Safety Assessment of Acrylamide/Acrylate Copolymer Ingredients as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	November 10, 2021
Panel Meeting Date:	December 6 – 7, 2021

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Scientific Analyst/Writer, CIR.

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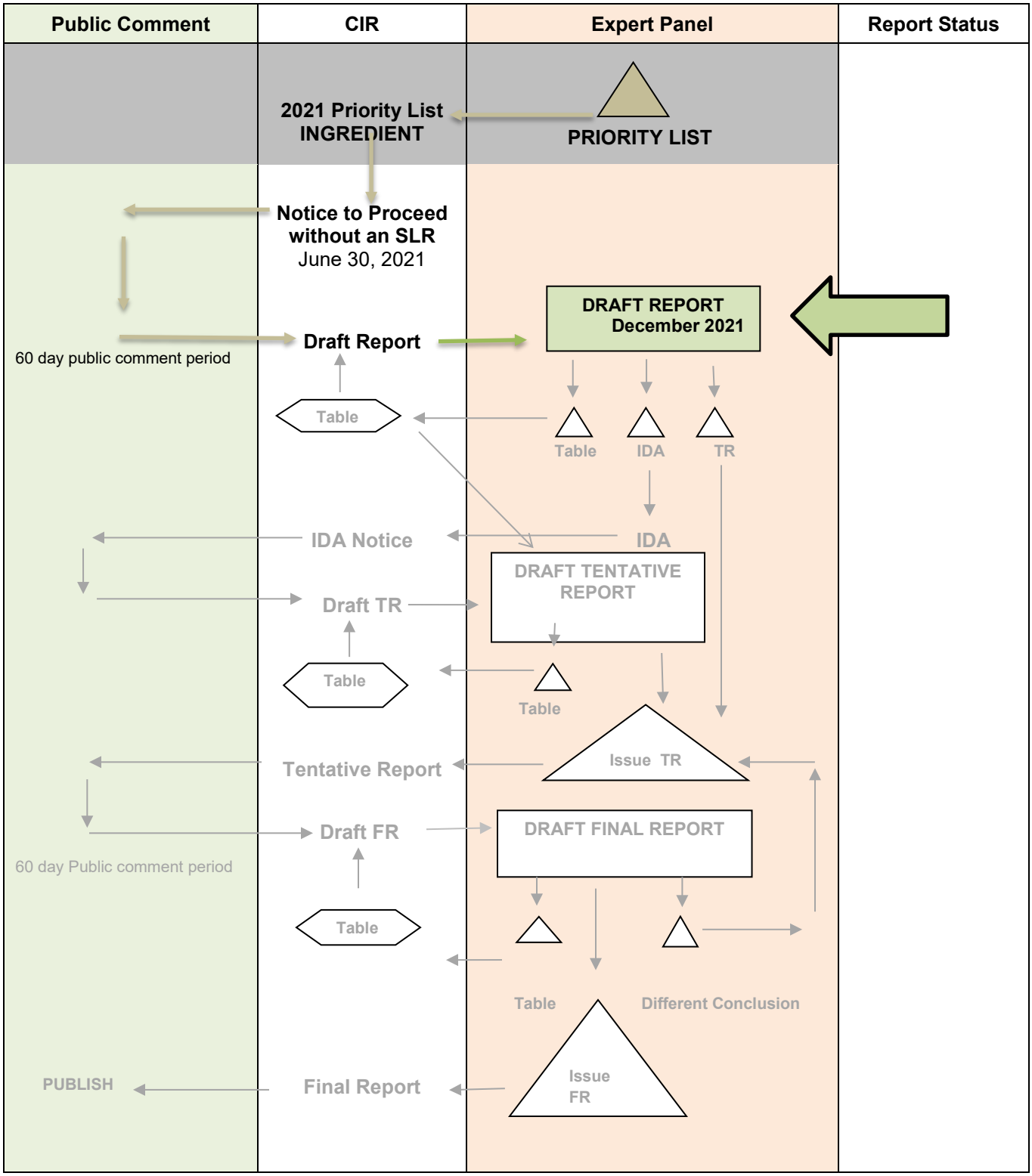
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Acrylamide/Acrylate Copolymers

MEETING December 2021



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, Scientific Analyst/Writer, CIR
Date: November 10, 2021
Subject: Safety Assessment of Acrylamide/Acrylate Copolymer Ingredients

Enclosed is the Draft Report of the Safety Assessment of Acrylamide/Acrylate Copolymer Ingredients in Cosmetics (*report_ArylamideAcrylateCopolymers_122021*). This is the first time the Panel is reviewing the safety of these 16 ingredients. On June 30, 2021, a Scientific Literature Review Notice to Proceed (NTP) was issued due to a lack of published data found upon search of these ingredients. Information sought included chemistry, manufacturing, composition, impurities, toxicity, and dermal irritation/sensitization data. Since the issuing of the NTP, an ample amount of data have been received and can be found below in Table 1.

Included in this packet are concentration of use data (*data1_ArylamideAcrylateCopolymers_122021*), 2021 VCRP frequency of use data (*VCRP_AcrylamideAcrylateCopolymers_122021*), a report history (*history_ AcrylamideAcrylateCopolymers_122021*), a data profile (*dataprofile_AcrylamideAcrylateCopolymers_122021*), the search strategy (*search_AcrylamideAcrylateCopolymers_122021*), and flow chart (*flow_AcrylamideAcrylateCopolymers_122021*).

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, or unsafe conclusion, and Discussion items should be identified. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

Table 1. Unpublished data submitted by Personal Care Products Council

Data Point	Data	Data Source
	Acrylamide/Ammonium Acrylate Copolymer	
Impurities	residual acrylamide present at 2 ppm not expected: 1,4-dioxane, ethylene oxide, solvent residues, free amines, nitrosamines;	*data2_ArylamideAcrylateCopolymers_122021*
Genotoxicity assay	Ames assay; non-genotoxic	*data2_ArylamideAcrylateCopolymers_122021*
In vitro dermal irritation assay	reconstructed human epidermis; tested at 32%; non-irritating	*data3_ArylamideAcrylateCopolymers_122021* English translation: *data4_AcrylamideAcrylatesCopolymers_122021*
Dermal irritation assay	20 subjects; tested at 5%; occlusive conditions; non-irritating	*data2_ArylamideAcrylateCopolymers_122021*
Dermal sensitization assay	performed in guinea pigs; tested at 32%; non-sensitizing	*data3_ArylamideAcrylateCopolymers_122021*
Dermal sensitization assay	HRIPT; 109 subjects; tested at 0.66%; non-irritating and non-sensitizing	*data3_ArylamideAcrylateCopolymers_122021*
Dermal sensitization assay	HRIPT; 50 subjects; tested at 5%; non-irritating and non-sensitizing	*data2_ArylamideAcrylateCopolymers_122021*

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In vitro eye irritation assay	HET-CAM; tested at 3%; non-irritating	*data2_ArylamideAcrylateCopolymers_122021*
Acrylates/Octylacrylamide Copolymer		
Acute dermal toxicity assay	performed in rats; LD_{50} greater than 2000 mg/kg	*data5_ArylamideAcrylateCopolymers_122021*
Acute oral toxicity assay	performed in rabbits; tested at 15%; LD_{50} greater than 2300 mg/kg	*data5_ArylamideAcrylateCopolymers_122021*
Acute inhalation toxicity assay	performed in rats; tested at 10%; LC_{50} greater than 3.4 mg/l	*data5_ArylamideAcrylateCopolymers_122021*
Subchronic inhalation toxicity assay	performed in rats; up to 828 µg/m^3; no adverse effects	*data5_ArylamideAcrylateCopolymers_122021*
Dermal irritation assay	performed in rabbits; tested at 15%; occlusive conditions; abraded and intact sites; mildly irritating	*data5_ArylamideAcrylateCopolymers_122021*
Guinea pig maximization test	occlusive conditions; tested at 5%; non-sensitizing	*data5_ArylamideAcrylateCopolymers_122021*
Dermal sensitization assay	HRIPT; tested at 15%; 50 subjects; non-irritating/non-sensitizing	*data5_ArylamideAcrylateCopolymers_122021*
Ocular irritation assay	performed in rabbits; tested at 15%; mildly irritating	*data5_ArylamideAcrylateCopolymers_122021*
Ocular irritation assay	performed in rabbits; non-irritating	*data5_ArylamideAcrylateCopolymers_122021*
Acrylates/t-Butylacrylamide Copolymer		
In vitro dermal irritation assay	reconstructed human epidermis; tested at 100%; non-irritating	*data3_ArylamideAcrylateCopolymers_122021* English translation: *data4_ArylamideAcrylateCopolymers_122021*
Dermal sensitization assay	HRIPT; tested at 13.34%; 96 subjects; semi-occlusive conditions; non-irritating and non-sensitizing	*data3_ArylamideAcrylateCopolymers_122021*
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer		
Chemistry	MW = approx. 24,000; percent of molecular weight less than 500 Da: 0.0001%; chemical structures given	*data6_ArylamideAcrylateCopolymers_122021*
Method of manufacturing	starting monomers polymerized in ethanol, solution is then refined and neutralized	*data6_ArylamideAcrylateCopolymers_122021*
Impurities	less than 2000 ppm residual monomers; no acrylamide detected	*data6_ArylamideAcrylateCopolymers_122021*
Acute oral toxicity	performed in rats; tested at 40%; LD_{50} greater than 2000 mg/kg	*data6_ArylamideAcrylateCopolymers_122021*
Genotoxicity	Ames assay; tested at 40%; non-genotoxic	*data6_ArylamideAcrylateCopolymers_122021*
Dermal irritation assay	performed in rabbits; tested at 40%; mildly irritating	*data6_ArylamideAcrylateCopolymers_122021*
In vitro dermal sensitization assay	EpiSkin; non-sensitizing	*data6_ArylamideAcrylateCopolymers_122021*
Dermal sensitization assay	guinea pig maximization assay; tested at 40%; non-sensitizing	*data6_ArylamideAcrylateCopolymers_122021*
In vitro ocular irritation assay	non-irritating	*data6_ArylamideAcrylateCopolymers_122021*
Ocular irritation assay Copolymer	performed in rabbits; tested at 40%; slightly irritating	*data6_ArylamideAcrylateCopolymers_122021*
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer		
Chemistry	MW = approx. 250,000; percent of molecular weight less than 500 Da: 0%; chemical structures given	*data6_ArylamideAcrylateCopolymers_122021*

Method of manufacturing	starting monomers polymerized in ethanol, solution is then refined and neutralized	*data6_ArylamideAcrylateCopolymers_122021*
Impurities	less than 3000 ppm residual monomers; no acrylamide detected	*data6_ArylamideAcrylateCopolymers_122021*
Acute oral toxicity assay	performed in rats; tested at 40%; LD_{50} greater than 2000 mg/kg	*data6_ArylamideAcrylateCopolymers_122021*
Short-term dermal toxicity assay	performed in rats; tested at 38%; 28-d; up to 1000 mg/kg bw/d; occlusive conditions; NOAEL = 1000 mg/kg bw/d	*data3_ArylamideAcrylateCopolymers_122021*
Dermal developmental toxicity assay	performed in pregnant rats; tested at 38%; g.d. 5-19; up to 1000 mg/kg bw/d; occlusive conditions; maternal and fetal NOAEL = 1000 mg/kg bw/d	*data3_ArylamideAcrylateCopolymers_122021*
Genotoxicity assay	Ames assay; tested at 38%; non-genotoxic	*data3_ArylamideAcrylateCopolymers_122021*
Genotoxicity assay	In vitro mammalian cell micronucleus assay; tested at 38%; non-genotoxic	*data3_ArylamideAcrylateCopolymers_122021*
Dermal irritation assay	performed in rabbits; tested at 38%; mildly irritating	*data3_ArylamideAcrylateCopolymers_122021*
Dermal sensitization assay	LLNA; tested at 38%; non-sensitizing	*data3_ArylamideAcrylateCopolymers_122021*
Ocular irritation assay	Performed in rabbits; tested at 40%; slightly irritating	*data6_ArylamideAcrylateCopolymers_122021*
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer		
Chemistry	MW = approx. 10,000; percent of molecular weight less than 500 Da: 0.0124%; chemical structures given	*data6_ArylamideAcrylateCopolymers_122021*
Method of manufacturing	starting monomers polymerized in ethanol, solution is then refined	*data6_ArylamideAcrylateCopolymers_122021*
Impurities	less than 200 ppm residual monomers; no acrylamide detected	*data6_ArylamideAcrylateCopolymers_122021*
Acute oral toxicity assay	performed in rats; tested at 70%; LD_{50} greater than 2000 mg/kg	*data6_ArylamideAcrylateCopolymers_122021*
Genotoxicity assay	Ames assay; tested at 70%; non-genotoxic	*data6_ArylamideAcrylateCopolymers_122021*
Dermal irritation assay	performed in rabbits; tested at 10%; mildly irritating	*data6_ArylamideAcrylateCopolymers_122021*
Dermal irritation assay	performed in guinea pigs; tested at 10%; cumulative exposure; non-irritating	*data6_ArylamideAcrylateCopolymers_122021*
Dermal irritation assay	40 subjects; tested at 5%; non-irritating	*data6_ArylamideAcrylateCopolymers_122021*
In vitro dermal irritation assay	EpiSkin method; tested at 50%; non-sensitizing	*data6_ArylamideAcrylateCopolymers_122021*
Dermal sensitization assay	guinea pig maximization assay; tested at 70%; non-sensitizing	*data6_ArylamideAcrylateCopolymers_122021*
In vitro ocular irritation assay	SkinEthic™ HCE method; tested at 50%; non-irritating	*data6_ArylamideAcrylateCopolymers_122021*
Ocular irritation assay	performed in rabbits: tested at 10%; non-irritating	*data6_ArylamideAcrylateCopolymers_122021*

t-Butylacrylamide/ Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer		
Chemistry	MW = approx. 5000; percent of molecular weight less than 500 Da: 0.0005%; chemical structures given	*data6_ArylamideAcrylateCopolymers_122021*
Method of manufacturing	starting monomers polymerized in ethanol, solution is then refined	*data6_ArylamideAcrylateCopolymers_122021*

Acrylamide-Acrylate Copolymer Ingredients – History

January 2021

- Updated concentration of use received

June 2021

- NTP issued

August 2021

The following data was received:

- Summary toxicity assays received for Acrylates/Octylacrylamide Copolymer
    - Acute dermal toxicity
    - Acute oral toxicity
    - Acute inhalation toxicity
    - Subchronic inhalation toxicity
    - Dermal irritation – animal
    - Dermal sensitization – animal
    - Dermal sensitization – human
    - Ocular irritation – animal
    - Ocular irritation (abraded and intact) – animal
- Summary data received for Dimethyl Acrylamide/ Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer
    - Chemistry
    - Impurities
    - Acute oral toxicity
    - Genotoxicity
    - Dermal irritation – animal
    - Dermal irritation (cumulative) – animal
    - Dermal irritation – human
    - In vitro dermal sensitization
    - Dermal sensitization – animal
    - In vitro ocular irritation
    - Ocular irritation – animal
- Summary data received for t-Butylacrylamide/ Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer
    - Chemistry
- Summary data received for Acrylamide/Ammonium Acrylate Copolymer:
    - Chemistry
    - Impurities
    - Genotoxicity assay
    - Dermal irritation – human
    - Dermal sensitization – human

    - In vitro ocular irritation

- Summary data received for AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer
    - Chemistry
    - Impurities
    - Acute oral toxicity
    - Genotoxicity
    - Dermal irritation - animal
    - In vitro dermal sensitization
    - Dermal sensitization – animal
    - In vitro ocular irritation
    - Ocular irritation – animal

- Summary data received for AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer
    - Chemistry
    - Impurities
    - Acute oral toxicity
    - Dermal irritation – animal
    - Ocular irritation – ocular

- In vitro dermal irritation assay – Acrylamide/Ammonium Acrylate Copolymer
- Dermal sensitization assay – animal – Acrylamide/Ammonium Acrylate Copolymer
- Dermal sensitization assay – human – Acrylamide/Ammonium Acrylate Copolymer
- In vitro dermal irritation assay - Acrylates/t-Butylacrylamide Copolymer
- Dermal sensitization assay – human - Acrylates/t-Butylacrylamide Copolymer
- Genotoxicity assay – Ames - AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide /Hydroxyethylacrylate Copolymer
- Genotoxicity assay – micronucleus assay - AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide /Hydroxyethylacrylate Copolymer
- Subchronic dermal toxicity assay - AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide /Hydroxyethylacrylate Copolymer
- Dermal developmental assay - AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide /Hydroxyethylacrylate Copolymer
- Dermal sensitization assay – animal - AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide /Hydroxyethylacrylate Copolymer

October 2021

- Expert Panel reviews Draft Report.

Acrylamide/Acrylate Copolymer Ingredients Profile – December 2021 – Writer, Priya Cherian																															
				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	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	Case Reports
Acrylamide/Ammonium Acrylate Copolymer	x		x												x				x		x		x	x		x			
Acrylamide/Sodium Acrylate Copolymer	x																												
Acrylates/Acrylamide Copolymer	x																												
Acrylates/*t*-Butylacrylamide Copolymer	x																		x					x			x		
Acrylates/Methacrylamide Copolymer	x																												
Acrylates/Octylacrylamide Copolymer	x						x	x	x			x								x			x	x					
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	x	x	x					x							x					x		x	x			x			
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer		x	x					x		x			x		x					x			x				x		
t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer		x																											
Butyl Acrylate/Isopropylacrylamide/ PEG-18 Dimethacrylate Crosspolymer:																													
Corn Starch/Acrylamide/Sodium Acrylate Copolymer	x																												
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer	x	x	x					x							x					x	x	x	x			x	x		
Dimethylacrylamide/Lauryl Methacrylate Copolymer	x																												
Potassium Acrylates/Acrylamide Copolymer																													
Sodium Acrylate/Hydroxyethyl Acrylamide Copolymer																													
Starch/Acrylates/Acrylamide Copolymer																													

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

Acrylamide/Acrylate Copolymer Ingredients Search Strategy

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Acrylamide/Ammonium Acrylate Copolymer	26100-47-0		x						x								
Acrylamide/Sodium Acrylate Copolymer	25085-02-3; 25987-30-8		x						x								
Acrylates/Acrylamide Copolymer	9003-06-9	x	x						x								
Acrylates/*t*-Butylacrylamide Copolymer									x								
Acrylates/Methacrylamide Copolymer									x								
Acrylates/Octylacrylamide Copolymer	129702-02-9	x							x								
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer		x							x								
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer		x							x								
t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer									x								
Butyl Acrylate/Isopropylacrylamide/ PEG-18 Dimethacrylate Crosspolymer:									x								
Corn Starch/Acrylamide/Sodium Acrylate Copolymer		x							x								
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer	103479-14-7	x							x								
Dimethylacrylamide/Lauryl Methacrylate Copolymer									x								
Potassium Acrylates/Acrylamide Copolymer		x							x								
Sodium Acrylate/Hydroxyethyl Acrylamide Copolymer									x								

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Starch/Acrylates/Acrylamide Copolymer									x								

Search Strategy

All search terms were searched in search engines without limiting parameters.

Typical Search Terms (this is informational – not for inclusion for search strategy that goes to the Panel)

- INCI names
- CAS numbers
- chemical/technical names
- additional terms will be used as appropriate

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)

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;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- 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
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- 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://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.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

Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/phytochem/search
- Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
- GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
- Sigma Aldrich plant profiler- http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html
- American Herbal Products Association Botanical Safety Handbook (database) - http://www.ahpa.org/Resources/BotanicalSafetyHandbook.aspx
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0

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ABBREVIATIONS

AMP	adenosine monophosphate
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
Da	Daltons
DART	developmental and reproductive toxicity
DMSO	dimethyl sulfoxide
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
EU	European Union
FDA	Food and Drug Administration
GD	gestation days
GRAS	generally recognized as safe
HCE	human corneal epithelium
HET-CAM	hen's egg test-chorioallantoic membrane
HRIPT	human repeat insult patch test
LC_{50}	lethal concentration 50
LD_{50}	median lethal dose
NOAEL	no-observable-adverse-effect-level
NR	not reported
OECD	Organisation for Economic Cooperation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
ppm	parts per million
TG	test guidelines
US	United States
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

This assessment reviews the safety of the following 16 acrylamide/acrylate copolymer ingredients as used in cosmetic formulations:

Acrylamide/Ammonium Acrylate Copolymer
Acrylamide/Sodium Acrylate Copolymer
Acrylates/Acrylamide Copolymer
Acrylates/*t*-Butylacrylamide Copolymer
Acrylates/Methacrylamide Copolymer
Acrylates/Octylacrylamide Copolymer
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer
t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer
Butyl Acrylate/Isopropylacrylamide/PEG-18 Dimethacrylate Crosspolymer
Corn Starch/Acrylamide/Sodium Acrylate Copolymer
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer
Dimethylacrylamide/Lauryl Methacrylate Copolymer
Potassium Acrylates/Acrylamide Copolymer
Sodium Acrylate/Hydroxyethyl Acrylamide Copolymer
Starch/Acrylates/Acrylamide Copolymer

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook (*wINCI *Dictionary)*, the majority of these ingredients are reported to function in cosmetics as binders, film formers, and hair fixatives (Table 1).[1] Other reported functions for this ingredient group include viscosity-increasing agent, hair-waving/straightening agent, emulsion stabilizer, skin-conditioning agent – miscellaneous, dispersing agent – non-surfactant, antistatic agent, and hair conditioning agent.

