced by binucleated cells (Fenech and Morley, 1986). The cyto B exposure time for the 4 hour treatment in the non-activated and the S9-activated studies will be 20 hours (± 30 minutes).

Cells will be collected by centrifugation, swollen with 0.075M KCl, washed with fixative (methanol: glacial acetic acid, 25:1 v/v), capped and may be stored overnight or longer at 2-8°C. To prepare slides, the cells will be collected by centrifugation and if necessary, the cells will be resuspended in fresh fixative. The suspension of fixed cells will be applied to glass microscope slides and air-dried. The slides will be stained with acridine orange and identified by the BioReliance study number and a code system to designate at least the date of harvest, treatment condition, dose level, and test phase.

Cell Cycle Kinetics Scoring (Preliminary Toxicity Test and Definitive Assay)
For the preliminary toxicity test, at least 500 cells, if possible, will be evaluated to determine the CBPI at each dose level and the control. For the micronucleus assay, at least 1,000 cells (500 cells per culture), if possible, will be evaluated to determine the CBPI at each dose level and the control. The CBPI will be determined using the following formula:

$$\text{CBPI} = \frac{1\text{X Mononucleate cells} + 2 \text{ x Binonucleate cells} + 3 \text{ x Multionucleate cells}}{\text{Total number of cells scored}}$$

% Cytostasis (cytotoxicity) = 100 -100 {(CBPIt-1) /(CBPIc-1)}

T = test article treatment culture
C = vehicle control culture

Micronucleus Scoring (Definitive Assay)
The slides from at least three test article treatment groups will be coded using random numbers by an individual not involved with the scoring process and scored for the presence of micronuclei based on cytotoxicity. Whenever possible, a minimum of 2000 binucleated cells from each concentration (if possible, 1000 binucleated cells from each culture) will be examined and scored for the presence of micronuclei.

Micronuclei in a binucleate cell (MN-BN) will be recorded if they meet the following criteria:

- the micronucleus should have the same staining characteristics as the main nucleus.
- the micronuclei should be separate from the main nuclei or just touching (no cytoplasmic bridges).
- the micronuclei should be of regular shape and approximately 1/3 or less than the diameter of the main nucleus.

9. CRITERIA FOR DETERMINATION OF A VALID TEST

Vehicle Controls
The frequency of cells with micronucleus induction in the vehicle control must be within the historical control range.

Positive Controls
The percentage of cells with micronucleus inducation must be statistically increased ($p \leq 0.05$, Fisher's exact test) in the positive control condition relative to the vehicle control.

10. EVALUATION OF TEST RESULTS

Toxicity induced by treatment is based upon CBPI and will be reported for the cytotoxicity and micronucleus portions of the study. The percent frequency of micronucleated binucleated (MN-BN) cells will be determined out of at least 2000 total binucleuted cells per dose levels, when possible, and reported for each treatment group.

Statistical analysis of the percentage of micronucleuted cells will be performed using the Fisher's exact test. The Fisher's test will be used to compare pairwise the percent micronucleuted cells of each treatment group with that of the vehicle control. In case of a positive result, the Cochran-Armtiage test will be used to measure dose-responsiveness.

A test article will be considered to have induced a positive response if it induces a statistically significant and dose-dependent increase the frequency of MN-BN cells ($p \leq 0.05$). If only one criterion is met (statistically significant OR dose-dependent increase), the result may be considered equivocal. If neither criterion is met, the results will be considered to be negative.

Other criteria also may be used in reaching a conclusion about the study results (e.g., comparison to historical control values, biological significance, etc.). In such cases, the Study Director will use sound scientific judgment and clearly report and describe any such considerations.

11. ELECTRONIC DATA COLLECTION SYSTEMS

Electronic systems used for the collection or analysis of data will include but not be limited to the following (version numbers are maintained in the system documentation):

System	Purpose
LIMS Labware System	Test Article Tracking
Excel (Microsoft Corporation)	Calculations
Kaye Lab Watch Monitoring system (Kaye GE)	Environmental Monitoring
BRIQS	Deviation and audit reporting

12. REPORT

A report of the results of this study will accurately describe all methods used for generation and analysis of the data. The report will include, but not limited to information about the following:

- Test article
- Vehicle
- Cells

- Test conditions
- Results
- Discussion of results
- Conclusion
- Appendices: Historical Control Data (negative and positive controls with ranges, means and standard deviations), copy of protocol and any amendment, contributing reports (if applicable), and, if provided by the Sponsor, copies of the analyses that characterized the test article, its stability and the stability and strength of the dosing preparations.
- Statement of Compliance
- Quality Assurance Statement
- Location of archived material
- CTD Tables (unless otherwise requested)

The report will be issued as a QA-audited draft. After receipt of the Sponsor's comments a final report will be issued. A GLP Compliance Statement signed by the Study Director will also be included in the final report and will note any exceptions if the characterization of the test article and/or the characterization of the dose formulations are not performed or provided. Six months after issuance of the draft report, if no communication regarding the study is received from the Sponsor or designated representative, the draft report may be issued as a final report. If all supporting documents have not been provided, the report will be written based on those that are provided.

13. RECORDS AND ARCHIVES

All raw data, the protocol, pertinent study email correspondence, slides and/or specimens (as applicable), and all reports for procedures performed at BioReliance will be maintained in the archives at BioReliance, Rockville, MD for at least five years. At that time, the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials will first be copied and the copy will be retained at the BioReliance archives for a minimum of 10 years. The raw data, reports, and other documents generated at locations other than BioReliance will be archived by the test site.

14. REFERENCES

Fenech, M. and Morley, A.A. (1986). Cytokinesis-block micronucleus method in human lymphocytes: effect of *in-vivo* ageing and low dose X-irradiation. Mutation Res., 161, 193-198.

Preston, R.J., Au, W., Bender, M.A., Brewen, J.G., Carrano, A.V., Heddle, J.A., McFee, A.F., Wolff, S. and Wassom, J.S. (1981). Mammalian in vivo and in vitro cytogenetic assays: a report of the Gene-Tox Program, Mutation Research, 87:143-188.

APPROVALS

Sponsor Approval

[redacted]

Dec-10-2012
Date

Study Director and Test Facility Management Approvals

BioReliance Study Director — Date: 18 Dec 2012

BioReliance Study Management — Date: 18-DEC-2012

APPENDIX II

Certificate of Analysis

[redacted]

CERTIFICATE OF ANALYSIS

CUSTOMER INFORMATION:

PRODUCT: Caprylhydroxamic Acid
LOT NO.: AM4744
SHIP DATE: 11/29/2012
QUANTITY: 12g

RESULTS OF ANALYSIS:

TEST PARAMETER	UNITS	RESULT
Infrared Spectrum		Conforming
CHA	%	98.09
Appearance		Conforming

Date of manufacture: June 12, 2011

Re-evaluation Date: June 12, 2013

CERTIFIED BY: [redacted]
Development Chemist

APPENDIX III

Historical Control Data

IN VITRO MICRONUCLEUS TEST USING HUMAN PERIPHERAL BLOOD LYMPHOCYTES (HPBL)

HISTORICAL CONTROL VALUES 2010-2012

NON-ACTIVATED ASSAY

Historical Values	Micronucleated Binucleated Cells		
	Negative Control[1]	Positive Controls	
		MMC[2]	VB[3]
Mean	0.263%	3.625%	1.729%
Standard Deviation	±0.166%	±1.273%	±0.853%
Range	0.1-1.0%	2.1-5.9%	1.0-4.9%

S9-ACTIVATED ASSAY

Historical Values	Micronucleated Binucleated Cells	
	Negative Control[1]	Positive Control
		CP[4]
Mean	0.233%	1.510%
Standard Deviation	±0.128%	±0.506%
Range	0.0-0.5%	0.8-2.9%

1 Includes distilled water and dimethyl sulfoxide (DMSO)
2 MMC = Mitomycin C, 0.125 to 0.8 µg/mL
3 VB = Vinblastine Sulfate, 5 to 150 ng/mL
4 CP = Cyclophosphamide, 1 to 15 µg/mL

APPENDIX IV

Common Technical Document (CTD) Summary Tables

2.6.7.8 Genotoxicity: In Vitro

Report Title:	The *In Vitro* Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL)			**Test Article:**	Caprylhydroxamic Acid
Test for Induction of:	Micronuclei	**No. of Independent Assays:**	1	**BioReliance Study No.:**	AD64VD.348REACH.BTL
Strains:	Human Peripheral Blood Lymphocytes (HPBL)	**No. of Replicate Cultures:**	2	**Sponsor No.:**	NA
Metabolizing System:	Aroclor-induced rat liver S9	**No. of Cells Analyzed/Culture:**	1000		
Vehicles:	**For Test Article:** DMSO	**For Positive Controls:**	Water (CP, VB)	**GLP Compliance:**	Yes
Treatment:	24 hr without S9; 4 hr with 20 hr recovery period with and without S9		**Date of Treatment:**	24 Jan 2013 (Definitive Assay)	

Cytotoxic Effects:	In the definitive micronucleus assay, substantial toxicity [50 to 60% cytokinesis-blocked proliferation index (CBPI) relative to the vehicle control] was observed at dose levels ≥ 175 µg/mL in the non-activated and S9-activated 4-hour exposure groups, and at dose levels ≥ 25 µg/mL in the non-activated 24-hour exposure group.
Genotoxic Effects:	None.

DMSO: Dimethyl sulfoxide
CP: Cyclophosphamide
VB: Vinblastine

Test Article: Caprylhydroxamic Acid (continued)

Metabolic Activation	Test Article	Concentration	CBPI[a]	Cytotoxicity[b] (% of Control)	Percentage of MNBN[c] Cells Per Total BN[d] Cells Counted
24-hr Continuous	DMSO	NA	1.509	NA	0.1
Treatment	Caprylhydroxamic Acid	7.5 µg/mL	1.407	20	0.2
Without	Caprylhydroxamic Acid	15 µg/mL	1.328	36	0.4
Activation	Caprylhydroxamic Acid	30 µg/mL	1.242	52	0.7
	VB	5 ng/mL	1.331	35	1.2**
4-hr Treatment	DMSO	NA	1.478	NA	0.2
With 20 hr Recovery	Caprylhydroxamic Acid	50 µg/mL	1.471	2	0.1
Without	Caprylhydroxamic Acid	88 µg/mL	1.382	20	0.2
Activation	Caprylhydroxamic Acid	175 µg/mL	1.226	53	0.1
	VB	10 ng/mL	1.377	21	1.2**
4-hr Treatment	DMSO	NA	1.404	NA	0.1
With 20 hr Recovery	Caprylhydroxamic Acid	50 µg/mL	1.377	7	0.2
With	Caprylhydroxamic Acid	88 µg/mL	1.356	12	0.2
Activation	Caprylhydroxamic Acid	175 µg/mL	1.201	50	0.1
	CP	7.5 µg/mL	1.224	45	1.4**

DMSO: Dimethyl sulfoxide; CP: Cyclophosphamide; VB: Vinblastine; NA: Not Applicable; Fisher's Exact Test: * $p \leq 0.05$; ** $p \leq 0.01$.

a. CBPI = cytokinesis-blocked proliferation index

b. Relative to vehicle control

c. MNBN = micronucleated binucleated cells

d. BN = binucleated cells

MatTek CORPORATION

FINAL REPORT

Evaluation of the Skin Irritation Potential of Diheptyl Succinate and Caprylhydroxamic Acid Using the EpiDerm Skin Irritation Test OECD TG439

Study Number: 034-18

Testing Facility:
MatTek Corporation
200 Homer Ave.
Ashland, MA 01721
Phone: (1-)508-881-6771

Study Director:
Kalyani Guntur, Ph.D
Research Scientist
Phone: (1-)508-881-6771 ext 225
E-mail: kguntur@mattek.com

Sponsor:
[redacted]

Date of Final Report: April 5, 2018

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Definition
%	Percent
°C	Degrees Celsius
µL	Microliter(s)
DPBS	Dulbecco's Phosphate-Buffered Saline
hr	Hour(s)
ID	Identification
mL	Milliliter(s)
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue)
OD	Optical Density
PC	Positive Control
SDS	Sodium dodecyl sulfate
SD	Standard deviation
TA1	Test article 1
TA2	Test article 2
NC	Negative Control
NI	Non-Irritant
I	Irritant
min	Minutes
GLP	Good Laboratory Practice
MSDS	Material Safety Data Sheet
g	Gram

COMPLIANCE STATEMENT

The report in support of the study entitled "Evaluation of Skin Irritation Potential of Diheptyl Succinate and Caprylhydroxamic Acid Using the EpiDerm Skin Irritation Test OECD TG439" was conducted and reported in compliance with existing OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Guidelines (OECD ENV/MC/CHEM(98)17) with the following exception:

The positive control test substance, 5% SDS, provided by MatTek was manufactured following GMP. This exception to the regulations did not have any effect on the quality or integrity of the data generated.

There were no deviations from the aforementioned regulations that affected the quality or integrity of the data or the interpretation of the results presented in this report.

Kalyani

April 05, 2018

Kalyani Guntur, PhD
Study Director
MatTek Corporation

Date

QUALITY ASSURANCE STATEMENT

The report entitled "Evaluation of the Skin Irritation Potential of Diheptyl Succinate and Caprylhydroxamic Acid Using the EpiDerm Skin Irritation Test OECD TG439" has been audited for compliance to the OECD regulations, the study plan and amendments, and MatTek standard operating procedures. The dates for all inspections are listed below.

Date of Audit	Type of Audit	Date Reported to Study Director	Date Reported to Testing Facility Management
February 6, 2018	Study Plan Audit	February 7, 2018	February 7, 2018
February 19, 2018	Protocol Amendment #1	February 19, 2018	February 19., 2018
February 26, 2018	Plan Amendment #2	February 26, 2018	February 26, 2018
February 28, 2018	Dosing	February 28, 2018	February 28, 2018
March 7, 2018	Raw Data and Draft Report Audit	March 7, 2018	March 7, 2018
March 28, 2018	Final Report	March 28, 2018	March 28, 2018

All audit findings have been adequately addressed. It is concluded that the draft report accurately describes the methods and standard operating procedures and the reported results accurately reflect the raw data for this study.

Joseph Stashick, RQAP-GLP
QA Manager

April 05, 2018
Date

1. SUMMARY

1.1 Purpose

Study 034-18 is intended to evaluate the skin irritation potential of topically applied Diheptyl Succinate and Caprylhydroxamic Acid using the EpiDerm Skin Irritation Test.

1.2 Study Design

EpiDerm (EPI-200), produced by MatTek Corporation, was used to evaluate skin irritation potential of two topically applied test articles. Tissue viability following topical application of test articles was evaluated via MTT assay.

1.3 Results

PC (5% SDS) decreased tissue viability to 2.714%. The tissue viability of tissues treated with test article 1 (TA1 (Diheptyl Succinate)) was 101.5% and test article 2 (TA2 (Caprylhydroxamic Acid)) was 102.6% and hence were classified as non-irritants (NI).

2. KEY STUDY PERSONNEL AND TEST SITES

2.1 Testing Facility

MatTek Corporation
200 Homer Ave.
Ashland, MA 01721
Phone: (1-)508-881-6771 / Fax: (1-)508-879-1532

Study Director:	Kalyani Guntur, PhD Phone:(1-)508-881-6771 ext. 225 E-mail: kguntur@mattek.com
Testing Facility Manager:	Patrick Hayden, PhD Phone: (1-)508-739-8220 E-mail: phayden@mattek.com
Major Contributor:	Alena Plotkin Associate Scientist

2.2 Sponsor

[redacted]

2.3 Archive Facility

MatTek Corporation
200 Homer Ave.
Ashland, MA 01721
Phone: (1-)508-881-6771/ Fax: (1-)508-879-1532

3. STUDY TIMELINE

Study Initiation date:	February 13, 2018
Experimental starting date:	February 26, 2018
Experimental completion date:	March 2, 2018
Study completion date:	April 5, 2018

4. REGULATORY COMPLIANCE

This study was conducted in accordance with basic principles of the following standards of GLP:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring [ENV/MC/CHEM(98/17)]

5. INTRODUCTION

The objective of the study was to evaluate the skin irritation potential of topically applied materials using the EpiDerm Skin Irritation Test. Tissue viability was determined via MTT assay.

6. TEST/CONTROL ARTICLE

6.1 Test Article

The test articles, Diheptyl Succinate and Caprylhydroxamic Acid were supplied by the Sponsor. The Certificate of Analysis provided by the Sponsor included identity, strength, purity, and composition. A description of the material, lot number, storage conditions, expiration date, physical properties, and any other relevant data was documented in the study file.

Test Article

Batch/Lot No:	GE4293
Identity:	Diheptyl Succinate
Purity:	100%
Expiration Date:	July 27 2020
Storage Condition:	Ambient
Description:	Clear liquid
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sponsor.
Supplier:	[redacted]

Batch/Lot No:	FM7477
Identity:	Caprylhydroxamic Acid
Purity:	100%
Expiration Date:	April 11 2018
Storage Condition:	Ambient
Description:	White powder
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sponsor.
Supplier:	[redacted]

6.2 Control Article

The control article used in this study is described below.

Control Article	
Batch/Lot No:	071817MAB
Identity:	5% SDS Solution
Purity:	≥ 99.0%
Expiration Date:	July 18, 2018
Storage Condition:	15 – 30°C
Description:	Sodium dodecyl sulfate
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by MatTek.
Supplier:	MatTek

6.3 Purity and Stability

The test article Diheptyl Succinate is considered stable when stored at ambient temperature for 36 months. According to the information supplied by the sponsor, the expiration date of the Diheptyl Succinate is July 27 2020. The information about purity is provided in the Certificate of Analysis by the Sponsor (see Appendix A). The test article Caprylhydroxamic Acid is considered stable when stored at ambient temperature for 24 months. According to the information supplied by the sponsor, the expiration date of the Diheptyl Succinate is April 11 2018. The information about purity is provided in the Certificate of Analysis by the Sponsor (see Appendix A).

The control article is considered stable when stored at 15 - 30°C. According to the information supplied by the manufacturer, the expiration date of the control article is July 18, 2018. Information on purity was provided by the product insert (see Appendix A).

6.4 Reserve Samples and Test and Control Article Disposition

6.4.1 Reserve Samples

Approximately 1 mL of Diheptyl Succinate and 1 g of Caprylhydroxamic Acid were removed prior to use and stored within the company archives.

6.4.2 Disposition

Per the sponsor's request, any remaining test article was destroyed at the end of the study.

Any empty test article containers were discarded upon completion of the study.

7. IDENTIFICATION OF THE TEST SYSTEM

The EpiDerm (EPI-200) human tissue model (Lot# 27961) produced by MatTek Corporation was used for this study.

8. MATERIALS

8.1 Solvents and Reagents

All media, reagents, and EpiDerm tissues were supplied by MatTek Corporation (Ashland, MA) and are list in the table below.

Material	Lot #	Expiration Date
EpiDerm (EPI-200)	27961	03/03/18
Assay medium (EPI-100-NMM)	022218TVKC	03/08/18
MTT concentrate	022018TVKA	04/20/18
MTT diluent	1930119	11/30/18
MTT extractant	011718MAA	01/17/19
DPBS	020618TVKD	02/06/19
DPBS (pH 7)	022618APC	02/26/19
Nylon Mesh	0526023-00	12/31/19

8.2 Assay Controls

One negative control (DPBS) and one positive control (5% SDS) were tested concurrently with the test articles to benchmark the effect of the test articles. MatTek supplied the assay controls used for this study. Documentation of quality of all assay controls is maintained within the study file.

9. METHODS

9.1 Preparation of Test Materials

Test materials were used as neat.

9.2 Exposure of EpiDerm Tissues for Skin Irritation Test

1. The procedure outlined in the protocol MK-24-007-0023 (Appendix B) was followed. Briefly, EpiDerm tissues were removed from packaging. Each insert was placed into one well of a 6-well plate containing 0.9 mL EPI-100-NMM. The tissues were equilibrated at 37°C/5%CO_2 for 1 hour ± 5 min.

2. Following 1-hr equilibration, the EPI-200 tissues were transferred from upper wells into the lower wells of the 6-well plate containing 0.9 mL EPI-100-NMM media. The tissues were equilibrated at 37°C/5%CO_2 overnight (18 ± 3 hours).

3. Following overnight equilibration, 30 µl of TA1, negative control (NC) and positive control (PC) (mesh was used for TA1, NC & PC) were applied topically to n=3 tissues per treatment group. N=3 tissues were prewet with 25 µl of DPBS. A 25 mg sharp application

spoon was filled with test material (TA2). The spoon was leveled by gentle scratching of the excess material away and then applied. The treatments were performed at an interval of 1 minute between tissues and the tissues were incubated at 37°C/5%CO_2 for 35 ± 1 minutes.

Table 1. Treatment groups

Group ID	MatTek Sample ID	Test Material	Number Replicates
1	NC	DPBS	N=3 tissues
2	PC	5% SDS	N=3 tissues
3	TA1	Diheptyl Succinate	N=3 tissues
4	TA2	Caprylhydroxamic Acid	N=3 tissues

4. After 35 min, all plates were removed from the incubator and place into the biological safety cabinet until the 60-min exposure period was completed for the first dosed tissue. The tissues were rinsed with sterile DPBS by filling and emptying the tissue inserts 15 times to remove any test material and transferred the tissues to 6-well plates pre-filled with 0.9 mL EPI-100-NMM media. The tissues were incubated in the incubator for next 24 ± 2 hours.

5. 24 hours post-treatment the tissues were re-feed with 0.9 mL EPI-100-NMM medium and incubated at 37°C/5%CO_2 for an additional 18 ± 2 hours.

6. At the end of 18 ± 2 hours post-incubation period the tissues were removed from culture and MTT analysis was performed.

9.3 MTT Analysis

1. MTT analysis was performed following procedure outlined in MK-24-007-0023 (Appendix B). Briefly, just prior to the end of 18 hours post-incubation period, 2 mL MTT concentrate was thawed (supplied by MatTek, part number MTT-100-CON) and added to 8 mL MTT diluent (supplied by MatTek, part number MTT-100-DIL) to prepare the MTT reagent. The reconstituted MTT reagent was protected from light by covering the tube with aluminum foil.

2. 300 µl of the MTT reagent was dispensed into the appropriate number of wells of a 24-well plate and was equilibrated to 37°C by placing the plate in a 37°C/5%CO_2 incubator.

3. The inserts were placed into the wells containing the pre-warmed MTT reagent and incubated at 37°C, 5% CO_2 for 3 hours ± 5 min. Viable tissues converted the MTT to a purple dye. The amount of conversion is proportional to the viability of the tissue.

4. At the end of the incubation, the tissues were removed from the MTT, blotted dry on a paper towel and moved to a clean 24-well plate.

5. 2 ml of extractant solution (supplied by MatTek, part number MTT-100-EXT) was pipetted into each insert, allowing it to overflow into the well below.

6. Extraction was performed for two hours at room temperature on a shaker. The plate was protected from light exposure and sealed to prevent extractant evaporation.

7. At the end of the extraction period, the extractant solution was combined from the apical compartment with that in the well below, the tissue inserts were removed and discarded.

8. The extractant solution was mixed well and 200 µl of each sample was added to a 96-well plate. Added 200 µl of sample to a second well in the 96-well plate and all samples were prepared in duplicate. The optical density (OD) of the extracted samples were determined at 570 nm using 200 µl of extractant as a blank using a spectrophotometer.

9.4 Statistical Analysis

Data collection and analysis were performed using the SpectraMax Pro GxP software.

10. DATA RETENTION/ARCHIVES

Following approval of the final report, all specimens, raw data and documents generated at MatTek Corporation during this study, together with the original copy of the approved study plan (including amendments) and the final report were transferred to the scientific archives of MatTek Corporation for a period of approximately 5 years. At the end of the storage period, MatTek Corporation will contact the sponsor for the authorization to transfer the data to the sponsor facilities at the expense of the sponsor. In the case that the sponsor wishes to continue to store the documents at MatTek Corporation, a charge will be levied on a per box per year basis.

11. RESULTS

11.1 Percent Viability

Table 2. Percent viability of test article treated tissue (TA) versus DPBS-treated tissue (NC)

Treatment group #	Conditions	Mean of OD	SD of OD	Mean of viability [%]	SD of viability	Prediction
NC	Negative Control (DPBS)	2.464	0.112	100.0	4.555	NI
PC	Positive Control (5%SDS)	0.067	0.007	2.714	0.284	I
TA1	Diheptyl Succinate	2.501	0.056	101.5	2.286	NI
TA2	Caprylhydroxamic Acid	2.527	0.085	102.6	3.470	NI

Figure 1. Tissue Viability (MTT) Data

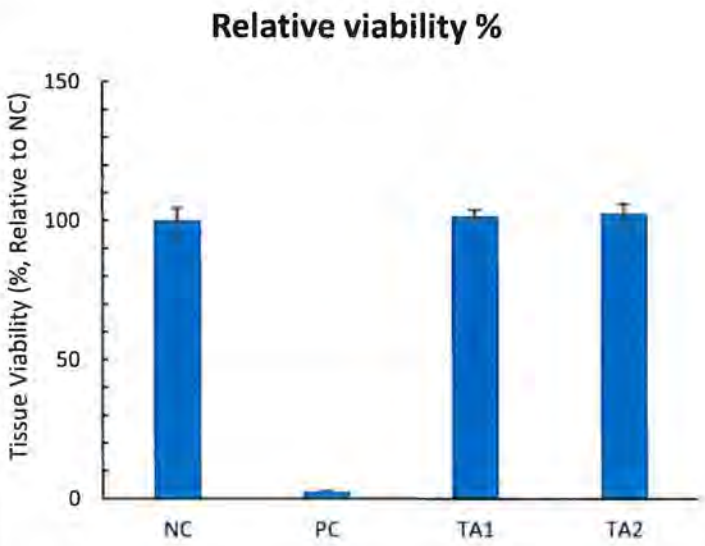

Table 3. Raw Data

Sample	Tissue	OD		Mean OD	% Viability
		Aliquot 1	Aliquot 2		
NC	1	2.582	2.596	2.589	105.1
	2	2.389	2.354	2.371	96.25
	3	2.434	2.429	2.431	98.69
PC	1	0.076	0.073	0.074	3.010
	2	0.062	0.059	0.060	2.444
	3	0.058	0.074	0.066	2.688
TA1	1	2.587	2.535	2.561	103.9
	2	2.514	2.473	2.493	101.2
	3	2.450	2.448	2.449	99.40
TA2	1	2.511	2.501	2.506	101.7
	2	2.440	2.469	2.455	99.63
	3	2.637	2.606	2.622	106.4

12. DISCUSSION AND CONCLUSIONS

- TA1 (Diheptyl Succinate) had tissue viability of 101.5%, and TA2 (Caprylhydroxamic Acid) had tissue viability of 102.6% and therefore were classified as non-irritants (NI).

13. STUDY REPORT APPROVAL SIGNATURES

We agree that the content of this report represent the raw data recorded and adhered to the study protocol, corporate SOPs and the regulatory requirements for this study.

IN AGREEMENT WITH THE STUDY REPORT

Kalyani

Kalyani Guntur, PhD
Study Director
MatTek Corporation

04/05/18
Date

Patrick Hayden

Patrick Hayden, PhD
Testing Facility Manager
MatTek Corporation

4/5/18
Date

[redacted]

APPENDIX A: COA

[redacted]

CERTIFICATE OF ANALYSIS

CUSTOMER INFORMATION:

PRODUCT: [redacted]CHA
LOT NO.: FM7477
SHIP DATE: 03/09/2018
QUANTITY:

RESULTS OF ANALYSIS:

TEST PARAMETER	UNITS	RESULT
Melt Range (Begin)	°C	79.8
Melt Range (End)	°C	80.4
CHA	%	98.57
Infrared Spectrum	Matches STD	OK

[redacted]

[redacted]

SAFETY DATA SHEET

Patent Pending

Product Id Number: 69065
Revision Date: 10/13/2017

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name: [redacted]
Synonym(s): INCI: Caprylhydroxamic Acid

[redacted]

E-Mail Address: [redacted]
For Emergency Information contact: Chemtrec - 1-800-424-9300
For Non-Emergency Information contact: 1-800-521-9891

2. HAZARDS IDENTIFICATION

2.1. Classification of the substance or mixture

Not classified as hazardous.

2.2. Label elements

Not classified as hazardous.

2.3. Other hazards
No additional information available

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical name	CAS No	Weight-%
Caprylhydroxamic Acid	7377-03-9	100

*The exact percentage (concentration) of composition has been withheld as trade secret.

4. FIRST AID MEASURES

Ingestion: If swallowed, observe victim for 24 hours; seek medical attention if indicated. If vomiting occurs spontaneously, keep head below hips to prevent aspiration

Skin: For skin contact, wipe away excess material with dry towel. Then wash affected areas with plenty of water, and mild soap if available, for several minutes. Get medical attention if irritation occurs.

Inhalation: If inhaled, remove from area to fresh air. Get medical attention if respiratory

[redacted] [redacted] [redacted]

irritation develops or if breathing becomes difficult.

Eyes: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if irritation occurs.

Notes to Physician: Treatment based on sound judgment of physician and individual reactions of patient.

5. FIRE FIGHTING MEASURES

Flammable Limits in Air - Upper (%): Maximum % by volume of vapor in air above which propagation of flame does not occur on contact with a source of ignition: Not Determined

Sensitivity to Mechanical Impact (Y/N): NO

Sensitivity to Static Discharge (Y/N): Sensitivity to static discharge is not expected.

Extinguishing Media: Water fog, carbon dioxide, foam, dry chemical

Special Firefighting Procedures: Fire-fighters should wear self-contained breathing apparatus and full protective clothing when fighting chemical fires. Use water spray to cool nearby containers and structures exposed to fire. Containers can build up pressure if exposed to heat (fire). Fire involving large amounts of material should not be approached because individual containers may rupture abruptly causing "fireball" effect.

6. ACCIDENTAL RELEASE MEASURES

Action to be taken if material is released or spilled: Wipe, scrape or soak up in an inert material and put in a container for disposal. Wash walking surfaces with detergent and water to reduce slipping hazard. Wear proper protective equipment as specified in the protective equipment section. Large quantity spills should be contained and pumped into drums for recovery or disposal.

7. HANDLING AND STORAGE

Precautions to be taken in handling and storage: Keep container closed when not in use.
Good hygienic practices should be observed. Work clothes should be washed separately at the end of each work day. Disposable clothing should be discarded with material.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Controls: None required under normal conditions.

Personal Protection Equipment

Respiratory Protection: Wear a respirator with filter.
Protective Gloves: Use gloves as a standard industrial handling procedure.
Eye and Face Protection: Wear safety glasses or goggles to protect against exposure.
Other Protective Equipment: None required under normal operating conditions.

[redacted] [redacted] [redacted]

Ventilation: Handle in well ventilated places.

9. PHYSICAL AND CHEMICAL PROPERTIES

Boiling Point:	343.32°C
Melting Point:	79-81°C
Physical State:	Crystalline Solid
Odor:	MILD CHARACTERISTIC ODOR
Color:	WHITE
Solubility in Water:	1.55 g/L at 23°C

10. STABILITY AND REACTIVITY

Stability:	Avoid strong oxidizing agents.
Hazardous Polymerization:	Does not occur
Hazardous Thermal Decomposition / Combustion Products:	Combustion/thermal decomposition of this matter might generate toxic gases (CO, NOx).
Incompatibility (Materials to Avoid):	None known.
Conditions to Avoid:	None known.

11. TOXICOLOGICAL INFORMATION

Product Information

Acute Toxicity

Inhalation	Specific test data for the substance or mixture is not available.
Eye contact	Specific test data for the substance or mixture is not available.
Skin contact	Specific test data for the substance or mixture is not available.
Ingestion	Specific test data for the substance or mixture is not available.

Information on likely routes of exposure

Symptoms No information available.

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Germ cell mutagenicity	Not mutagenic in AMES Test.
Carcinogenicity	No information available.
Reproductive toxicity	No information available.
STOT - single exposure	No information available.
STOT - repeated exposure	No information available.
Aspiration hazard	No information available.

[redacted] [redacted] [redacted]

Numerical measures of toxicity

Component Information Classification based on data available for ingredients

Chemical name	LD50 Oral	LD50 Dermal	LC50 Inhalation
Caprylhydroxamic Acid	> 10700 mg/kg (Rat)		

Component Tox Information
Caprylhydroxamic Acid (7377-03-9)

Skin sensitization: In an RIPT of 52 subjects, caprylhydroxamic acid did not indicate a potential for dermal irritation and/or sensitization.

Serious eye damage/eye irritation: Caprylhydroxamic acid is classified as non-irritating/minimally irritating in an EpiOcular MTT Viability Assa (non-animal). ET50 > 130.8 based on ocular toxicity or irritation potential of the test substance for MTT viability of EpiOcular samples.

12. ECOLOGICAL INFORMATION

Ecotoxicological Information: No data at this time.

Chemical Fate Information: No data at this time.

13. DISPOSAL CONSIDERATIONS

Disposal Method: As local regulations may vary; all waste must be disposed/recycled/reclaimed in accordance with federal, state, and local environmental control regulations.

14. TRANSPORT INFORMATION

Department of Transportation Information

Shipping Name: Not regulated as dangerous goods.
Hazard Class: Non-Regulated
Label(s): NONE
UN/NA Number: Not regulated as dangerous goods.
Placards: NONE

International Transportation Classifications

IATA Dangerous Goods Regulation (DGR) 58th Edition 2017 Non-Regulated
IMDG, International Maritime Dangerous Goods Non-Regulated as dangerous goods.

15. REGULATORY INFORMATION

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture

TSCA This material is manufactured for use as an additive in personal care products and is regulated under the FOOD, DRUG, and COSMETICS ACT (FDA) and is therefore exempt from TSCA inventory listing requirements.

[redacted] [redacted] [redacted]

15.2. Chemical Safety Assessment

No information available

15.3. OTHER REGULATORY INFORMATION:

Please contact your [redacted] representative for region- or country-specific compliance.

16. OTHER INFORMATION

Revision Date 10/13/2017

This safety data sheet complies with the requirements of Regulation (EC) No. 1272/2008

General Disclaimer
The information provided on this SDS is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.
End of Safety Data Sheet

*** END OF SDS ***

[redacted]

Certificate of Analysis

Product: [redacted] DHS Date: Mar/08/2018

Mfg Date: May/22/2017

LOT #: GE4293

ANALYSIS	RESULT	SPECIFICATION
Odor	Conforming	Equal to or Better Than Std.

[redacted]

[redacted]

SAFETY DATA SHEET

Patent Pending

Product Id Number: 63129
Revision Date: 07/21/2017

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name: [redacted]
Synonym(s): INCI: Diheptyl Succinate

Chemical Family / Use: For use in Cosmetic/Personal Care Products

[redacted]

E-Mail Address: [redacted]
For Emergency Information contact: Chemtrec - 1-800-424-9300
For Non-Emergency Information contact: 1-800-521-9891

2. HAZARDS IDENTIFICATION

2.1. Classification of the substance or mixture

Non-Hazardous

2.2. Label elements

Non-Hazardous

2.3. Other hazards
This substance does not meet the PBT/vPvB crtieria according to Regulation (EC) No 1907/2006 [REACH], Annex XIII

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical name	CAS No	Weight-%
Diheptyl Succinate	15872-89-6	100

*The exact percentage (concentration) of composition has been withheld as trade secret.

4. FIRST AID MEASURES

Ingestion: If swallowed, observe victim for 24 hours; seek medical attention if indicated. If vomiting occurs spontaneously, keep head below hips to prevent aspiration

Skin: For skin contact, wipe away excess material with dry towel. Then wash affected areas with plenty of water, and mild soap if available, for several minutes. Get

[redacted] [redacted] [redacted]

medical attention if irritation occurs.

Inhalation: If inhaled, remove from area to fresh air. Get medical attention if respiratory irritation develops or if breathing becomes difficult.

Eyes: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if irritation occurs.

Notes to Physician: Treatment based on sound judgment of physician and individual reactions of patient.

5. FIRE FIGHTING MEASURES

Flash Point: >185° C (open cup)
Flammable Limits In Air - Upper (%): Maximum % by volume of vapor in air above which propagation of flame does not occur on contact with a source of ignition: N/D

Sensitivity to Mechanical Impact (Y/N): NO

Sensitivity to Static Discharge (Y/N): Sensitivity to static discharge is not expected.

Extinguishing Media: Water fog, carbon dioxide, foam, dry chemical

Special Firefighting Procedures: Containers can build up pressure if exposed to heat (fire). Fire involving large amounts of material should not be approached because individual containers may rupture abruptly causing "fireball" effect. Fire-fighters should wear self-contained breathing apparatus and full protective clothing when fighting chemical fires. Use water spray to cool nearby containers and structures exposed to fire.

6. ACCIDENTAL RELEASE MEASURES

Action to be taken if material is released or spilled: Wipe, scrape or soak up in an inert material and put in a container for disposal. Wash walking surfaces with detergent and water to reduce slipping hazard. Wear proper protective equipment as specified in the protective equipment section. Large quantity spills should be contained and pumped into drums for recovery or disposal.

7. HANDLING AND STORAGE

Precautions to be taken in handling and storage: Keep container closed when not in use.
Good hygienic practices should be observed. Work clothes should be washed separately at the end of each work day. Disposable clothing should be discarded with material.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Controls: None required under normal conditions.

Personal Protection Equipment

Respiratory Protection: None required under normal conditions.
Protective Gloves: Use gloves as a standard industrial handling procedure
Eye and Face Protection: Wear safety glasses or goggles to protect against

[redacted] [redacted] [redacted]

exposure.

Other Protective Equipment: None Required

Ventilation: No unusual ventilation required.

9. PHYSICAL AND CHEMICAL PROPERTIES

Boiling Point:	294°C
Melting Point:	5°C
Physical State:	Liquid
Odor:	MILD
Color:	YELLOW AMBER
Solubility in Water:	INSOLUBLE
Viscosity:	22 cSt @ 40°C

10. STABILITY AND REACTIVITY

Stability:	Stable
Hazardous Polymerization:	Does not occur
Hazardous Thermal Decomposition / Combustion Products:	No unusual decomposition products known.
Incompatibility (Materials to Avoid):	None known.
Conditions to Avoid:	None known.

11. TOXICOLOGICAL INFORMATION

Product Information

Acute Toxicity

Inhalation	Specific test data for the substance or mixture is not available.
Eye contact	Specific test data for the substance or mixture is not available.
Skin contact	Specific test data for the substance or mixture is not available.
Ingestion	Specific test data for the substance or mixture is not available.

Information on likely routes of exposure

Symptoms No information available.

Delayed and Immediate effects as well as chronic effects from short and long-term exposure

Germ cell mutagenicity	OECD Test No. 471: Bacterial Reverse Mutation Test: Not mutagenic.
Carcinogenicity	No information available.
Reproductive toxicity	No information available.
STOT - single exposure	No information available.

[redacted] [redacted]

STOT - repeated exposure	No information available.
Aspiration hazard	No information available.

Numerical measures of toxicity

Component Information	Classification based on data available for ingredients

Component Tox Information
Diheptyl Succinate (15872-89-6)

Skin sensitization	In an RIPT of 50 subjects, diheptyl succinate did not indicate a potential for dermal irritation or sensitization.
Serious eye damage/eye irritation	In a Hen's Egg Test Chorioallantoic Membrane (non-animal alternative test method utilized to determine the potential ocular irritancy), the irritating potential of diheptyl succinate was determined none to slight.

12. ECOLOGICAL INFORMATION

Ecotoxicological Information:	Algae-ErC50 (72 hours)>100 mg/L[3]
Chemical Fate Information:	Tested as readily biodegradable.

13. DISPOSAL CONSIDERATIONS

Disposal Method:	As local regulations may vary; all waste must be disposed/recycled/reclaimed in accordance with federal, state, and local environmental control regulations.

14. TRANSPORT INFORMATION

Department of Transportation Information

Shipping Name:	Non-Regulated
Hazard Class:	Non-Regulated
Label(s):	NONE
UN/NA Number:	NONE
Placards:	NONE

International Transportation Classifications

IATA Dangerous Goods Regulation (DGR) 58th Edition 2017	Non-Regulated
IMDG, International Maritime Dangerous Goods	Non-Regulated as dangerous goods.

15. REGULATORY INFORMATION

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture

TSCA	This material is manufactured for use as an additive in personal care products and is regulated under the FOOD, DRUG, and COSMETICS ACT (FDA) and is therefore exempt from TSCA inventory listing requirements.

[redacted] [redacted] [redacted]

15.2. Chemical Safety Assessment

No information available

15.3. OTHER REGULATORY INFORMATION:

Please contact your [redacted] representative for region- or country-specific compliance.

16. OTHER INFORMATION

Revision Date 07/21/2017

This safety data sheet complies with the requirements of Regulation (EC) No. 1272/2008

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End of Safety Data Sheet

*** END OF SDS ***

Certificate of Analysis

MatTek CORPORATION

Product: EpiDerm™ Reconstructed Human Epidermis

Lot Number: 27961

Part#: EPI-200, EPI-212
Description: Reconstructed human epidermis tissue containing normal human keratinocytes. This product is for research use only. Not for use in animals, humans or diagnostic purposes.

I. Cell source

All cells used to produce EpiDerm™ are purchased or derived from tissue obtained by MatTek Corporation from accredited institutions. In all cases, consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the tissues or derivatives of the tissue for research purposes.

Keratinocyte Strain: 4F1188

II. Analysis for potential biological contaminants

The cells used to produce EpiDerm™ tissue are screened for potential biological contaminants. Tests for each potential biological contaminant listed below were performed according to the test method given. Results of "Not detected" indicate that testing for the potential biological contaminant was not observed as determined by the stated test method.

Keratinocytes:

HIV-1 virus - Oligonucleotide-directed amplification	Not detected
Hepatitis B virus - Oligonucleotide- directed amplification	Not detected
Hepatitis C virus - Oligonucleotide- directed amplification	Not detected
Bacteria, yeast, and other fungi - long term antibiotic, antimycotic free culture	Not detected

III. Analysis for tissue functionality and quality

Test	Specification	Acceptance criteria	Result and QA Statement	
Tissue viability	MTT QC assay, 4 hours, n=3	OD (540-570 nm) <1.0-3.0>	1.913 ± 0.143	Pass
Barrier function	ET-50 assay, 100 µl 1% Triton X-100, 4 time-points, n=3, MTT assay	ET-50 <4.77-8.72 hrs>	7.71 hrs	Pass
Sterility	Long term antibiotic and antimycotic free culture	No contamination	Sterile	Pass

Tissue viability and the barrier function test are within the acceptable ranges and indicate appropriate formation of the epidermal barrier, the presence of a functional stratum corneum, a viable basal cell layer, and intermediate spinous and granular layers. Results obtained with this lot conform to the requirements of the **OECD TG 431 and 439.**

Initials: TVK
Date: 2/28/18

Paul Kearney
Paul Kearney
Quality Assurance Director

February 28, 2018
Date

CAUTION: Whereas all information herein is believed to be correct, no absolute guarantee that human derived material is non-infectious can be made or is implied by this certificate of analysis. All tissues should be treated as potential pathogens. The use of protective clothing and eyeware and appropriate disposal procedures are strongly recommended.

MatTek Corporation
200 Homer Avenue, Ashland, MA - USA
+1-508-881-6771

www.mattek.com
information@mattek.com

Certificate of Analysis

MatTek CORPORATION

Catalog #: TC-SDS-5%

Lot number: 071817MAB

Description: 5% SDS Solution:

Skin irritant reference chemical (positive control) - Component of part number EPI-200-SIT. Used in the assay of MatTek tissue model cultures.
This product is for research use only. Not for use in animals, humans or diagnostic purposes.

I. Material Characteristics:

Base material:	Ultrapure Water
Other:	Sodium Dodecyl Sulfate (SDS), ≥ 99.0%
Storage Temperature:	15 -30°C
Expiration date:	July 18, 2018

II. Analysis for Purity / Contaminants of SDS (Powder):

Test	Specification	Results
Appearance (Color)	Colorless Or White	White
Appearance (Form)	Powder	Powder
Purity (GC Area %)	≥ 99.0 %	≥ 99.6 %
Loss On Drying	≤ 3.0%	≤ 0.1 %
Infrared Spectrum	Conforms To Structure	Conforms
Metal Trace Analysts (ICP)	Corresponds To Requirements	Passes
Aluminum (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Barium (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Bismuth (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Calcium (ICP)	≤ 10 mg/kg	≤ 10 mg/kg
Cadmium (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Cobalt (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Chromium (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Copper (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Iron (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Potassium (ICP)	≤ 200 mg/kg	≤ 200 mg/kg
Lithium (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Magnesium (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Manganese (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Molybdenum (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Nickel (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Lead (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Strontium (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Zinc (ICP)	≤ 5 mg/kg	≤ 5 mg/kg
Chloride (Cl)	≤ 200 mg/kg	≤ 200 mg/kg
Phosphate (PO4)	≤ 1 mg/kg	≤ 1 mg/kg

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Certificate of Analysis

MatTek CORPORATION

Extraneous Activities	Dnases, Rnases, Proteases, Phosphatases not detectable	Pass
Solubility (Method)	-	
Appearance (Solution)	Clear Colorless	Clear Colorless
Residue (Filter Test)	No Residue	No Residue
UV Absorbance (at 260 nm)	≤ 0.04	0.02
UV Absorbance (at 280 nm)	≤ 0.02	0.01

III. Analysis for Material Functionality:

Measurement of tissue viability following application of material (TC-SDS-5%) as positive control in the In Vitro EpiDerm™ Skin Irritation Test: < 50.0%

Pass

Initials: TVK
Date: 2/26/18

Paul Kearney
Paul Kearney
Quality Assurance Director

February 26, 2018
Date

CAUTION: The material listed above should be treated as potentially hazardous. The use of protective clothing and eyeware and appropriate disposal procedures is strongly recommended.

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QC-10 012-0154 Rev. New

APPENDIX B: PROTOCOL AND AMENDMENTS

MatTek CORPORATION

STUDY PLAN

Evaluation of the Skin Irritation Potential of Diheptyl Succinate and Caprylhydroxamic Acid Using the EpiDerm Skin Irritation Test OECD TG439

Study Number: #034-18

Testing Facility:
MatTek Corporation
200 Homer Ave.
Ashland, MA 01721
Phone: (1-)508-881-6771

Study Director:
Kalyani Guntur, Ph.D
Research Scientist
Phone: (1-)508-881-6771 ext 225
E-mail: kguntur@mattek.com

[redacted]

Date of Study Plan: February 5th, 2018

TABLE OF CONTENTS

1. PURPOSE

The purpose of the study is to evaluate the skin irritation potential of topically applied Diheptyl Succinate and Caprylhydroxamic Acid using the EpiDerm Skin Irritation Test

2. PERSONNEL INVOLVED IN THE STUDY

2.1 Testing Facility

MatTek Corporation
200 Homer Ave.
Ashland, MA 01721
Phone: (1-)508-881-6771 / Fax: (1-)508-879-1532

Study Director:	Kalyani Guntur, PhD Phone:(1-)508-881-6771 ext 225 E-mail: kguntur@mattek.com
Testing Facility Manager:	Patrick Hayden, PhD Phone: (1-)508-739-8220 E-mail: phayden@mattek.com

2.2 Sponsor

[redacted]

2.3 Archive Facility

MatTek Corporation
200 Homer Ave.
Ashland, MA 01721
Phone: (1-)508-881-6771/ Fax: (1-)508-879-1532

3. PROPOSED TIME SCHEDULE

Study Initiation date:	February 7 2018
Experimental starting date:	February 26, 2018
Experimental completion date:	March 5, 2018
Study completion date:	March 31, 2018

4. REGULATORY COMPLIANCE

This study will be conducted in accordance with basic principles of the following standards of GLP.

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring [ENV/MC/CHEM(98/17)]

5. TEST/CONTROL ARTICLE

5.1 Test Article

The test articles, Diheptyl Succinate and Caprylhydroxamic Acid, will be supplied by the Sponsor. The Certificate of Analysis provided by the Sponsor will include identity, strength, purity, and composition. A description of the material, lot number, storage conditions, expiration date, physical properties, and any other relevant data will be documented in the study file.

Test Article 1 (TA1)

Batch/Lot No:	TBA
Identity:	Diheptyl Succinate
Purity:	TBA
Expiration Date:	36 months after the date of manufacturing
Storage Condition:	Ambient
Description:	Clear liquid
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sponsor.
Supplier:	[redacted]

Test Article 2 (TA2)

Batch/Lot No:	TBA
Identity:	Caprylhydroxamic Acid
Purity:	TBA
Expiration Date:	24 months after the date of manufacturing
Storage Condition:	Ambient
Description:	White powder
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sponsor
Supplier:	[redacted]

5.2 Control Article

The manufacturer of the control article will be maintained in the raw data including a description of lot number, storage conditions, expiration date, physical properties, and any other relevant information.

Control Article

Batch/Lot No:	TBA
Identity:	SDS
Purity:	TBA
Expiration Date:	TBA
Storage Condition:	Room Temperature
Description:	Sodium dodecyl sulfate
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by MatTek
Supplier:	Sigma

5.3 Purity and Stability

Purity and stability of the test articles is the responsibility of the Sponsor. The Sponsor will provide pertinent data necessary for incorporation into the final report.

Purity and stability of the control article is the responsibility of the manufacturer of the reagent used. Any information received from the manufacturer (product inserts) will be maintained in the study file for incorporation into the final report.

5.4 Reserve Samples and Test and Control Article Disposition

5.4.1 Reserve Samples

Approximately 1 mL of Diheptyl Succinate and 1 g of Caprylhydroxamic Acid will be taken prior to first use and stored under the storage conditions mentioned in sections 5.1.

5.4.2 Disposition

Any remaining test article will be returned to the Sponsor, used in subsequent studies, or disposed of at the direction of the Sponsor.

Any empty test article containers will be discarded upon completion of the study.

6. IDENTIFICATION OF THE TEST SYSTEM

The EpiDerm (EPI-200) human tissue model (Lot# will be recorded in the study data and listed within the study report) produced by MatTek Corporation will be used.

7. QUALITY CONTROL IDENTIFICATION OF THE TEST SYSTEM

Quality control results will be obtained from the standardized quality control tests. Assay results will be accepted if the positive control compound, 1.0% Triton X-100, yields an ET-50 greater than 4.77 hours and less than 8.72 hours. Similarly, the negative control MTT optical density must be > 1.0. If any assay fails to meet these acceptance criteria, the study will be repeated.

8. MATERIALS

8.1 Solvents and Reagents

All media, reagents, and EpiDerm tissues will be supplied by MatTek Corporation (Ashland, MA). All items are covered under MatTek's Standard Operating Procedures (SOPs) used in the production of these items for commercial sale. All solvents and reagents used during this study will be recorded in the raw data and reported in the final report.

8.2 Assay Controls

Negative control (DPBS) and one positive control (5% SDS) will be run concurrently with the test articles to benchmark the effect of the test materials. MatTek will supply the assay controls used for this study. Documentation of receipt and quality of all assay controls will be maintained within the study file.

9. EXPERIMENTAL DESIGN

9.1 Preparation of test materials

1. Test materials will be used as neat.

9.2 Test for Interference of Chemicals with MTT Endpoint and Correction Procedures

1 The procedure outlined in the protocol MK-24-007-0023 (Appendix B) will be followed.

9.3 Test for Mesh Compatibility

1 The procedure outlined in the protocol MK-24-007-0023 (Appendix B) will be followed.

9.4 Exposure of EpiDerm tissues for skin irritation test

1 The procedure outlined in the protocol MK-24-007-0023 (Appendix B) will be followed. Briefly, remove EpiDerm tissues from packaging. Place each insert into one well of a 6-well plate containing 0.9 mL EPI-100-NMM. Equilibrate at 37 °C/5%CO_2 for 1 hour ± 5 min.

2 Following 1-hr equilibration, transfer one EPI-200 tissue from upper wells into the lower wells of the 6-well plate containing 0.9 mL EPI-100-NMM media. Equilibrate at 37 °C/5%CO_2 overnight (18 ± 3 hours).

3 Following overnight equilibration, apply 30 µl of TA1 (use mesh if compatible), negative control (NC) and positive control (PC) topically to n=3 tissues per treatment group; prewet n=3 tissues with 25µl of DPBS and apply 25 mg of TA2 (using sharp applicator spoon). Perform the treatments at an interval of 1 minute per tissue and incubate the tissues at 37 °C/5%CO_2 for 35 ± 1 minutes.

Table 1. Treatment groups

Group ID	MatTek Sample ID	Test Material	Number Replicates
1	NC	DPBS	N=3 tissues
2	PC	5% SDS	N=3 tissues
3	TA1	Diheptyl Succinate	N=3 tissues
4	TA2	Caprylhydroxamic Acid	N=3 tissues

4 After 35 min, remove all plates from the incubator, place them into the biological safety cabinet and wait until the period for 60 min is completed for the first dosed tissue. Rinse tissues with sterile DPBS by filling and emptying the tissue inserts 15 times to remove any test material and transfer the tissues to 6-well plates pre-filled with 0.9 mL EPI-100-NMM media. Incubate tissues in the incubator for next 24 ± 2 hours.

5 24 hours post-treatment re-feed the tissues with 0.9 mL EPI-100-NMM medium and incubate at 37 °C/5%CO_2 for an additional 18 ± 2 hours.

6 At the end of 18 ± 2 hours post-incubation period remove tissues from culture and perform MTT analysis.

9.5 MTT Analysis

1 MTT analysis will be performed following procedure outlined in MK-24-007-0023 (Appendix B). Briefly, just prior to the end of 18 hours post-incubation period, thaw the 2 mL MTT concentrate (supplied by MatTek, part number MTT-100-CON) and add to 8 mL MTT

diluent (supplied by MatTek, part number MTT-100-DIL) to prepare the MTT reagent. Protect the reconstituted MTT reagent from light by covering the tube with aluminum foil.

2. Dispense 300 µl of the MTT reagent into the appropriate number of wells of a 24 well plate and equilibrate to 37°C by placing the plate in a 37°C/5%CO_2 incubator.

3. Place the inserts into the wells containing the pre-warmed MTT reagent and incubate at 37°C, 5% CO_2 for 3 hours ± 5 min. Viable tissues will convert the MTT to a purple dye. The amount of conversion is proportional to the viability of the tissue.

4. At the end of the incubation, remove tissues from the MTT, blot dry on a paper towel and move to a clean 24-well plate.

5. Pipette 2 ml of extractant solution (supplied by MatTek, part number MTT-100-EXT) into each insert, allowing it to overflow into the well below.

6. Extract for two hours at room temperature on a shaker. The plate should be protected from light exposure and sealed to prevent extractant evaporation.

7. At the end of the extraction period, combine the extractant solution from the apical compartment with that in the well below, remove the tissue inserts and discard.

8. Mix the extractant solution well and add 200 µl of each sample to a 96-well plate. Add 200 µl of sample to a second well in the 96-well plate so that all samples are prepared in duplicate. Determine the optical density (OD) of the extracted samples at 570 nm using 200 µl of extractant as a blank using a spectrophotometer.

9.6 Data presentation

Percent viability will be calculated according to the following formula:

% viability = 100 x [Avg OD (sample) / AVG OD (negative control]

10. STATISTICAL ANALYSIS OF DATA

Data collection and analysis will be performed using the SpectraMax Pro software.

11. CHANGES TO STUDY PLAN

11.1 Amendments

Any planned changes to the study plan will be documented by Study Plan Amendment. The Study Plan Amendments will be in writing and signed by the Study Director and will be included in the final report.

11.2 Deviations

Any unplanned changes to the study plan, including those activities that occurred unintentionally will be in writing and contained within the study file. The impact on the outcome of the study will be assessed. Any study plan deviations will be communicated to the Sponsor Representative in a timely manner and will be included within the final report. Any other deviations that may affect the quality of the data will also be included within the final report.

12. QUALITY ASSURANCE

The Quality Assurance Unit (QAU) will audit the study in accordance with the study plan, amendments, MatTek Corporation's SOPs, and any regulatory requirements.