These ingredients are being reviewed together as they share structural similarities. Specifically, each of these ingredients comprise a copolymer, polymerized from at least 1 acrylamide monomer and 1 acrylate monomer. The Expert Panel for Cosmetic Ingredient Safety (Panel) has previously reviewed the safety of several other polyacrylamides (Polyacrylate 2, Polyacrylamide, and Acrylamide/Sodium Acryloyldimethyltaurate Copolymer). Polyacrylate 2 and Acrylamide/Sodium Acryloyldimethyltaurate Copolymer were considered safe as used in the present practices of use and concentration (as described in that safety assessment).[2,3] Polyacrylamide was considered safe as used in the present practices of use and concentration (as described in that safety assessment), if the level of acrylamide monomer in formulation is not greater than 5 ppm.[4] The full reports on these ingredients can be accessed on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ingredients).

This safety assessment includes 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 the Panel 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.

CHEMISTRY

Definition and Structure

All ingredients reviewed in this report comprise a copolymer, polymerized from at least 1 acrylamide monomer and 1 acrylate monomer. Acrylate monomers may comprise acrylic acid, methacrylic acid, or one of their esters.[1] The definitions and structures of these ingredients are provided in Table 1. For example, Acrylates/Methacrylamide Copolymer is a copolymer comprising methacrylamide and acrylate monomers as demonstrated in idealized Figure 1.

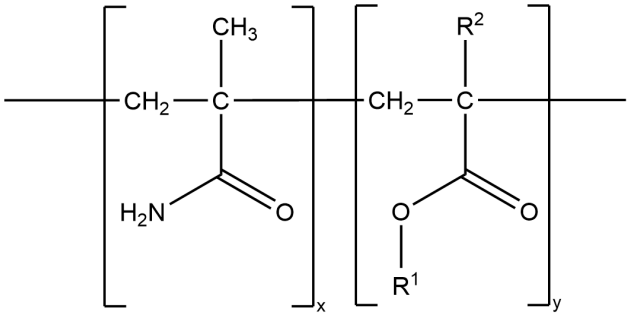


Figure 1. Acrylates/Methacrylamide Copolymer, wherein R^1 may be hydrogen, methyl, ethyl, propyl, or butyl; R^2 may be hydrogen or methyl; and, x and y are undefined.

Chemical Properties

According to a couple of suppliers, the acrylate/acrylamide copolymers reviewed in this report have large molecular weights ranging from 5000 to 250,000 g/mol.[5,6] Approximate molecular weights for these ingredients, where available, may be found in Table 2. Mean molecular weights, related weight distributions, and degrees of polymerization were neither found in the available literature nor submitted as unpublished data, for many of these ingredients.

Method of Manufacture

According to unpublished summary manufacturing data, the starting monomers of several of these ingredients (AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer, Dimethyl Acrylamide/ Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer, and *t*-Butylacrylamide/ Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer) are polymerized in ethanol, and then refined.[5-8] AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer and AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer solutions are reported to be neutralized with 2-amino-2-methyl-1-propanol.

Composition and Impurities

Acrylamide/Ammonium Acrylate Copolymer

For Acrylamide/Ammonium Acrylate Copolymer, less than 2% of oligomers are < 500 Da.[9] In addition, this ingredient is not expected to contain 1,4-dioxane, ethylene oxide, solvent residues (e.g., benzene), free amines, or nitrosamines. Residual acrylamide amounts may be present at levels of 2 ppm.

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer

According to a manufacturer, less than 2000 ppm residual monomers were present in AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer.[8] Acrylamide was not detected as an impurity.

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer

Residual monomers were present in amounts of less than 3000 ppm in AMP-Acrylate/C1-18 Alkyl Acrylate/C1-8 Acrylamide/Hydroxyethylacrylate Copolymer.[7] Acrylamide was not detected as an impurity.

Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer

Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer contains less than 200 ppm residual monomers.[5] Acrylamide was not detected as an impurity.

USE

Cosmetic

The safety of the cosmetic ingredients 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 these ingredients 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 2021 VCRP survey data, the ingredient with the highest number of uses, Acrylates/Octylacrylamide Copolymer, is reported to be used in 160 formulations, all other in-use ingredients are reported to be used in 14 formulations or less (Table 3).[10] The results of the concentration of use survey conducted by the Council in 2020 indicate that Acrylates/*t*-Butylacrylamide Copolymer, Acrylates/Octylacrylamide Copolymer, and Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer are used at up to 7% in leave-on formulations as aerosol hair sprays, mascaras, and tonics, dressings, and other hair grooming aids, respectively.[11] Use concentration data were reported for Dimethylacrylamide/Lauryl Methacrylate Copolymer, but no uses were received in the VCRP; it should be presumed that there is at least one use in every category for which a concentration is reported. The 6 ingredients not in use, according to the VCRP data and industry survey, are listed in Table 4.

Two ingredients are used in products that can be potentially ingested (Acrylamide/Sodium Acrylate Copolymer used in lipstick (concentration not reported) and Acrylates/Octylacrylamide Copolymer used in dentifrices (toothpaste) at up to 19.4%). Acrylates/Octylacrylamide Copolymer is also used in products used near the eye (eyeliners up to 4.6%, eye shadows up to 0.001%, and mascaras at up to 7%). In addition, mucous membrane exposure to these ingredients may occur (Acrylates/Acrylamide Copolymer is used in bath soaps and detergents (concentration not reported) and Corn Starch/Acrylamide/Sodium Acrylate Copolymer is used in bath oils, tablets, and salts (at up to 2%)).

Some of these ingredients are used in cosmetic sprays and could possibly be inhaled; for example, Acrylates/*t*-Butylacrylamide Copolymer is reported to be used at 7% in aerosol hair sprays and Acrylates/Octylacrylamide Copolymer was reportedly used in face powders (concentration not reported). 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.[12,13] 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.[14,15] Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the air.[16-18]

All of the acrylate/acrylamide copolymers named in this report are listed in the European Union inventory of cosmetic ingredients.[19] According to the European Commission, several of these ingredients (Acrylamide/Ammonium Acrylate Copolymer, Acrylamide/Sodium Acrylate Copolymer, Acrylates/Acrylamide Copolymer, AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, Corn Starch/Acrylamide/Sodium Acrylate Copolymer, Dimethyl Acrylamide/ Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer, Potassium Acrylates/Acrylamide Copolymer and Starch/ Acrylates/Acrylamide Copolymer) are not to exceed a maximum residual acrylamide content of 0.1 mg/kg in body care leave-on products and 0.5 mg/kg in other cosmetic products.[20] Restrictions were also noted for AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer and Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer. Restrictions state that these ingredients should not be used with nitrosating systems, must have a maximum purity of 99%, must not exceed a secondary amine content of 0.5%, must not exceed a nitrosamine content of 50 μg/kg, and must be kept in nitrite-free containers.

Non-Cosmetic

Acrylate/Acrylamide Copolymer and Acrylamide/Sodium Acrylate Copolymer

Acrylate/Acrylamide Copolymer and Acrylamide/Sodium Acrylate Copolymer are used as indirect, direct, and secondary food additives. CFR citation details regarding these uses and relevant limitations can be found in Table 5.

TOXICOKINETIC STUDIES

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

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute dermal, oral, and inhalation studies summarized below can be found in Table 6.

The acute dermal LD_{50} was reported to be greater than 2000 mg/kg in rabbits dosed with Acrylates/Octylacrylamide Copolymer.[21] Acute oral toxicity assays were performed in rats using several test substances (Acrylates/Octylacrylamide Copolymer (15% solids), a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer, and a 70% ethanol solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer).[5,7,8,21] Oral D_{50}s reported for these assays were greater than 2000 mg/kg, excluding Acrylates/Octylacrylamide Copolymer, in which the reported LD_{50} was greater than 2300 mg solids/kg bw. An LC_{50} of greater than 3.4 mg/l was reported in an acute inhalation toxicity assay performed in rats exposed to Acrylates/Octylacrylamide Copolymer.[21]

Short-Term Toxicity Studies

Dermal

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer

A 28-d dermal toxicity assay was performed in Wistar Han rats (5/sex/group).[22] The test substance (38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer in water; 2 ml/kg) was applied to the skin at doses of 0, 100, 300, and 1000 mg/kg bw/d, under semi-occlusive conditions, for 6 h/d. Clinical, hematological, urinary, and pathological parameters were evaluated. Very slight erythema was observed between days 26 and 29 in two females dosed with 1000 mg/kg of the test substance. No other skin reactions were observed. No relevant adverse test item-related effects were evaluated throughout the study. The no-observed-adverse-effect level (NOAEL) was determined to be 1000 mg/kg bw/d.

Subchronic Toxicity Studies

Inhalation

Acrylates/Octylacrylamide Copolymer

Sprague-Dawley rats (10/sex/group) were exposed to 0, 199, 491, or 828 μg/m^3 Acrylates/Octylacrylamide Copolymer in ethanol (mean particle aerodynamic diameter of 1.9 μ), via a full body chamber, for 4 h/d, 7 d/wk, for 13 wk.[21] Clinical, hematological, and histopathological parameters were observed. The test substance did not produce any adverse effects.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer

A dermal prenatal development toxicity assay was performed in pregnant female Wistar rats (24/group).[23] The test substance (38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer in water; 2 ml/kg) was applied to the skin, in doses of 0, 100, 300, and 1000 mg/kg bw/d, under semi-occlusive conditions, on gestation days 5 to 19. Each application lasted for a duration of 6 h. Maternal skin reactions, body weight, clinical parameters, and gross pathological effects were observed. In addition, litter parameters and external, visceral, and skeletal observations of fetuses were performed. No adverse effects were observed for any of the evaluated parameters. The NOAEL for maternal and fetal toxicity was determined to be 1000 mg/kg bw/d.

GENOTOXICITY

In Vitro

Acrylamide/Ammonium Acrylate Copolymer

The potential genotoxicity of Acrylamide/Ammonium Acrylate Copolymer (up to 5000 µg/plate) was evaluated via an Ames test *(Salmonella typhimurium* (strains not specified) and *Escherichia coli* WP2 (uvrA-)).[9] No other details regarding this study were provided. The test substance was considered to be non-genotoxic.

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer

A 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer was used in an Ames assay to determine potential genotoxicity.[8] No other details regarding this study were provided. The test substance was considered to be non-genotoxic.

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer

An Ames assay was performed was performed with and without metabolic activation using a mixture containing 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-18 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer (5, 15.81, 50, 158.1, 500, 1581, and 5000 µg/plate; dissolved in dimethyl sulfoxide (DMSO)) in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102.[24] Negative (DMSO) and positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, mitomycin c, benzo[a]pyrene, 2-aminoanthracene) were used, and yielded expected results. The test substance was not considered to be mutagenic.

The same test substance (up to 300 µg/ml; dissolved in DMSO) was evaluated in an in vitro mammalian cell micronucleus assay using human peripheral blood lymphocytes, with and without metabolic activation.[25] Negative (DMSO) and positive controls (mitomycin C, cyclophosphamide, vinblastine) were used, and yielded expected results. The test substance did not induce micronuclei in cultured human peripheral blood lymphocytes.

Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer

The genotoxic potential of a 70% ethanolic solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer was evaluated via an Ames assay.[5] No details regarding this assay were provided. The test material was considered to be non-genotoxic.

CARCINOGENICITY STUDIES

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

DERMAL IRRITATION AND SENSITIZATION

Details regarding the irritation and sensitization studies summarized below can be found in Table 7.

Reconstructed human epidermis cytotoxicity assays were performed using a mixture containing 32% Acrylamide/Ammonium Acrylate Copolymer and undiluted *t*-Butylacrylamide Copolymer.[26] Both test substances were considered to be non-irritating. In an animal assay, a neutralized, aqueous solution of Acrylates/Octylacrylamide Copolymer (15% solids) was applied to intact and abraded skin sites on New Zealand White rabbits, under occlusive conditions.[21] The test substance was considered to be mildly irritating. A primary skin irritation assay performed in rabbits using AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer yielded negative results.[8] Mild irritation was noted in a primary skin assay performed in rabbits using a 10% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer; however the same test substance was non-irritating in a cumulative irritation assay performed in guinea pigs.[5] Mild irritation was also noted in a primary irritation assay performed in rabbits using a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer.[7] No irritation was noted in a human dermal irritation assay using a 5% aqueous solution of Acrylamide/Ammonium Acrylate Copolymer.[9] A human dermal irritation assay performed using a 50% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer yielded negative results.[5]

In vitro EpiSkin® dermal irritation assays were performed on AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer and a 50% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer.[5,8] Both test substances were considered to be non-sensitizing. The skin sensitization potential of a mixture containing 32% Acrylamide/Ammonium Acrylate Copolymer was evaluated in guinea pigs (tested undiluted under occlusive conditions).[27] No signs of sensitization were observed. Guinea pig maximization assays were performed to evaluate the potential sensitization of Acrylates/Octylacrylamide Copolymer (5 - 100%), a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, and a 70% ethanolic solution Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer.[5,8,21] All test substances were considered to be non-sensitizing. Similarly, no signs of sensitization were observed in a local lymph node assay performed in mice using a mixture containing 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-18 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer (5, 10, 25, and 50% in dimethylformamide).[28] Several test substances (mixture containing 0.66% Acrylamide/Ammonium Acrylate Copolymer, aqueous solution of neutralized Acrylates/Octylacrylamide Copolymer (15% solids), and a formula containing 13.34% Acrylates/*t*-Butylacrylamide Copolymer) were evaluated for potential sensitization via human repeat insult patch tests (HRIPTs).[9,21,29] All test substances evaluated were considered to be non-irritating and non-sensitizing.

OCULAR IRRITATION STUDIES

The ocular irritation studies summarized below can be found in Table 8.

In vitro ocular irritation assays performed using a 3% solution of Acrylamide/Ammonium Acrylate Copolymer, AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, and a 50% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer yielded negative results.[5,8,9] Mild irritation was noted in an in in vitro ocular irritation assay performed using a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer.[5] No ocular irritation was noted in an ocular irritation assay performed on New Zealand White rabbits using Acrylates/Octylacrylamide Copolymer.[21] However, mild ocular irritation was observed in an ocular irritation performed in New Zealand white rabbits using a neutralized, aqueous solution of Acrylates/Octylacrylamide Copolymer (15% solids). Slight irritation was observed in an ocular irritation assay performed on rabbits using a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer.[7] No irritation was noted in an ocular irritation assay performed in rabbits using a 10% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer.[5]

SUMMARY

The majority of the acrylamide/acrylate copolymers reviewed in this report are reported to function as binders, film formers, and hair fixatives. According to manufacturers, several of these ingredients are reported to contain less than 3000 ppm residual monomers.

Based on 2021 FDA VCRP and data, Acrylates/Octylacrylamide Copolymer is reported to be used in 160 formulations. All other in-use formulations are reported to be used in 14 formulations or less. The results of the concentration of use survey conducted by the Council indicate that Acrylates/*t*-Butylacrylamide Copolymer, Acrylates/Octylacrylamide Copolymer, and Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer are used at up to 7% in leave-on formulations as aerosol hair sprays, mascaras, and tonics, dressings, and other hair grooming aids, respectively. According to the European Commission, several of the ingredients reviewed in this report may be used in cosmetics under certain restrictions. In addition, Acrylate/Acrylamide Copolymer and Acrylamide/Sodium Acrylate Copolymer are used as indirect, direct, and secondary food additives.

The acute dermal LD_{50} was reported to be greater than 2000 mg/kg in rabbits dosed with Acrylates/Octylacrylamide Copolymer. Acute oral toxicity assays were performed in rats using several test substances (Acrylates/Octylacrylamide Copolymer (15% solids), a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer, and a 70% Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer). LD_{50}s reported for these assays were greater than 2000 mg/kg, excluding Acrylates/Octylacrylamide Copolymer, in which the reported LD_{50} was greater than 2300 mg solids/kg bw. An LC_{50} of greater than 3.4 mg/l was reported in an acute inhalation toxicity assay performed in rats exposed to Acrylates/Octylacrylamide Copolymer.

In a 28-d dermal toxicity assay, Wistar rats were given 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer in water in doses of up to 1000 mg/kg bw/d. The NOAEL was determined to be 1000 mg/kg bw/d. The potential subchronic inhalation toxicity of Acrylates/Octylacrylamide Copolymer in ethanol (up to 828 $\mu g/m^3$) was evaluated in Sprague-Dawley rats, for 13 wks. No adverse effects were observed.

Potential dermal developmental toxicity of 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer in water (up to 1000 mg/kg bw/d; semi-occlusive conditions; gestation days 5 - 19) was evaluated in pregnant female Wistar rats. The NOAEL for maternal and fetal toxicity was determined to be 1000 mg/kg bw/d.

Ames assays were performed on several test substances (Acrylamide/Ammonium Acrylate Copolymer, a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, a mixture containing 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-18 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer, and a 70% ethanolic solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer). All test substances were considered to be non-genotoxic. In addition, negative results were obtained in an in vitro mammalian cell micronucleus assay performed in human peripheral blood lymphocytes using a mixture containing 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-18 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer.

Reconstructed human epidermis cytotoxicity assays were performed using a mixture containing 32% Acrylamide/Ammonium Acrylate Copolymer and undiluted *t*-Butylacrylamide Copolymer. Both test substances were considered to be non-irritating. In an animal assay, a neutralized, aqueous solution of Acrylates/Octylacrylamide Copolymer (15% solids) was applied to intact and abraded skin sites on New Zealand White rabbits, under occlusive conditions. The test substance was considered to be mildly irritating. A primary skin irritation assay performed in rabbits using AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer yielded negative results. Mild irritation was noted in a primary skin assay performed in rabbits using a 10% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer; however, the same test substance was non-irritating in a cumulative irritation assay performed in guinea pigs. Mild irritation was also noted in a primary irritation assay performed in rabbits using a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer. No irritation was noted in a human dermal irritation assay using a 5% aqueous solution of Acrylamide/Ammonium Acrylate Copolymer.[9] A human dermal irritation assay performed using a 50% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer yielded negative results.

In vitro EpiSkin® dermal irritation assays were performed on AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer and a 50% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer. Both test substances were considered to be non-sensitizing. The skin sensitization potential of a mixture containing 32% Acrylamide/Ammonium Acrylate Copolymer was evaluated in guinea pigs (tested undiluted under occlusive conditions). No signs of sensitization were observed. Guinea pig maximization assays were performed to evaluate the potential sensitization of Acrylates/Octylacrylamide Copolymer (5 - 100%), a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, and a 70% ethanolic solution Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer. All test substances were considered to be non-sensitizing. Similarly, no signs of sensitization were observed in a local lymph node assay performed in mice using a mixture containing 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-18 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer (up to 50% in dimethylformamide). Several test substances (mixture containing 0.66% Acrylamide/Ammonium Acrylate Copolymer, aqueous solution of neutralized Acrylates/Octylacrylamide Copolymer (15% solids), and a formula containing 13.34% Acrylates/*t*-Butylacrylamide Copolymer) were evaluated for potential sensitization via HRIPTs. All test substances evaluated were considered to be non-irritating and non-sensitizing.