Any portions of the study performed by the Sponsor or subcontractor(s) will be verified by their QAU(s). A Quality Assurance Statement from the Sponsor or subcontractor(s) will be provided to MatTek Corporation for inclusion in the final report.

Authorized designates of the Sponsor may inspect their study during regular working hours for quality assurance purposes.

13. REPORTS

A complete detailed audited draft report comprised of electronic files will be submitted to the Sponsor for review. Any revised draft reports will be unaudited and will be submitted to the Sponsor electronically for further review. Subsequent to the inclusion of any modifications or corrections (agreed upon by the Sponsor and MatTek Corporation), a submission ready electronic version (linked, searchable PDF) of the signed final report will be provided. All corrections to the final report will be performed by means of amendments. If no requested revisions or instructions to finalize have been communicated by the Sponsor three months after issuance of the Audited Draft Report, the Audited Draft Report will be issued as a signed Final Report. Any modifications requested by the sponsor after this finalization will be done via amended final report at an additional charge to the sponsor.

14. DATA RETENTION/ARCHIVES

Following dispatch of the final report, all specimens, raw data and documents generated at MatTek Corporation during this study, together with the original copy of the approved study plan (including amendments) and the final report will be transferred to the scientific archives of MatTek Corporation for a period of approximately 5 years. At the end of the storage period, MatTek Corporation will contact the sponsor for the authorization to transfer the data to the sponsor facilities at the expense of the sponsor. In the case that the sponsor wishes to continue to store the documents at MatTek Corporation, a charge will be levied on a per box per year basis.

15. STUDY PLAN APPROVAL SIGNATURES

We agree to conduct the study according to this study plan and to comply with its obligations

IN AGREEMENT WITH THE STUDY PLAN

Kalyani Gunjur, PhD
Study Director
MatTek Corporation

2/13/2018
Date

Patrick Hayden, PhD
Testing Facility Manager
MatTek Corporation

2/9/18
Date

[redacted]

APPENDIX A: DEFINITIONS AND ABBREVIATIONS

Abbreviation or specialist term	Explanation
GLP	Good Laboratory Practice
°C	Degree Celsius
Avg	Average
%	Percent
h or hr	Hours
MSDS	Material safety data sheet
min	Minute
No	Number
SOP	Standard operating procedure
ET-50	Effective time – 50%
NC	Negative control
PC	Positive control
TA1	Test Article 1
TA2	Test Article 2
nm	Nano meter
OD	Optical Density
mL	Milliliter
µL	Microliter
CO_2	Carbon dioxide
SDS	Sodium dodecyl sulfate
DPBS	Dulbecco's Phosphate Buffered Saline
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide; thiazolyl blue)
PDF	Portable document format
TBA	To be added
mg	milligram
MSDS	Material safety data sheet

APPENDIX B: IN VITRO EPIDERM SKIN IRRITATION TEST (EPI-200-SIT) MK-24-007-0023

MatTek CORPORATION

Protocol

In Vitro EpiDerm™ Skin Irritation Test (EPI-200-SIT)

For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm™ (EPI-200-SIT)

Note 1: This protocol is based on Zebet's SOP version 7.0 drafted by Manfred Liebsch and Dieter Traue (ZEBET at the BfR) and approved by Helena Kandárová (MatTek Corporation) which was used in the follow-up validation study of the Modified EpiDerm™ Skin Irritation Test (SIT).

The Modified EpiDerm™ Skin Irritation Test (SIT) was validated in 2007 in an international validation trial involving BASF SE (Ludwigshafen, Germany), IIVS Inc. (Gaithersburg, USA), ZEBET at the BfR (Berlin, Germany), and Zet-LSL (Linz, Austria). The study was conducted in line with requirements of OECD GD 34 and ECVAM Performance Standard document for applying human skin models to in vitro skin irritation.

Performing the EpiDerm SIT as outlined fulfills criteria set forth in OECD TG439.

The ECVAM Scientific Advisory Committee (ESAC) formally endorsed the scientific validity of the Modified EpiDerm™ Skin Irritation Test (SIT) at its November, 2008 meeting. ESAC concluded that the Modified EpiDerm™ SIT has sufficient accuracy and reliability for the prediction of skin irritating and non-irritating test substances and that it should be considered a validated, stand-alone in vitro replacement for animal skin irritation testing.

Note 2: A detailed video demonstrating use of this protocol is available via the Journal of Visualized Experiments (JoVE): http://www.mattek.com/pages/abstracts/528

Table of Contents

1. Rationale and Background

The potential of chemicals to induce skin irritation (hazard) is an important consideration in establishing procedures for the safe handling, packing and transport of chemicals. Skin irritation refers to the production of reversible damage to the skin following the application of a test substance for up to 4 hours [as defined by the United Nations (UN) Globally Harmonized System of Classification and Labeling of Chemicals (GHS)](1). Skin irritation in vivo is determined by modification of the Draize rabbit skin irritation test as described in the OECD TG 404 (2, 3). Because systemic reactions play a minor role in modulating local skin toxicity potential of chemicals, skin irritation potential may be predicted by *in vitro* systems, provided they are sufficiently complex to mimic skin barrier in vivo and cell reactivity.

The method described in this SOP is based on method initially developed and refined by L'Oreal for EPISKIN model (4, 5). The SOP was applied on EpiDerm™ model, with the aim to develop for both systems a common protocol able to predict skin irritation potential according to the EU classification system and replace the in vivo acute skin irritation test in rabbits (6, 7). Upon review of existing information by the ECVAM Skin Irritation Task Force and an ECVAM Workshop both EPISKIN and EpiDerm™ skin irritation tests (SIT) were regarded as sufficiently promising predictors for skin irritancy potential and ready to enter the formal validation study. Due to the under-prediction of several chemicals in the second Phase of the ECVAM validation study (8), ESAC recommended to increase sensitivity of the EpiDerm™ SIT to better match in vivo rabbit data (9).

Following the recommendation of ESAC (9), the EpiDerm™ skin irritation test was further optimized by MatTek Corporation during 2006 and 2007. The extended exposure time (60 min) and minor modification of exposure conditions improved the sensitivity of the assay. The applicability domain, prediction model (50% viability border for identification of irritants) as well as the endpoint (MTT cytotoxicity assay) did not change; thus the concept of common protocol was maintained (10).

The predictive capacity of the modified EpiDerm™ SIT was initially assessed by MatTek Corporation, USA in an intra-laboratory study (10). Transferability of the method was evaluated in 2007 in an external international validation study between 4 laboratories: ZEBET at the BfR, Berlin, Germany; BASF, Ludwigshafen, Germany; IIVS, Gaithersburg, MD and Zet-LSL, Linz, Austria (11, 12). The validation trial was in accordance with the principles and criteria documented in OECD Guidance Document No. 34 on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (13) and ECVAM (2007) Performance Standards for applying human skin models to *in vitro* skin irritation (14).

In 2008, ESAC concluded that the Modified EpiDerm™ SIT has sufficient accuracy and reliability for prediction of R38 skin irritating and no-label (non-skin irritating) test substances (15). The Modified EpiDerm™ SIT is an *in vitro* procedure that, depending on information requirements, allows determining the skin irritancy of chemicals as a stand-alone replacement test, as a screen, or within a testing strategy in combination with, if appropriate, a weight of evidence approach (16).

2. Specific Purpose of the Method

The *EpiDerm™ SIT was developed and* designed to predict skin irritation potential of neat test substances in the context of identification and classification of skin irritation hazard according to the EU classification system (R 38 or no label). Since the EU and GHS systems were harmonized in 2008, the procedure described in this SOP also allows for hazard identification of irritant substances in accordance with UN GHS. The Modified EpiDerm™ SIT allows discrimination between irritants of category 2 and non-irritants. The test does not discriminate between non-mandatory subcategories of the UN GHS, i.e. it does not distinguish between GHS category 2 and category 3 irritants.

3. Basis of the Method

The test consists of a topical exposure of the neat test chemical to a reconstructed human epidermis (RhE) model followed by a cell viability test. Cell viability is measured by dehydrogenase conversion of MTT [*(3-4,5-dimethyl thiazole 2-yl) 2,5-diphenyltetrazoliumbromide*], present in cell mitochondria, into a blue formazan salt that is quantitatively measured after extraction from tissues (**17**). The reduction of the viability of tissues exposed to chemicals in comparison to negative controls (treated with water) is used to predict the skin irritation potential. Recent comparative studies in RhE models employing various endpoints to predict skin irritancy of topical formulations have shown that the MTT endpoint had clear advantages, even over mechanistically based endpoints like the release of IL-1α (**18, 19**).

3.1 Test System Description

The reconstructed human epidermal model EpiDerm™ (EPI-200, MatTek, Ashland, USA) consists of normal human-derived epidermal keratinocytes, which have been cultured to form a multilayered highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers and a multilayered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found in vivo. A generic description of general and functional conditions that reconstructed human skin models need to comply with can be found in the new OECD Test Guideline 431 *In vitro* Skin Corrosion: Human Skin Model (**20**).

The EpiDerm™ tissues (surface 0.63 cm²) are cultured on specially prepared cell culture inserts and shipped world-wide as kits, containing 24 tissues on shipping agarose together with the necessary amount of culture media, DPBS, 6-well plates, and 24-well plates. In addition, the MTT kit (containing MTT concentrate, diluent, and extractant) is provided by MatTek per request.

3.1.1 Quality Control of the Test System

The EpiDerm™ System is manufactured according to defined quality assurance procedures. All biological components of the epidermis and the culture medium are tested by manufacturer for viral, bacterial, fungal and mycoplasma contamination. MatTek determines the ET-50 value following exposure to Triton X-100 (1%) for each EpiDerm™ lot. The ET-50 must fall within a range established based on a historical database of results. Histology is provided upon request.

3.1.2 Precautions

The epidermal cells are taken from healthy volunteers negative to HIV, and Hepatitis. Nevertheless, handling procedures for biological materials should be followed:
a) It is recommended to wear gloves during handling with the skin and kit components.
b) After use, the epidermis, the material and all media in contact with it should be decontaminated prior to disposal (e.g. using 10% bleach, special containers or autoclaving).

Note: Due to long post-incubation period, it is necessary to perform the test under aseptic conditions in the microbiological safety cabinet (laminar flow hood).

3.2 Assay Quality Controls

3.2.1 Assay Acceptance Criterion 1: Negative Control

The absolute OD of the negative control (NC) tissues (treated with sterile DPBS) in the MTT-test is an indicator of tissue viability obtained in the testing laboratory after shipping and storing procedures and under specific conditions of use.

The assay meets the acceptance criterion if the mean OD_{570} of the NC tissues is ≥ 1.0 and ≤ 2.8.

3.2.2 Assay Acceptance Criterion 2: Positive Control

A 5% SDS (in H_2O) solution (see 7.6.3) is used as positive control (PC) and tested concurrently with the test chemicals. Concurrent means here the PC has to be tested in each assay, but not more than one PC is required per testing day. Viability of positive control should be within 95±1 % confidence interval of the historical data.

The assay meets the acceptance criterion if the mean viability of PC tissues expressed as % of the negative control tissues is ≤ 20%.

3.2.3 Assay Acceptance Criterion 3: Standard Deviation (SD)

Since in each test skin irritancy potential is predicted from the mean viability determined on 3 single tissues, the variability of tissue replicates should be acceptably low.

The assay meets the acceptance criterion if the SD calculated from individual % tissue viabilities of the 3 identically treated replicates is < 18%.

Note: Chemicals that provide tissue viabilities in a range of 30 – 70 % may provide high SD. If the high SD (above acceptance limits) is typical for the chemical and the classification of the chemical is consistent in all independent runs, it is recommended to accept this result, although the Assay Acceptance Criterion 3 is not met.

4. Limitations of the Method

One limitation of this assay method is a possible interference of the test substance with the MTT endpoint. A colored test substance or one that directly reduces MTT (and thereby mimics dehydrogenase activity of the cellular mitochondria) may interfere with the MTT endpoint. However, these test substances are a problem only if at the time of the MTT test (i.e. 42 hours after test substance exposure) sufficient amounts of the test substance are still present on (or in) the tissues. In case of this unlikely event, the (true) metabolic MTT reduction and the contribution by a colored test material or (false) direct MTT reduction by the test material can be quantified by a procedure described in Section 7.3, and Annex D.

The method is not designed for testing of highly volatile test substances, gases and aerosols.

5. Brief Basic Procedure

On the day of receipt, EpiDerm™ tissues are conditioned by incubation to release transport-stress related compounds and debris overnight. After pre-incubation, tissues are topically exposed to the test chemicals for 60 minutes. Preferably, three tissues are used per test chemical (TC) and for the positive control (PC) and negative control (NC). Tissues are then thoroughly rinsed, blotted to remove the test substances, and transferred to fresh medium. After a 24 hr incubation period, the medium is collected for analysis of cytokines (*Note: This step is optional, since no improvement in assay performance was noted by using IL-1α or other cytokines as complementary endpoint*). Tissues are incubated for another 18 hours. Afterwards the MTT assay is performed by transferring the tissues to 24-well plates containing MTT medium (1 mg/ml). After a 3 hr MTT incubation, the blue formazan salt formed by cellular mitochondria is extracted with 2.0 ml/tissue of isopropanol (extractant solution, part # MTT-100-EXT) and the optical density of the extracted formazan is determined using a spectrophotometer at 570 nm. Relative cell viability is calculated for each tissue as % of the mean of the negative control tissues. Skin irritation potential of the test material is predicted if the remaining relative cell viability is below 50% (Section 6).

Note: Detailed video demonstrating the protocol is available via Journal of Visualized Experiments http://www.mattek.com/pages/abstracts/528 (21)

6. Data Interpretation Procedure (Prediction Model)

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

In vitro result	*In vivo* prediction
mean tissue viability ≤ 50%	Irritant (I), (R38 or GHS category 2)
mean tissue viability > 50%	non-irritant (NI)

7. Materials and Methods

7.1 Material provided by MatTek Corporation

7.1.1 EPI-200-SIT Kit Components

EPI-200-SIT kits are shipped from Boston each Monday. Upon receipt of the EpiDerm™ tissues, transfer the tissue inserts into the assay medium and pre-incubate the cultures overnight at 37±1°C, 5±1 % CO_2 in air and 95% relative humidity (RH) (for detailed procedure see section 7.7.1 Tissue conditioning). Place the rest of the assay medium into the refrigerator (5±3°C), MTT concentrate containing vial in the freezer (-20±5°C) and the MTT diluent in the refrigerator (5±3°C). Record lot numbers of all kit components into the Methods Documentation Sheet (MDS – see Annex B)

Standard Assay Kit Components (order under the part # EPI-200-SIT)

1	Sealed 24-well EpiDerm™ (EPI-200) plate	Contains 24 tissues in cell culture inserts, packaged on agarose
2	24-well plates (sterile)	Used for MTT viability assay
8	6-well plates (sterile)	Used for maintaining tissues during assay protocol
1 bottle, 100 ml	Assay Medium (EPI-100-NMM)	DMEM based medium
1 vial, 1 ml	5% SDS Solution (TC-SDS-5%)	Skin irritant reference chemical – Positive Control
1 bottle, 100 ml	DPBS Rinse Solution (TC-PBS)	Used for rinsing the inserts
25 pieces	Nylon Mesh circles 8 mm diameter, 200 µm pore (EPI-MESH)	Used for spreading test chemicals
1	MK-24-007-023	Complete EpiDerm™ Skin Irritation Test (SIT) protocol is sent electronically

MTT-100 Assay Kit Components (MTT-100 must be ordered separately)

1 vial, 2 ml	MTT concentrate (MTT-100-CON)	Frozen MTT concentrate
1 vial, 8 ml	MTT diluent (MTT-100-DIL)	For diluting MTT concentrate prior to use in the MTT assay
1 bottle, 60 ml	Extractant Solution (MTT-100-EXT)	For extraction of formazan crystals

7.1.2 Expiration and Component Storage Conditions

Part #	Description	Conditions	Shelf life
EPI-200-SIT	EpiDerm™ cultures	refrigerator (5±3°C)	96 hours
EPI-100-NMM	Assay medium	refrigerator (5±3°C)	7 days
MTT-100-DIL	MTT diluent	refrigerator (5±3°C)	2 months
MTT-100-CON	MTT concentrate	freezer (-20±5°C)	2 months

Note: Examine all kit components for integrity. If there is a concern, call MatTek Corporation immediately

7.2 Other Materials (not provided with the EPI-200-SIT kit)

• Laminar flow hood	For safe work under sterile conditions
• Humidified incubator (37±1°C, 5±1% CO_2, 95% relative humidity (RH))	For incubating tissues prior to and during assays
• Vacuum source/trap (optional)	For aspirating media and solutions
• Laboratory balance	For pipette verification and checking spoonful weight
• 96-well plate photometer	For reading OD
• Plate shaker	For extraction of formazan
• Stop-watch	For timing of application of test materials and other timed steps in protocol
• Sterile, blunt-edged forceps	For handling tissue inserts
• 500 ml plastic wash bottle	For rinsing tissue with DPBS
• 200 ml beaker	For collecting DPBS rinses
• 37±1°C water bath	For warming Media and MTT solution
• Mortar and pestle	For grinding granular solids
• Adjustable pipette / multi-step pipette	For pipetting 0.9 ml assay medium. For pipetting 300 µl MTT medium For pipetting 2 ml MTT extraction solution For pipetting 200 µl formazan extract from 24-well plate into 96-well plate for the plate photometer For application of 30 µl liquid test materials and 25 µl for application of DPBS when wetting the tissue surface before application of solid substances
• Positive displacement pipet, 30 µl	For application of gels, creams, viscous materials, semi-solid test materials and suspensions
• Sharp spoon (NaCl weight: 25 mg), e.g. Aesculap, Purchase No.: FK 623	For application of solids
• Bulb headed glass Pasteur pipette	To aid leveling the spoonful of solid test articles and for spreading on the tissue surface
• Dulbecco's Phosphate Buffered Saline (DPBS) (TC-PBS)	For rinsing tissues.
• Freeze-killed EpiDerm™ tissue (EPI-200-FRZN-EA)	Needed for colored test materials or for materials that directly reduce MTT.
• Extra 6-well plates - sterile (FALCON recommended)	For transferring tissue inserts to fresh media (instead of replacing the media using the same plate).
• Cryovials - polypropylene (NeoLab # 7-4581)	Collecting and freezing of media samples for each tissue
Adhesive tape (NeoLab # 7-2220 or # 2-5082) or Parafilm M	Covering plates during formazan extraction
• Cotton tip swabs (sterile)	For drying the tissue surface
• MTT-100 assay kit	Contains MTT - Thiazolyl Blue Tetrazolium Bromide reagent (Sigma, # M-5655) and isopropanol extractant. Note: The MTT-100 kit must be ordered separately.

7.3 Test for Interference of Chemicals with MTT Endpoint and Correction Procedures

As specified in Section 4, a test substance may interfere with the MTT endpoint if: a) it is colored and/or b) able to directly reduce MTT (for possible combination of interactions, see Annex D). The MTT assay is affected only if the test material is present in the tissues when the MTT viability test is performed.

Some non-colored test materials may change into colored materials in wet or aqueous conditions and thus stain tissues during the 60 min exposure. Therefore, before exposure, a functional check for this possibility should be performed (**Step 1**)

Step 1
Add **30 µl** (liquid) or **25 mg** (solid - using a sharp spoon as per Section **7.2**) of the test substance into 0.3 ml of deionized water. Perform the test in a transparent, preferably glass test-tube since plastic test tubes may react with the test articles during the incubation time. Incubate the mixture in the incubator (37±1°C, 5±1 % CO_2, 95% RH) for 60 min. At the end of the exposure time, shake the mixture and evaluate the presence and intensity of the staining (if any). If the solution changes color significantly, the test substance is presumed to have the potential to stain the tissue. A functional check on viable tissues should be performed (**Step 2**).

Step 2
To check the tissue-binding of a colored test article (or a chemical that changes into a colored substance), expose one viable tissue to **30 µl** of liquid test substance or **25 mg** of solid test substance. In parallel, expose a tissue to DPBS (negative control). Follow all procedures as described in this SOP Section 7.7 except incubate the tissue for 3-h incubation in culture media without MTT (37±1°C, 5±1% CO_2, 95% RH) instead of incubating in media containing MTT. After the 3 hour incubation, rinse the tissues and extract the tissues using 2.0 ml of isopropanol and measure the optical density (OD) at 570 nm.

Note: If the colored test substance does not completely rinse off, pipette 1.0 ml of the extracting agent into each well so that the MTT is extracted through the bottom of the tissue culture insert. After extraction is complete, remove the insert and add an additional 1.0 ml of extractant to bring the total volume to 2.0 ml.

Data correction procedure
If the extract from tissues treated by colored substance (or substance detected in step 1) has an OD between 5% and 30% of the negative control tissue (treated with PBS), the chemical should be further tested on more tissues using the procedure described above. The real MTT OD (unaffected by interference with the colored test materials) is calculated using following formula:

OD = OD colored tissue (MTT assay) – OD colored tissue (no MTT assay)

Note: If the extract from tissues treated by colored substance (or substance detected in step 1) has an OD <5% of the PBS treated control tissue and the tissue viability (determined in MTT assay) is not close to the classification cut-off (50%), correction of the results is not necessary.

If OD of extract from the tissue treated by colored substance (or substance detected in **Step 1**) is > 30% of the PBS treated control tissue, additional steps and expert judgment must be performed to determine if the test substance must be considered as incompatible with the test.

Step 3
All test materials (including those already evaluated in **Step 1** and **Step 2**) should be further evaluated for their potential to interfere with MTT assay. To test if a material directly reduces MTT, add **30 µl** (liquid) or **25 mg** (solid - using sharp spoon **7.2**) of the test substance to **1 ml** of the MTT medium and incubate in the incubator (37±1°C, 5±1 CO_2, 95% RH) for 60 min. Untreated MTT medium is used as control. If the MTT solution turns blue/purple, the test substance reduces MTT and additional functional check (**Step 4**) must be performed.

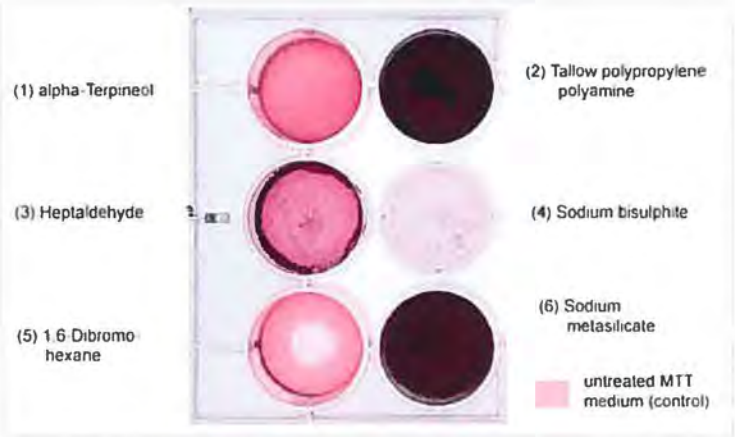

Figure 1: Example of test for direct MTT reduction ability (Step 3). Test substances (2) (3) and (6) have directly reduced MTT. In these cases, Step 4 (below) must be performed.

Step 4:
The procedure employs freeze-killed tissues that possess no metabolic activity but absorb and bind the test substance similar to viable tissues.

- Freeze-killed tissues can be ordered separately from MatTek Corporation (part # EPI-200-FRZN-EA). The frozen tissues may be stored indefinitely in the freezer (-20 ± 5°C).
- Each MTT reducing chemical is applied to two freeze-killed tissues. In addition, two freeze killed tissues are left untreated (Note: The untreated killed controls will show a small amount of MTT reduction due to residual reducing enzymes within the killed tissue). The entire assay protocol is performed on the frozen tissues in parallel to the assay performed with the live EpiDerm™ tissues. Data are then corrected as follows:

Data correction procedure

True viability = Viability of treated tissue – Interference from test chemical = OD tvt – OD kt
where OD kt = (mean OD tkt – mean OD ukt)

tvt = treated viable tissue
tkt = treated killed tissue
kt = killed tissues
ukt = untreated killed tissue (NC treated tissue)

If the interference by the test substance is greater than 30% of the negative control value, additional steps must be taken into account or the test substance may be considered incompatible with this test system (expert judgment).

If the interference by the test substance is < 30% of the negative control value, the net OD of the test substance treated killed control may be subtracted from the mean OD of the test substance treated viable tissues to obtain the true amount of MTT reduction that reflects metabolic conversion only.

Note 1: If the colored test material or the MTT reducing chemical is classified as irritant by the SIT (tissue viability <50 %), the correction procedures are not necessary.

Note 2: Frozen tissues (EPI-200-FRZN-EA) can be ordered separately from MatTek Corporation.

7.4 Test for Mesh Compatibility (liquid test substances only)

Capillary effects (surface tension effects) were observed if low volumes of lipophilic liquid test chemicals were applied on EpiDerm™ surface. Therefore, a nylon mesh (provided by MatTek) is used as a spreading support (see **7.7**). Twenty-five (25) nylon mesh samples are provided with the EPI-200-SIT kit. The mesh can be used for a wide range of liquid substances. However, some chemicals may react with the mesh and therefore the compatibility of each liquid chemical with nylon mesh has to be checked.

To test if a test chemical interacts with the mesh, place the mesh on a slide and apply 30 µl test substance. After 60 minutes exposure, check using a microscope (**Figure 3**), if an interaction between test substance and the mesh is noticed (**B**). In that case, the test substance has to be applied without using a mesh as a spreading aid.

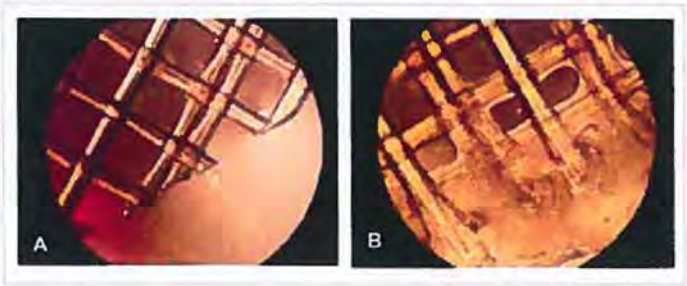

Figure 3: The mesh compatibility test showing: **A)** Normal mesh (no reaction with test material) and **B)** Damaged mesh (reaction with test materials)

7.5 Preparations

7.5.1 MTT solution (prepare freshly on the day of testing)

Thaw the MTT concentrate (MTT-100-CON) and dilute the concentrate with the MTT diluent (MTT-100-DIL). Store the remaining MTT solution in the dark at 5±3°C for later use on the same day (do not store overnight since MTT will degrade with time).

If you are not using the MTT-100 kit provided by MatTek, prepare the stock solution (5 mg/ml) of MTT (**see 7.2**) in DPBS. Stock solution can be stored frozen (-20±5°C) for up to 2 months. Before use, filter the stock solution and dilute the filtrate with the assay medium to final concentration (1 mg/ml). Record the preparation in the MDS. Do not store the diluted MTT solution overnight.

Safety precaution: MTT is toxic (Risk phrases: R26, R68, R22, R36, R37, R38). Wear protective gloves during manipulation with MTT and its solutions!

Note: MTT is light sensitive. Protect all solutions from light.

7.5.2 Dulbecco's PBS

Sterile ready-to-use DPBS should be used. About two liters are sufficient for all rinsing performed with one kit. If DPBS is prepared from 10x concentrates or powder, the pH needs to be adjusted to 7.0 and solution must be sterilized. Record the preparation in the MDS.

7.6 Test substances

Safety Instruction

1. For handling of known test substances, follow instructions given in the Material Safety Data Sheet.
2. If coded materials or unknown samples are supplied, no (or possibly incomplete) information regarding the safe handling will be provided. Therefore, all test materials must be treated as if they were irritating and toxic. Work must be performed in accordance with chemical safety guidelines (use ventilated cabinet, wear gloves, protect eyes and face).
3. Store all test substances according to recommendations. Follow all the storage conditions provided (temperature, protection from light, protection from oxidization by nitrogen, etc.).

Liquids: Dispense **30 µl** directly atop the tissue and gently spread the chemical using bulb headed Pasteur pipette. Try to avoid contact of the pipette with the tissue surface. Afterwards, carefully place the nylon on the tissues surface. If necessary, gently position the mesh using the bulb headed glass Pasteur pipette. Record the use of mesh as a spreading tool in the MDS.

Figure 4: Application of liquids

Semisolids

Dispense **30 µl** using a positive displacement pipette directly atop the tissue. If necessary spread the chemical with Pasteur pipette to match size of tissue. Record the use of spreading in the MDS.

Figure 5: Application of semisolids (positive displacement pipette - detail) and subsequent spreading using bulb headed Pasteur pipette

Solids

Crush and grind test material using a mortar with pestle. Shortly before application of the solid substance, moisten the tissue surface with **25 µl of sterile DPBS** to improve contact of the tissue surface with the test chemical. Fill **25 mg** sharp application spoon (see **Figure 6**) with fine ground test material. Level the spoon by gently scratching the excess material away with an appropriate aid, avoiding compression ("packing") of the test material. A bulb headed Pasteur pipette can be used to empty the spoon completely.

Gently shake the inserts to improve the spreading of the solid on the surface. If necessary, gently spread the chemical to match size using bulb headed sound (or bulb headed Pasteur pipette). Do not press on the tissue surface. Record observations of the solubility of the material into the MDS. Record in the MDS if grinding was not necessary.

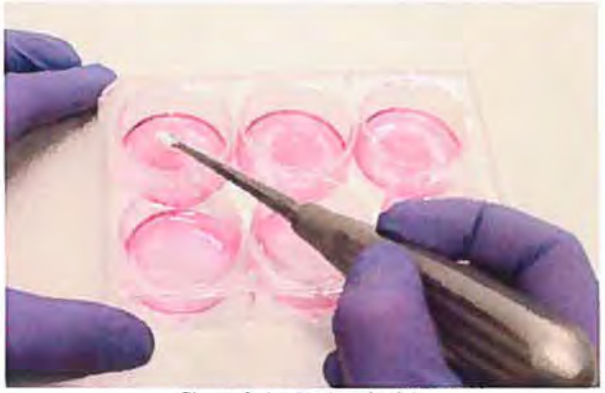

Figure 6: Application of solids

Waxes: For test substances with **waxy consistency**, the spoon application will not work. In these cases try to form a flat "cookie like" piece of about 8 mm diameter and place it atop the tissue, wetted with sterile **DPBS**. To improve the contact between the test substance and the tissue weigh down the "cookie" with a stainless steel aid like that shown in **Figure 7**.

Figure 7: The stainless steel aid used application of waxy materials

Note: Highly volatile toxic test substances may affect neighbouring tissues within the same 6-well treatment plate. In these cases, plates should be covered with an adhesive plate cover, or other measures should be taken, like testing the volatile substances on separate plates.

7.7 Experimental Procedure (see also ANNEX A)

7.7.1 DAY 0 - Day prior to dosing

1. Upon receipt of the shipment, examine all kit components for integrity. If there is a concern call MatTek Corporation immediately.

Contact persons:

Mitch Klausner (US) Phone: +1-508-881-6771 Email: mklausner@mattek.com	Paul Kearney (US) Phone: +1-508-881-6771 Email: phkearney@mattek.com	Helena Kandarova (EU) Phone: +1-508-881-6771 Email: hkandarova@mattek.com

2. Record all information about supplied material into the MDS.
3. Place the DPBS into the refrigerator (5±3°C) and the vial containing the MTT concentrate in the freezer (-20±5°C).

Tissue conditioning:

1. Let the assay medium reach room temperature (20-25°C). Do not pre-heat to 37°C.
2. Pipette 0.9 ml of the assay medium into each well of sterile 6-well plates (For 24 inserts prepare eight 6-well plates. Use one 6-well plate for pre-incubation of three inserts).
3. Under sterile conditions, open the plastic bag containing the 24-well plate with epidermal tissues. Under a sterile airflow, remove the sterile gauze and carefully (using sterile forceps) take out each insert containing the epidermal tissue. Remove any remaining agarose that adheres to the outer sides of the insert by gentle blotting on the sterile filter paper or gauze, and place the tissues in the empty, sterile 24-well plate.

Figure 8: Visual inspection of the epidermal tissues

4. Perform visual inspection of the inserts within the next 5 min. Record any tissue defects and excess moisture on the surface. Do not use tissues with defects or tissues with excessive moisture on the surface.
5. Dry the surface of the tissues with a sterile cotton tip swab and transfer tissues to a 6-well plate pre-filled with 0.9 ml medium. Place the plates for 60 ± 5 min into the incubator (37±1°C, 5±1% CO_2, 95 % RH).
6. At the end of the first (60 minute) pre-incubation period, transfer the inserts from upper wells into the lower wells of the 6-well plate. Further, pre-incubate the tissues (37±1°C, 5±1% CO_2, 95 % RH) overnight for 18 ± 3 hr.

Figure 9: Pre-incubation plate design

7. Place the plates back into incubator for overnight pre-incubation.
8. Place the rest of the assay medium into the refrigerator (5±3°C) and the vial containing the MTT concentrate in the freezer (-20 ±5°C).

Note 1: Any air bubbles trapped underneath the insert should be released.
Note 2: The visual quality check of the tissues has to be done quickly.
Note 3: Removal of the moisture collected on the tissues surface is important.

Do not use in the assay tissues which are extensively covered with liquid (>40% of surface).

9. If necessary, prepare sufficient amount of rinsing DPBS for the next day (approximately 1 L per 24 inserts)
10. Prepare and sterilize all devices which will be used in the assay:
    - 150 ml vials (autoclave or heat sterilization),
    - cotton tips (autoclave),
    - mesh (UV irradiation, do not autoclave),
    - bulb headed Pasteur pipettes (70% Ethanol or heat sterilization),
    - washing bottles (UV/ gamma irradiation or 70% Ethanol),
    - blotting paper (autoclave),
    - spatula or spoon for application of solids (70% Ethanol),

7.7.2 DAY 1 - Chemical Exposure

1. Place all devices, solution and chemicals necessary for the test into the sterile hood
   Checklist
   - sterile 150 ml vials pre-filled (for 1 chemical (≈3 inserts) one vial is required)
   - sterile cotton tips
   - sterile mesh
   - sterile bulb headed Pasteur pipettes
   - sterile washing bottles pre-filled with sterile DPBS
   - sterile blotting paper
   - sterile tips and pipettors
   - all vials with chemicals heated at room temperature (~25°C), NC (sterile DPBS) and PC (5% SDS)
   - digital timer
   - sharp, pointed tweezers
   - sharp spoon
   - 1 big vial for waste

Note: Wipe all non-sterile material bottles with 70% EtOH
Note: If you are not using the spoon for the application of solids, weight out 3x 25 mg of the test materials into the appropriate vials and prepare spatula for the application

2. Prepare a sufficient number of 6-well plates pre-filled with 0.9 ml of assay medium in the upper row (1 plate = 1 chemical)
3. Remove the pre-equilibrated, 6-well plates from the incubator approximately 5 min before exposure to chemicals will begin
4. Evaluate the surface of tissues and exclude completely wet tissues or tissues with any visible defects
5. Remove any moisture using sterile cotton tip
6. Before test chemical exposure, label all 6-well plate lids with the test material codes or names

Note: The assay is designed to test maximum of 6 chemicals (using n=3 tissues/test chemical) in one testing SET by ONE technician using application and washing interval of 1 minute. ***Deviations from this SOP may cause different outcomes.***

Test Substance Exposure

Note: Do not dose more than 18 tissues (= 6 test articles including PC and NC in a block (SET), in order to be able to perform all steps as required by this protocol

1. Apply **30 µl** (liquid) or **25 mg** (solid) of the undiluted test substance, NC or PC to three single tissues each according to **7.7.** Dose tissues at the time intervals needed later for rising off the test substances (optimal and highly recommended is 1 minute interval)
2. Keep the plates with dosed tissues in the laminar flow hood, until the last tissue is dosed
3. After dosing the last tissue, transfer **all plates for 35 ± 1 minutes** to the humidified incubator (37±1°C, 5±1% CO_2, 95 % RH)

Protocol: In Vitro EpiDerm™ Skin Irritation Test (EPI-200-SIT)

4. After 35 min, remove **all plates** from the incubator, place them into the sterile hood and wait until the period of 60 min is completed for the first dosed tissue
5. After the **60 ± 1 minutes** test substance exposure, rinse the tissues with sterile DPBS, filling and emptying the tissue insert **15 times** to remove any residual test material (**Figure 10**). Use constant stream of DPBS applied from 1.5 cm distance from the tissue surface. (The stream of DPBS should not be too soft, otherwise, the test article will not be removed. Optimal wash bottle, with pointed endings is shown in **Figure 10**)

Note: Washing procedure – tissues with mesh: wash the tissues 5 times from the wash bottle to remove excess of the test chemical. Then remove the mesh carefully with pointed, sharp forceps (see Figure 9 and 10) and only then continue with the washing procedure (10 times). If the test substance reacts with the insert, the mesh may stick to the edge of insert. This may complicate its removal.

6. After the 15th rinse from washing bottle, completely submerge the insert 3 times in 150 ml DPBS (shake to remove all rests of test material).

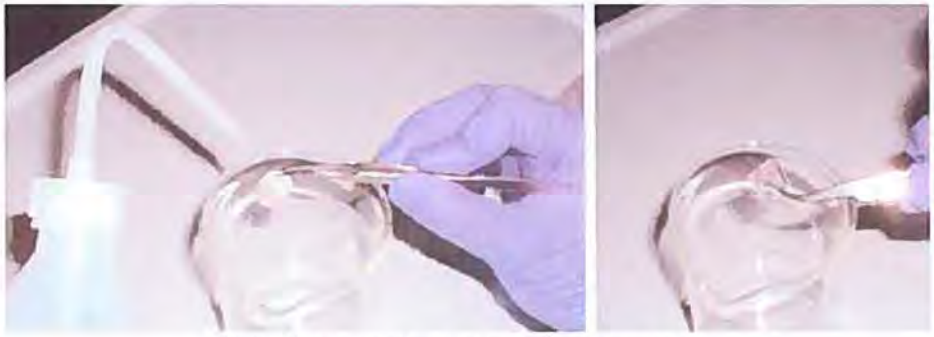

Figure 10: Removal of test articles - washing procedure

7. Finally, rinse the tissue once from inside and once from outside with sterile DPBS. Remove excess of DPBS by gentle shaking the insert, blot insert on sterile blotting paper (**Figure 11**)
8. Transfer the blotted tissue inserts to new 6 well plates pre-filled with 0.9 ml of fresh assay medium.
9. After all inserts are washed, **DO NOT FORGET to carefully dry** the surface of each tissue with a sterile cotton tipped swab (**Figure 11**). In case that traces of the chemical are still present on the surface, try to remove it with the sterile wetted cotton swab. Record this procedure in the MDS. You may evaluate visually tissue surface under a dissecting stereoscope.
10. Incubate tissues in the incubator for next 24±2 hours. Record start time of incubation in the MDS.

Figure 11: Completion of tissue washing - blotting and drying the tissue surface

7.7.3 DAY 2 - Change medium (mandatory - steps 1-2) Collect media for cytokine analysis (optional - steps 3-7)

1. At the end of the 24 ± 2 hr incubation period, pre-fill the lower row of the 6-well plates with 0.9 ml of fresh assay medium.
2. Transfer the inserts from the upper row of the 6-well plates into the lower row and place the 6-well plates back into the incubator for an additional 18 ± 2 hr post-incubation.
3. If the medium from the 24 hour incubation will be analyzed for cytokine or chemokine release, prepare a sufficient number of sterile vials (e.g. cryotubes, volume 1.5 ml). Alternatively, the media can be stored in a labeled 24-well plate (design shown below).

NCa	NCb	NCc	PCa	PCb	PCc
1a	1b	1c	2a	2b	2c
3a	3b	3c	4a	4b	4c
5a	5b	5c	6a	6b	6c

4. Mark the cryotubes or a 24-well plate with names or codes of the test substances and replicate code (e.g. a, b, c). Include the tissue lot number and date of the experiment. Use a water resistant marker.
5. Place the 6-well plates containing inserts on a plate shaker (500 rpm/min) for 5 minutes.
6. Transfer the medium (approximately 0.9 ml) from the 24-hour incubation plates into the cryotubes or 24-well plate. Use fresh pipette tips between samples.
7. Close the vials properly. If used, the 24-well plate should be sealed with parafilm. Store the samples at -20 ± 5°C (for up to 12 months) until analysis.

7.7.4 DAY 3 - MTT Viability Test

MTT Test

1. Prior to the MTT assay, label a sufficient number of 24-well plates.
2. Prepare MTT medium from frozen concentrate according to **7.5.1** and pipette 300 µl of MTT medium in each well.

Protocol: In Vitro EpiDerm™ Skin Irritation Test (EPI-200-SIT)

3. Remove inserts from the 6-well plates, blot the bottom of the inserts, and transfer them into the 24-well plates, pre-filled with 0.3 ml of MTT (1 mg/ml). Place the plates in the incubator (37±1°C, 5± 1% CO_2, 95 % RH), record the start time of MTT incubation in the MDS and incubate for **3 hours ± 5 min.**

Note: The 3 hours +/- 5 min MTT incubation time must be strictly adhered to. Deviations from the 3 hour time for MTT incubation will result in different MTT readings.

4. After MTT incubation is complete, gently aspirate the MTT medium from all the wells (e.g. using a suction pump plus toxic waste trap). Refill the wells with DPBS and aspirate again. Repeat this rinsing twice and make sure that tissues are dry after the last aspiration. Transfer inserts to new 24-well plates.
5. Immerse the inserts by gently pipetting 2 ml of isopropanol (extractant solution MTT-100-EXT) into each insert. The level will rise above the upper edges of the insert, thus completely covering the tissues from both sides.
6. Seal the 24-well plates (e.g. with Parafilm or place into a sealable plastic bag) to inhibit extractant evaporation. Record start time of extraction in the MDS and extract formazan for at least 2 hours at room temperature with gentle shaking on a plate shaker (~ 120 rpm).
7. As an alternative, overnight extraction is also possible. Seal plates as described above and extract at room temperature in the dark, without shaking. Before using the extracts, shake for at least 15 min on plate shaker. After the extraction period is complete, pierce the inserts with an injection needle (~gauge 20, ~ 0.9 mm diameter) and allow the extract to run into the well from which the insert was taken. Afterwards the insert can be discarded. Before transferring the extract to 96 well plates pipette up and down 3x until the extractant solution is homogenous.
8. For each tissue, transfer 2 × 200µl aliquots of the blue formazan solution into a 96-well flat bottom microtiter plate according to the fixed plate design given in spreadsheet (example is given in **Figure 12**). Use isopropanol (MTT-100-EXT) as blanks. Read OD in a 96-well plate spectrophotometer using a wavelength between 540 and 595 nm, preferably at **570 nm, without using a reference filter.**

PLATE 1

	1	2	3	4	5	6	7	8	9	10	11	12	
	BL	**BL**	**BL**	**BL**	**BL**	**BL**							A
Tissue 1	**NC**	**PC**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	B
	NC	**PC**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	C
Tissue 2	**NC**	**PC**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	D
	NC	**PC**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	E
Tissue 3	**NC**	**PC**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	F
	NC	**PC**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	G
													H

NC - Negative control ; PC – Positive control; C1, C2... - Chemical No. 1, Chemical No.2...; BL - Blank

Figure 12: Fixed 96 well-plate design (for OD reading in plate photometer, 2 aliquots per tissue)

In contrast to other photometers, in plate readers pipetting errors influence the OD. Therefore, 2 formazan extract aliquots shall be taken from each tissue extract. In the Excel data sheet, these 2 aliquots will be automatically reduced to one value by calculating the mean of the two aliquots. Thus, for calculations from each single tissue only one single mean OD-value is used.

The plate design is dictated by the plate design used in the Spreadsheet, which was used in the validation study for data collection and preliminary calculations. It is necessary to strictly keep the plate design given here. Otherwise, the calculation of results will be incorrect.

Note: Readings are performed without a reference filter, since the "classical" reference filter often used in the MTT test (630 nm) is still within the absorption curve of formazan. Since filters may have a ± tolerance in some cases the reference filter reduces the dynamics of the signal (OD) up to 30%.

7.8 Documentation

7.8.1 Method Documentation Sheet, MDS (see ANNEX B)

The Method Documentation Sheet allows a GLP compliant QC of the correct set up, calibration and function of the equipment, as well as all preparations.

If the test is performed as a GLP compliant procedure in a non-GLP environment filling in all information is mandatory. However, in a full GLP certified laboratory many records (in particular equipment calibrations, temperatures etc.) may be recorded centrally by other means. In this case, reference to the relevant laboratory notebook or other documentation is sufficient.

Per each experiment, make a hardcopy of the MDS, fill in and sign the requested information, starting on the day prior to testing and ending after the test has been conducted.

Note (1): If several tests are performed per week, pipette verification (weighing H_2O on a balance) is only necessary once at the beginning of each week. If adjustable pipettes are used, the correct adjustment shall be checked and recorded in the MDS on each test day.
Note (2): It is recommended at the beginning of the study to check the weight of a levelled application spoon of each solid test substance and record this weight in Annex C.

7.8.2 Test Data

A blank, password protected MS EXCEL workbook **EpiDerm-SIT-SPREAD.XLS** can be provided by ZEBET or MatTek Corporation. A copy should be made before the first data entry. The workbook consists of two single spreadsheets named IMPORT and SPREAD.

Data files of optical densities (ODs) generated by the microplate reader (without blank subtraction) are copied from the reader software to the Windows Clipboard and then pasted into the first spreadsheet of the EXCEL workbook (IMPORT – see **Figure 13**). The blank corrections, calculation of results and statistical parameters are done automatically in the second part of the workbook (SPREAD – see **Figure 14**).

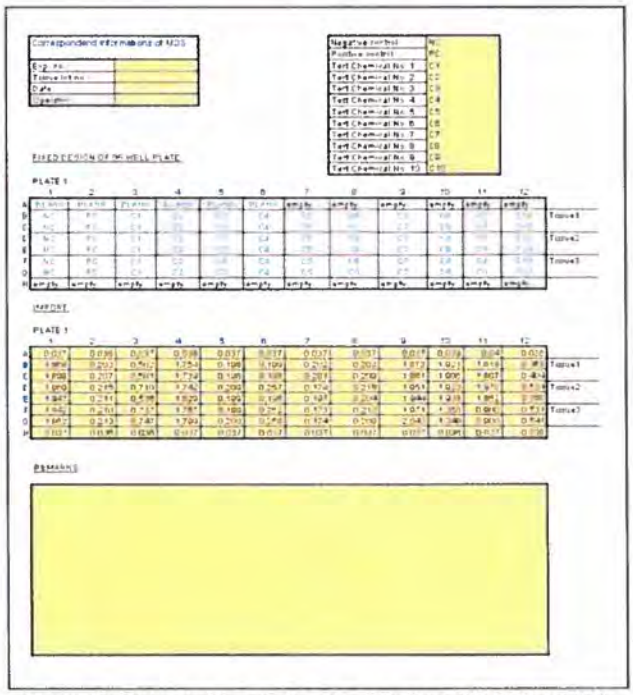

Figure 13: First part of the excel workbook EpiDerm-SIT-SPREAD.XLS

Use the fixed 96-well plate design as specified in the SOP. In addition to the entry of the reader raw data, some requested information has to be filled in the first map of the spreadsheet (tissue lot numbers, test material codes, date, lab personnel).

After data entry, the spreadsheet performs the following calculations

1. Blank correction
2. For each individual tissue treated with a test substance (TS), the positive control (PC) and the negative control (NC) the individual relative tissue viability is calculated according to the following formulas

$$\text{Relative viability TS (\%)} = [OD_{TS} / \text{Mean of } OD_{NC}] \times 100$$
$$\text{Relative viability NC (\%)} = [OD_{NC} / \text{mean of } OD_{NC}] \times 100$$
$$\text{Relative viability PC (\%)} = [OD_{PC} / \text{mean of } OD_{NC}] \times 100$$

3. For each test substance, negative control, and the positive control, the mean relative viability of the three individual tissues is calculated and used for classification according to the Prediction Model (see section 6)
4. The spreadsheet shows a graph of the results (% of relative viability ± SD) see **Figure 14**

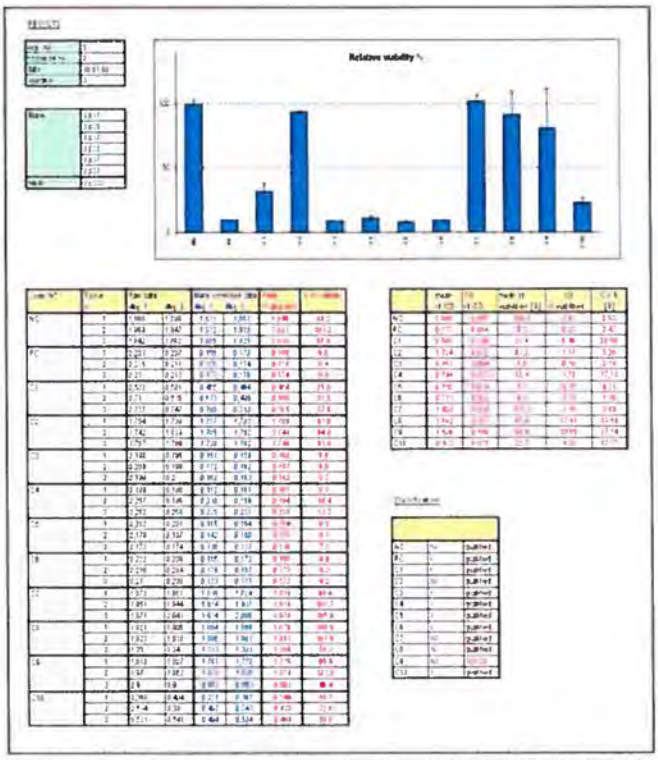

Figure 14: Second spreadsheet of the excel workbook EpiDerm-SIT-SPREAD.XLS

- **For each experiment, make a hardcopy of the raw data (i.e. plate reader data).**
- **For each experiment, save your secondary data in one copy "EpiDerm-SIT-SPREAD.XLS".**
- **Fill in the requested information in "EpiDerm-SIT-SPREAD.XLS".**
- **In addition, per each experiment, keep signed hardcopies of "EpiDerm-SIT-SPREAD.XLS" together with the signed hardcopy of the MDS.**

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ANNEX A: EpiDerm™ Skin Irritation Test: Flowchart

Transfer tissues from agarose to assay medium

↓

Incubate (37±1°C, 5±1 % CO_2 95 % RH) for 60 ± 5 min

↓

Transfer tissues to fresh assay medium

↓

Incubate (37±1°C, 5±1 % CO_2 95 % RH) overnight (18±3 h)

↓

Dose 3 tissues each with 30 µl / 25 mg TS, or PC, or NC

↓

Expose 60 min (37±1°C, 5±1 % CO_2 95 % RH)

↓

Stop exposure after 60 minutes by rinsing with DPBS

↓

Transfer tissues to fresh assay medium

↓

Incubate (37±1°C, 5±1 % CO_2 95 % RH) for 24 ±2 hours

↓

Collect medium for IL-1α analysis (*optional step*)

↓

Incubate (37±1°C, 5±1 % CO_2) for next 18±2 hours

↓

Blot tissues and perform MTT assay

↓

Read OD in a plate spectrophotometer at 550-570 nm

ANNEX B: Methods Documentation Sheet (MDS)

Assay:.....

Date: .....

Corresponding XLS Data File Name: .....

PERFORMED BY: .....**SIGNATURES:** .....

Time Protocol

Receipt of EpiDerm™ tissues (date, day, hour)

.....

ID:

Experimental schedule

Procedure	Date (dd-mm-yy)	Set 1		Set 2		Remark
		start	stop	start	stop	
Pre-incubation 1 (60 ± 5 min)						
Pre-incubation 2 (18 ± 3h)						
Exposure (60 ±1 min)						
Washing						
Post-incubation 1 - start (24 ± 2h)						
Medium change (24h after exposure ± 2h)						
Post-incubation 2 – start (18 ± 2h)						
MTT test (3h ± 5 min)						
Extraction (minimum 2h)						
Measurement						

Devices Verification

Incubator verification

Incubator #	CO_2 < 5±1 % >	Temperature < 37 ± 1°C >	Check water in reservoir (✓)

ID/ Date:

Refrigerator verification

Water bath verification

Refrigerator #	Temperature < 5°C ± 3°C >		Water bath #	Temperature < 37 ± 1°C >

ID/ Date: ID/ Date:

In case that your devices are controlled by central computer, fill in the following table instead of fields above:

Name of the device	device #	reference

ID/ Date:

Pipette verification (triplicate weightings)

Pipette 3 x H_2O into a small baker on a laboratory scale and record readings in g. Perform pipette verification only once per week and refer to it in all assays of this week. If adjustable pipettes are used, check adjustment daily.

	0.9 ml	2 ml	300 µl	200 µl	25 µl	30 µl
	……H_2O weight in g……					
1.						
2.						
3.						
Mean						
SD						

ID/ Date:

Kit Components *(**MTT-100 Kit Must Order Separately)*

EpiDerm™ (EPI-200-SIT) Lot no.:	Production date:
Assay medium (EPI-100-NMM) Lot no.:	Expiration date:
MTT concentrate (**MTT-100-CON); 2 ml Lot no.:	Expiration date:
MTT diluent (**MTT-100-DIL); 8 ml Lot no.:	Production date:
MTT extractant (**MTT-100-EXT), 60 ml Lot no.:	Expiration date:
DPBS (TC-PBS); 100 ml Lot no:	Expiration date:
5% SDS – Positive Control (TC-SDS-5%)	Expiration date:
Position of Ice-packs: (direct contact of the ice-packs with the skin must be avoided)	
Other remarks	

ID/ Date:

Visual Quality Control Of The Skin

Use scores: 1- very good, 2-good, 3- acceptable, 4- not acceptable

APPERANCE	KIT 1	KIT 2
MACRO		
No of excluded tissues with: - edge defects - air bubbles - extensive moisture on the surface		

Specific observations:

Solutions

Positive Control

Note: In case you are preparing your own MTT stock solution and/or DPBS fill in the following forms

MTT Stock Solution Preparation: 5 mg/ml
- MTT batch N° .....
- Weight .....
- DPBS batch N° ...
- DPBS Volume added .....
- Preparation date: ...
- Expiration date .....
- Stocking place: Refrigerator N° .....

DPBS Solution Preparation
- Preparation date: ...
  - pH adjustment (to 7.0) .....
  - Type of sterilisation .....
- Preparation date .....
- Expiration date .....

Dosing Procedure

Tick (✓) in the columns the type of application (pipette, patch, spoon...).
(P:pasty, V: viscous; W:waxy; ST:sticky; C:creamy)

Code	Liquid/ Solid (L/S)	Application (25 mg / 30 µl)					Remarks
		Pipette (✓)	Mesh (✓)	Spoon (✓)	Pin (✓)	Wetting (✓)	

ID/ Date:

Remarks to Single Tissues

If during the assay you observe any abnormality, have to substitute tissues, or deal with any technical problems, fill in following table

Record the tissue number, substance code and your observation or remark.

Substance code	Tissue No.	Remark

ID/ Date:

MTT Plate Configuration

PLATE 1

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24

PLATE 2

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24

ID/ Date

Spectrophotometrical Measurement

Plate Configuration For Reading (for transfer to Spreadsheet EpiDerm SIT.xls) :
Record the positions of substances on 96-well plate.
Strictly adhere to the fixed plate design of the SOP (version January 14, 2009)

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

Note: Turn on the reader 10 min before reading plate.