In vitro ocular irritation assays performed using a 3% solution of Acrylamide/Ammonium Acrylate Copolymer, AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer, and a 50% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer yielded negative results. Mild irritation was noted in an in in vitro ocular irritation assay performed using a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer. No ocular irritation was noted in an ocular irritation assay performed on New Zealand White rabbits using Acrylates/Octylacrylamide Copolymer. However, mild ocular irritation was observed in an ocular irritation performed in New Zealand white rabbits using a neutralized, aqueous solution of Acrylates/Octylacrylamide Copolymer (15% solids). Slight irritation was observed in an ocular irritation assay performed on rabbits using a 40% ethanolic solution of AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer. No irritation was noted in an ocular irritation assay performed in rabbits using a 10% aqueous solution of Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. INCI names, definitions, and reported functions of the Acrylamide/Acrylate Copolymer ingredients in this safety assessment[1, CIR Staff]

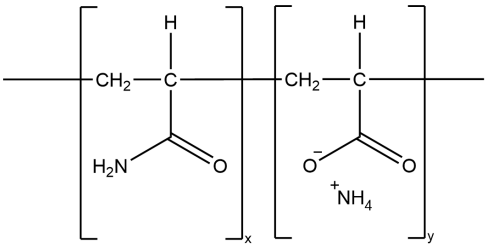
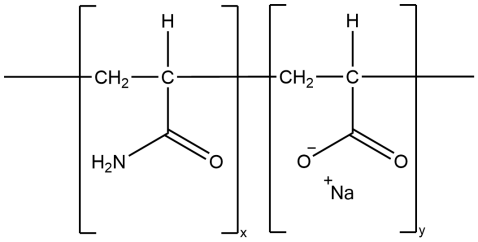
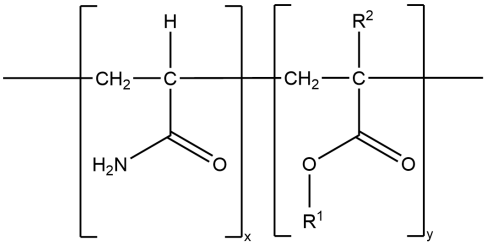
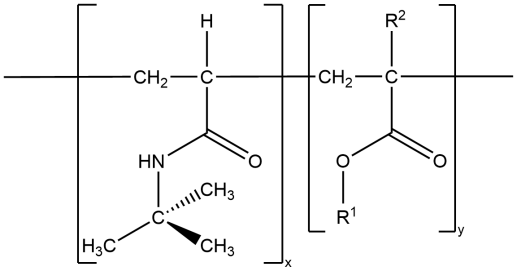
Ingredient (CAS No.)	Definition	Function
Acrylamide/Ammonium Acrylate Copolymer [26100-47-0]	Acrylamide/Ammonium Acrylate Copolymer is a copolymer of Acrylamide and Ammonium Acrylate monomers. 	Binders; Film Formers; Hair Fixatives
Acrylamide/Sodium Acrylate Copolymer [25085-02-3; 25987-30-8]	Acrylamide/Sodium Acrylate Copolymer is a copolymer of Acrylamide and sodium acrylate monomers. 	Binders; Film Formers; Viscosity Increasing Agents - Aqueous
Acrylates/Acrylamide Copolymer [9003-06-9]	Acrylates/Acrylamide Copolymer is a copolymer of Acrylamide and one or more monomers of Acrylic Acid, Methacrylic Acid or one of their simple esters. 	Binders; Film Formers; Hair Fixatives
Acrylates/*t*-Butylacrylamide Copolymer	Acrylates/t-Butylacrylamide Copolymer is a copolymer of t-Butylacrylamide and one or more monomers of Acrylic Acid, Methacrylic Acid, or one of their simple esters.  **wherein R^1 may be hydrogen, methyl, ethyl, propyl, or butyl; and R^2 may be hydrogen or methyl**	Binders; Film Formers; Hair Fixatives

Table 1. INCI names, definitions, and reported functions of the Acrylamide/Acrylate Copolymer ingredients in this safety assessment[1, CIR Staff]

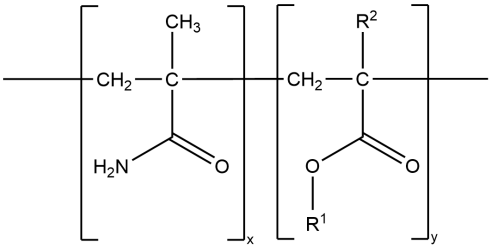
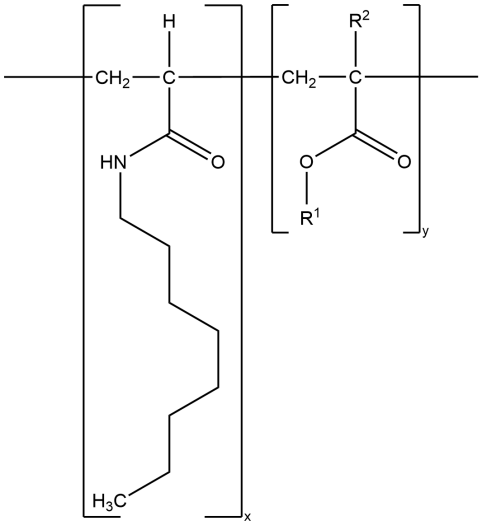
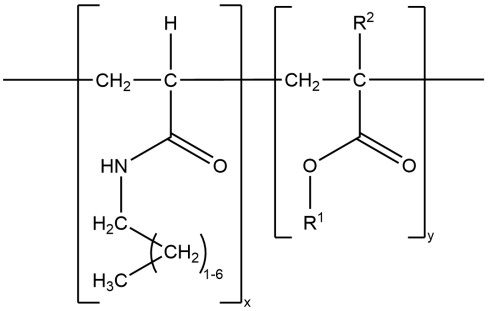
Ingredient (CAS No.)	Definition	Function
Acrylates/Methacrylamide Copolymer	Acrylates/Methacrylamide Copolymer is a copolymer of Methacrylamide and one or more monomers of Acrylic Acid, Methacrylic Acid or one of their simple esters.  **wherein R^1 may be hydrogen, methyl, ethyl, propyl, or butyl; and R^2 may be hydrogen or methyl**	Film Formers; Hair Fixatives
Acrylates/Octylacrylamide Copolymer [129702-02-9]	Acrylates/Octylacrylamide Copolymer is a copolymer of octylacrylamide and one or more monomers consisting of Acrylic Acid, Methacrylic Acid or one of their simple esters. 	Film Formers; Hair Fixatives
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer is a polymer of C1-18 Alkyl Acrylate, C1-8 Alkyl Acrylamide and the aminomethylpropanol salt of a monomer consisting of Acrylic Acid, Methacrylic Acid or one of their simple esters.  **wherein R^1 may be hydrogen, C1-18-alkyl, or a salt of 3-aminopropanol; and R^2 may be hydrogen or methyl**	Film Formers; Hair Fixatives

Table 1. INCI names, definitions, and reported functions of the Acrylamide/Acrylate Copolymer ingredients in this safety assessment[1, CIR Staff]

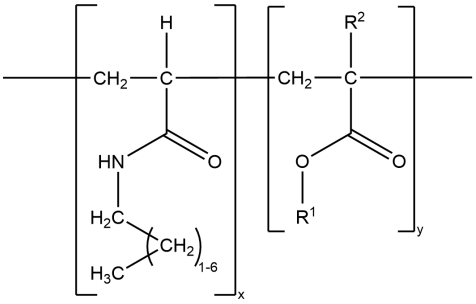
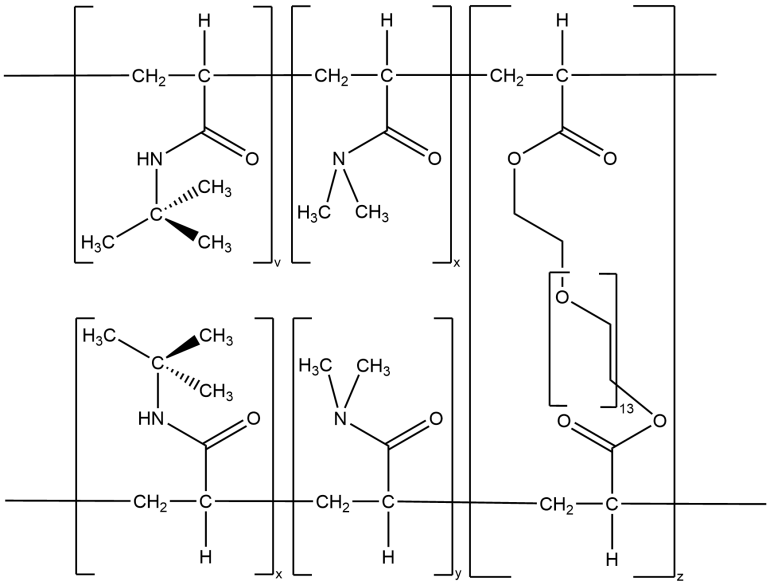
Ingredient (CAS No.)	Definition	Function
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer	AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer is a polymer of C1-18 Alkyl Acrylate or C1-18 alkyl methacrylate, C1-8 Alkyl Acrylamide, 2-Hydroxyethyl Acrylate, and the aminomethylpropanol salt of a monomer consisting of Acrylic Acid, Methacrylic Acid or one of their simple esters.  **wherein R^1 may be hydrogen, C1-18-alkyl, 2-hydroxyethyl, or a salt of 3-aminopropanol; and R^2 may be hydrogen or methyl**	Hair-Waving/Straightening Agents
t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer	t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer is a copolymer of t-Butylacrylamide and Dimethylacrylamide, cross-linked with PEG-14 diacrylate. 	Hair Fixatives

Table 1. INCI names, definitions, and reported functions of the Acrylamide/Acrylate Copolymer ingredients in this safety assessment[1, CIR Staff]

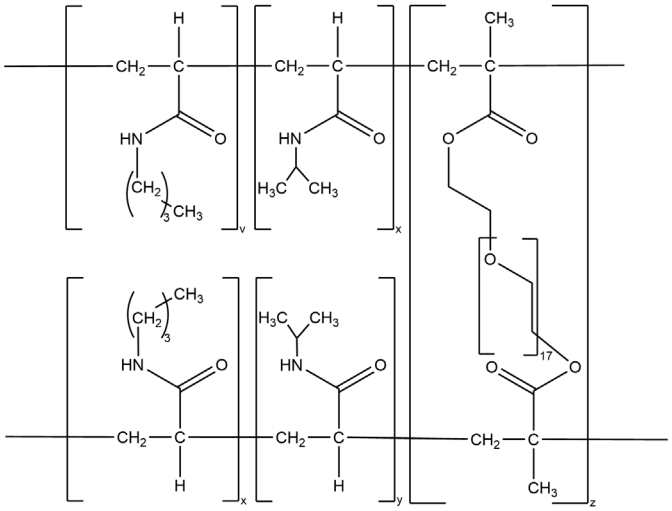
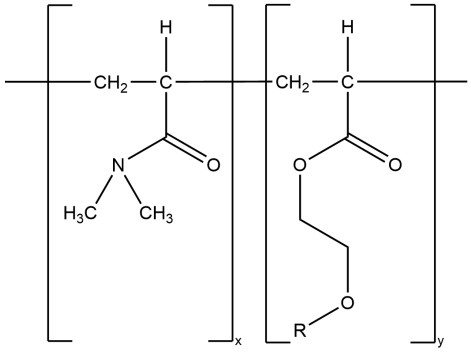
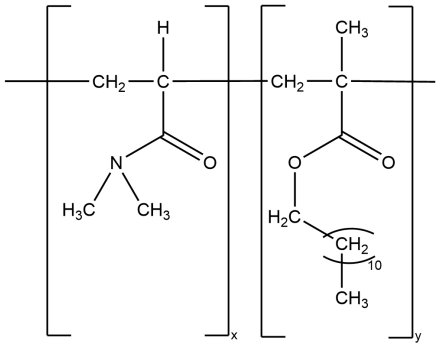
Ingredient (CAS No.)	Definition	Function
Butyl Acrylate/Isopropylacrylamide/PEG-18 Dimethacrylate Crosspolymer	Butyl Acrylate/Isopropylacrylamide/PEG-18 Dimethacrylate Crosspolymer is a crosslinked copolymer of Butyl Acrylate, Isopropylacrylamide and PEG-18 dimethacrylate monomers.	Emulsion Stabilizers; Film Formers; Skin-Conditioning Agents - Miscellaneous
		
Corn Starch/Acrylamide/Sodium Acrylate Copolymer	Corn Starch/Acrylamide/Sodium Acrylate Copolymer is a polymer of Zea Mays (Corn) Starch, Acrylamide and sodium acrylate monomers.	Dispersing Agents - Nonsurfactant; Emulsion Stabilizers; Film Formers; Hair Fixatives
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer	Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer is a copolymer of Dimethylacrylamide, 2-Hydroxyethyl Acrylate and Methoxyethyl Acrylate monomers.  wherein R may be hydrogen or methyl	Hair Fixatives
Dimethylacrylamide/Lauryl Methacrylate Copolymer [103479-14-7]	Dimethylacrylamide/Lauryl Methacrylate Copolymer is a copolymer of Dimethylacrylamide and Lauryl Methacrylate. 	Binders; Film Formers; Hair Fixatives

Table 1. INCI names, definitions, and reported functions of the Acrylamide/Acrylate Copolymer ingredients in this safety assessment[1, CIR Staff]

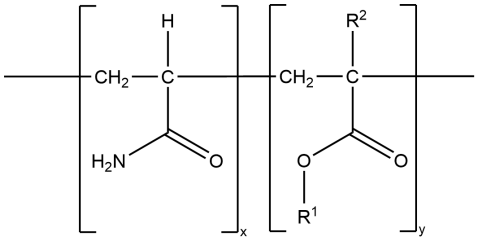
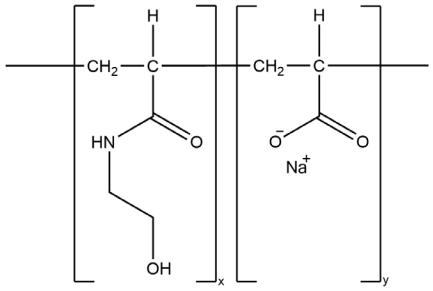
Ingredient (CAS No.)	Definition	Function
Potassium Acrylates/Acrylamide Copolymer	Potassium Acrylates/Acrylamide Copolymer is the potassium salt of Acrylates/Acrylamide Copolymer.  **wherein R^1 may be hydrogen, methyl, ethyl, propyl, or butyl; and R^2 may be hydrogen or methyl**	Film Formers
Sodium Acrylate/Hydroxyethyl Acrylamide Copolymer	Sodium Acrylate/Hydroxyethyl Acrylamide Copolymer is a copolymer of sodium acrylate and Hydroxyethyl Acrylamide that conforms generally to the formula: 	Antistatic Agents; Film Formers; Hair Conditioning Agents; Hair Fixatives
Starch/Acrylates/Acrylamide Copolymer	Starch/Acrylates/Acrylamide Copolymer is a polymer of starch, Acrylamide and a monomer consisting of Acrylic Acid, Methacrylic Acid or one of their simple ester.	Film Formers; Viscosity Increasing Agents - Aqueous

Table 2. Molecular weights of the acrylamide/acrylate copolymers

Ingredient	Approximate Molecular Weight (g/mol)	Reference
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	24,000 (percent molecular weight less than 500 Da: 0.0001%)	[8]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer	250,000 (percent molecular weight less than 500 Da: 0%)	[7]
t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer	5000 (percent molecular weight less than 500 Da: < 0.0005%)	[6]
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer	10,000 (percent molecular weight less than 500 Da: 0.0124%)	[5]

Da = Daltons

Table 3. Frequency (2021) and concentration (2020) of use according to duration and exposure[10,11]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Acrylamide/Ammonium Acrylate Copolymer		**Acrylamide/Sodium Acrylate Copolymer**		**Acrylates/Acrylamide Copolymer**	
Totals*	**1**	**NR**	**14**	**0.5 – 2.8**	**7**	**0.41**
Duration of Use						
Leave-On	*1*	*NR*	*13*	*NR*	*4*	*0.41*
Rinse-Off	*NR*	*NR*	*1*	*NR*	*3*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	2	NR	NR	NR
Incidental Inhalation-Spray	1[a]	NR	6[a]; 5[b]	2.8[a]	2[b]	NR
Incidental Inhalation-Powder	NR	NR	5[b]	0.5[c]	2[b]	NR
Dermal Contact	1	NR	10	0.5 – 2.8	7	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	2	2.8	NR	0.41
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	2	NR	3	NR
Baby Products	NR	NR	NR	NR	NR	NR
	Acrylates/*t*-Butylacrylamide Copolymer		**Acrylates/Methacrylamide Copolymer**		**Acrylates/Octylacrylamide Copolymer**	
Totals*	**4**	**0.06 – 7**	**2**	**NR**	**160**	**0.00097 – 19.4**
Duration of Use						
Leave-On	*4*	*0.06 – 7*	*NR*	*NR*	*160*	*0.00097 – 7*
Rinse Off	*NR*	*NR*	*2*	*NR*	*NR*	*4.9 – 19.4*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	NR	NR	17	0.00097 – 7
Incidental Ingestion	NR	NR	NR	NR	NR	19.4
Incidental Inhalation-Spray	1	0.06 – 7; 5[a]	NR	NR	123; 6[a]	0.5 – 3.2
Incidental Inhalation-Powder	NR	NR	NR	NR	3	NR
Dermal Contact	NR	NR	NR	NR	136	0.00097 – 4.9
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	4	0.06 – 7	2	NR	15	0.5 – 3.2
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	2	NR
Mucous Membrane	NR	NR	NR	NR	NR	19.4
Baby Products	NR	NR	NR	NR	NR	NR
	AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer		**Corn Starch/Acrylamide/Sodium Acrylate Copolymer**		**Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer**	
Totals*	**4**	**0.032 – 5**	**5**	**0.002 – 2**	**7**	**0.26 – 7**
Duration of Use						
Leave-On	*4*	*0.032 – 5*	1	*0.002*	7	*0.26 – 7*
Rinse Off	*NR*	*NR*	NR	*NR*	NR	*NR*
Diluted for (Bath) Use	*NR*	*NR*	4	*2*	NR	*NR*
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	2	1.3 – 3.9; 0.032 – 5[a]	1[b]	NR	2	0.26; 7[a]
Incidental Inhalation-Powder	NR	NR	1[b]	0.002[c]	NR	NR
Dermal Contact	2	0.032 – 0.05	5	0.002 – 2	NR	NR
Deodorant (underarm)	NR	0.05	NR	NR	NR	NR
Hair - Non-Coloring	2	0.3 – 5	NR	NR	5	0.26 – 7
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	2	NR
Mucous Membrane	NR	NR	4	2	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

Table 3. Frequency (2021) and concentration (2020) of use according to duration and exposure[10,11]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Dimethylacrylamide/Lauryl Methacrylate Copolymer					
Totals*	**NR**	**0.5**				
Duration of Use						
Leave-On	*NR*	*NR*				
Rinse Off	*NR*	*0.5*				
Diluted for (Bath) Use	*NR*	*NR*				
Exposure Type						
Eye Area	NR	NR				
Incidental Ingestion	NR	NR				
Incidental Inhalation-Spray	NR	NR				
Incidental Inhalation-Powder	NR	NR				
Dermal Contact	NR	0.5				
Deodorant (underarm)	NR	NR				
Hair - Non-Coloring	NR	NR				
Hair-Coloring	NR	NR				
Nail	NR	NR				
Mucous Membrane	NR	0.5				
Baby Products	NR	NR				

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

Table 4. Acrylate/Acrylamide Copolymers with no reported uses, according to the VCRP and Council survey

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer
t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer
Butyl Acrylate/Isopropylacrylamide/PEG-18 Dimethacrylate Crosspolymer
Potassium Acrylates/Acrylamide Copolymer
Sodium Acrylates/Hydroxyethyl Acrylamide Copolymer
Starch Acrylates/Acrylamide Copolymer

Table 5. CFR Citations for Acrylate/Acrylamide Copolymer and Acrylamide/Sodium Acrylate Copolymer

CFR Citation	Limitations
	Acrylate/Acrylamide Copolymer
21CFR176.110 Indirect food additives: paper and paperboard components	Acrylamide-acrylic acid resins may be safely used as components of articles in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food under the following limitations: -acrylamide-acrylic acid resins are produced by the polymerization of acrylamide with partial hydrolysis or by the copolymerization of acrylamide and acrylic acid -the acrylamide-acrylic acid resins contain less than 0.2% residual monomer -the resins are used as adjuvants in the manufacture of paper and paperboard in amounts not to exceed that necessary to accomplish the technical effect and not to exceed 2% by weight of the paper or paperboard
21CFR573.120 Food additives permitted in feed and drinking water of animals	Acrylamide-acrylic acid resin may be used safely under the following limitations: -the additive is produced by polymerization of acrylamide with partial hydrolysis, or by copolymerization of acrylamide and acrylic acid with the greater part of the polymer being composed of acrylamide units -the additive meets the following specifications: a) a minimum molecular weight of 3 million b) viscosity range: 3000 to 6000 centipoises at 77° F in a 1% aqueous solution as determined by LVF Brookfield Viscometer or equivalent using a number 6 spindle at 20 rpm c) residual acrylamide: not more than 0.05% -it is used as a thickener and suspending agent in non-medicated aqueous suspensions intended for addition to animal feeds
21CFR173.357 Secondary direct food additives permitted in food for human consumption	May be used as a fixing material in the immobilization of glucose isomerase enzyme preparations for use in the manufacture of high fructose corn syrup in accordance with CFR 184.1372
	Acrylamide/Sodium Acrylate Copolymer
21CFR172.710 Food additives permitted for direct addition to food for human consumption	Sodium acrylate and acrylamide copolymer with a minimum average molecular weight of 10,000,000 in which 30% of the polymer is comprised of acrylate units and acrylamide units, for use as a drift control agent in herbicide formulations applied to crops at a level not to exceed 0.5 oz of the additive per acre

Table 5. CFR Citations for Acrylate/Acrylamide Copolymer and Acrylamide/Sodium Acrylate Copolymer

CFR Citation	Limitations
21CFR173.310 Secondary direct food additives permitted in food for human consumption	Boiler water additives may be safely used in the preparation of steam that will contact food under the following conditions: -the amount of additive is not in excess of that required for its functional purposed, and the amount of steam in contact with food does not exceed that required to produce the intended effect in or on food -acrylamide-sodium acrylate resin may not contain more that 0.05% by weight of acrylamide monomer
40CFR180.960 Polymers; exemptions from the requirement of a tolerance	Exempted from the requirement of a tolerance under FFDCA section 408
Acrylate/Acrylamide Copolymer and Acrylamide/Sodium Acrylate Copolymer	
21CFR173.5 Secondary direct food additives permitted in food for human consumption	Acrylate-acrylamide resins may be safely used in food under the following conditions: 1. the additive consists of one of the following: a. acrylamide-acrylic resin (hydrolyzed polyacrylamide) is produced by the polymerization of acrylamide with partial hydrolysis, or by copolymerization of acrylamide and acrylic acid, with the greater part of the polymer being composed of acrylamide units b. sodium polyacrylate-acrylamide resin is produced by the polymerization and subsequent hydrolysis of acrylonitrile in a sodium silicate-sodium hydroxide aqueous solution, with the greater part of the polymer being composed of acrylate units 2. the additive contains not more than 0.05% of residual monomer calculated as acrylamide 3. the additive is used or intended for use as follows: a. the additive is used as a flocculent in the clarification of beet sugar juice and liquor of cane sugar juice and liquor or corn starch hydrolysate in an amount not to exceed 5 ppm by weight of the juice or 10 ppm by weight of liquor or the corn starch hydrolysate b. the additive is used to control organic and mineral scale in beet sugar juice and liquor or cane sugar juice and liquor in an amount not to exceed 2.5 ppm by the weight of the juice or liquor

Table 6. Acute toxicity studies

Test Substance	Animals	No./Group	Concentration/Dose/Protocol	LD_{50}/LC_{50}/Results	Reference
DERMAL					
Acrylates/Octylacrylamide Copolymer	albino rabbits (strain not reported)	10	2000 mg/kg; occlusion not reported; animals observed for 14 d	greater than 2000 mg/kg	[21]
ORAL					
Acrylates/Octylacrylamide Copolymer (aqueous solution ; 15% solids)	Charles River albino rats	2/sex/group	1000, 1500, 2300 mg solids/kg bw; method of oral administration not reported	greater than 2300 mg/kg	[21]
AMP-Acrylates/C1-18 Alkyl Acrylate/ C1-8 Alkyl Acrylamide Copolymer (40% ethanol solution)	rats (strain not reported)	NR	2000 mg/kg	greater than 2000 mg/kg	[8]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/ Hydroxyethylacrylate Copolymer (40% ethanol solution)	rats (strain not reported)	NR	2000 mg/kg	greater than 2000 mg/kg	[7]
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer (70% ethanol solution)	rats (strain not reported)	NR	2000 mg/kg	greater than 2000 mg/kg	[5]
INHALATION					
Acrylates/Octylacrylamide Copolymer (aqueous solution; 10% solids)	Sprague-Dawley rats	5/sex	whole body chamber (exposure concentration of 3.4 mg polymer/l; particle size 5.5 µm; 84% of the aerosol was less than 10 µ in size); animals observed for 14 d	greater than 3.4 mg/l	[21]

NR = not reported

Table 7. Dermal irritation and sensitization

Test Article	Dose/Concentration	Test Population	Procedure	Results	Reference
IRRITATION					
In Vitro					
Acrylamide/Ammonium Acrylate Copolymer (mixture containing 32%)	10 µl; administered neat	reconstructed human epidermis	reconstructed human epidermis cytotoxicity assay; application time 15 min; incubation time 42 h	non-irritating	[26]
Acrylates/t-Butylacrylamide Copolymer	10 mg; 100%	reconstructed human epidermis	reconstructed human epidermis cytotoxicity assay; application time 15 min; incubation time 42 h	non-irritating	[26]
Animal					
Acrylates/Octylacrylamide Copolymer (neutralized, aqueous solution ; 15% solids)	0.5 ml; applied neat	6 New Zealand White rabbits (sex not reported)	test substance applied to intact and abraded skin sites; occlusive conditions; duration of application was not reported.	erythema observed 24 and 72 h after application, in both intact and abraded sites; test substance considered to be mildly irritating.; primarily irritation score of 2.9	[21]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	NR	rabbits (strain and number of animals not reported)	primary skin irritation assay; Draize method; details not provided	non-irritating	[8]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer (40% ethanol solution)	NR	rabbits (strain and number of animals not reported)	primary skin irritation assay; Draize method; details not provided	mildly irritating; PII = 0	[7]
Dimethyl Acrylamide/Hydroxyethyl Acrylate/ Methoxyethyl Acrylate Copolymer (10% aqueous solution)	applied neat	rabbits (strain and number of animals not reported)	primary skin irritation assay; Draize method; details not provided	mildly irritating; PII = 0	[5]
Dimethyl Acrylamide/Hydroxyethyl Acrylate/ Methoxyethyl Acrylate Copolymer (10% aqueous solution)	applied neat	guinea pigs (strain and number of animals not reported)	cumulative skin irritation assay; details not provided	non-irritating	[5]
Human					
Acrylamide/Ammonium Acrylate Copolymer (5% aqueous solution)	applied neat	20 subjects	test substance applied to skin, under occlusive conditions, for 48 h	non-irritating	[9]
Dimethyl Acrylamide/Hydroxyethyl Acrylate/ Methoxyethyl Acrylate Copolymer (50% aqueous solution)	applied neat	40 subjects	patch test; no other details reported	non-irritating	[5]
SENSITIZATION					
In Vitro					
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	NR	reconstructed human epidermis	EpiSkin® method	non-sensitizing	[8]
Dimethyl Acrylamide/Hydroxyethyl Acrylate/ Methoxyethyl Acrylate Copolymer (50% aqueous solution)	applied neat	reconstructed human epidermis	EpiSkin® method	non-sensitizing	[5]
Animal					
Acrylamide/Ammonium Acrylate Copolymer (mixture containing 32%)	applied neat; 0.5 ml (dermal induction); 0.25 ml (dermal challenge)	22 female Dunkin-Hartley guinea pigs	For the intradermal induction, animals were treated with an injection of Freund's Complete Adjuvant and 0.9 % saline. Animals then received a dermal induction application of the test substance, under occlusive conditions for 48 h. A challenge patch was performed 29 d later, using the undiluted test material, under occlusive conditions, for 48 h	non-sensitizing	[27]

Table 7. Dermal irritation and sensitization

Test Article	Dose/Concentration	Test Population	Procedure	Results	Reference
Acrylates/Octylacrylamide Copolymer (aqueous solution and powder form)	intradermal induction: 5% aqueous solution; dermal induction: powder applied neat ; dermal challenge: aqueous solution (100% solids and 50% solids)	20 female guinea pigs/group (strain not reported)	guinea pig maximization assay; animals were exposed to a two-part induction phase: -part 1: injection with of solution containing Acrylates/Octylacrylamide Copolymer (5%) with and without Freund's Complete Adjuvant) -part 2: dermal induction with Acrylates/Octylacrylamide Copolymer powder (8 cm^2 patch; moistened) for 48 h; use of occlusion not reported Animals were then exposed to a challenge phase: -1 saturated, occlusive patch (4 cm^2) of an aqueous solution of Acrylates/Octylacrylamide Copolymer (100% solids) and 1 saturated, occlusive patch (4 cm^2) of an aqueous solution of Acrylates/Octylacrylamide Copolymer (50% solids); both patches were left on for 24 h	non-irritating and non-sensitizing	[21]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer (40% ethanol solution)	applied neat	guinea pigs (strain not reported)	guinea pig maximization assay; no other details provided	non-sensitizing	[8]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-18 Alkyl Acrylamide/ Hydroxyethylacrylate Copolymer in dimethylformamide (mixture containing 38%)	25 µl; 5, 10, 25, 50, and 75%	female CBA/J mice (4/group)	LLNA in accordance with OECD TG 429; positive control: α-hexylcinnamaldehyde in acetone/olive oil; negative control: *N,N*-dimethylformamide; 3-d applications	non-sensitizing; stimulation index: 0.8 – 1.5% (comparable to negative control); no local ear skin irritation; EC3 value was not calculable	[28]
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer (70% ethanolic solution)	applied neat	guinea pig (strain not reported)	guinea pig maximization assay; no other details provided	non-sensitizing	[6]
Human					
Acrylamide/Ammonium Acrylate Copolymer (mixture containing 0.66%)	applied neat	109 subjects	HRIPT; induction phase consisted of 3 applications of the test substance, under occlusive conditions, each wk, for 3 wk; after a 2-wk rest period, challenge patch was applied to an untreated skin site, under occlusive conditions; all patches were applied for 48 h	Mild patch test responses occasionally accompanied by mild papular responses were observed in 28 subjects during the induction and/or challenge phase. The test substance was considered to be non-irritating and non-sensitizing.	[30]
Acrylamide/Ammonium Acrylate Copolymer (5% aqueous solution)	applied neat	50 subjects	HRIPT; details not provided	non-irritating and non-sensitizing	[9]
Acrylates/Octylacrylamide Copolymer (neutralized, aqueous solution; 15% solids)	applied neat	25 subjects/sex	HRIPT; use of occlusion not reported; 24-h patch application	Thirty subjects responded to the application of the test material with very slight to mild erythema. The test substance as considered to be non-irritating and non-sensitizing.	[21]
Acrylates/ *t*-Butylacrylamide Copolymer (formula containing 13.34%)	applied neat; 0.2 ml	96 subjects	HRIPT; semi-occlusive conditions; 2 cm x 2 cm patch	non-irritating and non-sensitizing	[29]

HRIPT: human repeat insult patch test; LLNA: local lymph node assay; NR = not reported; OECD TG: Organisation for Economic Co-operation and Development Test Guidelines

Table 8. Ocular irritation studies

Test Article	Concentration	Test Population	Procedure	Results	Reference
			IN VITRO		
Acrylamide/Ammonium Acrylate Copolymer (3% in water and 0.5% sodium chloride)	applied neat	NR	HET-CAM assay	non-irritating	[9]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	100%	NR	SkinEthic™ HCE (human corneal epithelium) assay	non-irritating	[8]
Dimethyl Acrylamide/ Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer (50% aqueous solution)	applied neat	NR	SkinEthic™ HCE (human corneal epithelium) assay	non-irritating	[5]
			ANIMAL		
Acrylates/Octylacrylamide Copolymer	100%	6 New Zealand White rabbits	ocular irritation assay; irritation of cornea, iris, and conjunctiva observed on days 1, 2, and 3 post-instillation	Non-irritating	[21]
Acrylates/Octylacrylamide Copolymer (neutralized, aqueous solution ; 15% solids)	applied neat	6 New Zealand White rabbits	ocular irritation assay	Iritis and mild conjunctival irritation were noted in 3/6 and 6/6 animals, respectively. Effects were fully reversible within 24 h. The test substance was considered to be mildly irritating.	[21]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer (40% ethanolic solution)	applied neat	NR	ocular irritation assay performed according to the Draize method	slightly irritating	[8]
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/ Hydroxyethylacrylate Copolymer (40% ethanol solution)	applied neat	rabbits (strain and number of animals not reported)	ocular irritation assay performed according to the Draize method	Slightly irritating	[7]
Dimethyl Acrylamide/ Hydroxyethyl Acrylate/ Methoxyethyl Acrylate Copolymer (10% aqueous solution)	applied neat	rabbits (strain and number of animals not specified)	ocular irritation assay performed according to the Draize method	Non-irritating	[5]

HET-CAM = hen's egg test chorioallantoic membrane; NR = not reported

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Concentration of Use by FDA Product Category – Acrylate/Acrylamide Copolymers*

Acrylamide/Ammonium Acrylate Copolymer
Acrylamide/Sodium Acrylate Copolymer
Acrylates/Acrylamide Copolymer
Acrylates/t-Butylacrylamide Copolymer
Acrylates/Methacrylamide Copolymer
Acrylates/Octylacrylamide Copolymer
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydoxyethylacrylate Copolymer
t-Butyacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer
Butyl Acrylate/Isopropylacrylamide/PEG-18 Dimethacrylate Crosspolymer
Corn Starch Acrylamide/Sodium Acrylate Copolymer
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer
Dimethylacrylamide/Lauryl Methacrylate Copolymer
Potassium Acrylates/Acrylamide Copolymer
Sodium Acrylate/Hydroxyethyl Acrylamide Copolymer
Starch/Acrylates/Acrylamide Copolymer

Ingredient	**Product Category**	**Maximum Concentration of Use**
Acrylamide/Sodium Acrylate Copolymer	Tonics, dressings, and other hair grooming aids	2.8%
Acrylamide/Sodium Acrylate Copolymer	Body and hand products Not spray	 0.5%
Acrylamide/Sodium Acrylate Copolymer	Moisturizing products Not spray	 2.8%
Acrylates/Acrylamide Copolymer	Tonics, dressings, and other hair grooming aids Not spray	 0.41%
Acrylates/t-Butylacrylamide Copolymer	Hair sprays Aerosols Pump sprays	 7% 0.06%
Acrylates/t-Butylacrylamide Copolymer	Tonics, dressings, and other hair grooming aids	5%
Acrylates/Octylacrylamide Copolymer	Eyeliners	2-4.6%
Acrylates/Octylacrylamide Copolymer	Eye shadows	0.00097-0.001%
Acrylates/Octylacrylamide Copolymer	Mascaras	7%
Acrylates/Octylacrylamide Copolymer	Hair sprays Aerosols	 0.5-3.2%
Acrylates/Octylacrylamide Copolymer	Tonics, dressings, and other hair grooming aids Mousse	 0.66%
Acrylates/Octylacrylamide Copolymer	Dentifrices Toothpaste	 19.4%

Acrylates/Octylacrylamide Copolymer	Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	4.9%
Acrylates/Octylacrylamide Copolymer	Other skin care preparations	0.75%
Acrylates/Octylacrylamide Copolymer	Suntan products Not spray	 1.5%
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	Hair sprays Aerosol Pump spray	 1.3% 3.9%
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	Tonics, dressings, and other hair grooming aids	5%
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	Deodorants Not spray	 0.05%
AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer	Skin fresheners	0.032%
Corn Starch/Acrylamide/Sodium Acrylate Copolymer	Bath oils, tablets, and salts	2%
Corn Starch/Acrylamide/Sodium Acrylate Copolymer	Body and hand products Not spray	 0.002%
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer	Hair sprays Aerosol	 0.26%
Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer	Tonics, dressings and other hair grooming aids	7%
Dimethylacrylamide/Lauryl Methacrylate Copolymer	Bath soaps and detergents	0.5%

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

Information collected in 2020
Table prepared: January 25, 2021

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: August 17, 2021

SUBJECT: Acrylamide/Ammonium Acrylate Copolymer

Anonymous. 2021. Summary Information Acrylamide/Ammonium Acrylate Copolymer.

August 2021

Summary Information Acrylamide/Ammonium Acrylate Copolymer

Polymer molecular weight: Several millions of Daltons (theoretical calculation).

% of oligomers < 500 Da: < 2%

Impurities: The following not expected: 1.4-Dioxane, Ethylene oxide, Solvent residues (benzene, ...), Free amines, nitrosamines

Monomers: Residual Acrylamide - 2ppm

Toxicity Data

Genotoxicity data: Ames test: "The test substance does not induce reverse mutation on four *Salmonella typhimurium* strains and one *Escherichia coli* WP2(uvrA-) (pKM 101) strain (tested up to 5000 µg/plate)"

Dermal irritation and sensitization data

occlusive patch test 48h on 20 volunteers: «non-irritant» for 48-hour exposures, at a concentration of 5% in water

HRIPT on 50 volunteers: no induction of sensitization reaction and considered as very well tolerated (Global Irritation Index = 0.00) when tested up to 5% in water

Eye Irritation (in vitro)

HET CAM test: Nonirritating at 3% in dilution in water with 0.5% NaCl

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: August 23, 2021

SUBJECT: Acrylamide/Acrylate Copolymers

Anonymous. 2007. Etude De Cytotoxicite sur Epiderme Human Reconstuit (mixture containing 32% Acrylamide/Ammonium Acrylate Copolymer).

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Anonymous. 2010. Evaluation of the Contact Sensitization Potential of Different Test Articles in Normal Healthy Subjects (HRIPT) (mixture containing 0.66% Acrylamide/Ammonium Acrylate Copolymer.

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Rapport n° : [redacted]

Distrib[redacted]or Quote

mixture containing 32% Acrylamide/Ammonium Acrylate Copolymer

ETUDE DE CYTOTOXICITE SUR EPIDERME HUMAIN RECONSTRUIT
BUT : Connaissance
Résumé et conclusion pour la matière première : 53845

CARACTÉRISTIQUES DE LA MATIÈRE PREMIÈRE ÉTUDIÉE

- Code de la matière première étudiée : 53845
- N° de lot : 114270
- Aspect physique et couleur : Lait translucide
- Nature : Polymère
- [redacted] : /
- Test d'interaction MTT : Négatif

PROTOCOLE

- Référence du protocole expérimental : P61/007 - V01
- Concentration testée : Tel quel
- Quantité déposée : 10 µL
- Temps d'application et de post-incubation : 15 min ± 0,5 min et 42h ± 1h
- Lots d'épidermes reconstruits : test 1 : lot N° : [redacted]
  test 2 : lot N° :
  test 3 : lot N° :
- Conditions particulières : Aucune
- Observations au cours du test : /

DATES

- Début des expériences : | 2 | 7 | | 0 | 2 | | 0 | 7 |
- Fin des expériences : | 0 | 2 | | 0 | 8 | | 0 | 7 |

ACCEPTABILITÉ

- DO du témoin négatif ≥ 0,600
- Viabilité du Sodium Dodecyl Sulfate (5%) ≤ 35%
- Ecart-type % viabilité < 18%

} Essai validé

RESULTATS : % VIABILITÉ et CONCENTRATION IL-1α

	% viabilité					IL-1α (pg/ml)				
Matière première	**V1 Essai 1**	**V2 Essai 2**	**V3 Essai 3**	**Moyenne**	**Ecart-type**	**V1 Essai 1**	**V2 Essai 2**	**V3 Essai 3**	**Moyenne**	**Ecart-type**
53845	84,7	85,2	100,0*	90,0	8,7	88,2	7,1	0,0**	31,8	49,0

* La viabilité a été ramenée à 100%, une viabilité supérieure à 100% n'indiquant pas une réponse biologique différente d'une viabilité égale à 100% dans le cadre du test MTT

**Le résultat a été ramené à 0,0 car une valeur négative n'est pas différente de 0.

CONCLUSION

D'après les conditions expérimentales adoptées, l'étude visant à évaluer la tolérance primaire cutanée sur un modèle d'épiderme humain reconstruit laisse supposer que la matière première **53845** est **potentiellement non irritante**.

Remarque: L'étude des MP sur épiderme reconstruit est basée sur le modèle prédictif *in vitro* établit lors des études préliminaires d'optimisation du protocole d'irritation cutanée des produits chimiques (Cotovio et al., 2005)* et l'étude ECVAM. Cette méthode est en attente de validation réglementaire.

[redacted]

Date : 04/09/07 Date : 04/09/07

Conclusion:(Google translate): According to the experimental conditions adopted, the study aimed at evaluating the primary skin tolerance on a reconstructed human epidermis model suggests that the raw material 53845 is potentially non-irritating.

[redacted]

[redacted]

test material was a mixture containing 32% Acrylamide/Ammonium Acrylate Copolymer

[redacted]

SKIN SENSITISATION TEST IN THE GUINEA PIG

AFTER GUILLOT ET AL

PROJECT NUMBER: [redacted]

Date Started: 10 May 1989

Experimental Procedures:

Date Completed: 16 June 1989

AUTHOR: [redacted]

STUDY SPONSOR:

ISSUED BY:

[redacted]

PROJECT NUMBER: [redacted]

[redacted]

QUALITY ASSURANCE UNIT REPORT

The routine inspection of short term toxicity studies at [redacted] is carried out as a continuous process designed to ensure that where possible all critical phases of a particular study type are inspected at least once per month. Dates of inspection for this study type are given below:

STUDY TYPE	DATE(S) OF INSPECTION
Sensitisation:	**08/08/89, 24/08/89**

This report has been checked by [redacted] and accurately records the original laboratory data generated during the study. In addition, the Quality Assurance Unit regularly reviews study reports of this type. Dates of audit for this report type are given below.

Sensitisation Report Audit: **09/08/89**

[redacted]
QUALITY ASSURANCE MANAGER ..... [redacted] .....

DATE: .....5 October 89.....

PROJECT NUMBER: [redacted]

AUTHENTICATION

I, the undersigned, hereby declare that this study was performed under my supervision, as Study Director, according to the procedures herein described. The work was performed in compliance with U.K., Japanese and OECD principles of Good Laboratory Practice, and this report provides an accurate and faithful record of the results obtained.

[redacted] .... DATE: 05/10/89

Study Director

Approved for Issue:

[redacted] DATE: 4/10/89

Head of General Toxicology

for [redacted]

The following scientific and supervisory personnel were involved in the study under the overall supervision of the Study Director.

[redacted]

SUMMARY OF RESULTS

STUDY SPONSOR : [redacted]

PROJECT NUMBER : [redacted]

REFERENCE : [redacted]

1. A study was performed to assess the skin sensitisation potential of the test material in the albino guinea pig. The method used was based on that described by Guillot et al (J. Soc. Cosmet. Chem. 1977 28 357-365).

2. Twenty-two female albino guinea pigs were used for the main study. The following dose volumes of the undiluted test material were used in the induction and challenge phases.

Topical Induction	:	0.50 ml/animal
Topical Challenge	:	0.25 ml/animal

3. The incidence of skin responses noted at challenge are summarised, as follows:

REFERENCE	*NUMBER OF ANIMALS*	*SENSITISATION RESPONSE* - *(negative)*	+ *(positive)*	*% SENSITISATION*
SPAD 189089	*22*	*22*	*0*	*0/22*

4. The test material, SPAD 189089 PAS 5161 (FLE 247568) No. DE LOT 81060 produced a 0% (0/22) sensitisation rate and was classified as a non-sensitiser (Grade 0) to guinea pig skin under the conditions of the study.

[redacted]

SKIN SENSITISATION TEST IN THE GUINEA PIG

AFTER GUILLOT ET AL

PROJECT NUMBER: [redacted]

INTRODUCTION

This study was performed according to [redacted] Standard Protocol Number [redacted] and was designed to assess the contact sensitisation potential of the test material.

The study was designed to follow the method described by Guillot et al, J. Soc. Cosmet. Chem. 1977 28, 357-365.

The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The test system was chosen because the guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method.

METHODS

1. Animals and Animal Husbandry

Twenty-four female albino Dunkin-Hartley guinea pigs were used for the study. Main study animals were supplied by [redacted] At the start of the main study the animals weighed 374 - 447g, and were approximately six to ten weeks old. After a minimum acclimatisation period of five days, each animal was selected at random and given a number unique within the study which was written both on a small area of clipped rump using a black water proof marker-pen, and on the cage card.

The animals were housed in groups of up to four in solid-floor polypropylene cages furnished with softwood shavings. Free access to mains tap water and food (Guinea Pig FD1 Diet, [redacted] was allowed throughout the study.

The animal room was maintained at a temperature of 19 - 24°C and relative humidity of 60 - 70%. The rate of air exchange was approximately 15 changes per hour and the lighting was controlled by a time switch to give 12 hours continuous light and 12 hours darkness.

2. Test Material and Experimental Preparation

The test material was supplied by [redacted]

Description	:	white paste
Container	:	glass screw-top bottle
Label	:	[redacted]
Date of arrival	:	3 April 1989
Storage conditions	:	room temperature

For the purpose of this study the following dose volumes of the test material were used as required:

Topical Induction	:	0.50 ml (undiluted as supplied)
Topical Challenge	:	0.25 ml (undiluted as supplied)

The identification and stability of the test material were not determined as part of this study.