Check plate photometer filter
Tick correct (✓) filter setting

Reading Filter 570 (550-570) nm	
No Reference Filter	

ID/ Date

Archivation

Raw data saved in/as

Spreadsheet saved in/as

MDS saved in/as

ANNEX C: Characterization of Test Substances

Code	Physical state (S/L)	Color	Change of the color in aqueous environment (+ / -)	MTT reduction (+ / -)	Reaction with mesh (+ / -)	Remarks			
						Known R-phrases	Known S-phrases	Special Storage Requirements	Other

Solid Substances Only

Code	Weight of substance (when applied by spoon) in mg			Grinding (yes / no)	Remarks
	1	2	3		

ANNEX D: Test for Interference of Chemicals with MTT Endpoint and Correction Procedures

Possible interactions between test chemicals and test system

	Water coloration (Step 1, Section 7.3)	Tissue Staining (Step 2, Section 7.3)	MTT Interaction (Step 3, Section 7.3)	Test Conditions
Case 1	-	-	-	A
Case 2	+	-	-	A
Case 3	-	+	-	B
Case 4	+	+	-	B
Case 5	-	-	+	C
Case 6	+	-	+	C
Case 7	-	+	+	B+C
Case 8	+	+	+	B+C

Test Conditions:
A – Perform all steps according to the basic SOP. Correction of results using additional controls is not needed.
B – Perform Step 2, Section 7.3 in addition to the basic SOP. Correction of results with viable tissue is needed.
C – Perform Step 4, Section 7.3 in addition to the basic SOP. Correction of results with frozen tissue is needed.

B+C:
A colored chemical (or a chemical that may turn colored after interaction with water) may cause both tissue staining and false MTT reduction. If the experimentator is interested only in the correction of final results, combination of test condition C is sufficient for this purpose, since the frozen tissues absorb approximately the same amount of chemicals (and color) as the viable tissue. However, since the frozen tissues are more hydrated than viable tissues, tissue staining by water soluble colorants (or non-colored chemicals that will turn in aqueous conditions into colored) can be overestimated. In addition, conditions may arise when information on the amount of non-specific coloration is required. For increased precision of the above- mentioned case, Steps 2 & 4 (Section 7.3) need to be performed.

MatTek CORPORATION

PROTOCOL AMENDMENT 1

Evaluation of the Skin Irritation Potential of Diheptyl Succinate and Caprylhydroxamic Acid Using the EpiDerm Skin Irritation Test OECD TG439

Study Number: 034-18

Testing Facility:
MatTek Corporation
200 Homer Ave
Ashland MA 01721
Phone: (1-)508-881-6771

Study Director:
Kalyani Guntur, Ph.D
Research Scientist
Phone: (1-)508-881-6771 ext 225
E-mail: kguntur@mattek.com

[redacted]

Date of Protocol: February 5th, 2018

CHANGE 1: SECTION 5.1 TEST ARTICLE

Change From:

Batch/Lot No:	TBA
Identity:	Diheptyl Succinate
Purity:	TBA
Expiration Date:	36 months after the date of manufacturing
Storage Condition:	Ambient
Description:	Clear liquid
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sponsor
Supplier:	INOLEX, Inc.

Batch/Lot No:	TBA
Identity:	Caprylhydroxamic Acid
Purity:	TBA
Expiration Date:	24 months after the date of manufacturing
Storage Condition:	Ambient
Description:	White powder
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sponsor
Supplier:	[redacted]

Change To:

Batch/Lot No:	GE4293
Identity:	Diheptyl Succinate
Purity:	100%
Expiration Date:	36 months after the date of manufacturing
Storage Condition:	Ambient
Description:	Clear liquid
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sponsor
Supplier:	[redacted]

Batch/Lot No:	FM7477
Identity:	Caprylhydroxamic Acid
Purity:	100%
Expiration Date:	24 months after the date of manufacturing
Storage Condition:	Ambient
Description:	White powder
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sponsor
Supplier:	[redacted]

Reason: Information was not available earlier

CHANGE 2: SECTION 5.2 CONTROL ARTICLE

Change From:

Batch/Lot No:	TBA
Identity:	SDS
Purity:	TBA
Expiration Date:	TBA
Storage Condition:	Room Temperature
Description:	Sodium dodecyl sulfate
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by MatTek
Supplier:	Sigma

Change To:

Batch/Lot No:	STBG6142V
Identity:	SDS
Purity:	99.6%
Expiration Date:	10/01/19
Storage Condition:	Room Temperature
Description:	Sodium dodecyl sulfate
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sigma
Supplier:	Sigma

Reason: Information was not available earlier

STUDY PROTOCOL AMENDMENT APPROVAL SIGNATURES

The date the Study Director signs is the effective date of this amendment

Kalyani

Name, Degree KALYANI GUNTUR, PhD
Study Director
MatTek Corporation

2/13/2018
Date

Patrick D. Hayden

Name, Degree PATRICK HAYDEN, PhD
Testing Facility Manager
MatTek Corporation

2/9/18
Date

[redacted]

MatTek CORPORATION

PLAN AMENDMENT 2

Evaluation of the Skin Irritation Potential of Diheptyl Succinate and Caprylhydroxamic Acid Using the EpiDerm Skin Irritation Test OECD TG439

Study Number: 034-18

Testing Facility:
MatTek Corporation
200 Homer Ave
Ashland, MA 01721
Phone: (1-)508-881-6771

Study Director:
Kalyani Guntur, Ph.D
Research Scientist
Phone: (1-)508-881-6771 ext 225
E-mail: kguntur@mattek.com

[redacted]

Date of Protocol: February 5th, 2018

Study 034-18 Plan Amendment 2

CHANGE 1: SECTION 9.4 EXPOSURE OF EPIDERM TISSUES FOR SKIN IRRITATION TEST. ITEM #3

Change From:

prewet n=3 tissues with 25µl of DPBS and apply 25 mg of TA (using sharp applicator spoon)

Change To:

prewet n=3 tissues with 25µl of DPBS Fill 25 mg sharp application spoon with test material. Level the spoon by gently scratching the excess material away Apply the test material

Reason: Earlier version of the test material application method was not accurate

CHANGE 2: SECTION 5.2 CONTROL ARTICLE

Change From:

Batch/Lot No:	STBG6142V
Identity:	SDS
Purity:	99.6%
Expiration Date:	10/01/19
Storage Condition:	Room Temperature
Description:	Sodium dodecyl sulfate
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by Sigma
Supplier:	Sigma

Change To:

Batch/Lot No:	071817MAB
Identity:	5% SDS Solution
Purity:	≥ 99.0%
Expiration Date:	July 18 2018
Storage Condition	15 – 30°C
Description:	Sodium dodecyl sulfate
Handling Precautions:	Relevant occupational safety information will be detailed in the MSDS provided by MatTek
Supplier:	MatTek

Reason: Updated information is available

MatTek Corporation
200 Homer Ave., Ashland, MA - USA
+1-508-881-6771 | information@mattek.com
www.mattek.com

STUDY PROTOCOL AMENDMENT APPROVAL SIGNATURES

The date the Study Director signs is the effective date of this amendment

Kalyani Guntur, PhD
Study Director
MatTek Corporation

2/27/18
Date

Patrick Hayden, PhD
Testing Facility Manager
MatTek Corporation

3/3/18
Date

[redacted]

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: February 26, 2019

SUBJECT: Caprylhydroxamic Acid

Consumer Product Testing Co. 2014. Repeated insult patch test (eyeliner containing 0.105% Caprylhydroxamic Acid).

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

Consumer Product Testing

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: [redacted]

TEST MATERIAL: [redacted]

Eyeliner containing 0.105% Caprylhydroxamic Acid

EXPERIMENT REFERENCE NUMBER: [redacted]

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.

QUALITY ASSURANCE UNIT STATEMENT

Study Number: [redacted]

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Quality Assurance Representative

01/11/2014
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: Fifty-seven (57) qualified subjects, male and female, ranging in age from 19 to 70 years, were selected for this evaluation. Fifty-four (54) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: [redacted]

Study Schedule:

Panel #	Initiation Date	Completion Date
[redacted]	December 4, 2013	January 9, 2014

[a]With parental or guardian consent

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 1" x 1" absorbent pad portion of a clear adhesive dressing and allowed to volatilize for approximately 15 minutes. This was then applied to the appropriate treatment site to form a semi-occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of twenty-four hours following each Tuesday and Thursday removal, and forty-eight hours following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic twenty-four and seventy-two hours post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	=	No visible skin reaction	E	=	Edema
0.5	=	Barely perceptible	D	=	Dryness
1	=	Mild	S	=	Staining
2	=	Moderate	P	=	Papules
3	=	Marked	V	=	Vesicles
4	=	Severe	B	=	Bullae
			U	=	Ulceration
			Sp	=	Spreading

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: There were no deviations.

Results: The results of each participant are appended (Table 1).

Observations remained within normal limits throughout the test interval.

Subject demographics are presented in Table 2.

Summary: Under the conditions of this study, test material, [redacted] did not indicate a potential for dermal irritation or allergic contact sensitization.

[redacted]

Table 1
Panel #[redacted]

Individual Results

[redacted]

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0.5	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0.5	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch

Table 1
(continued)
Panel #[redacted]

Individual Results

[redacted]

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	DID NOT COMPLETE STUDY									
36	0	0	0	0	0	DID NOT COMPLETE STUDY						
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	-	0	0	DID NOT COMPLETE STUDY								
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	-	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1" Induction and Challenge Patch
- = Subject not present for supervised removal

Table 2
Panel #[redacted]

Subject Demographics

Subject Number	Initials	Age	Sex
1	TJV	60	F
2	LCL	50	F
3	VRO	66	F
4	JAP	42	F
5	CCM	34	F
6	SSF	40	F
7	R-B	49	F
8	EMB	20	F
9	PAM	61	F
10	MAM	63	F
11	JFK	70	F
12	TMM	50	F
13	BIM	63	F
14	MSA	27	F
15	DMM	48	F
16	WRB	61	M
17	SAW	55	F
18	J-R	34	M
19	R-Z	48	F
20	SRD	64	F
21	REV	55	F
22	RER	50	F
23	LRC	43	F
24	EHV	52	F
25	KMB	28	F
26	RET	30	F
27	G-D	46	M
28	AMB	48	F
29	L-I	59	F

Table 2
(continued)
Panel #[redacted]

Subject Demographics

Subject Number	Initials	Age	Sex
30	DAS	29	F
31	J-T	55	F
32	JMA	22	F
33	JJS	52	M
34	LAC	65	F
35	GDG	22	M
36	ABK	49	F
37	JMC	54	F
38	JMS	35	M
39	DAF	50	F
40	RMS	30	F
41	PAI	59	F
42	JAB	25	M
43	WEA	50	F
44	SJB	26	F
45	MJC	27	M
46	C-C	37	F
47	SAS	45	F
48	M-S	66	F
49	JMS	19	M
50	APM	47	M
51	R-R	36	M
52	S-I	19	F
53	M-T	54	F
54	DMS	35	F
55	C-O	26	F
56	J-R	21	M
57	M-Q	53	F

Consumer Product Testing Co.
ESL 1973

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: Lotion Lot: 647-081-7J

water 72.35%
caprylic/capric triglyceride 5%
isopropyl myristate 5%
Arachidyl alcohol (and) behenyl alcohol (and) arachidyl glucoside 4.5%
petrolatum 4%
cetyl alcohol 3%
stearyl alcool 3%
glycerin 3%
caprylhydroxamic acid 0.15%

EXPERIMENT REFERENCE NUMBER: C17-5522.07

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-5522.07

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Nicole Bocoley
Quality Assurance Representative

1-17-18
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred fourteen (114) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. One hundred four (104) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: Lotion Lot: 647-081-7J

Study Schedule:

Panel #	Initiation Date	Completion Date
20170442	November 15, 2017	January 6, 2018

[a]With parental or guardian consent

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	**=**	**No visible skin reaction**	**E**	**=**	**Edema**
0.5	**=**	**Barely perceptible**	**D**	**=**	**Dryness**
1	**=**	**Mild**	**S**	**=**	**Staining**
2	**=**	**Moderate**	**P**	**=**	**Papules**
3	**=**	**Marked**	**V**	**=**	**Vesicles**
4	**=**	**Severe**	**B**	**=**	**Bullae**
			U	**=**	**Ulceration**
			Sp	**=**	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: Due to the New Year holiday, Subjects #20 – 51, Panel 20170442, were evaluated on Day 1 and Day 2 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Subject #88, Panel 20170442, had a late challenge schedule, but was unable to report on Day 1 post challenge application due to inclement weather. She kept her patch in place and reported on Day 2 and Day 3 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Results: The results of each participant are appended (Table 1).

Subject demographics are presented in Table 2.

It was noted that Subject #10 exhibited barely perceptible (0.5) to moderate (2) erythema and edema after the second Induction exposure, resulting in the discontinuation of subsequent patch applications at the fifth exposure. This pattern of skin reactivity is indicative of a pre-existing hypersensitivity to one or more ingredients in this formulation.

Scattered, transient barely perceptible (0.5) to moderate (2) erythema with occasional edema responses were noted throughout the test interval. Neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. No evidence of induced allergic contact sensitization was observed.

Summary: Under the conditions of this study, test material, Lotion Lot: 647-081-7J, indicated no clinically significant potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20170442

Individual Results

Lotion Lot: 647-081-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	DID NOT COMPLETE STUDY								
3	0	0	0	0	0	0	0	0	0	0	---DNC---	
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	**0.5**	**0.5**	**1**[E1]	**2**[E2X]						
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	**1**[E1]	**0.5**	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0**
21	0	0	0	0	0	0	0	0	0	0	0	0**
22	0	0	0	0	0	0	0	0	0	0	0	0**
23	0	0	0	0	0	0	0	0	0	0	0	0**
24	0	0	0	0	0	0	0	0	0	0	0	0**
25	0	0	0	0	0	0	0	0	0	0	0	0**
26	0	0	0	0	0	0	0	0	0	0	0	0**
27	0	0	0	0	0	0	0	0	0	0	0	0**
28	0	0	0	0	0	0	0	0	0	0	0	0**
29	0	0	0	0	0	0	0	0	0	0	0	0**

Day 1* = Supervised removal
** = Subjects 20-29 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
X = Patching discontinued
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

Lotion Lot: 647-081-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0**
31	0	0	0	0	0	0	0	0	0	0	0	0**
32	0	0	0	0	0	0	0	0	0	0	0	0**
33	0	0	0	0	0	0	0	0	0	0	0	0**
34	0	0	0	0	0	0	0	0	0	0	0	0**
35	0	0	0	0	0	0	0	0	0	0	0	0**
36	0	0	0	0	0	0	0	0	0	0	0	0**
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0**
39	0	0	0	0	0	0	0	0	0	0	0	0**
40	0	0	0	0	0	0	0	0	0	0	0	0**
41	0	0	0	DID NOT COMPLETE STUDY								
42	0	0	0	0	0	0	0	0	**0.5**	**0.5**	**0.5**	**1[E1]****
43	0	0	0	0	0	0	0	0	0	0	0	0**
44	0	0	0	0	0	0	0	0	DNC			
45	0	0	0	0	0	0	0	0	0	0	0	0**
46	0	0	0	0	0	0	0	0	0	0	0	0**
47	0	0	0	0	0	0	0	0	0	0	0	0**
48	DID NOT COMPLETE STUDY											
49	0	0	0	0	0	0	0	0	0	0[m]	0	0**
50	0	0	0	0	0	0	0	0	0	0	0	0**
51	0	0	0	0	0	0	0	0	0	0	0	0**
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
** = Subjects 30–51 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
m = Additional makeup day granted at the discretion of the clinic supervisor
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

Lotion Lot: 647-081-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	DID NOT COMPLETE STUDY									
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	**0.5**	0	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	DID NOT COMPLETE STUDY										
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	DNC			
87	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

Lotion Lot: 647-081-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
88	0	0	0	0	0	0	0	0	0	0	0†	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	**0.5**	**0.5**	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	**0.5**	0[D1]	0	0	0	0	0	0	---DNC---	
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0[m]	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0[m]	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
† = Late challenge – Subject unable to return due to inclement weather, evaluated on Day 2 and Day 3 post challenge, per deviation
D = Dryness

Table 2
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
1	SAV	75	M
2	K-G	17	F
3	RMM	74	F
4	AML	71	F
5	KMR	73	F
6	IGR	16	F
7	DJD	16	F
8	DJD	16	F
9	REV	78	F
10	LMK	77	F
11	KMM	17	F
12	JAP	76	F
13	JIA	16	M
14	RID	75	F
15	IGG	17	M
16	BAJ	78	F
17	BLJ	77	M
18	L-W	71	F
19	DFS	17	M
20	BIH	73	F
21	ANP	77	F
22	LJW	77	F
23	M-P	74	F
24	EMS	74	F
25	RWL	77	M
26	M-C	75	F
27	CMS	72	F
28	DPT	44	M
29	JMM	35	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
30	AMV	22	F
31	CRC	16	F
32	I-M	63	M
33	A-S	51	F
34	RAD	44	M
35	CRD	47	F
36	F-H	24	F
37	TAT	49	F
38	J-V	51	F
39	JCG	24	M
40	KMG	24	F
41	HAB	40	M
42	SMC	49	F
43	J-C	50	M
44	R-M	48	F
45	NLM	29	F
46	RJV	42	F
47	J-M	17	F
48	YMA	43	F
49	DCA	71	F
50	LTB	42	F
51	KDV	16	F
52	JBD	63	F
53	G-G	55	F
54	L-M	33	F
55	W-Z	44	F
56	SNS	64	F
57	C-P	30	F
58	RPK	79	M

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
59	BGM	65	F
60	V-M	61	M
61	G-M	44	F
62	L-S	65	F
63	T-R	48	F
64	SDW	48	F
65	MSA	46	F
66	DAT	49	F
67	EPL	43	F
68	GCL	67	F
69	GVC	19	F
70	G-B	68	F
71	MAM	67	F
72	Y-M	35	F
73	JMR	28	M
74	MJS	72	F
75	LEW	71	M
76	L-T	64	F
77	CAG	34	F
78	ALB	37	M
79	KSD	29	F
80	S-J	70	F
81	SEC	66	F
82	JAM	59	F
83	CFD	75	M
84	LAA	51	M
85	SJA	32	F
86	JMR	20	M
87	X-R	38	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
88	T-N	28	F
89	CJR	21	M
90	R-O	45	F
91	JAS	43	F
92	LAM	53	F
93	RMA	51	F
94	WLK	63	M
95	M-D	74	F
96	G-D	77	M
97	LOH	70	M
98	ACK	73	F
99	JBS	62	F
100	SEP	64	F
101	HSP	66	M
102	LJC	44	F
103	JAA	32	F
104	REC	70	F
105	E-R	24	F
106	WRB	65	M
107	EAC	50	M
108	KRM	55	F
109	PCL	54	F
110	RDS	58	F
111	DLW	48	F
112	HCT	58	M
113	F-G	72	M
114	MAH	71	F

Consumer Product Testing Co.
ESL 1975

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: W/O Thick Balm Lot: 617-109-7J

water 66.35%
sunflower seed oil 10%
isopropyl palmitate 10%
petrolatum 5%
octyldodecanol (and) octyldodecyl xyloside (and) PEG-30 dipolyhydroxystearate 3.5%
glycerin 3%
beeswax 2%
caprylhydroxamic acid 0.15%

EXPERIMENT REFERENCE NUMBER: C17-5522.08

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-5522.08

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Nicole Bowley
Quality Assurance Representative

1-17-18
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred fourteen (114) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. One hundred four (104) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: W/O Thick Balm Lot: 617-109-7J

Study Schedule:

Panel #	Initiation Date	Completion Date
20170442	November 15, 2017	January 6, 2018

[a]With parental or guardian consent

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	**=**	**No visible skin reaction**	**E**	**=**	**Edema**
0.5	**=**	**Barely perceptible**	**D**	**=**	**Dryness**
1	**=**	**Mild**	**S**	**=**	**Staining**
2	**=**	**Moderate**	**P**	**=**	**Papules**
3	**=**	**Marked**	**V**	**=**	**Vesicles**
4	**=**	**Severe**	**B**	**=**	**Bullae**
			U	**=**	**Ulceration**
			Sp	**=**	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: Due to the New Year holiday, Subjects #20 – 51, Panel 20170442, were evaluated on Day 1 and Day 2 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Subject #88, Panel 20170442, had a late challenge schedule, but was unable to report on Day 1 post challenge application due to inclement weather. She kept her patch in place and reported on Day 2 and Day 3 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Results: The results of each participant are appended (Table 1).

Subject demographics are presented in Table 2.

It was noted that Subject #10 exhibited mild (1) to moderate (2) erythema and edema after the fourth Induction exposure, resulting in the discontinuation of subsequent patch applications at the fifth exposure. This pattern of skin reactivity is indicative of a pre-existing hypersensitivity to one or more ingredients in this formulation.

Scattered, transient barely perceptible (0.5) to moderate (2) erythema with occasional edema responses were noted throughout the test interval. Neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. No evidence of induced allergic contact sensitization was observed.

Summary: Under the conditions of this study, test material, W/O Thick Balm Lot: 617-109-7J, did not indicated a clinically significant potential for dermal irritation or allergic contact sensitization. One subject did exhibit a pattern of skin reactivity indicative of a pre-existing hypersensitivity to one or more ingredients in this formulation.

Table 1
Panel #20170442

Individual Results

W/O Thick Balm Lot: 617-109-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	DID NOT COMPLETE STUDY								
3	0	0	0	0	0	0	0	0	0	0	---DNC---	
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	**1**[E1]	**2**[E2X]						
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	**0.5**	**0.5**	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0**
21	0	0	0	0	0	0	0	0	0	0	0	0**
22	0	0	0	0	0	0	0	0	0	0	0	0**
23	0	0	0	0	0	0	0	0	0	0	0	0**
24	0	0	0	0	0	0	0	0	0	0	0	0**
25	0	0	0	0	0	0	0	0	0	0	0	0**
26	0	0	0	0	0	0	0	0	0	0	0	0**
27	0	0	0	0	0	0	0	0	0	0	0	0**
28	0	0	0	0	0	0	0	0	0	0	0	0**
29	0	0	0	0	0	0	0	0	0	0	0	0**

Day 1* = Supervised removal
** = Subjects 20-29 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
X = Patching discontinued
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

W/O Thick Balm Lot: 617-109-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0**
31	0	0	0	0	0	0	0	0	0	0	0	0**
32	0	0	0	0	0	0	0	0	0	0	0	0**
33	0	0	0	0	0	0	0	0	0	0	0	0**
34	0	0	0	0	0	0	0	0	0	0	0	0**
35	0	0	0	0	0	0	0	0	0	0	0	0**
36	0	0	0	0	0	0	0	0	0	0	0	0**
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0**
39	0	0	0	0	0	0	0	0	0	0	0	0**
40	0	0	0	0	0	0	0	0	0	0	0	0**
41	0	0	0	DID NOT COMPLETE STUDY								
42	0	0	0	0	0	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	0	**1[E1]****
43	0	0	0	0	0	0	0	0	0	0	0	0**
44	0	0	0	0	0	0	0	0	DNC			
45	0	0	0	0	0	0	0	0	0	0	0	0**
46	0	0	0	0	0	0	0	0	0	0	0	0**
47	0	0	0	0	0	0	0	0	0	0	0	0**
48	DID NOT COMPLETE STUDY											
49	0	0	0	0	0	0	0	0	0	0[m]	0	0**
50	0	0	0	0	0	0	0	0	0	0	0	0**
51	0	0	0	0	0	0	0	0	0	0	0	0**
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
** = Subjects 30–51 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
m = Additional makeup day granted at the discretion of the clinic supervisor
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

W/O Thick Balm Lot: 617-109-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	DID NOT COMPLETE STUDY									
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	DID NOT COMPLETE STUDY										
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	DNC			
87	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

W/O Thick Balm Lot: 617-109-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
88	0	0	0	0	0	0	0	0	0	0	0†	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	**0.5**	**0.5**	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	---DNC---	
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0[m]	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0[m]	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
† = Late challenge – Subject unable to return due to inclement weather, evaluated on Day 2 and Day 3 post challenge, per deviation

Table 2
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
1	SAV	75	M
2	K-G	17	F
3	RMM	74	F
4	AML	71	F
5	KMR	73	F
6	IGR	16	F
7	DJD	16	F
8	DJD	16	F
9	REV	78	F
10	LMK	77	F
11	KMM	17	F
12	JAP	76	F
13	JIA	16	M
14	RID	75	F
15	IGG	17	M
16	BAJ	78	F
17	BLJ	77	M
18	L-W	71	F
19	DFS	17	M
20	BIH	73	F
21	ANP	77	F
22	LJW	77	F
23	M-P	74	F
24	EMS	74	F
25	RWL	77	M
26	M-C	75	F
27	CMS	72	F
28	DPT	44	M
29	JMM	35	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
30	AMV	22	F
31	CRC	16	F
32	I-M	63	M
33	A-S	51	F
34	RAD	44	M
35	CRD	47	F
36	F-H	24	F
37	TAT	49	F
38	J-V	51	F
39	JCG	24	M
40	KMG	24	F
41	HAB	40	M
42	SMC	49	F
43	J-C	50	M
44	R-M	48	F
45	NLM	29	F
46	RJV	42	F
47	J-M	17	F
48	YMA	43	F
49	DCA	71	F
50	LTB	42	F
51	KDV	16	F
52	JBD	63	F
53	G-G	55	F
54	L-M	33	F
55	W-Z	44	F
56	SNS	64	F
57	C-P	30	F
58	RPK	79	M

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
59	BGM	65	F
60	V-M	61	M
61	G-M	44	F
62	L-S	65	F
63	T-R	48	F
64	SDW	48	F
65	MSA	46	F
66	DAT	49	F
67	EPL	43	F
68	GCL	67	F
69	GVC	19	F
70	G-B	68	F
71	MAM	67	F
72	Y-M	35	F
73	JMR	28	M
74	MJS	72	F
75	LEW	71	M
76	L-T	64	F
77	CAG	34	F
78	ALB	37	M
79	KSD	29	F
80	S-J	70	F
81	SEC	66	F
82	JAM	59	F
83	CFD	75	M
84	LAA	51	M
85	SJA	32	F
86	JMR	20	M
87	X-R	38	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
88	T-N	28	F
89	CJR	21	M
90	R-O	45	F
91	JAS	43	F
92	LAM	53	F
93	RMA	51	F
94	WLK	63	M
95	M-D	74	F
96	G-D	77	M
97	LOH	70	M
98	ACK	73	F
99	JBS	62	F
100	SEP	64	F
101	HSP	66	M
102	LJC	44	F
103	JAA	32	F
104	REC	70	F
105	E-R	24	F
106	WRB	65	M
107	EAC	50	M
108	KRM	55	F
109	PCL	54	F
110	RDS	58	F
111	DLW	48	F
112	HCT	58	M
113	F-G	72	M
114	MAH	71	F

Consumer Product Testing Co.
EST. 1975

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: Wipe Juice Lot: 647-080-7J water 94.85% (and) propanediol 3% (and) polysorbate 20 2% (and) caprylhydroxamic acid 0.15%

EXPERIMENT REFERENCE NUMBER: C17-5522.06

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-5522.06

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Nicole Bowley
Quality Assurance Representative

1-17-18
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred fourteen (114) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. One hundred four (104) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: Wipe Juice Lot: 647-080-7J

Study Schedule:

Panel #	Initiation Date	Completion Date
20170442	November 15, 2017	January 6, 2018

[a]With parental or guardian consent

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	=	**No visible skin reaction**	**E**	=	**Edema**
0.5	=	**Barely perceptible**	**D**	=	**Dryness**
1	=	**Mild**	**S**	=	**Staining**
2	=	**Moderate**	**P**	=	**Papules**
3	=	**Marked**	**V**	=	**Vesicles**
4	=	**Severe**	**B**	=	**Bullae**
			U	=	**Ulceration**
			Sp	=	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: Due to the New Year holiday, Subjects #20 – 51, Panel 20170442, were evaluated on Day 1 and Day 2 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Subject #88, Panel 20170442, had a late challenge schedule, but was unable to report on Day 1 post challenge application due to inclement weather. She kept her patch in place and reported on Day 2 and Day 3 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Results: The results of each participant are appended (Table 1).

Subject demographics are presented in Table 2.

Scattered, transient barely perceptible (0.5) to mild (1) erythema with occasional edema responses were noted throughout the test interval. Neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. No evidence of induced allergic contact sensitization was observed.