3. Procedure

The sensitising properties of the test material were assessed using the sensitisation method of Guillot et al, J. Soc. Cosmet. Chem. 1977 28 357-365.

The dose volumes of the test material for each stage of the main study were determined by a 'sighting study' in which two female guinea pigs were treated with two dose levels of the test material.

A group of twenty-two female guinea pigs was used for the main study. The bodyweight of each animal was recorded at the start and end of the study (see Appendix I).

Three main procedures were involved in the main study; (a) the verification of the absence of significant individual reactions, (b) an induction of a response and (c) a challenge of the response.

3. Procedure (contd)

a) Main Study: Verification of the Absence of Individual Reactions

The hair from an area approximately 40 mm x 50 mm on upper thoracic region of each animal was removed with veterinary clippers.

On day one a quantity of 0.50 ml of the undiluted test material, was applied on absorbent lint (approximate size 20 mm x 20 mm) to the skin of each animal just behind the left scapulum. The patch was covered with an occlusive dressing of surgical adhesive tape (BLENDERM: approximate size 40 mm x 50 mm), held in place with an elastic sleeve (TUBIGRIP size B). This occlusive dressing was kept in place for 48 hours.

The treated site was evaluated for erythema and oedema, six, twenty-four and forty-eight hours following removal of the patches, using the following scale:

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	*Value*
No erythema	*0*
Very slight erythema (barely perceptible)	*1*
Well-defined erythema	*2*
Moderate to severe erythema	*3*
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	*4*

Oedema Formation	
No oedema	*0*
Very slight oedema (barely perceptible)	*1*
Slight oedema (edges of area well-defined by definite raising)	*2*
Moderate oedema (raised approximately 1 millimetre)	*3*
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	*4*

3. Procedure (contd)

b) Main Study: Topical Induction

On day one, all animals received an intradermal injection (0.1 ml) of Freund's Complete Adjuvant plus 0.9% saline solution in the ratio of 1:1, just behind the right scapulum. 0.5 ml of the undiluted test material was applied immediately behind this area on absorbent lint (approximate size 20 mm x 20 mm). The patch was covered with an occlusive dressing as previously described (3a) and was kept in place for 48 hours. The undiluted test material was re-applied to the same area of skin under occlusive dressings on days 3, 5, 8, 10, 12 and 15. All dressings were completely removed on day 17.

c) Main Study: Topical Challenge

On day 29, an area, approximately 50 - 70 mm x 50 mm on both flanks of each animal, was clipped free of hair with veterinary clippers.

A quantity of 0.25 ml of the undiluted test material, was applied to the shorn right flank of each animal on an absorbent lint patch (approximate size 20 mm x 20 mm), covered by a strip of surgical adhesive tape (BLENDERM: approximate size 40 mm x 50 mm). The patches were occluded with an overlapping length of aluminium foil and secured by a strip of elastic adhesive bandage (ELASTOPLAST: approximate size 250 mm x 70 mm), wound in a double layer around the torso of the animal. The dressing was held in place for forty-eight hours.

On day 31, the dressings were removed and the treated area identified using a black indelible marker-pen. The skin reactions were quantified using the scale described previously, six, twenty-four and forty-eight hours after removal of the occlusive patch.

4. Evaluation of Data

A positive macroscopic reaction is exhibited if, after challenge, any of the following are observed:

a) a focal reaction

b) a vesicular effect

4. Evaluation of Data (contd)

c) erythematous and/or oedematous reactions with scores equal to or greater than two units in comparison with the first application (corresponding to the verification of the absence of significant individual reactions).

A doubtful response is exhibited if the erythematous and/or oedematous reactions, after challenge, showed a difference of only one unit in comparison with the first application. Doubtful reactions are further interpreted using the histopathological findings.

A negative response is exhibited if, after challenge, the scores for erythema and/or oedema are equal to the scores noted at the first application.

5. Expression of Results

The reaction is positive if the animal shows significant macroscopic reactions, which are confirmed histologically, as a sensitisation reaction.

The reaction is negative if the animal does not show any macroscopic response or if the histological examination does not confirm the macroscopic reading.

The reaction is doubtful if a significant macroscopic response is found, but cannot be confirmed histologically.

To classify the sensitisation response the percentage of sensitised animals was compared with the following scale:

% OF SENSITISED ANIMALS	*GRADE*	*SENSITISATION POTENTIAL*
0	*0*	*non-sensitiser*
> 0 to < 10	*I*	*weak sensitiser*
11 to 25	*II*	*mild sensitiser*
26 to 50	*III*	*moderate sensitiser*
51 to 75	*IV*	*strong sensitiser*
76 to 100	*V*	*extreme sensitiser*

ARCHIVES

On completion of the study, all raw laboratory data, specimens, slides and a copy of the final report were transferred to [redacted], where they will be retained for a period of ten years.

6. R E S U L T S

TOPICAL SIGHTING TEST

GUILLOT CONTACT SENSITISATION STUDY IN THE GUINEA PIG

TOPICAL SIGHTING TEST - MACROSCOPIC OBSERVATIONS

REFERENCE: [redacted]

ANIMAL IDENTIFICATION AND SEX	DOSE (ml)	EVALUATION OF SKIN REACTIONS (HOURS AFTER REMOVAL OF DRESSINGS)					
		6		24		48	
		E	Oe	E	Oe	E	Oe
A	0.5	1	0	0	0	0	0
FEMALE	0.25	0	0	0	0	0	0
B	0.5	1	0	1	0	1D	0
FEMALE	0.25	0	0	0	0	0	0

E = erythema Oe = oedema D = desquamation

7. C O N C L U S I O N

CONCLUSION

The test material, [redacted] produced a 0% (0/22) sensitisation rate and was classified as a non-sensitiser (Grade 0) to guinea pig skin, under the conditions of this study.

EVALUATION OF THE CONTACT SENSITIZATION POTENTIAL OF DIFFERENT TEST ARTICLES IN NORMAL HEALTHY SUBJECTS (HRIPT)

SUMMARY

[redacted]

[redacted]

[redacted]

Study Objective: The main objective of this study is to confirm the absence of sensitization potential of a test article when patched under repetitive occlusive conditions in a population of one hundred (100) human subjects.

Secondarily, the potential irritant effect of the product may be evaluated during the Induction phase of the protocol.

Method: Study under dermatological control.
Induction: 3 occlusive patches on the back, each week for 3 weeks.
Patches applied for 48 hours.
Rest phase: 2 weeks
Challenge: One (1) 48-hour patch on the original and alternate site.
Skin grading 30 minutes, 48 hours and 96 hours after removal of the patches.

Principal Investigator: [redacted]

Medical Investigator:

Conclusion:

Not sensitizing ☒	Non-irritating ☒
Sensitizing ☐	Irritation acceptable (normal) for product type ☐
Additional data needed ☐	Irritation higher than normal for product type ☐

Signatures

3/8/10
Date of Final Report

[redacted]

[redacted]

FINAL REPORT

[redacted]

EVALUATION OF THE CONTACT SENSITIZATION POTENTIAL OF DIFFERENT TEST ARTICLES IN NORMAL HEALTHY SUBJECTS (HRIPT)

[redacted]

SUBMITTED TO:

[redacted]

Start Date: October 05, 2009
End Date: November 20, 2009

I, the undersigned, certify that this document accurately describes the conduct and results of this investigation and that the study was conducted in the spirit of GCP and ICH E6 guidelines.

[redacted]

3/8/10
Date of Final Report

[redacted]

QUALITY ASSURANCE STATEMENT

This study was conducted in accordance with the spirit of Good Clinical Practice regulations described in CFR 21, Part 50 (Protection of Human Subjects - Informed Consent), Part 56 (Institutional Review Boards) and the International Conference on Harmonization – Good Clinical Practice Guidelines, May 9, 1997, Federal Register.

For Purposes of this clinical study:

- ☒ Informed Consent was obtained.
- ☐ Informed Consent was not obtained.
- ☒ An IRB review was neither requested nor required.
- ☐ An IRB was convened and approval to conduct the proposed clinical research was granted.

The Quality Assurance Department conducted in-study inspections (audits) on a random sampling of subjects during the study. Written status reports of the inspections and findings were submitted to Management.

Date of Inspection	Type of Inspection	Date Reported to Management
10/05/2009	Day 1 procedures including study organization and management, qualification of subjects, consenting process and patching procedures.	10/05/2009
10/07/2009 10/14/2009 10/21/2009 10/26/2009	Induction phase including scoring of the test sites and patching procedures.	10/08/2009 10/14/2009 10/21/2009 10/26/2009
11/09/2009 11/16/2009	Day 1 of Challenge phase including explanation of Challenge procedure and patch application.	11/09/2009 11/24/2009
11/11/2009 11/18/2009	48-Hour read of Challenge phase.	11/11/2009 11/18/2009
11/13/2009 11/20/2009	96-Hour read of Challenge phase.	11/13/2009 11/20/2009
02/23/2010	Final Review of Data Tables	02/23/2010
02/04/2010	Review of Draft Report	02/04/2010
03/05/2010	Review of Final Report	03/05/2010

This study report has been reviewed to ensure that it correctly describes the methods of testing and that the reported results accurately reflect the data obtained during the clinical study [redacted]

On the basis of the audits conducted, this report is considered to be a true and accurate reflection of the source data obtained.

[redacted]

3/8/10
Date

03/08/2010
Date

[redacted]

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TEXT TABLES

POST-TEXT TABLE

[redacted]

[redacted]

CLINICAL SAFETY EVALUATION

EVALUATION OF THE CONTACT SENSITIZATION POTENTIAL OF DIFFERENT TEST ARTICLES IN NORMAL HEALTHY SUBJECTS (HRIPT)

1. SUMMARY

A continuous wear (Jordan-King) patch test procedure was conducted is to confirm the absence of sensitization potential of **Test Article:** [redacted] when patched under repetitive occlusive conditions in a population of one hundred (100) human subjects. Secondarily, the potential irritant effect of the product may be evaluated during the Induction phase of the protocol.

Of the one hundred and thirty-nine (139) subjects enrolled in the study, one hundred and nine (109) subjects satisfactorily completed the test procedure. Eighteen (18) test subjects (Subject Nos.: 4, 14, 28, 29, 31, 50, 59, 78, 81, 93, 96, 103, 108, 113, 126, 132, 137 and 138) were discontinued due to noncompliance (e.g., either missed a visit or unwilling to follow procedures outlined in protocol). One (1) test subject (Subject No. 17) was discontinued due to an adverse event (development of a yeast infection which treatment involved exclusionary medication). One (1) test subject (Subject No. 44) was discontinued due to a serious adverse event (hospitalized for contracting the H1N1 virus). One (1) test subject (Subject No. 56) was discontinued due to tape reaction. One (1) test subject (Subject No. 92) was discontinued due to technician error (patches were placed over a scar). Eight (8) subjects (Subject Nos.: 5, 6, 20, 55, 61, 70, 75 and 106) could not be contacted to determine the reason for discontinuation; therefore, these subjects were considered "lost to follow-up".

Under the conditions of a Human Repeated Insult Patch Test Procedure (Jordan-King; occlusive patch conditions), **Test Article:** [redacted] produced generally transient, mild (1-level) patch test responses (specific and non-specific) on twenty-eight (28/109 or 26% of the test population) test subjects during the Induction and/or Challenge phases of the study. The skin reactivity observed with test article [redacted] was considered neither evidence of clinically meaningful irritation nor allergic in nature.

2. OBJECTIVE

The main objective of this study was to confirm the absence of sensitization potential of a test article when patched under repetitive occlusive conditions in a population of one hundred (100) human subjects.

3. STUDY PERSONNEL

Principal Investigator: [redacted]
Medical Investigator:
Study Coordinator:

4. SPONSOR

[redacted]

5. SPONSOR'S REPRESENTATIVE

[redacted]

[redacted]

6. TESTING FACILITY

[redacted]

7. EXPERIMENTAL DESIGN

7.1 INFORMED CONSENT

The investigator (or his designee) explained the nature of the study, its purpose and associated procedures, the expected duration and the potential benefits and risks of participation to each subject prior to his/her entry into the study. Each subject was provided with a copy of the informed consent form, had ample opportunity to ask questions and was informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. No subject entered the study before his/her informed consent form was obtained.

7.2 SUBJECT SELECTION

One hundred thirty-nine (139) subjects, 81 females and 58 males, ranging in age from 18 to 68 years were empanelled for this study.

7.2.1 INCLUSION CRITERIA

Subjects included in the study:

1. Were males and females between 18 to 70 years of age in reasonably good health (no physical required). The 60-70 age bracket did not exceed 10% of the total number of subjects;
2. Were able to read and sign the informed consent statement;
3. Female subjects of childbearing age must have been surgically sterile, post-menopausal or using an acceptable method of birth control. Females using an acceptable method of birth control must have been using the same regimen for 3 months prior to enrollment and must have continued the same method throughout the duration of the study as well as 3 months following the completion of the study;
4. Were of any race or skin type provided their skin pigmentation did not interfere with evaluations;
5. Minor deviations in normal medical history not considered to be clinically significant by the investigator and the sponsor were permitted;
6. Refrained from sunbathing, using tanning salons, swimming or using hot tubs during the entire study; and
7. Agreed to try and keep the patches as dry as possible.

7.2.2 NON-INCLUSION CRITERIA

Subjects not included in the study:

1. Were participating in another research study, had participated in a study involving their back within the past 28 days and/or had participated in more than 3 L'Oreal patch studies within the last year;
2. Were using immunosuppressive drugs and/or undergoing an organ transplant;
3. Were using topical or systemic anti-inflammatory drugs for a defined medical condition (e.g. aspirin, ibuprofen, corticosteroids);
4. Applied any anti-inflammatory drug to their back within the last 2 months prior to study initiation;
5. Had clinically significant active dermatitis or skin disease anywhere on the body (excluding facial acne);
6. Had asthma or diabetes (insulin-dependant only);

[redacted]

7. Were receiving allergy injections, had their final injection within the week prior to starting the study or were expecting to begin allergy injections during the course of the study;
8. Received treatment for malignancy (of any kind) within the last 6 months prior to study enrollment;
9. Suffered from immune deficiency or autoimmune diseases;
10. Were lactating or pregnant;
11. Had a bilateral mastectomy, a mastectomy within the last year and/or axillary lymph nodes (both arms) removed for any reason;
12. Had skin disorders (scars, moles or other blemishes/abnormalities) in the test area which, in the opinion of the investigator, might have interfered with the evaluation of the test articles;
13. Had intensive exposure to sunlight (natural and artificial) within 1 month prior to study enrollment;
14. Had undergone intensive treatment with retinoids within 3 months prior to study enrollment;
15. Received vaccinations within 3 weeks prior to study enrollment or planned on being vaccinated during the course of the study;
16. Had a clinically significant dermographism or skin disorder or were taking a medication which, in the opinion of the investigator, might have interfered with the evaluation of the test articles or placed the subject at undue risk;
17. Were unwilling or did not have the ability to sign the informed consent or comply with protocol guidelines;
18. Had been deprived of their freedom by administrative or legal decision or were under guardianship;
19. Were unable to be contacted in an emergency;
20. Had a history of skin cancer;
21. Had a documented history of contact allergy;
22. Had excessive skin reactivity to patch materials; and/or
23. Were employees of the testing facility.

7.2.3 SUBJECT DEMOGRAPHICS

Demographic information is summarized in Text Table 7-1.

Text Table 7-1 Demographics of Subjects

		Enrolled N=139	Completed N=109
Age of Subjects (years)	Mean	40.7	42.2
	SD	12.6	12.6
	Median	42.0	44.0
	Range	18-68	18-68
	Range (60-70)	6 (4.3%)	6 (5.5%)
Gender of Subjects	Female	81 (58.3%)	66 (60.6%)
	Male	58 (41.7%)	43 (39.4%)
Ethnicity	African American	70 (50.4%)	50 (45.9%)
	Caucasian	48 (34.5%)	41 (37.6%)
	Hispanic	15 (10.8%)	12 (11.0%)
	Asian/Pacific Islander	4 (2.9%)	4 (3.7%)
	Native American	2 (1.4%)	2 (1.8%)

[redacted]

Discontinued subjects' data are shown up to the point of discontinuation but are not used in the Results and Discussion or Conclusion sections of this final report.

7.3 TEST ARTICLE

[redacted]

The testing facility confirmed receipt of the test article and used the test article only within the framework of this clinical study and in accordance with the study protocol. Responsibility of the identity, purity, strength, composition and stability of the test article remained with the sponsor. The test article was stored at room temperature in a secured location until use. After 90 days from the conclusion of the study the test articles will be disposed according to [redacted]

The investigator or his designee will sign the appropriate forms (after all subjects have been completed) to document the destruction of the used and remaining test articles. The testing facility will keep a record of the test article destruction.

[redacted]

7.5 QUALITY ASSURANCE

This study was conducted in accordance with the spirit of Good Clinical Practice Regulations (21 CFR 50: Protection of Human Subjects-Informed Consent and ICH-GCP Consolidated Guidelines, May 9, 1997 Federal Register). The study data and Final Report were reviewed and signed by Quality Assurance staff of RCTS, Inc. The investigator will allow representatives of the Sponsor's monitoring team (and of the Food and Drug Administration) to inspect all study records and Case Report Forms at regular intervals throughout the study. These inspections are for the purpose of verifying the adherence to the protocol, the completeness and exactness of the data entered in the report form and compliance with regulations.

8. METHOD

The study was initiated on October 05, 2009.

8.1.1 Screening/Induction 1/Day 1

At the Screening/Day 1 visit, potential subjects received all necessary written and verbal information and signed an informed consent form prior to entering the study. Subjects who fulfilled all of the inclusion and none of the exclusion criteria outlined in the study protocol were allowed to participate in the study and received a unique subject number.

Prior to test article application the test site was evaluated to ensure no dermatological condition, or anything that would interfere with the evaluation of the test site, was present. The site was initially wiped with a cotton ball treated with 70% isopropyl alcohol after which approximately 0.1 mL of the test article, or enough to fill the chamber, was placed onto an occlusive Finn Chamber patch (8 mm Finn Chambers on Scanpor), and the patch

[redacted]

applied to the back of each subject above the waist, between the left scapula and the spinal mid-line. The test article was tested neat (as received). The times of patch preparation and patch application were documented for each subject at each application day.

8.1.2 Inductions 2-9/Days 3-20

On Days 3-20, subjects arrived at the testing facility at which time they were queried as to any adverse events they might have experienced or any concomitant medications they might have taken since their last visit to the testing facility. The patch was then removed by the testing facility personnel approximately 48 hours after Monday and Wednesday application and approximately 72 hours after Friday application. Skin reactions were noted approximately 15-30 minutes after patch removal. The test site was scored by a trained evaluator just prior to the next patch application using the following 5-point scale:

0 = No visible reaction
1 = Mild erythema (pink)
2 = Moderate erythema (definite redness)
3 = Marked erythema (very intense redness)
4 = Severe erythema (deep red)

Inflammatory Responses ≥1 with accompanying palpable reactivity (edema, papules, etc.) were also graded using the following International Contact Dermatitis Group (ICDRG) scale:

0 = No reaction
+ = Weak positive reaction (erythema, edema, possible papules)
++ = Strong positive reaction (erythema, edema, papules, vesicles)
+++ = Extreme positive reaction (intense erythema, edema, vesicles may coalesce to form a blister)

All other observed dermal sequelae (i.e., dryness, hypo- or hyperpigmentation) were appropriately recorded and described as mild, moderate or severe.

Following evaluation, the test site was cleansed with a cotton ball wet with deionized water and a fresh patch containing 0.1 mL of the test article was applied to the subject's back. In general, this procedure was repeated every Monday, Wednesday and Friday until nine (9) applications of the test article had been made.

Procedurally, if a subject developed a 2-level (moderate) erythema reaction or greater during the Induction phase, or if the skin responses warranted a change in site, the test article amount was increased to 0.2 mL and the patch was applied to a previously unpatched, adjacent site, under semi-occlusive (Trumed™ patches containing needlepunch absorbent and Alpharma Scantape) patch conditions. Residual scores were recorded through to the end of the study for all previously exposed sites. When practical, reactions of 2-level or more were confirmed/validated by a second scorer and were reported to the sponsor via email. If the reactivity seen was considered a pre-existing allergy, the subject was examined by the Board Certified Dermatologist. If a 2-level reaction (or greater) occurred at the new site, no further applications were made; however, all subjects were subsequently patched with the test material at a naïve site during the Challenge phase of the study unless, in the opinion of the Principal Investigator, it was unwise to do so.

8.1.3 Day 22 (read only)

On Day 22 subjects returned to the testing facility and a trained evaluator examined the test site and recorded the degree of erythema and any other dermal sequelae present. At the conclusion of the Day 22 visit no further patches were applied and the subjects began a 10-20 day rest period following the final Induction application.

8.2 CHALLENGE PHASE

The Challenge phase was initiated on November 09, 2009. The final Challenge patch reading was made on November 20, 2009.

[redacted]

8.2.1 Day 1 of Challenge Phase

Approximately 10-20 days following the application of the last Induction patch subjects returned to the testing facility for the Challenge phase of the study. The same test article evaluated in the Induction phase was applied in the Challenge phase under the same testing conditions. Application consisted of applying 0.1mL of the test article to a patch and applying the patch to the same site as during the induction phase as well as a naïve site located away from the original application site (opposite side of the upper back). During the challenge phase the test article remained in contact with the skin for a period of approximately 48 hours.

8.2.2 Days 3 and 5 of Challenge Phase (48 and 96 hours after patch application)

Subjects returned to the testing facility forty-eight (48) hours after Challenge patch application for supervised patch removal. The site was scored 48- and 96-hours after test article application (i.e., 30 minutes after patch removal and 48-hours after patch removal) using the same 5-point scale as used for the Induction phase. All subjects were instructed to report any delayed skin reactivity that might have occurred after the final Challenge patch reading. When warranted, selected test subjects returned to the testing facility for additional examinations and scoring to determine possible increases or decreases in Challenge patch reactivity.

9. PROTOCOL AMENDMENT

No amendments were made to the original protocol.

10. ADVERSE EVENTS (AEs)

Two (2) non-serious adverse events were reported during the course of the study:

- Subject Number 4 reported that she had experienced a moderate breakout on the upper left side of her back beyond 24 hours of the first application of the test article (total amount of test article received prior to adverse event = 0.1 ml). The subject removed the patch her self and decided to discontinue herself from the study. The event lasted 1 hour before subsiding. It is unknown if this adverse event is related to the test article.
- Subject Number 17 reported that she developed a yeast infection beyond 24 hours of the first application of the test article (total amount of test article received prior to adverse event = 0.1 ml). The subject was prescribed 400mg Motrin® (twice a day) and 150mg Diflucan® (once). The subject also took 750 mg Hydrocodone as needed. This subject was discontinued from the study due to taking exclusionary medication. Contact with the subject could not be re-established in order to determine the duration of the event. This adverse event is definitely unrelated to the test article.

Four (4) serious adverse events were reported during the course of the study:

- Subject Number 14 [redacted]on 10/9/09 that she was admitted to the hospital on 10/05/09 after being involved in a car accident (total amount of test article received prior to adverse event = 0.1 ml). Her patches were removed by the attending physician. She was discharged after 5 hours of hospitalization and prescribed 500mg of Hydrocodone and 30mg of Flextra® to be taken as needed for pain. The subject took the medication sporadically over a three week period before the pain completely subsided. The Sponsor was notified of this event on 10/12/09 via email. This adverse event is definitely unrelated to the test article.
- Subject Number 44 [redacted]on 10/12/09 that she was diagnosed with the H1N1 virus on 10/12/09. She was admitted to the hospital on 10/12/09 (total amount of test article received prior to adverse event = 0.1 ml). She was hospitalized for 2 days and prescribed 500mg of Levaquin® to be taken once daily for 10 to 14 days and Tamiflu® 75mg twice daily to be taken for 5 days. The Sponsor was notified of this event on 10/12/09 via email. This adverse event is definitely unrelated to the test article.
- Subject Number 139 [redacted]on 11/16/09 that she was admitted to the hospital on 11/11/09 (during the study's rest period) due to chest pain. She was diagnosed with hypertension and was monitored at the hospital for 2 days. No medications were administered at that time. While the subject was in the hospital, it was also determined that she was diabetic. She was administered 4cc of insulin while she was hospitalized.

[redacted]

She was discharged on 11/12/09 and she was instructed to see her regular physician in order to determine any additional medications she would need for these conditions. Several attempts were made to obtain the names and dosage of the maintenance medications she was prescribed after her hospitalization, however, contact with the subject could not be re-established. The Sponsor was notified of these events on 11/17/09 via email. These adverse events are definitely unrelated to the test article.

11. PROTOCOL DEVIATIONS

Protocol deviations can be located in Post-Text Table II, Page 14, of this report.

In the opinion of the Principal Investigator, the data for the above mentioned subjects are considered valid for the purposes of determining irritation and contact sensitization potential of the test article.

12. RESULTS AND DISCUSSION

(See Post-Text Table I for Individual Scores)

Generally transient, mild (1-level) patch test responses (specific and non-specific), occasionally accompanied by mild papular responses, were observed on twenty-eight (28/109 or 26% of the test population) test subjects during the Induction and/or Challenge phases of the study. One (1) test subject (Subject No. 85) exhibited mild (1-level) erythema accompanied by pustules during the Induction phase of the study. One (1) test subject (Subject No. 69) displayed mild dryness without erythema during the Induction phase of the study. The skin reactivity observed with test article [redacted] was considered neither evidence of clinically meaningful irritation nor allergic in nature.

A summary table for the frequency of clinical observations for the Induction and Challenge phases is shown below:

		Frequency Table																	
		Induction Exposure No.																	
Clinical Score:	ICRDG Score:	1	ICDRG	2	ICDRG	3	ICDRG	4	ICDRG	5	ICDRG	6	ICDRG	7	ICDRG	8	ICDRG	9	ICDRG
0	0	107	1	103	1	104	1	100	0	104	0	102	1	104	1	105	1	106	1
1	+	2	0	6	0	5	0	9	0	5	0	7	0	5	0	4	0	3	0
2	++	0	0	0	0	0	0	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	0	0	0	0	0	0
4	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]
Total	Total	109	1	109	1	109	1	109	0	109	0	109	1	109	1	109	1	109	1

		Frequency Table							
		Challenge							
Clinical Score:	ICRDG Score:	48 Hr				96 Hr			
		Left	ICDRG	Right	ICDRG	Left	ICDRG	Right	ICDRG
0	0	103	0	105	0	109	0	108	0
1	+	6	0	4	0	0	0	1	0
2	++	0	0	0	0	0	0	0	0
3	+++	0	0	0	0	0	0	0	0
4	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]
Total	Total	109	0	109	0	109	0	109	0

13. CONCLUSIONS

Under the conditions of a Human Repeated Insult Patch Test Procedure (Jordan-King; occlusive patch conditions), **Test Article:** [redacted] produced generally transient, mild (1-level) patch test responses (specific and non-specific) on twenty-eight (28/109 or 26% of the test population) test subjects during the Induction and/or Challenge phases of the study. The skin reactivity observed with test article [redacted] was considered neither evidence of clinically meaningful irritation nor allergic in nature.

[redacted]

[redacted]

[redacted]

Subjects' Individual Scores

Subj. No.	Subj. Initals	Subj. Age	Subj. Gender	Subj. Ethnicity	Induction Exposure No.																Challenge							
					1	ICDRG	2	ICDRG	3	ICDRG	4	ICDRG	5	ICDRG	6	ICDRG	7	ICDRG	8	ICDRG	9	ICDRG	48 Hr				96 Hr			
																							Left	ICDRG	Right	ICDRG	Left	ICDRG	Right	ICDRG
1	[redacted]				0		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		0	
3					0		1		0		0		0		0		0		0		0		0		0		0		0	
4					Disc																									
5					0		0		0		0		0		0		0		0		0		Disc							
6					Disc																									
7					0		0		0		0		0		0		0		0		0		0		0		0		0	
8					0		0		0		1		0		1		0		0		0		0		0		0		0	
9					0		0		0		0		0		0		0		0		0		0		0		0		0	
10					0		0		1		0		0		0		0		0		0		0		0		0		0	
11					0		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		0	
13					0		0		0		0		0		0		0		0		0		0		0		0		0	
14					Disc																									
15					0		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		0	
17					0		Disc																							
18					0		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		0	
20					0		0		0		0		0		0		0		Disc											
21					0		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		0	
23					0		0		0		0		0		0		0		0		1		0		0		0		0	
24					0		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		0	
26					0		0		0		0		0		0		0		0		0		0		0		0		0	
27					0		0		1		0		0		0		0		0		0		0		0		0		0	
28					0		0		0		0		0		0		0		0		Disc									
29					0		0		0		0		0		Disc															
30					0		0		0		0		0		0		0		0		0		0		0		0		0	
31					0		0		0		0		0		0		Disc													
32					0		0		0		0		0		1p	0	0		0		0		0		0		0		0	
33					0		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		0	
35					0		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		0	
37					0		0		0		0		0		0		0		0		0		0		0		0		0	
38					0		0		1		0		0		0		0		0		0		0		0		0		0	
39					0		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		0	
41					0		0		0		0		0		0		0		0		0		0		0		0		0	
42					0		0		0		0		1		0		0		0		0		0		0		0		0	
43					0		0		0		0		0		0		0		0		0		0		0		0		0	
44					Disc																									
45					0		0		0		0		0		0		0		0		0		0		0		0		0	
46					0		0		0		0		0		1		1		0		0		0		0		0		0	
47					0		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		0	
49					0		0		0		0		0		0		0		0		0		0		0		0		0	
50					0		0		0		0		0		0		0		0		0		0		0		Disc			
51					0		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		0	
53					0		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		0	
55					0		1		0		0		0		0		0		0		1		Disc							
56					0		0		0		Disc																			
57					0		1		0		0		0		0		0		0		0		0		0		0		0	
58					0		0		0		0		0		0		0		0		0		0		0		0		0	
59					0		Disc																							
60					0		0		0		0		0		0		0		0		0		0		0		0		0	
61					0		0		0		0		0		Disc															
62					0		0		0		0		0		0		0		0		0		1		1		0		0	
63					0		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		0	
65					0		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		0	
67					0		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		0	

Clinical Observation Scale:
0 = No evidence of any effect
1 = Mild (Erythema covering most of the contact site, pink in color)
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4 = Severe (Deep-red erythema. May or may not show signs of vesiculation or weeping)

ICDRG Scoring Scale:
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+ = Weak positive reaction (erythema, edema, possible papules)
++ = Strong positive reaction (erythema, edema, papules, vesicles)
+++ = Extreme positive reaction (intense erythema, edema, vesicles may coalesce to form a blister)

Disc = Discontinued
p = Mild papular response

[redacted]

[redacted]

[redacted]

Subj. No.	Subj. Initals	Subj. Age	Subj. Gender	Subj. Ethnicity	Induction Exposure No.																Challenge							
					1	ICDRG	2	ICDRG	3	ICDRG	4	ICDRG	5	ICDRG	6	ICDRG	7	ICDRG	8	ICDRG	9	ICDRG	48 Hr				96 Hr			
																							Left	ICDRG	Right	ICDRG	Left	ICDRG	Right	ICDRG
69	MJ	48	Male	African American	0		0		0		0		0		0		0d		0		0		0		0		0		0	
70	[redacted]				0		Disc																							
71					0		0		0		0		0		0		0		0		0		0		0		0		0	
72					0		0		0		0		1		0		0		0		0		0		0		0		0	
73					0		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		0	
75					0		0		0		0		Disc																	
76					0		0		0		0		0		0		0		0		0		0		0		0		0	
77					0		0		0		0		0		0		0		0		0		0		0		0		0	
78					Disc																									
79					0		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		0	
81					0		0		0		Disc																			
82					0		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		0	
84					0		0		0		0		0		0		0		0		0		0		0		0		0	
85					1Pu	0	1Pu	0	1Pu	0	0		0		0		0		0		0		0		0		0		0	
86					0		0		0		0		0		0		0		0		1		0		1		0		0	
87					0		0		0		0		0		0		0		0		0		0		0		0		0	
88					0		0		0		0		0		0		0		0		0		0		0		0		0	
89					0		0		1		1		1		0		1		0		0		0		0		0		0	
90					0		0		0		0		0		0		0		0		0		0		0		0		0	
91					0		0		0		1		1		1		1		1		0		1		0		0		0	
92					Disc																									
93					0		0		Disc																					
94					0		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		0	
96					0		Disc																							
97					0		0		0		0		0		1		1p	0	1p	0	1p	0	1		1		0		0	
98					0		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		0	
100					0		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		0	
102					0		0		0		0		0		0		0		0		0		1		0		0		0	
103					0		Disc																							
104					0		0		0		0		0		0		0		0		0		0		0		0		0	
105					0		0		0		0		0		0		0		0		0		0		0		0		0	
106					Disc																									
107					0		1		0		1		0		0		0		0		0		0		0		0		0	
108					0		0		0		0		0		0		0		Disc											
109					0		0		0		1		1		1		1		0		0		1		0		0		0	
110					1		1		1		0		0		0		0		0		0		0		0		0		0	
111					0		0		0		0		0		1		0		0		0		0		0		0		0	
112					0		1		0		1		0		0		0		0		0		0		1		0		1	
113					Disc																									
114					0		0		0		0		0		0		0		1		0		0		0		0		0	
115					0		0		0		0		0		0		0		0		0		0		0		0		0	
116					0		0		0		0		0		0		0		0		0		0		0		0		0	
117					0		0		0		0		0		0		0		0		0		0		0		0		0	
118					0		0		0		1		0		0		0		0		0		0		0		0		0	
119					0		0		0		0		0		0		0		0		0		0		0		0		0	
120					0		0		0		0		0		0		0		0		0		0		0		0		0	
121					0		0		0		0		0		0		0		0		0		0		0		0		0	
122					0		0		0		1		0		0		0		0		0		0		0		0		0	
123					0		0		0		0		0		0		0		0		0		0		0		0		0	
124					0		0		0		0		0		0		0		0		0		0		0		0		0	
125					0		0		0		0		0		0		0		0		0		0		0		0		0	
126					Disc																									
127					0		0		0		0		0		0		0		0		0		0		0		0		0	
128					0		0		0		0		0		0		0		0		0		0		0		0		0	
129					0		0		0		0		0		0		0		0		0		0		0		0		0	
130					0		0		0		0		0		0		0		0		0		0		0		0		0	
131					0		0		0		0		0		0		0		0		0		0		0		0		0	
132					Disc																									
133					0		0		0		0		0		0		0		1		0		0		0		0		0	
134					0		0		0		0		0		0		0		0		0		1		0		0		0	
135					0		0		0		0		0		0		0		0		0		0		0		0		0	
136					0		0		0		0		0		0		0		0		0		0		0		0		0	
137					Disc																									
138					Disc																									
139					0		0		0		0		0		0		0		0		0		0		0		0		0	

Scale: 0 = No evidence of any effect
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ICDRG Scale:
0 = No reaction
+ = Weak positive reaction (erythema, edema, possible papules)
++ = Strong positive reaction (erythema, edema, papules, vesicles)
+++ = Extreme positive reaction (intense erythema, edema, vesicles may coalesce to form a blister)

Disc = Discontinued
d = Mild dryness
p = Mild papular response
Pu = Pustules

Frequency Table

Clinical Score:	ICRDG Score:	Induction Exposure No.																			Challenge							
		1	ICDRG	2	ICDRG	3	ICDRG	4	ICDRG	5	ICDRG	6	ICDRG	7	ICDRG	8	ICDRG	9	ICDRG		48 Hr				96 Hr			
																					Left	ICDRG	Right	ICDRG	Left	ICDRG	Right	ICDRG
0	0	107	1	103	1	104	1	100	0	104	0	102	1	104	1	105	1	106	1		103	0	105	0	109	0	108	0
1	+	2	0	6	0	5	0	9	0	5	0	7	0	5	0	4	0	3	0		6	0	4	0	0	0	1	0
2	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0		0	0	0	0	0	0	0	0
4	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]		0	[redacted]	0	[redacted]	0	[redacted]	0	[redacted]
Total	Total	109	1	109	1	109	1	109	0	109	0	109	1	109	1	109	1	109	1		109	0	109	0	109	0	109	0

Protocol Deviations

Subject Number	Deviation
43	Panelist had a change in birth control within the last 3 months. She went from having an IUD to taking birth control pills.
7, 10, 12, 18, 19, 23, 24, 30, 34, 38, 41, 45, 47, 52, 54, 55, 57, 58, 60, 62, 63, 69, 71, 72, 73, 74, 76, 77, 80, 83, 86, 88, 99, 100, 112, 116, 124, 127, 128, 134, 135, 136	Missed 1 visit.
139	Subject was diagnosed with diabetes and given insulin while study was in progress. Allowed to stay on study

Rapport n° : [redacted]

Dis[redacted] or Quote

test material 100% Acrylates/t-Butylacrylamide Copolymer

ETUDE DE CYTOTOXICITE SUR EPIDERME HUMAIN RECONSTRUIT
BUT : Connaissance
Résumé et conclusion pour la matière première : 71596

CARACTÉRISTIQUES DE LA MATIÈRE PREMIÈRE ÉTUDIÉE

- Code de la matière première étudiée : 71596
- Aspect physique et couleur : Poudre blanche
- [redacted] : /

N° de lot : 01240229U0
Nature : Polymère
Test d'interaction MTT : Negatif

PROTOCOLE

- Référence du protocole expérimental : P61/007 - V01
- Concentration testée : Tel quel
- Quantité déposée : 10 mg
- Temps d'application : 15min ± 0,5min
- Temps d'incubation : 42h ± 1h
- Lots d'épidermes reconstruits : test 1 : lot N° : [redacted]
  test 2 : lot N° :
- Conditions particulières : Aucune
- Observations au cours du test :

DATES

- Début des expériences : 17/10/06
- Fin des expériences : 22/01/07

ACCEPTABILITÉ

- DO du témoin négatif ≥ 0,600
- Viabilité du Sodium Dodecyl Sulfate (5%) ≤ 35% et EC < 18%

} Essai validé

Google translate conclusion: According to the experimental conditions adopted, the study aiming to evaluate the primary tolerance skin on a model of reconstructed human epidermis suggests that the raw material 71596 is potentially non-irritating.

RESULTATS : % VIABILITÉ et CONCENTRATION IL-1α

	% viabilité				IL-1α (pg/ml)			
Matière première	V1 Essai 1	V2 Essai 2	Moyenne	Ecart-type	V1 Essai 1	V2 Essai 2	Moyenne	Ecart-type
71596	100,0	100,0	100,0	/	0,0**	0,0**	0,0**	/**

Les résultats ont été ramenés à 100%, une viabilité supérieure à 100% n'indiquant pas une réponse biologique différente d'une viabilité égale à 100% dans le cadre du test MTT. Il n'y a donc pas lieu de calculer l'écart-type.
**Les résultats ont été ramenés à 0,0 car une valeur négative n'est pas différente de 0. Il n'y a donc pas lieu de calculer l'écart-type.

CONCLUSION

D'après les conditions expérimentales adoptées, l'étude visant à évaluer la tolérance primaire cutanée sur un modèle d'épiderme humain reconstruit laisse supposer que la matière première **71596** est **potentiellement non irritante**.

Remarque: L'étude des MP sur épiderme reconstruit est basée sur le modèle prédictif *in vitro* établit lors des études préliminaires d'optimisation du protocole d'irritation cutanée des produits chimiques (Cotovio et al., 2005)* et l'étude ECVAM. Cette méthode est en attente de validation réglementaire.

*: [redacted]

EXPERIMENTATEUR (S)

[redacted]

Date : 31.01.07

Signature : [redacted]

RESPONSABLE

[redacted]

Date : 22/2/07

Signature : [redacted]

[redacted]

Human Repeated Insult Patch Test with Challenge

(2cm X 2cm SEMI-OCCLUSIVE 48H)

Sponsor: [redacted] formula containing 13.34% Acrylates/t-Butylacrylamide Copolymer

Document type: Clinical Study Report

Investigational Product: Formula No. [redacted]

Batch No.: [redacted]

Product Type:

Study Monitor:

Investigator:

Investigating Site:

Investigating Laboratory:

Number of Pages: 45

Document Version: Final Date: October 21, 2015

[redacted]

[redacted]

SIGNATURES

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

[redacted]

October 21, 2015
Date

Principal Investigator
Dermatologist

[redacted]

October 21, 2015
Date

Vice President, Clinical Operations

[redacted]

October 21, 2015
Date

Manager, Dermatologic Safety Testing

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

[redacted]

STATEMENT OF TKL RESEARCH, INC QUALITY CONTROL

[redacted]

Date of Inspection	Type of Inspections	Date Reported to Management
08/07/2015	Initial Binder Review Prior to Study Start	08/07/2015
08/13/2015	Screeners/Consents 100% Review	08/13/2015
08/14/2015		08/14/2015
08/18/2015		08/18/2015
08/24/2015		08/24/2015
09/28/2015	After Study Binder Review	09/28/2015
09/29/2015	Tables and Data Listings	09/29/2015
10/21/2015	Final Study Report	10/21/2015

[redacted]

Quality Control

October 21, 2015

Date

[redacted]

Study Title: Human Repeated Insult Patch Test with Challenge

Sponsor:

Protocol #:

Contract Research Organization:

TKL Study Report #:

Investigating Site:

Dates of Study: August 10, 2015 – September 25, 2015

STUDY PERSONNEL

Principal Investigator

Vice President, Clinical Operations

Manager, Dermatologic Safety Testing

[redacted]

TABLE OF CONTENTS

[redacted]

SUMMARY

One investigational product, Formula No. J1181050, was evaluated as supplied to determine if the application of the investigational product, Formula No. J1181050, did not cause a delayed contact allergic response in volunteer subjects with normal skin using a semi-occlusive human repeat insult patch test. Ninety six (96) subjects completed the study, a deviation from the protocol-specified requirement of 100 completed subjects. This deviation did not affect the validity of the study.

Under the conditions employed in this study, Formula No. J1181050 was non-sensitizing and non-irritating.