Summary: Under the conditions of this study, test material, Wipe Juice Lot: 647-080-7J, indicated no clinically significant potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20170442

Individual Results

Wipe Juice Lot: 647-080-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	DID NOT COMPLETE STUDY								
3	0	0	0	0	0	0	0	0	0	0	---DNC---	
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0**
21	0	0	0	0	0	0	0	0	0	0	0	0**
22	0	0	0	0	0	0	0	0	0	0	0	0**
23	0	0	0	0	0	0	0	0	0	0	0	0**
24	0	0	0	0	0	0	0	0	0	0	0	0**
25	0	0	0	0	0	0	0	0	0	0	0	0**
26	0	0	0	0	0	0	0	0	0	0	0	0**
27	0	0	0	0	0	0	0	0	0	0	0	0**
28	0	0	0	0	0	0	0	0	0	0	0	0**
29	0	0	0	0	0	0	0	0	0	0	0	0**

Day 1* = Supervised removal
** = Subjects 20-29 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

Wipe Juice Lot: 647-080-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0**
31	0	0	0	0	0	0	0	0	0	0	0	0**
32	0	0	0	0	0	0	0	0	0	0	0	0**
33	0	0	0	0	0	0	0	0	0	0	0	0**
34	0	0	0	0	0	0	0	0	0	0	0	0**
35	0	0	0	0	0	0	0	0	0	0	0	0**
36	0	0	0	0	0	0	0	0	0	0	0	0**
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0**
39	0	0	0	0	0	0	0	0	0	0	0	0**
40	0	0	0	0	0	0	0	0	0	0	0	0**
41	0	0	0	DID NOT COMPLETE STUDY								
42	0	0	0	0	0	0	**0.5**	0	**0.5**	0	0	**1[E1]****
43	0	0	0	0	0	0	0	0	0	0	0	0**
44	0	0	0	0	0	0	0	0	DNC			
45	0	0	0	0	0	0	0	0	0	0	0	0**
46	0	0	0	0	0	0	0	0	0	0	0	0**
47	0	0	0	0	0	0	0	0	0	0	0	0**
48	DID NOT COMPLETE STUDY											
49	0	0	0	0	0	0	0	0	0	0[m]	0	0**
50	0	0	0	0	0	0	0	0	0	0	0	0**
51	0	0	0	0	0	0	0	0	0	0	0	0**
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
** = Subjects 30–51 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
m = Additional makeup day granted at the discretion of the clinic supervisor
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

Wipe Juice Lot: 647-080-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	DID NOT COMPLETE STUDY									
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	DID NOT COMPLETE STUDY										
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	DNC			
87	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

Wipe Juice Lot: 647-080-7J

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
88	0	0	0	0	0	0	0	0	0	0	0†	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	**0.5**	**0.5**	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	---DNC---	
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0[m]	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0[m]	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
† = Late challenge – Subject unable to return due to inclement weather, evaluated on Day 2 and Day 3 post challenge, per deviation

Table 2
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
1	SAV	75	M
2	K-G	17	F
3	RMM	74	F
4	AML	71	F
5	KMR	73	F
6	IGR	16	F
7	DJD	16	F
8	DJD	16	F
9	REV	78	F
10	LMK	77	F
11	KMM	17	F
12	JAP	76	F
13	JIA	16	M
14	RID	75	F
15	IGG	17	M
16	BAJ	78	F
17	BLJ	77	M
18	L-W	71	F
19	DFS	17	M
20	BIH	73	F
21	ANP	77	F
22	LJW	77	F
23	M-P	74	F
24	EMS	74	F
25	RWL	77	M
26	M-C	75	F
27	CMS	72	F
28	DPT	44	M
29	JMM	35	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
30	AMV	22	F
31	CRC	16	F
32	I-M	63	M
33	A-S	51	F
34	RAD	44	M
35	CRD	47	F
36	F-H	24	F
37	TAT	49	F
38	J-V	51	F
39	JCG	24	M
40	KMG	24	F
41	HAB	40	M
42	SMC	49	F
43	J-C	50	M
44	R-M	48	F
45	NLM	29	F
46	RJV	42	F
47	J-M	17	F
48	YMA	43	F
49	DCA	71	F
50	LTB	42	F
51	KDV	16	F
52	JBD	63	F
53	G-G	55	F
54	L-M	33	F
55	W-Z	44	F
56	SNS	64	F
57	C-P	30	F
58	RPK	79	M

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
59	BGM	65	F
60	V-M	61	M
61	G-M	44	F
62	L-S	65	F
63	T-R	48	F
64	SDW	48	F
65	MSA	46	F
66	DAT	49	F
67	EPL	43	F
68	GCL	67	F
69	GVC	19	F
70	G-B	68	F
71	MAM	67	F
72	Y-M	35	F
73	JMR	28	M
74	MJS	72	F
75	LEW	71	M
76	L-T	64	F
77	CAG	34	F
78	ALB	37	M
79	KSD	29	F
80	S-J	70	F
81	SEC	66	F
82	JAM	59	F
83	CFD	75	M
84	LAA	51	M
85	SJA	32	F
86	JMR	20	M
87	X-R	38	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
88	T-N	28	F
89	CJR	21	M
90	R-O	45	F
91	JAS	43	F
92	LAM	53	F
93	RMA	51	F
94	WLK	63	M
95	M-D	74	F
96	G-D	77	M
97	LOH	70	M
98	ACK	73	F
99	JBS	62	F
100	SEP	64	F
101	HSP	66	M
102	LJC	44	F
103	JAA	32	F
104	REC	70	F
105	E-R	24	F
106	WRB	65	M
107	EAC	50	M
108	KRM	55	F
109	PCL	54	F
110	RDS	58	F
111	DLW	48	F
112	HCT	58	M
113	F-G	72	M
114	MAH	71	F

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: March 8, 2019

SUBJECT: Caprylhydroxamic Acid

Anonymous 2019. Summary of an HRIPT of a facial cream containing 0.15% Caprylhydroxamic Acid.

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

2019

Product Number	% Caprylhydroxamic Acid	Product Type	HRIPT Test Yes/No	Occlusivity	Completed Subjects	Did formula induce an allergic response	Number of Subjects Exhibiting Low Level Reaction During Induction	Number of Subjects Exhibiting High Level Reaction During Induction	Number of Subjects Exhibiting Low Level Reaction During Challenge	Number of Subjects Exhibiting High Level Reaction During Challenge	pass/fail	comments
1	0.15	LEAVE ON facial cream	YES	OCCLUSIVE	109	NO	0	0	1	0	PASS	Did not induce Allergic Contact Sensitization

Product Number 1	
Induction Phase Grading Scale	
Grade	Response
-	No evidence of any effect
1	Mild (pink, uniform erythema covering
2	Moderate (pink-red erythema uniform
3	Marked (Bright red erythema with/without petechiae, papules,
4	Severe (Deep red Erytheme with/without vesiculation or weeping)

Challenge Phase Grading Scale	
Score	Interpretation
-	Negative
-?	Doubtful reaction (slight erythema)
+	Weak (non-vesicular) reaction
++	Strong (oedematous or vesicular) reaction
+++	Extreme (bullous or ulcerative)
NT	Not tested
IR	Irritant reaction of different types

Details of Test Methodology and Results	
0	panelist discontinued due to reactions

48 hrs	patch duration
9	induction patches
3	weeks induction
2	week rest period
Inducted/orginial and virgin site	challenge Patch
30 mins, 24 hrs , 48 hrs	challenge readings
0.02ml	Amount of product applied
Test Material Concentration/Dilution	As is /No Dilution

Grading Scale interpretation	
Low Level Reactions	0 or 1
High Level Reaction	2 and above

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: March 14, 2019

SUBJECT: Caprylhydroxamic Acid

Anonymous. 2019. Summary of an HRIPT on a product containing 0.195% Caprylhydroxamic Acid.

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

March 11, 2019

HRIPT on a product containing Caprylhydroxamic Acid

A leave-on eye brow thickening powder containing the ingredient Caprylhydroxamic Acid at 0.195% was tested for its potential to induce primary/cumulative irritation or sensitization in healthy human subjects using HRIPT procedure, as described below.

HRIPT procedure

HRIPT was carried out by Consumer Product Testing Co. from October 27, 2010 to December 10, 2010, in accordance with Good Clinical Practice regulations (CFR 21, Part 50 and Part 56).

A total of 52 subjects completed the test. Approximately 0.2 grams of the neat product was applied to 1 x 1 inch (equivalent to 6.45 cm^2) absorbent pad portion of clear adhesive dressing under semi-occlusive conditions. This pad was moistened with several drops of water to ensure adherence of test material. This was then placed on the appropriate treatment site at the upper back between the scapulae.

The HRIPT consists of three phases: induction phase, rest phase and challenge phase. During the induction phase, patches were applied on the subject's back and were removed 24 hours after each application. A trained examiner scored skin responses when subjects returned to the testing facility for next patch application. Patches were applied at the same site 3 times a week (Monday, Wednesday and Friday) for 3 consecutive weeks. Around 2 weeks (rest phase) after application of the last induction patch, challenge patches were applied to adjacent virgin sites. Patches were removed after 24 hours. Test sites were scored at 24 and 72 hours after application

Calculations for dose/cm^2

Amount of product applied = 200 mg
Concentration of ingredient = 0.195%
Amount of ingredient applied = 0.39 mg
Applied area = 6.45 cm^2
Dose per unit area = 0.06 mg/cm^2

Conclusion

Under the conditions of the test, the product (containing Caprylhydroxamic Acid at 0.195%, applied at 0.06 mg/cm^2) did not show potential to induce dermal irritation or allergic contact sensitization.

Consumer Product Testing Co.
EST. 1975

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: CHA blend #3 Lot: GH5355 hexanediol 30% (and) propanediol 65% (and) caprylhydroxamic acid 5%

EXPERIMENT REFERENCE NUMBER: C17-5522.05

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-5522.05

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Nicole Bowley
Quality Assurance Representative

1-17-18
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred fourteen (114) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. One hundred four (104) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: CHA blend #3 Lot: GH5355

Study Schedule:

Panel #	Initiation Date	Completion Date
20170442	November 15, 2017	January 6, 2018

[a]With parental or guardian consent

Methodology:

Prior to the initiation of this study, the test material was prepared as a 6% dilution, using distilled water.

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	**=**	**No visible skin reaction**	**E**	**=**	**Edema**
0.5	**=**	**Barely perceptible**	**D**	**=**	**Dryness**
1	**=**	**Mild**	**S**	**=**	**Staining**
2	**=**	**Moderate**	**P**	**=**	**Papules**
3	**=**	**Marked**	**V**	**=**	**Vesicles**
4	**=**	**Severe**	**B**	**=**	**Bullae**
			U	**=**	**Ulceration**
			Sp	**=**	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: Due to the New Year holiday, Subjects #20 – 51, Panel 20170442, were evaluated on Day 1 and Day 2 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Subject #88, Panel 20170442, had a late challenge schedule, but was unable to report on Day 1 post challenge application due to inclement weather. She kept her patch in place and reported on Day 2 and Day 3 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Results: The results of each participant are appended (Table 1).

Subject demographics are presented in Table 2.

Scattered, transient barely perceptible (0.5) to moderate (2) erythema with occasional edema responses were noted throughout the test interval. Neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. No evidence of induced allergic contact sensitization was observed.

Summary: Under the conditions of this study, test material, CHA blend #3 Lot: GH5355, indicated no clinically significant potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20170442

Individual Results

CHA blend #3 Lot: GH5355

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	DID NOT COMPLETE STUDY								
3	0	0	0	0	0	0	0	0	0	0	---DNC---	
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	**2**[AE1]	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0**
21	0	0	0	0	0	0	0	0	0	0	0	0**
22	0	0	0	0	0	0	0	0	0	0	0	0**
23	0	0	0	0	0	0	0	0	0	0	0	0**
24	0	0	0	0	0	0	0	0	0	0	0	0**
25	0	0	0	0	0	0	0	0	0	0	0	0**
26	0	0	0	0	0	0	0	0	0	0	0	0**
27	0	0	0	0	0	0	0	0	0	0	0	0**
28	0	0	0	0	0	**0.5**	0	0	0	0	0	0**
29	0	0	0	0	0	0	0	0	0	0	0	0**

Day 1* = Supervised removal
** = Subjects 20-29 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
A = Changed to adjacent site
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #3 Lot: GH5355

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0**
31	0	0	0	0	0	0	0	0	0	0	0	0**
32	0	0	0	0	0	0	0	0	0	0	0	0**
33	0	0	0	0	0	0	0	0	0	0	0	0**
34	0	0	0	0	0	0	0	0	0	0	0	0**
35	0	0	0	0	0	0	0	0	0	0	0	0**
36	0	0	0	0	0	0	0	0	0	0	0	0**
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0**
39	0	0	0	0	0	0	0	0	0	0	0	0**
40	0	0	0	0	0	0	0	0	0	0	0	0**
41	0	0	0	DID NOT COMPLETE STUDY								
42	0	0	0	0	**0.5**	0	0	0	**0.5**	**1**	**0.5**	**1[E1]****
43	0	0	0	0	0	0	0	0	0	0	0	0**
44	0	0	0	0	0	0	0	0	DNC			
45	0	0	0	0	0	0	0	0	0	0	0	0**
46	0	0	0	0	0	0	0	0	0	0	0	0**
47	0	0	0	0	0	0	0	0	0	0	0	0**
48	DID NOT COMPLETE STUDY											
49	0	0	0	0	0	0	0	0	0	0[m]	0	0**
50	0	0	0	0	0	0	0	0	0	0	0	0**
51	0	0	0	0	0	0	0	0	0	0	0	0**
52	0	0	0	**0.5**	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
** = Subjects 30–51 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
m = Additional makeup day granted at the discretion of the clinic supervisor
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #3 Lot: GH5355

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	DID NOT COMPLETE STUDY									
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	**1**	**0.5**	**0.5**	**0.5**	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	DID NOT COMPLETE STUDY										
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	DNC			
87	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #3 Lot: GH5355

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
88	0	0	0	0	0	0	0	0	0	0	0†	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	**0.5**	**0.5**	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	**0.5**	**0.5**	0	0	0	0	0	0	---DNC---	
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0[m]	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0[m]	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
† = Late challenge – Subject unable to return due to inclement weather, evaluated on Day 2 and Day 3 post challenge, per deviation

Table 2
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
1	SAV	75	M
2	K-G	17	F
3	RMM	74	F
4	AML	71	F
5	KMR	73	F
6	IGR	16	F
7	DJD	16	F
8	DJD	16	F
9	REV	78	F
10	LMK	77	F
11	KMM	17	F
12	JAP	76	F
13	JIA	16	M
14	RID	75	F
15	IGG	17	M
16	BAJ	78	F
17	BLJ	77	M
18	L-W	71	F
19	DFS	17	M
20	BIH	73	F
21	ANP	77	F
22	LJW	77	F
23	M-P	74	F
24	EMS	74	F
25	RWL	77	M
26	M-C	75	F
27	CMS	72	F
28	DPT	44	M
29	JMM	35	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
30	AMV	22	F
31	CRC	16	F
32	I-M	63	M
33	A-S	51	F
34	RAD	44	M
35	CRD	47	F
36	F-H	24	F
37	TAT	49	F
38	J-V	51	F
39	JCG	24	M
40	KMG	24	F
41	HAB	40	M
42	SMC	49	F
43	J-C	50	M
44	R-M	48	F
45	NLM	29	F
46	RJV	42	F
47	J-M	17	F
48	YMA	43	F
49	DCA	71	F
50	LTB	42	F
51	KDV	16	F
52	JBD	63	F
53	G-G	55	F
54	L-M	33	F
55	W-Z	44	F
56	SNS	64	F
57	C-P	30	F
58	RPK	79	M

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
59	BGM	65	F
60	V-M	61	M
61	G-M	44	F
62	L-S	65	F
63	T-R	48	F
64	SDW	48	F
65	MSA	46	F
66	DAT	49	F
67	EPL	43	F
68	GCL	67	F
69	GVC	19	F
70	G-B	68	F
71	MAM	67	F
72	Y-M	35	F
73	JMR	28	M
74	MJS	72	F
75	LEW	71	M
76	L-T	64	F
77	CAG	34	F
78	ALB	37	M
79	KSD	29	F
80	S-J	70	F
81	SEC	66	F
82	JAM	59	F
83	CFD	75	M
84	LAA	51	M
85	SJA	32	F
86	JMR	20	M
87	X-R	38	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
88	T-N	28	F
89	CJR	21	M
90	R-O	45	F
91	JAS	43	F
92	LAM	53	F
93	RMA	51	F
94	WLK	63	M
95	M-D	74	F
96	G-D	77	M
97	LOH	70	M
98	ACK	73	F
99	JBS	62	F
100	SEP	64	F
101	HSP	66	M
102	LJC	44	F
103	JAA	32	F
104	REC	70	F
105	E-R	24	F
106	WRB	65	M
107	EAC	50	M
108	KRM	55	F
109	PCL	54	F
110	RDS	58	F
111	DLW	48	F
112	HCT	58	M
113	F-G	72	M
114	MAH	71	F

Consumer Product Testing Co.
EST. 1975

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: [redacted]CHA blend #5 Lot: GK9324 caprylhydroxamic acid 7.5% (and) propanediol 92.5%

EXPERIMENT REFERENCE NUMBER: C17-5522.04

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-5522.04

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Nicole Bowley
Quality Assurance Representative

1-17-18
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred fourteen (114) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. One hundred four (104) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: CHA blend #5 Lot: GK9324

Study Schedule:

Panel #	Initiation Date	Completion Date
20170442	November 15, 2017	January 6, 2018

[a]With parental or guardian consent

Methodology:

Prior to the initiation of this study, the test material was prepared as a 4% dilution, using distilled water.

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	=	**No visible skin reaction**	**E**	=	**Edema**
0.5	=	**Barely perceptible**	**D**	=	**Dryness**
1	=	**Mild**	**S**	=	**Staining**
2	=	**Moderate**	**P**	=	**Papules**
3	=	**Marked**	**V**	=	**Vesicles**
4	=	**Severe**	**B**	=	**Bullae**
			U	=	**Ulceration**
			Sp	=	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: Due to the New Year holiday, Subjects #20 – 51, Panel 20170442, were evaluated on Day 1 and Day 2 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Subject #88, Panel 20170442, had a late challenge schedule, but was unable to report on Day 1 post challenge application due to inclement weather. She kept her patch in place and reported on Day 2 and Day 3 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Results: The results of each participant are appended (Table 1).

Subject demographics are presented in Table 2.

Scattered, transient barely perceptible (0.5) to mild (1) erythema with occasional edema responses were noted throughout the test interval. Neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. No evidence of induced allergic contact sensitization was observed.

Summary: Under the conditions of this study, test material, [redacted] Lot: GK9324, indicated no clinically significant potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20170442

Individual Results

[redacted]Lot: GK9324

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	DID NOT COMPLETE STUDY								
3	0	0	0	0	0	0	0	0	0	0	---DNC---	
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	**0.5**	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0**
21	0	0	0	0	0	0	0	0	0	0	0	0**
22	0	0	0	0	0	0	0	0	0	0	0	0**
23	0	0	0	0	0	0	0	0	0	0	0	0**
24	0	0	0	0	0	0	0	0	0	0	0	0**
25	0	0	0	0	0	0	0	0	0	0	0	0**
26	0	0	0	0	0	0	0	0	0	0	0	0**
27	0	0	0	0	0	0	0	0	0	0	0	0**
28	0	0	0	0	0	0	0	0	0	0	0	0**
29	0	0	0	0	0	0	0	0	0	0	0	0**

Day 1* = Supervised removal
** = Subjects 20-29 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

[redacted] Lot: GK9324

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0**
31	0	0	0	0	0	0	0	0	0	0	0	0**
32	0	0	0	0	0	0	0	0	0	0	0	0**
33	0	0	0	0	0	0	0	0	0	0	0	0**
34	0	0	0	0	0	0	0	0	0	0	0	0**
35	0	0	0	0	0	0	0	0	0	0	0	0**
36	0	0	0	0	0	0	0	0	0	0	0	0**
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0**
39	0	0	0	0	0	0	0	0	0	0	0	0**
40	0	0	0	0	0	0	0	0	0	0	0	0**
41	0	0	0	DID NOT COMPLETE STUDY								
42	0	0	0	0	**0.5**	**0.5**	**0.5**	**0.5**[D1]	**0.5**	0	0	**1**[E1]**
43	0	0	0	0	0	0	0	0	0	0	0	0**
44	0	0	0	0	0	0	0	0	DNC			
45	0	0	0	0	0	0	0	0	0	0	0	0**
46	0	0	0	0	0	0	0	0	0	0	0	0**
47	0	0	0	0	0	0	0	0	0	0	0	0**
48	DID NOT COMPLETE STUDY											
49	0	0	0	0	0	0	0	0	0	0[m]	0	0**
50	0	0	0	0	0	0	0	0	0	0	0	0**
51	0	0	0	0	0	0	0	0	0	0	0	0**
52	0	0	0	**0.5**	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
** = Subjects 30–51 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
m = Additional makeup day granted at the discretion of the clinic supervisor

E = Edema
D = Dryness

Table 1
(continued)
Panel #20170442

Individual Results

[redacted] Lot: GK9324

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	DID NOT COMPLETE STUDY									
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	**0.5**	**0.5**	**0.5**	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	DID NOT COMPLETE STUDY										
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	DNC			
87	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

[redacted] Lot: GK9324

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
88	0	0	0	0	0	0	0	0	0	0	0†	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	**0.5**	**1**[E1]	**1**[E1]	**0.5**	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	---DNC---	
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0[m]	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0[m]	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
† = Late challenge – Subject unable to return due to inclement weather, evaluated on Day 2 and Day 3 post challenge, per deviation
E = Edema

Table 2
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
1	SAV	75	M
2	K-G	17	F
3	RMM	74	F
4	AML	71	F
5	KMR	73	F
6	IGR	16	F
7	DJD	16	F
8	DJD	16	F
9	REV	78	F
10	LMK	77	F
11	KMM	17	F
12	JAP	76	F
13	JIA	16	M
14	RID	75	F
15	IGG	17	M
16	BAJ	78	F
17	BLJ	77	M
18	L-W	71	F
19	DFS	17	M
20	BIH	73	F
21	ANP	77	F
22	LJW	77	F
23	M-P	74	F
24	EMS	74	F
25	RWL	77	M
26	M-C	75	F
27	CMS	72	F
28	DPT	44	M
29	JMM	35	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
30	AMV	22	F
31	CRC	16	F
32	I-M	63	M
33	A-S	51	F
34	RAD	44	M
35	CRD	47	F
36	F-H	24	F
37	TAT	49	F
38	J-V	51	F
39	JCG	24	M
40	KMG	24	F
41	HAB	40	M
42	SMC	49	F
43	J-C	50	M
44	R-M	48	F
45	NLM	29	F
46	RJV	42	F
47	J-M	17	F
48	YMA	43	F
49	DCA	71	F
50	LTB	42	F
51	KDV	16	F
52	JBD	63	F
53	G-G	55	F
54	L-M	33	F
55	W-Z	44	F
56	SNS	64	F
57	C-P	30	F
58	RPK	79	M

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
59	BGM	65	F
60	V-M	61	M
61	G-M	44	F
62	L-S	65	F
63	T-R	48	F
64	SDW	48	F
65	MSA	46	F
66	DAT	49	F
67	EPL	43	F
68	GCL	67	F
69	GVC	19	F
70	G-B	68	F
71	MAM	67	F
72	Y-M	35	F
73	JMR	28	M
74	MJS	72	F
75	LEW	71	M
76	L-T	64	F
77	CAG	34	F
78	ALB	37	M
79	KSD	29	F
80	S-J	70	F
81	SEC	66	F
82	JAM	59	F
83	CFD	75	M
84	LAA	51	M
85	SJA	32	F
86	JMR	20	M
87	X-R	38	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
88	T-N	28	F
89	CJR	21	M
90	R-O	45	F
91	JAS	43	F
92	LAM	53	F
93	RMA	51	F
94	WLK	63	M
95	M-D	74	F
96	G-D	77	M
97	LOH	70	M
98	ACK	73	F
99	JBS	62	F
100	SEP	64	F
101	HSP	66	M
102	LJC	44	F
103	JAA	32	F
104	REC	70	F
105	E-R	24	F
106	WRB	65	M
107	EAC	50	M
108	KRM	55	F
109	PCL	54	F
110	RDS	58	F
111	DLW	48	F
112	HCT	58	M
113	F-G	72	M
114	MAH	71	F

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: CHA blend #2 Lot: GK9325 glyceryl caprylate 75% (and) glycerin 15% (and) caprylhydroxamic acid 10%

EXPERIMENT REFERENCE NUMBER: C17-5522.01

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
ESTD 1975

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-5522.01

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Nicole Bowley
Quality Assurance Representative

1-17-18
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred fourteen (114) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. One hundred four (104) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: CHA blend #2 Lot: GK9325

Study Schedule:

Panel #	Initiation Date	Completion Date
20170442	November 15, 2017	January 6, 2018

[a]With parental or guardian consent

Methodology:

Prior to the initiation of this study, the test material was prepared as a 3% dilution, using distilled water.

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	**=**	**No visible skin reaction**	**E**	**=**	**Edema**
0.5	**=**	**Barely perceptible**	**D**	**=**	**Dryness**
1	**=**	**Mild**	**S**	**=**	**Staining**
2	**=**	**Moderate**	**P**	**=**	**Papules**
3	**=**	**Marked**	**V**	**=**	**Vesicles**
4	**=**	**Severe**	**B**	**=**	**Bullae**
			U	**=**	**Ulceration**
			Sp	**=**	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: Due to the New Year holiday, Subjects #20 – 51, Panel 20170442, were evaluated on Day 1 and Day 2 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Subject #88, Panel 20170442, had a late challenge schedule, but was unable to report on Day 1 post challenge application due to inclement weather. She kept her patch in place and reported on Day 2 and Day 3 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Results: The results of each participant are appended (Table 1).

Subject demographics are presented in Table 2.

Scattered, transient barely perceptible (0.5) to mild (1) erythema with occasional edema responses were noted throughout the test interval. Neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. No evidence of induced allergic contact sensitization was observed.