[redacted]

1 INTRODUCTION

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

2 STUDY OBJECTIVE

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

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

3 STUDY DESIGN

3.1 OVERALL STUDY DESIGN

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

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

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

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

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

3.2 DISCUSSION OF DESIGN

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

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

[redacted]

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

3.3 STUDY PROCEDURES

3.3.1 Screening / Day 1

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

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

3.3.2 Induction

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

[redacted]

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

3.3.3 Rest Period

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

3.3.4 Challenge

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

3.3.5 Rechallenge

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

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

3.3.6 Study Flow Chart

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

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

Week 3
1-7 Same as Week 2

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

Week 5
1-7 Rest Period

[redacted]

Week 6
1 Staff applied patches
3 Staff removed patches, graded
5 Staff graded

3.4 SELECTION OF SUBJECTS

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

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

3.4.1 Inclusion Criteria

Subjects included in the study were those who:

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

2. Were able to give written consent,

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

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

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

3.4.2 Non-inclusion Criteria

Subjects excluded from the study were those who:

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

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

3. Had been deprived of their freedom by a legal or administrative decision, or people undergoing an emergency medical treatment (article L 209-5 French Law),

4. Were minors or subjects protected by law, as well as those admitted into a health, social, or mental institution (article L 209-6 French law),

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

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

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

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

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

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

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

12. Had been presenting cutaneous hyperactivity or skin disorder,

13. Had strong reactions to sticking plaster of patches,

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

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

3.4.3 Informed Consent

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

3.4.4 Interruption or Discontinuation of Treatment

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

[redacted]

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

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

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

Withdrawals

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

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

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

[redacted]

3.5 INVESTIGATIONAL PRODUCT (IP)

3.5.1 Investigational Product Specifications

IP Category	:	[redacted]
Formula No.	:	
Batch No.	:	
Description	:	
Amount Applied	:	0.2 mL
Patch Type	:	Semi-Occlusive
Evaporation	:	No
Dilution	:	No
Storage Conditions	:	Room Temperature
Special Instructions	:	None

3.5.2 Description of Patch Conditions

The product was evaluated under semi-occlusive patch conditions. It was applied to a 2 cm x 2 cm Webril™ pad. The pad is affixed to the skin with hypoallergenic tape (Micropore). Investigational product was sufficient to cover the patch (200 µL or mg) was applied to the patch and secured with the Micropore tape. Liquids are applied to the patch using an Eppendorf single channel adjustable pipette set at the appropriate amount to be applied to the patch, usually 20 µL. Creams, semi-solids, and solids are weighed by applying product to a patch that has been pre-weighed on a pre-calibrated weight balance. The product and patch are then weighed on the pre-calibrated weight balance to determine the appropriate amount of product, usually 20 mg. The weighed patch is used as a visual guide to prepare patches.

3.5.3 Storage, Handling, and Documentation of the Investigational Product

Receipt of the investigational product used in this study was documented in a general log book which serves as a permanent record of the receipt, storage, and disposition of all investigational products received by [redacted] On the basis of information provided by the Sponsor, the investigational product was considered reasonably safe for evaluation on human subjects. The investigational product was kept locked in product storage rooms at the [redacted]

3.5.4 Treatment Compliance

All patches were applied and removed by clinical study staff. Whereas bathing was allowed (low tub bath/frontal showers), the patched area was not to be soaked and was to be kept as dry as possible, per the instructions given to each subject (see Section 3.3.1). A Dermatologist Investigator-trained, experienced evaluator assessed study compliance. Records of patch applications and visit schedule compliance were recorded on the subjects' CRFs.

[redacted]

3.6 SAFETY EVALUATIONS

3.6.1 Local Tolerability Assessments

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

3.6.2 Adverse Events

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

Expected Adverse Events

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

3.7 QUALITY CONTROL

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

4 DATA MANAGEMENT

4.1 DOCUMENTATION

[redacted]

[redacted]

[redacted]

4.2 DATABASE MANAGEMENT AND QUALITY CONTROL

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

5 INTERPRETATION OF THE RESULTS

5.1 SAMPLE SIZE

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

5.2 POPULATIONS

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

5.3 CRITERIA OF EVALUATION OF SKIN COMPATIBILITY

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

5.4 DERMAL SENSITIZATION POTENTIAL

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

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

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

6 RESULTS

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

[redacted]

6.1 SUBJECTS EVALUATED

6.1.1 Subject Disposition

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

Text Table 6-1 Subject Disposition

Number of subjects enrolled	130
Number of subjects treated	130
Number of subjects discontinued	34
Lost to follow-up	28
Voluntary withdrawal	5
Adverse events (015: hip surgery)	1
Number of subjects completed	96

Source: Table 1, Appendix I

6.1.2 Protocol Deviations

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

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

This report does not include the summary in French as specified in the protocol. This is in agreement with the Sponsor and the deviation does not affect the validity of the study.

Ninety six (96) subjects completed the study, a deviation from the protocol-specified requirement of 100 completed subjects. This deviation did not affect the validity of the study.

There were four (4) late start groups enrolled on this study with start dates of Wednesday, August 12, 2015, Friday, August 14, 2015, Monday, August 17, 2015 and Wednesday, August 19, 2015. These are deviations from the protocol-specified requirement of no late start groups and the study not beginning on a Monday. These deviations did not affect the validity of the study.

Subject Nos. 088-095 and 106-130 were on rest for 12 days and subject nos. 096-099 were on rest for 10 days, a deviation from the protocol-specified 14 day Rest Period. This deviation did not affect the validity of the study.

6.1.3 Protocol Amendments

No amendments to the protocol were issued for this study.

[redacted]

6.1.4 Baseline Demographic and Background Characteristics

All subjects met the inclusion and none of the non-inclusion criteria. Demographic information is summarized in Table 2, Appendix I; these data are supported by Data Listing 2, Appendix II. The study population contained 34 (26.2%) males and 96 (73.8%) females, of whom 54 (41.5%) were Caucasian, 41 (31.5%) were Hispanic, 31 (23.8%) were Black, 2 (1.5%) were of Other Ethnicity, 1 (0.8%) was Asian, and 1 (0.8%) was American Indian. Subject ages ranged from 18 to 65 years; the mean was 47 years.

6.2 SAFETY RESULTS

6.2.1 Induction and Challenge Responses

Ninety nine (99) subjects completed the Induction Phase and were included in determining the presence of significant irritation. Ninety six (96) subjects completed the Challenge Phase of the study and were included in the sensitization analysis.

There were no reaction of concern observed during this study.

There was no requirement for a Rechallenge Phase to be conducted. A summary of the repeated insult patch test responses during the Induction and Challenge Phases of the study is provided in Table 3 of Appendix I, a by-subject listing of the sensitization response data is provided in Data Listing 3, Appendix II.

6.2.2 Overall Experience of Adverse Events

There was one (1) non-product related adverse event (AE) reported during this study. Please see Data Listing 4, Appendix II for more details.

7 CONCLUSIONS

Under the conditions employed in this study, Formula No. J1181050 was non-sensitizing and non-irritating.

8 REFERENCES

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5. Declaration of Helsinki adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, and amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975; 35th World Medical Assembly, Venice, Italy, October 1983; 41st World Medical Assembly,

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APPENDIX I

SUMMARY TABLES

[redacted]

[redacted]

Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	130
Subjects completed induction phase	99 (76.2)
Subjects completed all phases	96 (73.8)
Total subjects discontinued	34 (26.2)
Lost to follow-up	28 (21.5)
Voluntary withdrawal	5 (3.8)
Adverse events	1 (0.8)

Note: All percentages are relative to total subjects enrolled.

See data listing 1 for further detail.

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[redacted]

Table 2: Summary of Subject Demographics
All Enrolled Subjects

Age	
N (%) 18 to 44	47 (36.2)
N (%) 45 to 59	70 (53.8)
N (%) 60 to 65	13 (10.0)
Mean (SD)	47.1 (11.7)
Median	49.6
Range	18.2 to 65.8
Gender	
N (%) Male	34 (26.2)
N (%) Female	96 (73.8)
Race	
Amer Ind	1 (0.8)
Asian	1 (0.8)
Black	31 (23.8)
Caucasian	54 (41.5)
Hispanic	41 (31.5)
Other	2 (1.5)

See data listing 2 for further detail.

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Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

[redacted]

	Induction Reading										Challenge Phase 48hr		Challenge Phase 96hr	
Response (EAM)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Make Up**	**O**	**N**	**O**	**N**
00	125	116	109	104	103	103	102	99	98	0	96	96	96	96
Total evaluable	125	116	109	104	103	103	102	99	98	0	96	96	96	96
Number absent	0	0	0	0	0	0	0	0	1		0	0	0	0
Number discontinued	5	14	21	26	27	27	28	31	31		34	34	34	34

Maximum Elicited Response During Induction
All Subjects Completing Induction (N= 99)

Response	n(%) Subjects
00	99 (100.0%)

See Table 3.1 for key to symbols and scores

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Table 3.1: Key To Symbols and Scores

Score or Symbol	Response or Description of Reaction
	Erythema Results (E)
0	No visible erythema
1	Mild erythema (faint pink)
2	Moderate erythema (well defined)
3	Severe erythema
4	Caustic erythema - erosive aspect and/or necrotic aspect
	Allergic Results (A)
0	No reaction
1	Weak positive reaction: erythema, infiltration, possibly papules
2	Strong positive reaction: erythema, vesicles, papules, infiltration
3	Extreme positive reaction: intense erythema, infiltration, vesicles may coalesce to form a blister
	Additional Comments (M)
E	- Edema from 0 to 3
P	- Papules
V	- Vesicles
B	- Bullae
S	- Spreading of reaction beyond the patch area
Pe	- Petichiae
SV	- Soap effect
F	- Fissuring
D	- Desquamation
Dr	- Dryness
C	- Skin coloration - hyperpigmentation
H	- Hypopigmentation
Fr	- Follicular reaction
NA	- Not applied
T	Tape reaction
*	Additional free comments (First new site reading)
N9G	No ninth grading
Cr	Exudation and/or surface encrustation
X	Succeeding patch not applied and succeeding grade (in brackets) denotes a residual reaction
--	Subject absent

[redacted]

APPENDIX II

DATA LISTINGS

[redacted]

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
001	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
002	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
003	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
004	08/10/15	08/10/15	--	08/17/15	I2	L	8
005	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
006	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
007	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
008	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
009	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
010	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
011	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
012	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
013	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
014	08/10/15	08/10/15	--	08/19/15	I3	L	10
015	08/10/15	08/10/15	--	09/14/15	I9	AE	36
016	08/10/15	08/10/15	--	08/14/15	I1	L	5
017	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
018	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
019	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
020	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
021	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
022	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
023	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
024	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
025	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
026	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
027	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
028	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
029	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
030	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
031	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
032	08/10/15	08/10/15	--	08/14/15	I1	L	5
033	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
034	08/10/15	08/10/15	--	08/28/15	I7	L	19
035	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
036	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
037	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

Generated on 10/20/15:12:12 [redacted]

[redacted]

[redacted]

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
038	08/10/15	08/10/15	--	08/17/15	I2	L	8
039	08/10/15	08/10/15	--	08/17/15	I2	L	8
040	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
041	08/10/15	08/10/15	--	08/12/15	I0	S	3
042	08/10/15	08/10/15	--	08/12/15	I0	S	3
043	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
044	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
045	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
046	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
047	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
048	08/10/15	08/10/15	--	08/14/15	I9	L	5
049	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
050	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
051	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
052	08/10/15	08/10/15	--	08/14/15	I1	L	5
053	08/10/15	08/10/15	--	08/17/15	I2	L	8
054	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
055	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
056	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
057	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
058	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
059	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
060	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
061	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
062	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
063	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
064	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
065	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
066	08/10/15	08/10/15	--	08/14/15	I1	L	5
067	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
068	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
069	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
070	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
071	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
072	08/10/15	08/10/15	--	08/21/15	I4	L	12
073	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
074	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

Generated on 10/20/15:12:12 by [redacted]

[redacted]

[redacted]

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
075	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
076	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
077	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
078	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
079	08/10/15	08/10/15	--	08/19/15	I3	L	10
080	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
081	08/10/15	08/10/15	--	08/14/15	I1	L	5
082	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
083	08/10/15	08/10/15	--	08/17/15	I2	S	8
084	08/10/15	08/10/15	--	08/17/15	I2	S	8
085	08/10/15	08/10/15	--	08/26/15	I6	L	17
086	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
087	08/10/15	08/10/15	09/14/15	09/18/15	C	C	40
088	08/12/15	08/12/15	--	08/14/15	I0	L	3
089	08/12/15	08/12/15	09/14/15	09/18/15	C	C	38
090	08/12/15	08/12/15	--	08/17/15	I1	L	6
091	08/12/15	08/12/15	09/14/15	09/18/15	C	C	38
092	08/12/15	08/12/15	09/14/15	09/18/15	C	C	38
093	08/12/15	08/12/15	--	08/21/15	I3	L	10
094	08/12/15	08/12/15	09/14/15	09/18/15	C	C	38
095	08/12/15	08/12/15	--	08/19/15	I2	L	8
096	08/14/15	08/14/15	--	08/17/15	I0	L	4
097	08/14/15	08/14/15	--	08/19/15	I1	L	6
098	08/14/15	08/14/15	09/14/15	09/18/15	C	C	36
099	08/14/15	08/14/15	09/14/15	09/18/15	C	C	36
100	08/17/15	08/17/15	09/21/15	09/25/15	C	C	40
101	08/17/15	08/17/15	09/21/15	09/25/15	C	C	40
102	08/17/15	08/17/15	09/21/15	09/25/15	C	C	40
103	08/17/15	08/17/15	09/21/15	09/25/15	C	C	40
104	08/17/15	08/17/15	09/21/15	09/25/15	C	C	40
105	08/17/15	08/17/15	09/21/15	09/25/15	C	C	40
106	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
107	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
108	08/19/15	08/19/15	--	09/07/15	I7	S	20
109	08/19/15	08/19/15	--	08/24/15	I1	L	6
110	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
111	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

Generated on 10/20/15:12:12 [redacted]

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	**Screened**	**1st Applic**	**Chall Applic**	**Ended**	**Last Reading #**	**Completion Status**	**Days in Study**
112	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
113	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
114	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
115	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
116	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
117	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
118	08/19/15	08/19/15	--	09/07/15	I7	L	20
119	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
120	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
121	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
122	08/19/15	08/19/15	--	08/21/15	I0	L	3
123	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
124	08/19/15	08/19/15	--	08/24/15	I1	L	6
125	08/19/15	08/19/15	--	08/28/15	I3	L	10
126	08/19/15	08/19/15	--	08/28/15	I3	L	10
127	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
128	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
129	08/19/15	08/19/15	09/21/15	09/25/15	C	C	38
130	08/19/15	08/19/15	--	09/21/15	I9	L	34

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

Generated on 10/20/15:12:12 by [redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	[redacted]
001	
002	
003	
004	
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007	
008	
009	
010	
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012	
013	
014	
015	
016	
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030	
031	
032	
033	
034	
035	
036	
037	

Generated on 10/20/15:12:12 by [redacted]

[redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	[redacted]
038	
039	
040	
041	
042	
043	
044	
045	
046	
047	
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071	
072	
073	
074	

Generated on 10/20/15:12:12 by [redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	[redacted]
075	
076	
077	
078	
079	
080	
081	
082	
083	
084	
085	
086	
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107	
108	
109	
110	
111	

Generated on 10/20/15:12:12 by [redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	[redacted]
112	
113	
114	
115	
116	
117	
118	
119	
120	
121	
122	
123	
124	
125	
126	
127	
128	
129	
130	

Generated on 10/20/15:12:12 [redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

[redacted]

Subject No.	Induction Reading 1 EAM	2 EAM	3 EAM	4 EAM	5 EAM	6 EAM	7 EAM	8 EAM	9 EAM	MU	Challenge Phase 48-hour O	48-hour N	96-hour O	96-hour N	120-hour(*) O	120-hour(*) N
001	00	00	00	00	00	00	00	00	00		00	00	00	00		
002	00	00	00	00	00	00	00	00	00		00	00	00	00		
003	00	00	00	00	00	00	00	00	00		00	00	00	00		
004	00	00	--	--	--	--	--	--	--		--	--	--	--		
005	00	00	00	00	00	00	00	00	00		00	00	00	00		
006	00	00	00	00	00	00	00	00	00		00	00	00	00		
007	00	00	00	00	00	00	00	00	00		00	00	00	00		
008	00	00	00	00	00	00	00	00	00		00	00	00	00		
009	00	00	00	00	00	00	00	00	00		00	00	00	00		
010	00	00	00	00	00	00	00	00	00		00	00	00	00		
011	00	00	00	00	00	00	00	00	00		00	00	00	00		
012	00	00	00	00	00	00	00	00	00		00	00	00	00		
013	00	00	00	00	00	00	00	00	00		00	00	00	00		
014	00	00	00	--	--	--	--	--	--		--	--	--	--		
015	00	00	00	00	00	00	00	00	00		--	--	--	--		
016	00	--	--	--	--	--	--	--	--		--	--	--	--		
017	00	00	00	00	00	00	00	00	00		00	00	00	00		
018	00	00	00	00	00	00	00	00	00		00	00	00	00		
019	00	00	00	00	00	00	00	00	00		00	00	00	00		

(*) when required
E = Erythema results A = Allergic results M = Additional comments MU = Make-up visit
See Table 3.1 for Key to Symbols and Scores
Generated on 10/20/15:12:12 by DETAIL5.SAS/USES: LRESPONS, PRODLIST

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

[redacted]

	Induction Reading										Challenge Phase					
											48-hour		96-hour		120-hour(*)	
Subject No.	1 EAM	2 EAM	3 EAM	4 EAM	5 EAM	6 EAM	7 EAM	8 EAM	9 EAM	MU	O	N	O	N	O	N
020	00	00	00	00	00	00	00	00	00		00	00	00	00		
021	00	00	00	00	00	00	00	00	00		00	00	00	00		
022	00	00	00	00	00	00	00	00	00		00	00	00	00		
023	00	00	00	00	00	00	00	00	00		00	00	00	00		
024	00	00	00	00	00	00	00	00	00		00	00	00	00		
025	00	00	00	00	00	00	00	00	00		00	00	00	00		
026	00	00	00	00	00	00	00	00	00		00	00	00	00		
027	00	00	00	00	00	00	00	00	00		00	00	00	00		
028	00	00	00	00	00	00	00	00	00		00	00	00	00		
029	00	00	00	00	00	00	00	00	00		00	00	00	00		
030	00	00	00	00	00	00	00	00	00		00	00	00	00		
031	00	00	00	00	00	00	00	00	00		00	00	00	00		
032	00	--	--	--	--	--	--	--	--		--	--	--	--		
033	00	00	00	00	00	00	00	00	00		00	00	00	00		
034	00	00	00	00	00	00	00	--	--		--	--	--	--		
035	00	00	00	00	00	00	00	00	00		00	00	00	00		
036	00	00	00	00	00	00	00	00	00		00	00	00	00		
037	00	00	00	00	00	00	00	00	00		00	00	00	00		
038	00	00	--	--	--	--	--	--	--		--	--	--	--		

(*) when required
E = Erythema results A = Allergic results M = Additional comments MU = Make-up visit
See Table 3.1 for Key to Symbols and Scores
Generated on 10/20/15:12:12 by DETAIL5.SAS/USES: LRESPONS, PRODLIST

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

[redacted]

Subject No.	Induction Reading 1 EAM	2 EAM	3 EAM	4 EAM	5 EAM	6 EAM	7 EAM	8 EAM	9 EAM	MU	Challenge Phase 48-hour O	48-hour N	96-hour O	96-hour N	120-hour(*) O	120-hour(*) N
039	00	00	--	--	--	--	--	--	--		--	--	--	--		
040	00	00	00	00	00	00	00	00	00		00	00	00	00		
041	--	--	--	--	--	--	--	--	--		--	--	--	--		
042	--	--	--	--	--	--	--	--	--		--	--	--	--		
043	00	00	00	00	00	00	00	00	00		00	00	00	00		
044	00	00	00	00	00	00	00	00	00		00	00	00	00		
045	00	00	00	00	00	00	00	00	00		00	00	00	00		
046	00	00	00	00	00	00	00	00	00		00	00	00	00		
047	00	00	00	00	00	00	00	00	00		00	00	00	00		
048	00	00	00	00	00	00	00	00	00		--	--	--	--		
049	00	00	00	00	00	00	00	00	00		00	00	00	00		
050	00	00	00	00	00	00	00	00	00		00	00	00	00		
051	00	00	00	00	00	00	00	00	00		00	00	00	00		
052	00	--	--	--	--	--	--	--	--		--	--	--	--		
053	00	00	--	--	--	--	--	--	--		--	--	--	--		
054	00	00	00	00	00	00	00	00	00		00	00	00	00		
055	00	00	00	00	00	00	00	00	00		00	00	00	00		
056	00	00	00	00	00	00	00	00	00		00	00	00	00		
057	00	00	00	00	00	00	00	00	00		00	00	00	00		

(*) when required
E = Erythema results A = Allergic results M = Additional comments MU = Make-up visit
See Table 3.1 for Key to Symbols and Scores
Generated on 10/20/15:12:12 by DETAIL5.SAS/USES: LRESPONS, PRODLIST

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

[redacted]

Subject No.	Induction Reading 1 EAM	2 EAM	3 EAM	4 EAM	5 EAM	6 EAM	7 EAM	8 EAM	9 EAM	MU	Challenge Phase 48-hour O	N	96-hour O	N	120-hour(*) O	N
058	00	00	00	00	00	00	00	00	00		00	00	00	00		
059	00	00	00	00	00	00	00	00	00		00	00	00	00		
060	00	00	00	00	00	00	00	00	00		00	00	00	00		
061	00	00	00	00	00	00	00	00	00		00	00	00	00		
062	00	00	00	00	00	00	00	00	00		00	00	00	00		
063	00	00	00	00	00	00	00	00	00		00	00	00	00		
064	00	00	00	00	00	00	00	00	00		00	00	00	00		
065	00	00	00	00	00	00	00	00	00		00	00	00	00		
066	00	--	--	--	--	--	--	--	--		--	--	--	--		
067	00	00	00	00	00	00	00	00	00		00	00	00	00		
068	00	00	00	00	00	00	00	00	00		00	00	00	00		
069	00	00	00	00	00	00	00	00	00		00	00	00	00		
070	00	00	00	00	00	00	00	00	00		00	00	00	00		
071	00	00	00	00	00	00	00	00	00		00	00	00	00		
072	00	00	00	00	--	--	--	--	--		--	--	--	--		
073	00	00	00	00	00	00	00	00	00		00	00	00	00		
074	00	00	00	00	00	00	00	00	00		00	00	00	00		
075	00	00	00	00	00	00	00	00	00		00	00	00	00		
076	00	00	00	00	00	00	00	00	00		00	00	00	00		