Summary: Under the conditions of this study, test material, CHA blend #2 Lot: GK9325, indicated no clinically significant potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20170442

Individual Results

CHA blend #2 Lot: GK9325

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	DID NOT COMPLETE STUDY								
3	0	0	0	0	0	0	0	0	0	0	---DNC---	
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	**0.5**	**0.5**	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0**
21	0	0	0	0	0	0	0	0	0	0	0	0**
22	0	0	0	0	0	0	0	0	0	0	0	0**
23	0	0	0	0	0	0	0	0	0	0	0	0**
24	0	0	0	0	0	0	0	0	0	0	0	0**
25	0	0	0	0	0	0	0	0	0	0	0	0**
26	0	0	0	0	0	0	0	0	0	0	0	0**
27	0	0	0	0	0	0	0	0	0	0	0	0**
28	0	0	0	0	0	**0.5**	0	0	0	0	0	0**
29	0	0	0	0	0	0	0	0	0	0	0	0**

Day 1* = Supervised removal
** = Subjects 20-29 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #2 Lot: GK9325

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0**
31	0	0	0	0	0	0	0	0	0	0	0	0**
32	0	0	0	0	0	0	0	0	0	0	0	0**
33	0	0	0	0	0	0	0	0	0	0	0	0**
34	0	0	0	0	0	0	0	0	0	0	0	0**
35	0	0	0	0	0	0	0	0	0	0	0	0**
36	0	0	0	0	0	0	0	0	0	0	0	0**
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0**
39	0	0	0	0	0	0	0	0	0	0	0	0**
40	0	0	0	0	0	0	0	0	0	0	0	0**
41	0	0	0	DID NOT COMPLETE STUDY								
42	0	0	0	0	0	**0.5**	**0.5**	0	**0.5**	**1**	**0.5**	**1[E1]****
43	0	0	0	0	0	0	0	0	0	0	0	0**
44	0	0	0	0	0	0	0	**0.5**	DNC			
45	0	0	0	0	0	0	0	0	0	0	0	0**
46	0	0	0	0	0	0	0	0	0	0	0	0**
47	0	0	0	0	0	0	0	0	0	0	0	0**
48	DID NOT COMPLETE STUDY											
49	0	0	0	0	0	0	0	0	0	0[m]	0	0**
50	0	0	0	0	0	0	0	0	0	0	0	0**
51	0	0	0	0	0	0	0	0	0	0	0	0**
52	0	0	0	**0.5**	**0.5**	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
** = Subjects 30–51 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
m = Additional makeup day granted at the discretion of the clinic supervisor
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #2 Lot: GK9325

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	DID NOT COMPLETE STUDY									
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	**0.5**	**0.5**	**0.5**	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	DID NOT COMPLETE STUDY										
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	DNC			
87	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #2 Lot: GK9325

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
88	0	0	0	0	0	0	0	0	0	0	0†	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	**1[E1]**	**1[E1]**	**1[E1]**	**0.5**	**0.5**	**0.5**	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	---DNC---	
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0[m]	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0[m]	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
† = Late challenge – Subject unable to return due to inclement weather, evaluated on Day 2 and Day 3 post challenge, per deviation

E = Edema

Table 2
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
1	SAV	75	M
2	K-G	17	F
3	RMM	74	F
4	AML	71	F
5	KMR	73	F
6	IGR	16	F
7	DJD	16	F
8	DJD	16	F
9	REV	78	F
10	LMK	77	F
11	KMM	17	F
12	JAP	76	F
13	JIA	16	M
14	RID	75	F
15	IGG	17	M
16	BAJ	78	F
17	BLJ	77	M
18	L-W	71	F
19	DFS	17	M
20	BIH	73	F
21	ANP	77	F
22	LJW	77	F
23	M-P	74	F
24	EMS	74	F
25	RWL	77	M
26	M-C	75	F
27	CMS	72	F
28	DPT	44	M
29	JMM	35	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
30	AMV	22	F
31	CRC	16	F
32	I-M	63	M
33	A-S	51	F
34	RAD	44	M
35	CRD	47	F
36	F-H	24	F
37	TAT	49	F
38	J-V	51	F
39	JCG	24	M
40	KMG	24	F
41	HAB	40	M
42	SMC	49	F
43	J-C	50	M
44	R-M	48	F
45	NLM	29	F
46	RJV	42	F
47	J-M	17	F
48	YMA	43	F
49	DCA	71	F
50	LTB	42	F
51	KDV	16	F
52	JBD	63	F
53	G-G	55	F
54	L-M	33	F
55	W-Z	44	F
56	SNS	64	F
57	C-P	30	F
58	RPK	79	M

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
59	BGM	65	F
60	V-M	61	M
61	G-M	44	F
62	L-S	65	F
63	T-R	48	F
64	SDW	48	F
65	MSA	46	F
66	DAT	49	F
67	EPL	43	F
68	GCL	67	F
69	GVC	19	F
70	G-B	68	F
71	MAM	67	F
72	Y-M	35	F
73	JMR	28	M
74	MJS	72	F
75	LEW	71	M
76	L-T	64	F
77	CAG	34	F
78	ALB	37	M
79	KSD	29	F
80	S-J	70	F
81	SEC	66	F
82	JAM	59	F
83	CFD	75	M
84	LAA	51	M
85	SJA	32	F
86	JMR	20	M
87	X-R	38	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
88	T-N	28	F
89	CJR	21	M
90	R-O	45	F
91	JAS	43	F
92	LAM	53	F
93	RMA	51	F
94	WLK	63	M
95	M-D	74	F
96	G-D	77	M
97	LOH	70	M
98	ACK	73	F
99	JBS	62	F
100	SEP	64	F
101	HSP	66	M
102	LJC	44	F
103	JAA	32	F
104	REC	70	F
105	E-R	24	F
106	WRB	65	M
107	EAC	50	M
108	KRM	55	F
109	PCL	54	F
110	RDS	58	F
111	DLW	48	F
112	HCT	58	M
113	F-G	72	M
114	MAH	71	F

Consumer Product Testing Co.
EST. 1975

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: CHA blend #1 Lot: GK9326 caprylhydroxamic acid 15% (and) phenoxyethanol 70% (and) methylpropanediol 7.5% (and) water 7.5%

EXPERIMENT REFERENCE NUMBER: C17-5522.03

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1973

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-5522.03

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Nicole Bowley
Quality Assurance Representative

1-17-18
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred fourteen (114) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. One hundred four (104) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: CHA blend #1 Lot: GK9326

Study Schedule:

Panel #	Initiation Date	Completion Date
20170442	November 15, 2017	January 6, 2018

[a]With parental or guardian consent

Methodology:

Prior to the initiation of this study, the test material was prepared as a 2% dilution, using distilled water.

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	**=**	**No visible skin reaction**	**E**	**=**	**Edema**
0.5	**=**	**Barely perceptible**	**D**	**=**	**Dryness**
1	**=**	**Mild**	**S**	**=**	**Staining**
2	**=**	**Moderate**	**P**	**=**	**Papules**
3	**=**	**Marked**	**V**	**=**	**Vesicles**
4	**=**	**Severe**	**B**	**=**	**Bullae**
			U	**=**	**Ulceration**
			Sp	**=**	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: Due to the New Year holiday, Subjects #20 – 51, Panel 20170442, were evaluated on Day 1 and Day 2 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Subject #88, Panel 20170442, had a late challenge schedule, but was unable to report on Day 1 post challenge application due to inclement weather. She kept her patch in place and reported on Day 2 and Day 3 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Results: The results of each participant are appended (Table 1).

Subject demographics are presented in Table 2.

Scattered, transient barely perceptible (0.5) to moderate (2) erythema with occasional edema responses were noted throughout the test interval. Neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. No evidence of induced allergic contact sensitization was observed.

Summary: Under the conditions of this study, test material, CHA blend #1 Lot: GK9326, indicated no clinically significant potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20170442

Individual Results

CHA blend #1 Lot: GK9326

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	DID NOT COMPLETE STUDY								
3	0	0	0	0	0	0	0	0	0	0	---DNC---	
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	**2**[AE1]	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0**
21	0	0	0	0	0	0	0	0	0	0	0	0**
22	0	0	0	0	0	0	0	0	0	0	0	0**
23	0	0	0	0	0	0	0	0	0	0	0	0**
24	0	0	0	0	0	0	0	0	0	0	0	0**
25	0	0	0	0	0	0	0	0	0	0	0	0**
26	0	0	0	0	0	0	0	0	0	0	0	0**
27	0	0	0	0	0	0	0	0	0	0	0	0**
28	0	0	0	0	0	**0.5**	0	0	0	0	0	0**
29	0	0	0	0	0	0	0	0	0	0	0	0**

Day 1* = Supervised removal
** = Subjects 20-29 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
A = Changed to adjacent site
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #1 Lot: GK9326

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0**
31	0	0	0	0	0	0	0	0	0	0	0	0**
32	0	0	0	0	0	0	0	0	0	0	0	0**
33	0	0	0	0	0	0	0	0	0	0	0	0**
34	0	0	0	0	0	0	0	0	0	0	0	0**
35	0	0	0	0	0	0	0	0	0	0	0	0**
36	0	0	0	0	0	0	0	0	0	0	0	0**
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0**
39	0	0	0	0	0	0	0	0	0	0	0	0**
40	0	0	0	0	0	0	0	0	0	0	0	0**
41	0	0	0	DID NOT COMPLETE STUDY								
42	0	0	0	0	0	**0.5**	**0.5**	0	**0.5**	**1**	**0.5**	**1[E1]****
43	0	0	0	0	0	0	0	0	0	0	0	0**
44	0	0	0	0	0	0	0	0	DNC			
45	0	0	0	0	0	0	0	0	0	0	0	0**
46	0	0	0	0	0	0	0	0	0	0	0	0**
47	0	0	0	0	0	0	0	0	0	0	0	0**
48	DID NOT COMPLETE STUDY											
49	0	0	0	0	0	0	0	0	0	0[m]	0	0**
50	0	0	0	0	0	0	0	0	0	0	0	0**
51	0	0	0	0	0	0	0	0	0	0	0	0**
52	0	0	0	**0.5**	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
** = Subjects 30–51 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
m = Additional makeup day granted at the discretion of the clinic supervisor
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #1 Lot: GK9326

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	DID NOT COMPLETE STUDY									
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	**0.5**	**0.5**	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	DID NOT COMPLETE STUDY										
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	DNC			
87	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #1 Lot: GK9326

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
88	0	0	0	0	0	0	0	0	0	0	0†	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	**1**[E1]	**1**[E1]	**1**[E1]	**0.5**	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	---DNC---	
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0[m]	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0[m]	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
† = Late challenge – Subject unable to return due to inclement weather, evaluated on Day 2 and Day 3 post challenge, per deviation
E = Edema

Table 2
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
1	SAV	75	M
2	K-G	17	F
3	RMM	74	F
4	AML	71	F
5	KMR	73	F
6	IGR	16	F
7	DJD	16	F
8	DJD	16	F
9	REV	78	F
10	LMK	77	F
11	KMM	17	F
12	JAP	76	F
13	JIA	16	M
14	RID	75	F
15	IGG	17	M
16	BAJ	78	F
17	BLJ	77	M
18	L-W	71	F
19	DFS	17	M
20	BIH	73	F
21	ANP	77	F
22	LJW	77	F
23	M-P	74	F
24	EMS	74	F
25	RWL	77	M
26	M-C	75	F
27	CMS	72	F
28	DPT	44	M
29	JMM	35	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
30	AMV	22	F
31	CRC	16	F
32	I-M	63	M
33	A-S	51	F
34	RAD	44	M
35	CRD	47	F
36	F-H	24	F
37	TAT	49	F
38	J-V	51	F
39	JCG	24	M
40	KMG	24	F
41	HAB	40	M
42	SMC	49	F
43	J-C	50	M
44	R-M	48	F
45	NLM	29	F
46	RJV	42	F
47	J-M	17	F
48	YMA	43	F
49	DCA	71	F
50	LTB	42	F
51	KDV	16	F
52	JBD	63	F
53	G-G	55	F
54	L-M	33	F
55	W-Z	44	F
56	SNS	64	F
57	C-P	30	F
58	RPK	79	M

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
59	BGM	65	F
60	V-M	61	M
61	G-M	44	F
62	L-S	65	F
63	T-R	48	F
64	SDW	48	F
65	MSA	46	F
66	DAT	49	F
67	EPL	43	F
68	GCL	67	F
69	GVC	19	F
70	G-B	68	F
71	MAM	67	F
72	Y-M	35	F
73	JMR	28	M
74	MJS	72	F
75	LEW	71	M
76	L-T	64	F
77	CAG	34	F
78	ALB	37	M
79	KSD	29	F
80	S-J	70	F
81	SEC	66	F
82	JAM	59	F
83	CFD	75	M
84	LAA	51	M
85	SJA	32	F
86	JMR	20	M
87	X-R	38	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
88	T-N	28	F
89	CJR	21	M
90	R-O	45	F
91	JAS	43	F
92	LAM	53	F
93	RMA	51	F
94	WLK	63	M
95	M-D	74	F
96	G-D	77	M
97	LOH	70	M
98	ACK	73	F
99	JBS	62	F
100	SEP	64	F
101	HSP	66	M
102	LJC	44	F
103	JAA	32	F
104	REC	70	F
105	E-R	24	F
106	WRB	65	M
107	EAC	50	M
108	KRM	55	F
109	PCL	54	F
110	RDS	58	F
111	DLW	48	F
112	HCT	58	M
113	F-G	72	M
114	MAH	71	F

Consumer Product Testing Co.
EST. 1973

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: CHA blend #4 Lot: GK9322 caprylhydroxamic acid 15% (and) caprylyl glycol 71% (and) glycerin 14%

EXPERIMENT REFERENCE NUMBER: C17-5522.02

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-5522.02

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Nicole Bowley
Quality Assurance Representative

1-17-18
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred fourteen (114) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. One hundred four (104) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: CHA blend #4 Lot: GK9322

Study Schedule:

Panel #	Initiation Date	Completion Date
20170442	November 15, 2017	January 6, 2018

[a]With parental or guardian consent

Methodology: Prior to the initiation of this study, the test material was prepared as a 2% dilution, using distilled water.

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	**=**	**No visible skin reaction**	**E**	**=**	**Edema**
0.5	**=**	**Barely perceptible**	**D**	**=**	**Dryness**
1	**=**	**Mild**	**S**	**=**	**Staining**
2	**=**	**Moderate**	**P**	**=**	**Papules**
3	**=**	**Marked**	**V**	**=**	**Vesicles**
4	**=**	**Severe**	**B**	**=**	**Bullae**
			U	**=**	**Ulceration**
			Sp	**=**	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: Due to the New Year holiday, Subjects #20 – 51, Panel 20170442, were evaluated on Day 1 and Day 2 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Subject #88, Panel 20170442, had a late challenge schedule, but was unable to report on Day 1 post challenge application due to inclement weather. She kept her patch in place and reported on Day 2 and Day 3 post challenge application. It was the Principal Investigator's opinion that this did not affect test results, since observations remained negative.

Results: The results of each participant are appended (Table 1).

Subject demographics are presented in Table 2.

Scattered, transient barely perceptible (0.5) to moderate (2) erythema with occasional edema responses were noted throughout the test interval. Neither the number of responses or the peak level of these responses were inconsistent with similar diluted formulations evaluated under repetitive, occlusive patch conditions. No evidence of induced allergic contact sensitization was observed.

Summary: Under the conditions of this study, test material, CHA blend #4 Lot: GK9322, indicated no clinically significant potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20170442

Individual Results

CHA blend #4 Lot: GK9322

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	DID NOT COMPLETE STUDY								
3	0	0	0	0	0	0	0	0	0	0	---DNC---	
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	2[AE1]	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0**
21	0	0	0	0	0	0	0	0	0	0	0	0**
22	0	0	0	0	0	0	0	0	0	0	0	0**
23	0	0	0	0	0	0	0	0	0	0	0	0**
24	0	0	0	0	0	0	0	0	0	0	0	0**
25	0	0	0	0	0	0	0	0	0	0	0	0**
26	0	0	0	0	0	0	0	0	0	0	0	0**
27	0	0	0	0	0	0	0	0	0	0	0	0**
28	0	0	0	0	0	0	0	0	0	0	0	0**
29	0	0	0	0	0	0	0	0	0	0	0	0**

Day 1* = Supervised removal
** = Subjects 20-29 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
A = Changed to adjacent site
E = Edema

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #4 Lot: GK9322

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0**
31	0	0	0	0	0	0	0	0	0	0	0	0**
32	0	0	0	0	0	0	0	0	0	0	0	0**
33	0	0	0	0	0	0	0	0	0	0	0	0**
34	0	0	0	0	0	0	0	0	0	0	0	0**
35	0	0	0	0	0	0	0	0	0	0	0	0**
36	0	0	0	0	0	0	0	0	0	0	0	0**
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0**
39	0	0	0	0	0	0	0	0	0	0	0	0**
40	0	0	0	0	0	0	0	0	0	0	0	0**
41	0	0	0	DID NOT COMPLETE STUDY								
42	0	0	0	0	0	**0.5**	**0.5**	**0.5**	**0.5**	0	0	**0.5****
43	0	0	0	0	0	0	0	0	0	0	0	0**
44	0	0	0	0	0	0	0	0	DNC			
45	0	0	0	0	0	0	0	0	0	0	0	0**
46	0	0	0	0	0	0	0	0	0	0	0	0**
47	0	0	0	0	0	0	0	0	0	0	0	0**
48	DID NOT COMPLETE STUDY											
49	0	0	0	0	0	0	0	0	0	0[m]	0	0**
50	0	0	0	0	0	0	0	0	0	0	0	0**
51	0	0	0	0	0	0	0	0	0	0	0	0**
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
** = Subjects 30–51 evaluated Day 1 and Day 2 post challenge application, per deviation
DNC = Did not complete study
m = Additional makeup day granted at the discretion of the clinic supervisor

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #4 Lot: GK9322

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	DID NOT COMPLETE STUDY									
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	**0.5**	**0.5**	**0.5**	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	DID NOT COMPLETE STUDY										
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	DNC			
87	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
DNC = Did not complete study

Table 1
(continued)
Panel #20170442

Individual Results

CHA blend #4 Lot: GK9322

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day1*	Day 3
88	0	0	0	0	0	0	0	0	0	0	0†	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	**1**[E1]	**1**[E1]	**1**[E1]	**0.5**	**0.5**	**0.5**	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	---DNC---	
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0[m]	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0[m]	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study
† = Late challenge – Subject unable to return due to inclement weather, evaluated on Day 2 and Day 3 post challenge, per deviation

E = Edema

Table 2
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
1	SAV	75	M
2	K-G	17	F
3	RMM	74	F
4	AML	71	F
5	KMR	73	F
6	IGR	16	F
7	DJD	16	F
8	DJD	16	F
9	REV	78	F
10	LMK	77	F
11	KMM	17	F
12	JAP	76	F
13	JIA	16	M
14	RID	75	F
15	IGG	17	M
16	BAJ	78	F
17	BLJ	77	M
18	L-W	71	F
19	DFS	17	M
20	BIH	73	F
21	ANP	77	F
22	LJW	77	F
23	M-P	74	F
24	EMS	74	F
25	RWL	77	M
26	M-C	75	F
27	CMS	72	F
28	DPT	44	M
29	JMM	35	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
30	AMV	22	F
31	CRC	16	F
32	I-M	63	M
33	A-S	51	F
34	RAD	44	M
35	CRD	47	F
36	F-H	24	F
37	TAT	49	F
38	J-V	51	F
39	JCG	24	M
40	KMG	24	F
41	HAB	40	M
42	SMC	49	F
43	J-C	50	M
44	R-M	48	F
45	NLM	29	F
46	RJV	42	F
47	J-M	17	F
48	YMA	43	F
49	DCA	71	F
50	LTB	42	F
51	KDV	16	F
52	JBD	63	F
53	G-G	55	F
54	L-M	33	F
55	W-Z	44	F
56	SNS	64	F
57	C-P	30	F
58	RPK	79	M

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
59	BGM	65	F
60	V-M	61	M
61	G-M	44	F
62	L-S	65	F
63	T-R	48	F
64	SDW	48	F
65	MSA	46	F
66	DAT	49	F
67	EPL	43	F
68	GCL	67	F
69	GVC	19	F
70	G-B	68	F
71	MAM	67	F
72	Y-M	35	F
73	JMR	28	M
74	MJS	72	F
75	LEW	71	M
76	L-T	64	F
77	CAG	34	F
78	ALB	37	M
79	KSD	29	F
80	S-J	70	F
81	SEC	66	F
82	JAM	59	F
83	CFD	75	M
84	LAA	51	M
85	SJA	32	F
86	JMR	20	M
87	X-R	38	F

Table 2
(continued)
Panel #20170442

Subject Demographics

Subject Number	Initials	Age	Gender
88	T-N	28	F
89	CJR	21	M
90	R-O	45	F
91	JAS	43	F
92	LAM	53	F
93	RMA	51	F
94	WLK	63	M
95	M-D	74	F
96	G-D	77	M
97	LOH	70	M
98	ACK	73	F
99	JBS	62	F
100	SEP	64	F
101	HSP	66	M
102	LJC	44	F
103	JAA	32	F
104	REC	70	F
105	E-R	24	F
106	WRB	65	M
107	EAC	50	M
108	KRM	55	F
109	PCL	54	F
110	RDS	58	F
111	DLW	48	F
112	HCT	58	M
113	F-G	72	M
114	MAH	71	F

Clinical
Research
Laboratories, Inc.

Final Report

Repeated Insult Patch Test

CLIENT: [redacted]

ATTENTION: Mr. Jeffrey Parker
Personal Care Technology Manager

TEST MATERIAL: caprylohydroxamic acid

CRL STUDY NUMBER: CRL16108

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscatiello, Ph.D.
Executive Vice President/COO

George J. Neumaier, M.D.
Diplomate American Board
of Dermatology

REPORT DATE: March 31, 2008

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical Research Laboratories, Inc.

Good Clinical Practice Quality Assurance Audit Statement

Clinical Study Number: CRL16108

Start Date: February 11, 2008

Completion Date: March 21, 2008

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, Inc. Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance Representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted, as indicated below.

Karen Hayley
Signature of QA Auditor

March 31, 2008
Date

Clinical Research Laboratories, Inc.

FINAL REPORT

REPEATED INSULT PATCH TEST

PURPOSE

The purpose of this study was to determine the dermal irritation and sensitization potential of a test material.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc.
371 Hoes Lane
Piscataway, New Jersey 08854
732-981-1616

TEST MATERIAL

The following test material was provided by [redacted] and was received by Clinical Research Laboratories, Inc. on February 6, 2008:

Test Material	Test Condition	Patch Type
caprylohydroxamic acid	Test as received	Semi-occlusive*

The test material was coded with the following CRL identification number:

CRL16108

STUDY DATES

This study was initiated on February 11, 2008 and was completed on March 21, 2008.

* Semi-occlusive Strip (TruMed Technologies Inc., Burnsville, Minnesota)

Clinical Research Laboratories, Inc.

PANEL SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects" and a HIPAA Authorization Form in compliance with 45 CFR Parts 160 and 164. All subjects completed a Subject Profile/Medical History Form provided by Clinical Research Laboratories, Inc. prior to the study (Subject Demographics - Appendix I). Subjects who met the following criteria were impaneled:

- Male and female panelists between the ages of 18 and 70;
- Subjects who have completed a Panelist Profile/Medical History;
- Subjects who are in general good health as determined by a Panelist Profile/Medical History;
- Subjects who do not exhibit any skin diseases that might be confused with a skin reaction from the test material;
- Subjects who agree to avoid exposure of the test sites to the sun and to refrain from visits to tanning salons during the course of this study;
- Subjects willing to sign an Informed Consent Form in conformance with 21 CFR Part 50: "Protection of Human Subjects";
- Subjects who have completed a HIPAA Authorization Form in conformance with 45 CFR Parts 160 and 164;
- Females who are not pregnant or lactating;
- Subjects who demonstrate dependability and intelligence in following directions;
- Subjects who are not currently using any systemic or topical corticosteroids, anti-inflammatory drugs or antihistamines;
- Subjects who do not exhibit skin disorder, sunburn, scars, excessive tattoos, etc. in the test area.

Clinical Research Laboratories, Inc.

TEST METHOD

Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material, which was prepared as described in the Test Material section of the report, was applied to the upper back (between the scapulae) and was allowed to remain in direct skin contact for a period of 24 hours.

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the Induction Period. This schedule may have been modified to allow for missed visits or holidays. If a subject was unable to report on an assigned test date, the test material was applied on 2 consecutive days during the Induction Phase and/or a makeup day was added at the end of the Induction Phase.

The sites were graded by a CRL technician for dermal irritation 24 hours after removal of the patches by the subjects on Tuesday and Thursday and 48 hours after removal of the patches on Saturday, unless the patching schedule was altered as described above.

The sites were graded according to the following scoring system:

Dermal Scoring Scale

0 No visible skin reaction
± Barely perceptible erythema
1+ Mild erythema
2+ Well defined erythema
3+ Erythema and edema
4+ Erythema and edema with vesiculation

If a "2+" reaction or greater occurred, the test material was applied to an adjacent virgin site. If a "2+" reaction or greater occurred on the new site, the subject was not patched again during the Induction Phase but was challenged on the appropriate day of the study. At the discretion of the Study Director, patch sites with scores less than a "2+" may have been changed.

Following approximately a 2-week rest period, the challenge patches were applied to previously untreated test sites on the back. After 24 hours, the patches were removed by a CRL technician and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 and 72 hours. Subjects exhibiting reactions during the Challenge Phase of the study may have been asked to return for a 96-hour reading.

Clinical Research Laboratories, Inc.

RESULTS

This study was initiated with 56 subjects. Four subjects discontinued study participation for reasons unrelated to the test material. A total of 52 subjects completed the study.

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

CONCLUSION

Based on the test population of 52 subjects and under the conditions of this study, the test material identified as caprylohydroxamic acid did not demonstrate a potential for eliciting dermal irritation or sensitization.

RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, Inc. as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

Clinical Research Laboratories, Inc.

TABLE I

Summary of Dermal Scores

Test Material:	caprylohydroxamic acid

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	X	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	X	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	DISCONTINUED							
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent

Clinical Research Laboratories, Inc.

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	caprylohydroxamic acid

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	DISCONTINUED									
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	DISCONTINUED										
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0

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TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	caprylohydroxamic acid

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
51	DISCONTINUED											
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0

Clinical Research Laboratories, Inc.

Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1	JD	01639	54	F
2	AI	21944	55	F
3	TK	21092	38	F
4	NP	06660	48	F
5	BW	17003	57	F
6	MV	06227	52	F
7	JS	16720	23	F
8	EP	19636	58	F
9	CC	21631	37	F
10	BW	23307	47	F
11	RP	16060	25	F
12	PP	22202	55	M
13	JP	17665	35	F
14	GT	20882	55	M
15	MJ	04746	39	F
16	AK	22180	24	F
17	GN	21424	25	F
18	AD	20275	44	F
19	RA	22910	36	F
20	AC	21579	63	F
21	MC	06209	46	F
22	FR	21069	38	F
23	YP	11690	51	F
24	LL	08362	54	F
25	NJ	21262	53	F
26	SB	22235	61	M
27	SP	15058	52	F
28	SR	15278	43	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
29	DR	15821	46	F
30	AM	06580	37	F
31	CP	19477	50	F
32	EI	20400	37	F
33	LP	17996	39	M
34	AS	02343	58	F
35	RR	03308	68	F
36	SS	05426	45	F
37	SM	23337	27	F
38	AV	22838	28	F
39	JS	03048	43	F
40	PD	23040	28	M
41	FJ	18919	54	M
42	SP	17681	50	F
43	AC	06211	19	M
44	JM	02090	25	F
45	SM	01067	55	F
46	RS	19634	29	F
47	OS	21264	27	M
48	LN	19575	37	F
49	SJ	06312	31	F
50	CK	22181	31	M
51	JF	23342	22	F
52	MM	15443	46	F
53	AR	19479	24	F
54	BL	16445	60	F
55	LZ	08758	43	F
56	SE	20804	49	F

MB Research Laboratories

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Study Title	:	Bovine Corneal Opacity and Permeability Test (BCOP)
Test Article	:	CAPRYLHYDROXAMIC ACID
Author	:	Debra A. Hall, LATG, Study Director
Study Completed On	:	January 7, 2011
Performing Laboratory	:	MB Research Laboratories 1765 Wentz Road P.O. Box 178 Spinnerstown, PA 18968
MB Research Project #	:	MB 10-19564.09
MB Research Protocol #	:	441-05
Sponsor	:	[redacted]
Citation	:	Debra A. Hall, LATG (2011) Unpublished Report by MB Research Laboratories

MB Research Labs

Study Title	:	**BCOP**
Project #	:	**MB 10-19564.09**
Test article	:	**CAPRYLHYDROXAMIC ACID**
Protocol	:	**441-05**

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted in accordance with applicable Good Laboratory Practices regulations of the EPA, 40 CFR Part 160, and the FDA, 21 CFR Part 58.

STUDY DIRECTOR: Debra A. Hall 1-7-11

Debra A. Hall, LATG Date
MB RESEARCH LABORATORIES

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MB Research Labs

PROJECT NUMBER	:	**MB 10-19564.09**
TEST ARTICLE	:	**CAPRYLHYDROXAMIC ACID**
SPONSOR	:	[redacted]
TITLE	:	**Bovine Corneal Opacity and Permeability Test (BCOP)**
PROTOCOL #	:	**441-05**

ABSTRACT

Objective: To determine the potential for ocular irritation using an alternative to the Draize methodology. This protocol is based on the methodology described in *Bovine Corneal Opacity and Permeability Test: An In-Vitro Assay of Ocular Irritancy*, (1992); Gautheron, Pierre; Dukic, Martine; Alix, Danielle and Sina, Joseph F.; Fundamental and Applied Toxicology 18, 442-449 and includes an analysis based on OECD Guideline for the Testing of Chemicals #437, adopted September 7, 2009.

Method Synopsis: Five corneas were dosed with 0.75 ml of a 20% solution of CAPRYLHYDROXAMIC ACID. Opacity measurements and sodium fluorescein permeability were determined.

Summary: The corrected mean opacity score was 10.5. The corrected mean optical density (permeability) score was 0.108.

Conclusion: The *in vitro* score was calculated as 12.12.

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MB Research Labs

Study Title	:	**BCOP**
Project #	:	**MB 10-19564.09**
Test article	:	**CAPRYLHYDROXAMIC ACID**
Protocol	:	**441-05**

OBJECTIVE

To determine the potential for ocular irritation using an alternative to the Draize methodology. This protocol is based on the methodology described in *Bovine Corneal Opacity and Permeability Test: An In-Vitro Assay of Ocular Irritancy*, (1992); Gautheron, Pierre; Dukic, Martine; Alix, Danielle and Sina, Joseph F.; Fundamental and Applied Toxicology 18, 442-449.

TEST ARTICLE

Identity	:	CAPRYLHYDROXAMIC ACID
Test Article Characterization	:	See Appendix A for Test Article Characterization.
Stability	:	See Appendix A for stability information.
Source	:	[redacted]
Date Received	:	10/15/10
Storage	:	Room temperature and humidity.
Description	:	White powder
Sample Preparation	:	2 g of test article were mixed with Minimal Essential Media (MEM) to a total volume of 10 ml, then dosed from a stir plate. (White liquid)

TEST DATES

Study Initiation:	(date protocol signed)	:	12/14/10
Experimental Start Date	(1st exposure to test substance)	:	12/16/10
Experimental Term Date	(last date data collected)	:	12/16/10
Draft Report Submitted	(if applicable)	:	01/06/11
Final Report Signed	(submission of final report)	:	01/07/11

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Study Title	:	**BCOP**
Project #	:	**MB 10-19564.09**
Test article	:	**CAPRYLHYDROXAMIC ACID**
Protocol	:	**441-05**

EXPERIMENTAL DESIGN

Test System

The bovine eyes were received from Spear Products on 12/16/10 and transported to MB Research in Hank's Balanced Salt Solution in a refrigerated container.

Pretest Procedures

Fresh assay solutions were prepared prior to use. Minimum Essential Media (MEM) solution was prepared by stirring together one jar of MEM powder (sufficient to make one liter of solution), 2.2 g Sodium Bicarbonate, 0.292 g L-Glutamine, 10 ml of Fetal Bovine Serum (FBS) and 1000 ml distilled water. The MEM solution was kept in a incubator for the duration of testing. Hanks Balanced Salt Solution (HBSS) was prepared by stirring together one jar of HBSS powder (sufficient to make one liter), 0.35 g Sodium Bicarbonate and 1000 ml distilled water. HBSS was maintained at room temperature.

The eyes were examined within one hour after receipt. Any eye with a cornea exhibiting evidence of vascularization, pigmentation, opacity or scratches was discarded.

Corneas from eyes that were free of defects were dissected from the surrounding tissues. A 2-3 mm rim of sclera was left attached to each cornea. The corneas were then placed in a container of fresh Hank's Balanced Salt Solution (HBSS).

The dissected corneas were mounted in specially designed holders that were separated into anterior and posterior chambers and filled separately. Each cornea was mounted allowing the epithelium of the cornea to project into the anterior chamber. The posterior chamber was filled with MEM solution ensuring contact with the endothelium. The anterior chamber was filled with MEM solution, ensuring contact with the epithelium. Each cornea was visually inspected again to insure there were no defects.

The entire holder with the cornea was then incubated at 32°C (± 2 °C) and allowed to equilibrate for at least one hour, but not longer than 2 hours.

Following the equilibration, the holders containing the corneas were removed from the incubator. The MEM solution was removed from both chambers and the chambers refilled with fresh MEM solution. At this time, five corneas were selected for dosing with the test article and two were selected as controls.

A pre-exposure determination of opacity was made for each control by measuring each against the blanks supplied by the opacitometer. A pre-exposure determination of opacity was made for each test cornea by measuring against each control cornea (a total of 10 determinations).

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EXPERIMENTAL DESIGN (cont'd)

Study Procedure

Following the pretest observations, the MEM solution was removed from the anterior chamber and 0.75 ml of the test article mixture was applied to the epithelium of each of the five treated corneas.

The holders and corneas were then placed in the incubator at 32°C (± 2 °C) in a horizontal position to insure contact of the test article with the corneas. After four hours, the test substance (or MEM solution in the controls) was removed from the epithelium of the cornea and anterior chamber of the holder by washing with MEM solution. The anterior and posterior chambers of the holders were then refilled with fresh MEM solution and opacity measurements were made taken with each treated cornea compared to each of the two control corneas. Opacity measurement of the cornea was made using an OP-KIT opacitometer produced by Electro-Design Corporation of Riom, France.

Immediately following the four hour opacity measurement, the MEM solution was removed from the anterior chamber and replaced with 1.0 ml of 0.5% solution of sodium fluorescein in Dulbecco's Phosphate Buffered Saline (DPBS). Each holder was then returned to the 32°C (± 2 °C) incubator in a horizontal position insuring contact of the fluorescein with the cornea.

After 90 minutes, the fluid from the posterior chamber was removed and the amount of dye that passed through the cornea was measured as the optical density at 490 nm by spectrophotometric analysis.

EXPERIMENTAL DESIGN (cont'd)

Analysis of Data

The Corrected Mean Opacity Score was calculated using the control and treated cornea opacity values as determined from the OP-KIT. The corrected Mean Optical Density Score was calculated using the control and treated Optical Density values from the fluorescein permeability analysis. The *in vitro* score was calculated as follows:

***In Vitro* Score = Corrected Mean Opacity Score + 15 (Corrected Mean Optical Density Score)**

The general classification scheme of Gautheron et al. (1992) indicated that the *in vitro* scores can be interpreted as follows:

In-Vitro Score	Classification
0 - 25	Mild Irritant
25.1 to 55	Moderate Irritant
55.1 and above	Severe Irritant

OECD Guideline #437 defines a substance, which produces an In Vitro score of ≥ 55.1 as a corrosive or severe irritant.

Further interpretation of irritancy may also be made within the classification as more information on responses from various chemicals becomes available.

Retention of Data

Upon signing the final report, all raw data, supporting documentation and reports are submitted to the Archivist by the Study Director. The raw data is filed at MB Research by project number. The final report is filed at MB Research by Sponsor name and MB project number.

Any remaining test article will be discarded following submission of the report.

Amendment to the Protocol

There were no amendments to the protocol.

MB Research Labs

Study Title : BCOP
Project # : MB 10-19564.09
Test article : CAPRYLHYDROXAMIC ACID
Protocol : 441-05

RESULTS

INDIVIDUAL CONTROL SCORES FOR BCOP

Cornea #:	Pretest	4 Hours	O.D. Scores
C3	0	0	0.017
C4	0	0	0.024
MEAN	0	0	0.021
Corrected Mean Control Opacity Score[1]	0		

INDIVIDUAL TEST SCORES

CORNEA #	Pretest Scores				4 Hour Scores				O.D. Scores
1	C3	-3	C4	-3	C3	7	C4	7	0.170
2	C3	-5	C4	-5	C3	8	C4	8	0.101
3	C3	-3	C4	-3	C3	7	C4	7	0.150
4	C3	-5	C4	-5	C3	5	C4	5	0.107
5	C3	-4	C4	-4	C3	6	C4	5	0.117

CALCULATED SCORES

Cornea #	Corrected Opacity Scores				Corrected O.D.
4 Hour Scores					
1	C3	10	C4	10	0.149
2	C3	13	C4	13	0.080
3	C3	10	C4	10	0.129
4	C3	10	C4	10	0.086
5	C3	10	C4	9	0.096
Corrected Mean Optical Density =					0.108
Corrected Mean Opacity Score[2] =					10.5

Calculated In Vitro Score
10.5 + 15 (0.108)
10.5 + (1.62)
12.12

[1]Corrected Mean Control Opacity Score = 4 hour mean score minus pretest mean score
[2]Corrected Mean Opacity Score = mean treated opacity score minus corrected mean control opacity score

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MB Research Labs

Study Title	:	**BCOP**
Project #	:	**MB 10-19564.09**
Test article	:	**CAPRYLHYDROXAMIC ACID**
Protocol	:	**441-05**

DISCUSSION

The corrected mean opacity score was 10.5. The corrected mean optical density (permeability) score was 0.108.

CONCLUSION

The *in vitro* score was calculated as 12.12.

FINAL REPORT

Approved by: Debra A. Hall 1-7-11

Debra A. Hall, LATG
Study Director

Date

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MB Research Labs

Study Title : BCOP
Project # : MB 10-19564.09
Test article : CAPRYLHYDROXAMIC ACID
Protocol : 441-05

QUALITY ASSURANCE EVALUATION

The Quality Assurance Unit has inspected a critical phase of this study, audited the raw data and the report and determined that the methods and results contained herein accurately reflect the raw data. A summary of the compliance inspections is presented below.

Date of Inspection	Phase	Performed By	Date Inspection Results Reported	
			Sty. Dir.	Mgmt.
12/16/10	Dose Administration	Matt Lowrie	12/16/10	12/16/10
12/21/10	Raw data audit	Matt Lowrie	12/21/10	12/21/10
01/06/11	Draft report audit	Krista A. Stayer	01/06/11	01/07/11
01/07/11	Final report audit	Krista A. Stayer	01/07/11	01/07/11

Krista A. Stayer 1/7/11
Krista A. Stayer Date
Quality Assurance Unit

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SPONSOR
TEST ARTICLE CHARACTERIZATION INFORMATION

In compliance with Good Laboratory Practice (GLP) regulations, a characterization of the test article is required and should include identity, strength, purity, composition, stability and uniformity. This data must be reviewed by the Study Director prior to study initiation and will be included in the final report. (EPA 40 CFR 160.105 and 792.105; FDA 21 CFR 58.105, OECD 6.2).

In addition, the test article characterization should be performed in compliance with the Good Laboratory Practices.

Any exceptions to the GLP requirements will be indicated in the Compliance Statement of the final report.

Accordingly, please supply the following information for each test article submitted:

Proprietary is defined for this form as known by the Sponsor, but confidential.

JE=AA 12/09/10

Test Article Identity caprylhydroxamic acid (CAS# 7377-03-9)_____Lot/Batch#_____

Storage ■ Room Temperature ☐ Refrigerate(2-8°C) ☐ Other: _____

Stability Room temperature _____ ☐ unknown ☐ proprietary

Purity 100% _____ ☐ unknown ☐ proprietary

Strength Not a mixture _____ ☐ unknown ☐ proprietary

Composition 100% caprylhydroxamic acid_____ ☐ unknown ☐ proprietary

Uniformity Homogeneous _____ ☐ unknown ☐ proprietary

■ This characterization **was** conducted under GLPs

■ This characterization **was** conducted under GMPs

☐ This characterization **was not** conducted under GLPs **or** GMPs.

[redacted]

~~DAS per Sponsor's e-mail on 10/26/10, this is the same GLs.~~ JE=AA 12/09/10

MB Research Laboratories

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Study Title	:	MatTek EpiOcular™ MTT Viability Assay
Test Article	:	[redacted]CHA (Caprylhydroxamic Acid), Lot/Batch# HJ4059
Positive Control	:	0.3% Triton® X-100, Lot# 122109TTB
Negative Control	:	Tissue culture water, Lot# 128K2318 (TCH_2O)
Author	:	Michelle Piehl, Ph.D, Study Director
Study Completed On	:	February 18, 2010
Performing Laboratory	:	MB Research Laboratories 1765 Wentz Road P.O. Box 178 Spinnerstown, PA 18968
MB Research Project #	:	MB 10-18732.19
MB Research Protocol #	:	720-03
Sponsor	:	[redacted]
Citation	:	Michelle Piehl, Ph.D (2010) Unpublished Report by MB Research Laboratories

MB Research Laboratories

Study Title : MatTek EpiOcular™ Assay
Project # : MB 10-18732.19
Protocol : 720-03

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted in accordance with the Good Laboratory Practice requirements of EPA, 40 CFR 160 and 792, FDA 21 CFR 58, and as specified in Principles on Good Laboratory Practices, published by the Organization for Economic Cooperation & Development (OECD), 1997, with the following exception:

Test article characterization was not conducted according to the Good Laboratory Practices.

STUDY DIRECTOR:

2-18-10

Michelle Piehl, Ph.D — Date
MB RESEARCH LABORATORIES

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MB Research Laboratories

PROJECT NUMBER : MB 10-18732.19
SPONSOR : [redacted]
TITLE : MatTek EpiOcular™ MTT Viability Assay
PROTOCOL # : 720-03

ABSTRACT

OBJECTIVE: To provide an estimate of eye irritation potential using an alternative to the Draize Rabbit Eye Test. The exposure time needed for a test article to reduce viability to 50% can be correlated to an estimated Draize Rabbit Eye Score (Modified Maximum Average Score (MMAS)) or a "Predicted Irritancy Class".

METHOD SYNOPSIS: MatTek EpiOcular™ tissue samples were treated in duplicate with the test article and positive control for various exposure times listed below. Negative controls, treated with tissue culture water, were tested at 16 minutes only. Following treatment, the viability of the tissues was determined using Methyl thiazole tetrazolium (MTT) uptake and reduction. The absorbance of each sample was measured at 540 nm using a reference wavelength of 690 nm. The viability was then expressed as a percent of negative control values. The mean percent viability for each time point was used to calculate an ET_{50}, which represents the time at which the EpiOcular™ tissue viability was reduced 50% compared to control tissues. The ET_{50} scores were converted to an irritancy classification using the Standard Method.

SUMMARY/CONCLUSION:

Test Article Identity	Exposure Times (min)	ET_{50} (min)	Irritancy Classification
[redacted]CHA (Caprylhydroxamic Acid), Lot/Batch# HJ4059	16, 64, 256	130.8	Non-Irritating, Minimal
0.3% Triton® X-100 (Positive Control)	15, 45	31.5	Within Range (12.2 - 37.5)

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MB Research Laboratories

Study Title : MatTek EpiOcular™ Assay
Project # : MB 10-18732.19
Protocol : 720-03

OBJECTIVE

To provide an estimate of eye irritation potential using an alternative to the Draize Rabbit Eye Test. The exposure time needed for a test article to reduce viability to 50% can be correlated to an estimated Draize Rabbit Eye Score (Modified Maximum Average Score (MMAS)) or a "Predicted Irritancy Class".

TEST ARTICLE

Identity	:	[redacted] CHA (Caprylhydroxamic Acid), Lot/Batch# HJ4059
Provided by	:	[redacted]
Test Article Characterization	:	See Appendix B for Test Article Characterization.
Stability	:	See Appendix B for stability information.
Date Received	:	01/12/10
Storage	:	Room temperature and humidity.
Description	:	White powder
Sample Preparation	:	Used as received.

POSITIVE CONTROL

Identity	:	0.3% Triton® X-100, Lot# 122109TTB
Supplied by	:	MatTek
Date Received	:	01/26/10
Expiration Date	:	12/21/10
Storage	:	Refrigerated at approximately 4°C.
Description	:	Clear liquid
Sample Preparation	:	Used as received

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MB Research Laboratories

Study Title : MatTek EpiOcular™ Assay
Project # : MB 10-18732.19
Protocol : 720-03

NEGATIVE CONTROL

Identity	:	Tissue culture water, Lot# 128K2318 (TCH_2O)
Supplied by	:	Sigma
Date Received	:	06/03/09
Expiration Date	:	12/2010
Storage	:	Room temperature and humidity.
Description	:	Clear liquid
Sample Preparation	:	Used as received.

TEST DATES

Study Initiation	(date protocol signed)	:	01/25/10
Experimental Start Date	(1st date data collected - OECD)	:	01/26/10
Experimental Start Date	(1st exposure to test substance)	:	01/27/10
Experimental Term Date	(last date data collected)	:	01/29/10
Draft Report Signed	(if applicable)	:	02/16/10
Final Report Signed	(study completion)	:	02/18/10

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EXPERIMENTAL DESIGN

EpiOcular™ Tissue Samples:

EpiOcular™ tissues, Lot 13101 Kits M, N & O, were received from MatTek on 01/26/10 and refrigerated at approximately 4°C. Before use, tissues were incubated (37°C ± 1°C, 5% ± 1% CO_2) with assay medium (MatTek) for a one-hour equilibration. Equilibration medium was replaced with fresh medium before dosing.

Test Article Reduction of MTT:

100 mg of the test article were mixed with 1 ml of MTT solution (1 mg/ml Methyl thiazole tetrazolium diluted in Dulbecco's Modified Eagle's Medium (DMEM)). A negative control, 100 µl of tissue culture water, was tested concurrently. The solutions were incubated at room temperature in the dark for 60 minutes. After incubation, the solutions were visually inspected for purple coloration, which indicates that the test article reduced MTT. Since tissue viability is based on MTT reduction, direct reduction by a test article can exaggerate viability, making a test article seem less irritating than it really is. The test article did not reduce MTT and the assay continued as per the protocol.

Dosing:

At the request of the Sponsor, the test article was dosed neat. 100 mg of the test article were applied to the top of each EpiOcular™ tissue. Initially, duplicate EpiOcular™ tissues were exposed to the test article for 16 minutes. The MTT viability at the 16-minute time point for the test article was greater than 90%, so additional tissues were treated for 64 and 256 minutes. A negative control was tested using tissue culture water at 16 minutes. A positive control (0.3% Triton® X-100) was tested at 15 and 45 minutes. Each treatment with test article or control was conducted in duplicate.

Tissue Viability (MTT Reduction):

At the end of the selected exposure periods, each EpiOcular™ tissue was rinsed with phosphate buffered saline (PBS), soaked for 10 minutes in assay media and transferred to a 24-well plate containing 300 µl of MTT solution. The tissues were then returned to the incubator for a three-hour MTT incubation period.

Following the MTT incubation period, each EpiOcular™ tissue was rinsed with PBS and then treated overnight with 2.0 ml of extractant solution (isopropanol) per well. An aliquot of the extracted MTT formazan was measured at 540 nm using a plate reader, subtracting the absorbance at a reference wavelength of 690 nm.

MB Research Laboratories

Study Title : MatTek EpiOcular™ Assay
Project # : MB 10-18732.19
Protocol : 720-03

EXPERIMENTAL DESIGN (cont'd)

Analysis of Data:

The mean absorbance value for each time point was calculated from the optical density (OD) of the duplicate samples and expressed as percent viability for each sample using the following formula:

% viability = 100 X (OD sample/OD negative control)

The ET_{50}, the time at which the EpiOcular™ tissue viability was reduced 50% compared to control tissues, was then determined using a macro in Microsoft Excel 5.0, provided by MatTek, using the equation:

$$V = a + b \log t$$

Where V = percentage viability, t = time in minutes, and a and b are constants that can be determined by using the viability data for two different exposure times of the test article to the tissue. These exposure times must yield viabilities that flank 50%.

Correlation of *In vitro* and *In vivo* Results:

As per MatTek, as a general guideline, the following groups can be used to assign expected *in vivo* irritancy responses[1] based on the ET_{50} results obtained using the EpiOcular™ MTT Viability Assay:

Draize Score	Irritancy Classification	Example	EpiOcular™ ET_{50} (min)	
			Standard Method*	Specific Gravity Method**
0 –15	Non-Irritating, Minimal	PEG-75 Lanolin, Tween® 20	> 60	> 256-26.5
15.1 – 25	Mild	3% SodiumDodecyl Sulfate	30-60	< 26.5-11.7
25.1 – 50	Moderate	5% Triton® X-100	3-29.99	< 11.7-3.45
50.1 - 110	Severe, Extreme	5% Benzalkonium Chloride	< 3	< 3.45

* = ET_{50} ranges as defined by the MatTek protocol "Neat Method for Ocular Irritation"
** = ET_{50} ranges as defined by the MatTek protocol "Dilution Method for Ocular Irritation"

These groups are based on correlation with an analysis of historical animal test data[2] using the following equation derived by MatTek:

$$\text{Draize} = -4.74 + \frac{(101.7)}{\sqrt{ET_{50}}}$$

[1] J. H. Kay and J. C. Calandra. Interpretation of eye irritation tests. *J Soc Cosmet Chem* (13):281-289, 1962.
[2] M. L. Stern, M. Klausner, R. Alvarado, K. Renskers, and M. S. Dickens. Evaluation of the EpiOcular™ Tissue Model as an Alternative to the Draize Eye Irritation Test. *Toxicol In Vitro* 12 (4 (August)):455-461, 1998.

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EXPERIMENTAL DESIGN (cont'd)

Retention of Data:

Upon signing the final report, all raw data, supporting documentation and reports are submitted to the Archivist by the Study Director. The raw data is filed at MB Research by project number. The final report is filed at MB Research by Sponsor name and MB project number.

Any remaining test article will be discarded following submission of the report.

Amendment to the Protocol:

There were no amendments to the protocol.

Deviation to the Good Laboratory Practices:

The test article characterization was not conducted according to the Good Laboratory Practices. This is not expected to have any impact on the study.

MB Research Laboratories

Study Title : MatTek EpiOcular™ Assay
Project # : MB 10-18732.19
Protocol : 720-03

RESULTS AND DISCUSSION

The test article provided by Inolex Chemical Company was tested using the MatTek EpiOcular™ MTT Viability Assay (see Appendix A for data). At the request of the Sponsor, the test article was dosed neat. The ET_{50} score was converted to an irritancy classification using the Standard Method. The ET_{50} of the positive control, 0.3% Triton® X-100, was 31.5, which fell within MatTek's acceptance range of 12.2 - 37.5 minutes.

The summarized data and irritation classifications are as follows:

Test Article Identity	ET_{50} (min)	Irritancy Classification Standard Method
[redacted] CHA (Caprylhydroxamic Acid), Lot/Batch# HJ4059	130.8	Non-Irritating, Minimal
0.3% Triton® X-100 (Positive Control)	31.5	Within Range (12.2 - 37.5)

FINAL REPORT

Approved by: 2-18-10

Michelle Piehl, Ph.D — Date
Study Director

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MB Research Laboratories

Study Title : MatTek EpiOcular™ Assay
Project # : MB 10-18732.19
Protocol : 720-03

APPENDIX A

EXPERIMENTAL DATA

Test Article: [redacted]**CHA (Caprylhydroxamic Acid) Lot/Batch # HJ4059**
dose: **100 mg**
conc: **Neat**

TIME (min)	OD 1	OD 2	MEAN (OD)	SD	VIABILITY %	ERROR %
16.0	1.331	1.413	1.372	0.058	99.0	4.2
64.0	1.208	1.380	1.294	0.122	93.4	8.8
256.0	0.115	0.143	0.129	0.020	9.3	1.4
neg control	1.370	1.402	1.386	0.023	100.0	1.6

ET_{50} (mins) **130.8**

Irritancy Classification: Non-Irritating, Minimal

Positive Control: **0.3% Triton® X-100**
dose: **100 µl**
conc: **Neat**

TIME (min)	OD 1	OD 2	MEAN (OD)	SD	VIABILITY %	ERROR %
15.0	1.232	1.263	1.248	0.022	90.0	1.6
45.0	0.440	0.410	0.425	0.021	30.7	1.5
neg control	1.370	1.402	1.386	0.023	100.0	1.6

ET_{50} (mins) **31.5**

Irritancy Classification: Within Range (12.2-37.5)

MB Research Laboratories

Study Title : MatTek EpiOcular™ Assay
Project # : MB 10-18732.19
Protocol : 720-03

QUALITY ASSURANCE EVALUATION

The Quality Assurance Unit has inspected a critical phase of this study, audited the raw data and the report and determined that the methods and results contained herein accurately reflect the raw data. A summary of the compliance inspections is presented below.

Date of Inspection	Phase	Performed By	Date Inspection Results Reported	
			Mgmt.	Sty. Dir.
01/27/10	Dose Administration	Krista A. Stayer	01/27/10	01/27/10
02/09/10	Raw data audit	Matt Lowrie	02/09/10	02/09/10
02/15/10	Draft report audit	Matt Lowrie	NA	02/15/10
02/17/10	Final report audit	Matt Lowrie	02/17/10	02/17/10

Matt Lowrie 19FEB10

Matt Lowrie, B.S. Date
Quality Assurance Unit

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TEST ARTICLE CHARACTERIZATION INFORMATION

In compliance with Good Laboratory Practice (GLP) regulations, a characterization of the test article is required and should include identity, strength, purity, composition, stability and uniformity. This data must be reviewed by the Study Director prior to study initiation and will be included in the final report. (EPA 40 CFR 160.105 and 792.105; FDA 21 CFR 58.105, OECD 6.2).

In addition, the test article characterization should be performed in compliance with the Good Laboratory Practices.

Any exceptions to the GLP requirements will be indicated in the Compliance Statement of the final report.

Accordingly, please supply the following information for each test article submitted:

(1) **Test Article Identity** [redacted] CHA (capryllhydroxamic acid)

Storage Ambient room temp

Stability Ambient room temp

Purity 100%

Strength N/A

Composition 100% capryllhydroxamic acid

Uniformity N/A

Note: "N/A" is defined as "not applicable" to the test article, and/or the purposes of the study. If specific information regarding the characterization of the test article is not available, please fully document "not available".

☐ This characterization **was** conducted under GLPs

☒ This characterization **was not** conducted under GLPs

☐ This characterization **was** conducted under GMPs.

BY: [signature]
(signature)

12/17/09
(date)

[redacted]

T:\MARKETING\Information\TAC Form 5-21-09.doc

(1) Please add: Lot/Batch # HJ 4059, as per Sponsor's e-mail on 01/13/10.
AA 01/13/10

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: March 6, 2019

SUBJECT: Scientific Literature Review: Safety Assessment of Caprylhydroxamic Acid (release date February 21, 2019)

The Council respectfully submits the following comments on the scientific literature review, Safety Assessment of Caprylhydroxamic as Used in Cosmetics.

Key Issue

Although the NICNAS exposure assessment is included in the Cosmetic Use section, NICNAS also completed a risk assessment and calculated a margin of exposure (MOE). Based on their assessment they concluded that a concentration of 0.5% was too high and reduced the maximum use concentration to 0.3%. Even at 0.3% (estimated dose 0.702 mg/kg bw/day), the MOE was 71. NICNAS accepted the MOE of 71 because they used a dermal penetration value of 100% which they considered conservative. This additional information should be included in the CIR report in the Cosmetic Use or a risk assessment section.

Additional Considerations

Introduction - Please indicate that NICNAS is an Australian regulatory body.

Method of Manufacture, Summary - The CIR report should not imply that Caprylhydroxamic Acid used in cosmetics is made in a special manner.

Cosmetic Use, Summary - As exposure varies greatly by product type, please state the actual product categories for the highest reported leave-on and rinse-off use concentrations.

Non-Cosmetic Use - If available, please indicate the type of creatures, e.g., birds, ruminants, for which Caprylhydroxamic Acid is used as a growth-promoting feed additive.

ADME, Animal, Oral - The statement that Caprylhydroxamic Acid "was rapidly hydrolyzed by liver homogenates in rats" suggests that they also did an *in vitro* study, in addition to the *in vivo* study. This is also suggested by the figures in the Japanese study. Although the abstract may have not made it clear that they did both an *in vitro* and *in vivo* study, "liver homogenates" would not be part of an *in vivo* study.

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Acute, Oral, Summary - Although NICNAS does state that the oral LD_{50} in rats is >8820 mg/kg, they cite this value to RTECS (available from the On-Line Infobase) which indicates that the LD_{50} is 8820 mg/kg for mice and 10700 mg/kg in rats. RTECS cites these values to a 1936 Russian study. Does the CIR Expert Panel consider these values reliable? If these values are left in the CIR report, the source should be made clear.

Subchronic, Oral; DART - It would be helpful to also state the doses of Caprylhydroxamic Acid used in these studies.

Genotoxicity, In Vitro - Was metabolic activation used in the second Ames test (references 2, 3)?

Enzymatic Activity - Please revise: "Data specific to the enzymatic activity of Caprylhydroxamic Acid were found in the published literature." This suggests that Caprylhydroxamic Acid has enzymatic activity - which is not described. The Summary is clearer about what was studied: "Hydroxamic acids are capable of the inhibition of a variety of enzymes..." The Enzymatic Activity section would be clearer if it said: "Data concerning the effects of Caprylhydroxamic Acid on enzyme activity were not found in the published literature."

Provocative Testing, Summary - Please state the concentration of Caprylhydroxamic Acid in the moisturizer (about 0.075-0.15%).