(*) when required
E = Erythema results A = Allergic results M = Additional comments MU = Make-up visit
See Table 3.1 for Key to Symbols and Scores
Generated on 10/20/15:12:12 by DETAIL5.SAS/USES: LRESPONS, PRODLIST

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

[redacted]

Subject No.	Induction Reading 1 EAM	2 EAM	3 EAM	4 EAM	5 EAM	6 EAM	7 EAM	8 EAM	9 EAM	MU	Challenge Phase 48-hour O	48-hour N	96-hour O	96-hour N	120-hour(*) O	120-hour(*) N
077	00	00	00	00	00	00	00	00	00		00	00	00	00		
078	00	00	00	00	00	00	00	00	00		00	00	00	00		
079	00	00	00	--	--	--	--	--	--		--	--	--	--		
080	00	00	00	00	00	00	00	00	00		00	00	00	00		
081	00	--	--	--	--	--	--	--	--		--	--	--	--		
082	00	00	00	00	00	00	00	00	00		00	00	00	00		
083	00	00	--	--	--	--	--	--	--		--	--	--	--		
084	00	00	--	--	--	--	--	--	--		--	--	--	--		
085	00	00	00	00	00	00	--	--	--		--	--	--	--		
086	00	00	00	00	00	00	00	00	00		00	00	00	00		
087	00	00	00	00	00	00	00	00	00		00	00	00	00		
088	--	--	--	--	--	--	--	--	--		--	--	--	--		
089	00	00	00	00	00	00	00	00	00		00	00	00	00		
090	00	--	--	--	--	--	--	--	--		--	--	--	--		
091	00	00	00	00	00	00	00	00	00		00	00	00	00		
092	00	00	00	00	00	00	00	00	00		00	00	00	00		
093	00	00	00	--	--	--	--	--	--		--	--	--	--		
094	00	00	00	00	00	00	00	00	00		00	00	00	00		
095	00	00	--	--	--	--	--	--	--		--	--	--	--		

(*) when required
E = Erythema results A = Allergic results M = Additional comments MU = Make-up visit
See Table 3.1 for Key to Symbols and Scores
Generated on 10/20/15:12:12 by DETAIL5.SAS/USES: LRESPONS, PRODLIST

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

[redacted]

Subject No.	Induction Reading 1 EAM	2 EAM	3 EAM	4 EAM	5 EAM	6 EAM	7 EAM	8 EAM	9 EAM	MU	Challenge Phase 48-hour O	48-hour N	96-hour O	96-hour N	120-hour(*) O	120-hour(*) N
096	--	--	--	--	--	--	--	--	--		--	--	--	--		
097	00	--	--	--	--	--	--	--	--		--	--	--	--		
098	00	00	00	00	00	00	00	00	00		00	00	00	00		
099	00	00	00	00	00	00	00	00	00		00	00	00	00		
100	00	00	00	00	00	00	00	00	00		00	00	00	00		
101	00	00	00	00	00	00	00	00	00		00	00	00	00		
102	00	00	00	00	00	00	00	00	00		00	00	00	00		
103	00	00	00	00	00	00	00	00	00		00	00	00	00		
104	00	00	00	00	00	00	00	00	00		00	00	00	00		
105	00	00	00	00	00	00	00	00	00		00	00	00	00		
106	00	00	00	00	00	00	00	00	00		00	00	00	00		
107	00	00	00	00	00	00	00	00	00		00	00	00	00		
108	00	00	00	00	00	00	00	--	--		--	--	--	--		
109	00	--	--	--	--	--	--	--	--		--	--	--	--		
110	00	00	00	00	00	00	00	00	N9G		00	00	00	00		
111	00	00	00	00	00	00	00	00	00		00	00	00	00		
112	00	00	00	00	00	00	00	00	00		00	00	00	00		
113	00	00	00	00	00	00	00	00	00		00	00	00	00		
114	00	00	00	00	00	00	00	00	00		00	00	00	00		

(*) when required
E = Erythema results A = Allergic results M = Additional comments MU = Make-up visit
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[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

[redacted]

Subject No.	Induction Reading 1 EAM	2 EAM	3 EAM	4 EAM	5 EAM	6 EAM	7 EAM	8 EAM	9 EAM	MU	Challenge Phase 48-hour O	N	96-hour O	N	120-hour(*) O	N
115	00	00	00	00	00	00	00	00	00		00	00	00	00		
116	00	00	00	00	00	00	00	00	00		00	00	00	00		
117	00	00	00	00	00	00	00	00	00		00	00	00	00		
118	00	00	00	00	00	00	00	--	--		--	--	--	--		
119	00	00	00	00	00	00	00	00	00		00	00	00	00		
120	00	00	00	00	00	00	00	00	00		00	00	00	00		
121	00	00	00	00	00	00	00	00	00		00	00	00	00		
122	--	--	--	--	--	--	--	--	--		--	--	--	--		
123	00	00	00	00	00	00	00	00	00		00	00	00	00		
124	00	--	--	--	--	--	--	--	--		--	--	--	--		
125	00	00	00	--	--	--	--	--	--		--	--	--	--		
126	00	00	00	--	--	--	--	--	--		--	--	--	--		
127	00	00	00	00	00	00	00	00	00		00	00	00	00		
128	00	00	00	00	00	00	00	00	00		00	00	00	00		
129	00	00	00	00	00	00	00	00	00		00	00	00	00		
130	00	00	00	00	00	00	00	00	00		--	--	--	--		

(*) when required
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See Table 3.1 for Key to Symbols and Scores
Generated on 10/20/15:12:12 by DETAIL5.SAS/USES: LRESPONS, PRODLIST

Final Report

Study Title	Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*
Test Article	E212966 mixture containing 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer
Author	[redacted]
Sponsor	
Study Monitor	
Test Facility	
Covance Client Identifier	
Covance Study Number	
Report Issued	February 2013
Page Number	1 of 50

[redacted]

STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

E212966: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*

I, the undersigned, hereby declare that the work was performed under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

This study was conducted in accordance with the following:

- The United Kingdom (GLP Monitoring Authority, Medicines and Healthcare products Regulatory Agency [MHRA]) Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No.3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations, 2004.

- The OECD Principles on Good Laboratory Practice ENV/MC/CHEM (98) 17 (Revised in 1997, Issued January 1998).

- Directive 2004/10/EC of the European Parliament and the Council of 11 February 2004 on the Harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L50 of 20.2.2004).

[redacted]

1 February 2013
Date

QUALITY ASSURANCE STATEMENT

E212966: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*

This study has been reviewed by the GLP Quality Assurance Unit of Covance and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the Study Director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates			Date Reported to SD and SD Management
From	To	Phase	
16 Oct 2012	16 Oct 2012	Protocol Review	16 Oct 2012
03 Dec 2012	06 Dec 2012	Draft Report and Data Review	06 Dec 2012
31 Jan 2013	31 Jan 2013	Final Report Review	31 Jan 2013

Process

Inspection Dates			Date Reported to SD and SD Management
From	To	Phase	
23 Oct 2012	23 Oct 2012	Colony Counting	23 Oct 2012
26 Oct 2012	26 Oct 2012	Dose Preparation	26 Oct 2012
26 Oct 2012	26 Oct 2012	S9 Quality Control Checks	30 Oct 2012

[redacted]

01 February 2013
Date

Quality Assurance Unit

REVIEWING SCIENTIST'S STATEMENT

E212966: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.

[redacted]

28 January 2013
Date

RESPONSIBLE PERSONNEL

E212966: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*

The following personnel were responsible for key elements of the study:

Study Director	[redacted]
Laboratory Supervisor	
Study Monitor [1]	

ARCHIVE STATEMENT

E212966: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*

The raw data, including documentation, study protocol, final report and study correspondence resulting from this study will be retained in the test facility archives for ten years from the date of report finalisation. After completion of this period, the Sponsor will be contacted in order to determine their requirements for further retention or disposition of the archived materials (excluding facility records, non-transferable electronic data and facility copies of protocol/final report, which will be retained by [redacted] in accordance with test facility SOPs). Where continued retention is requested, the archived materials may subsequently be transferred to alternative [redacted] Archive locations. In this event, the Sponsor will be informed, and documented chain of custody records will be maintained.

CONTENTS

SUMMARY

E212966 was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

All E212966 treatments in this study were performed using formulations prepared in anhydrous analytical grade dimethyl sulphoxide (DMSO).

Experiment 1 treatments of all the tester strains were performed in the absence and in the presence of S-9, using final concentrations of E212966 at 5, 15.81, 50, 158.1, 500, 1581 and 5000 μg/plate, plus negative (vehicle) and positive controls. Following these treatments, evidence of toxicity was observed in strain TA102 at 5000 μg/plate in the absence and presence of S-9.

Experiment 2 treatments of all the tester strains were performed in the absence and in the presence of S-9. The maximum test concentration of 5000 μg/plate was retained for all strains. Narrowed concentration intervals were employed covering the ranges 78.13 – 5000 μg/plate (strain TA102) or 156.3 – 5000 μg/plate (strains TA98, TA100, TA1535 and TA1537), in order to examine more closely those concentrations of E212966 approaching the maximum test concentration and considered therefore most likely to provide evidence of any mutagenic activity. In addition, all treatments in the presence of S-9 were further modified by the inclusion of a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected using this assay system. Following these treatments, evidence of toxicity was observed in strain TA102 at 5000 μg/plate in the absence and presence of S-9.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed.

Negative (vehicle) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies all fell within, or slightly above, acceptable ranges for negative control treatments, and were significantly elevated by positive control treatments.

Following E212966 treatments of all the test strains in the absence and presence of S-9, no increases in revertant numbers were observed that were statistically significant when the data were analysed at the 1% level using Dunnett's test. This study was considered therefore to have provided no evidence of any E212966 mutagenic activity in this assay system.

It was concluded that E212966 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 5000 μg/plate (the maximum recommended concentration according to current regulatory guidelines), in the absence and in the presence of a rat liver metabolic activation system (S-9).

INTRODUCTION

The Ames test is a rapid, reliable and economical method of evaluating the mutagenic potential of a test article by measuring genetic activity in one or more histidine-requiring strains of *Salmonella typhimurium* in the absence and presence of a liver metabolising system (Ames *et al*., 1975). A large database has been accumulated with this assay, confirming its ability to detect genetically active compounds of most chemical classes with around 80-90% sensitivity and specificity (Gatehouse *et al*., 1990). The following bacterial strains were used in this study:

Table 1: Bacterial Strains

Organism	Strain	Type of mutation in the histidine gene
S. typhimurium	TA98	frame-shift
S. typhimurium	TA100	base-pair substitution
S. typhimurium	TA1535	base-pair substitution
S. typhimurium	TA1537	frame-shift
S. typhimurium	TA102	base-pair substitution

With the exception of strain TA102, these strains require biotin as well as histidine for growth. In strain TA102 the critical mutation in the histidine gene is located on a multicopy plasmid pAQ1. This strain is particularly sensitive to the activities of oxidative and cross-linking mutagens. The pKM101 plasmid derivatives (TA98, TA100 and TA102) have increased sensitivity to certain mutagens as the pKM101 plasmid codes for an error-prone DNA repair system (Maron and Ames, 1983).

When exposed to a mutagen, some of the bacteria in the treated population, through chemical interaction with the compound, undergo genetic changes which cause them to revert to a non-histidine-requiring state and thus grow in the absence of exogenous histidine. Different tester strains are used because each is mutated by particular chemical classes of compound. A compound that is mutagenic in one strain need not be so in another (Garner, 1979).

The objective of this study was therefore to evaluate the potential mutagenic activity of E212966 by examining its ability to revert five histidine-requiring strains of *Salmonella typhimurium* in the absence and in the presence of a rat liver metabolising system (S-9). The procedures used in this study were in accordance with OECD Guideline 471 (1997).

This study was performed according to the protocol, with the exception of the minor deviation detailed in Appendix 8, which did not prejudice the validity of this study.

The study was initiated on 15 October 2012. Experimental work started on 18 October 2012 and was completed on 29 October 2012. The study completion date is considered to be the date the Study Director signs the final report.

MATERIALS

Test article

E212966, batch number C2120726D, was a yellowish liquid. It was received on 2 October 2012 and stored at 15-25°C protected from light in a tightly closed container. The major components were AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate copolymer (38 parts), [redacted] (assumed 100% purity, tested as supplied) and the expiry date was given as 25 July 2015. The certificate of analysis, provided by the Sponsor, is presented in Appendix 7. The test article information and certificate of analysis provided by the Sponsor are considered an adequate description of the characterisation, purity and stability of the test article. Determinations of stability and characteristics of the test article were the responsibility of the Sponsor.

Preliminary solubility data indicated that E212966 was soluble in anhydrous analytical grade dimethyl sulphoxide (DMSO) at concentrations of at least 100 mg/mL. A maximum concentration of 5000 µg/plate was selected for Experiment 1, in order that initial treatments were performed up to this maximum recommended concentration according to current regulatory guidelines (Gatehouse *et al*., 1990; OECD, 1997). A maximum concentration of 5000 µg/plate was also selected for Experiment 2.

Test article stock solutions were prepared by formulating E212966 under subdued lighting in DMSO with the aid of vortex mixing, to give the maximum required treatment concentration. Subsequent dilutions were made using DMSO. The test article solutions were protected from light and used within approximately 6 hours of initial formulation as shown in Table 2:

Table 2: E212966 Concentrations Tested

Experiment	S-9	Concentration of treatment solution (mg/mL)	Final concentration (μg/plate)
Mutation Experiment 1	- and +	0.05000	5.000
		0.1581	15.81
		0.5000	50.00
		1.581	158.1
		5.000	500.0
		15.81	1581
		50.00	5000
Mutation Experiment 2	- and +	0.7813*[a]	78.13[a]
		1.563*	156.3
		3.125*	312.5
		6.250*	625.0
		12.50*	1250
		25.00*	2500
		50.00*	5000

* Concentration of treatment solutions used for the Experiment 2 pre-incubation treatments were twice that stated above, in order to permit treatments at the final concentration stated, whilst volume additions were reduced to 0.05 mL.

[a] Concentration employed for treatment of strain TA102 only.

0.1 mL volume additions of test article solution were used for all plate-incorporation treatments, 0.05 mL volume additions were used for all pre-incubation treatments.

Controls

Control treatments were performed using the same addition volumes per plate as the test article treatments, 0.1 mL for plate incorporation treatments and 0.05 mL for pre-incubation treatments. Negative controls comprised treatments with the vehicle DMSO. The positive control chemicals were supplied and used according to Table 3:

Table 3: Positive Controls

Chemical*	Stock ** concentration (µg/mL)	Final concentration (µg/plate)	Strain(s)	S-9
2-nitrofluorene (2NF)	50	5	TA98	–
Sodium azide (NaN_3)	20	2	TA100, TA1535	–
9-aminoacridine (AAC)	500	50	TA1537	–
Mitomycin C (MMC)	2	0.2	TA102	–
Benzo[a]pyrene (B[a]P)	100***	10	TA98	+
2-aminoanthracene (AAN)	50***	5	TA100, TA1535, TA1537	+
	200***	20	TA102	+

* Obtained from Sigma-Aldrich Chemical Co, Poole, UK.

** Stock solutions were formulated in purified water (NaN_3 and MMC), or in DMSO (2NF, AAC, AAN and B[a]P). All stock solutions were stored in aliquots protected from light at 2-8°C, with the exception of B[a]P which was stored in aliquots at <-50ºC and MMC which was prepared freshly on the day of use or stored in aliquots at <-50ºC.

*** Concentrations were twice that stated for the pre-incubation methodology (0.05 mL per plate).

Metabolic activation system

The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was obtained from Molecular Toxicology Incorporated, USA where it was prepared from male Sprague Dawley rats induced with Aroclor 1254. The batches of MolTox™ S-9 were stored frozen in aliquots at <-50°C, and thawed just prior to use (Booth *et al*., 1980). Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens, and cytochrome P-450-catalysed enzyme activities (alkoxyresorufin-O-dealkylase activities). The quality control statement, relating to the batch of S-9 preparation used, is included in Appendix 6 of this report.

Treatments were carried out both in the absence and presence of S-9 by addition of either buffer solution or 10% S-9 mix respectively, prepared according to the following table:

Table 4: Buffer solution and 10% S-9 mix

Ingredient	Final content per mL in:	
	10% S-9 mix	Buffer solution
Sodium phosphate buffer pH 7.4	100 µMoles	100 µMoles
Glucose-6-phosphate (disodium)	5 µMoles	-
β-Nicotinamide adenine dinucleotide phosphate (NADP) (disodium)	4 µMoles	-
Magnesium chloride	8 µMoles	-
Potassium chloride	33 µMoles	-
Water	To volume	To volume
S-9	100 µL	-

Amino acid supplement

The amino acid L-histidine HCl (in 250 mM $MgCl_2$) and d-biotin were added at the time of plating, by supplementing the S-9 mix or buffer solution for plate incorporation treatments or the molten agar for pre-incubation treatments. Concentrations of each supplement were as follows:

Table 5: Amino acid supplement

Supplement	Final concentration (µg/plate)
L-histidine HCl	20
d-biotin	24.4

Bacteria

Five strains of *Salmonella typhimurium* bacteria (TA98, TA100, TA1535, TA1537 and TA102) were used in this study. Strains TA98, TA1535 and TA1537 were originally obtained from the UK NCTC. Strains TA100 and TA102 were derived from cultures originally obtained from Covance Laboratories Inc., USA. For all assays, bacteria were cultured at 37±1°C for 10 hours in nutrient broth, containing ampicillin (TA98, TA100) or ampicillin and tetracycline (TA102) as appropriate, to provide bacterial cultures in the range of approximately 10^8 to 10^9 cells/mL, based on cell count data from testing of each strain batch. Incubation was carried out with shaking in an anhydric incubator, set to turn on using a timer switch. All treatments were completed within 6 hours of the end of the incubation period.

The inocula were taken from master plates or vials of frozen cultures, which had been checked for strain characteristics (histidine dependence, *rfa* character, *uvrB* character and resistance to ampicillin or ampicillin plus tetracycline). Checks were carried out according to Maron and Ames (Maron and Ames, 1983) and De Serres and Shelby (De Serres and Shelby, 1979).

METHODS

Test system

The test system was suitably labelled (using a colour-coded procedure) to clearly identify the study number, test article (when appropriate), positive and negative control groups.

Mutation Experiments

E212966 was tested for mutation (and toxicity) in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102), in two separate experiments, at the concentrations detailed previously, using triplicate plates without and with S-9. Negative (vehicle) controls were included in quintuplicate, and positive controls were included in triplicate in both assays without and with S-9. These platings were achieved by the following sequence of additions to 2.5 mL molten agar at 46±1°C:

- 0.1 mL bacterial culture
- 0.1 mL test article solution or control
- 0.5 mL 10% S-9 mix or buffer solution

followed by rapid mixing and pouring on to Vogel-Bonner E agar plates. When set, the plates were inverted and incubated at 37±1°C in the dark for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible revertant colonies were counted (see Colony counting).

As the results of Experiment 1 were negative, treatments in the presence of S-9 in Experiment 2 included a pre-incubation step. Quantities of test article or control solution (reduced to 0.05 mL), bacteria and S-9 mix detailed above, were mixed together and incubated for 20 minutes at 37±1°C, with shaking, before the addition of 2.5 mL molten agar at 46±1°C. Plating of these treatments then proceeded as for the normal plate-incorporation procedure. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay.

Volume additions for the Experiment 2 pre-incubation treatments were reduced to 0.05 mL due to the vehicle (DMSO) employed in this study. This, and some other organic vehicles, are known to be near to toxic levels when added at volumes

of 0.1 mL in this assay system when employing the pre-incubation methodology. By reducing the addition volume to 0.05 mL per plate, it was hoped to minimise or eliminate any toxic effects of the vehicle that may have otherwise occurred.

Colony counting

Colonies were counted electronically using a Sorcerer Colony Counter (Perceptive Instruments) or manually where confounding factors such as bubbles or splits in the agar affected the accuracy of the automated counter. The background lawn was inspected for signs of toxicity.

Analysis of results

Treatment of data

Individual plate counts from each experiment were recorded separately and the mean and standard deviation of the plate counts for each treatment were determined. Control counts were compared with the accepted normal ranges for our laboratory for numbers of spontaneous revertants on vehicle control plates (Appendix 4) and numbers of induced revertants on positive control plates (Appendix 5). Data were considered acceptable if the mean vehicle control counts fell within the historical 99% confidence intervals for group means and/or each vehicle control plate count fell within the historical 99% reference ranges, and the positive control plate counts were comparable with the historical 99% reference ranges. The ranges that are quoted are based on a large volume of historical control data accumulated from experiments where the correct strain and assay functioning are considered to have been confirmed. Data for our laboratory are consistent with ranges of spontaneous revertants per plate considered acceptable elsewhere (De Serres and Shelby, 1979).

For evaluation of test article and positive control data there are many statistical methods in use, and several are acceptable (Venitt *et al.*, 1983; Mahon *et al.*, 1989). Dunnett's test was used to compare the counts at each concentration with the control. The presence or otherwise of a concentration response was checked by non-statistical analysis, up to limiting levels (for example toxicity, precipitation or 5000 μg/plate).

Acceptance criteria

The assay was to be considered valid if the following criteria were met:

1. The negative control counts fell within the normal ranges as defined in Appendix 4.

2. The positive control chemicals induced increases in revertant numbers of ≥2.0-fold (in strains TA98, TA100, or TA102) or ≥3.0-fold (in strains TA1535 or TA1537) the concurrent vehicle control confirming discrimination between different strains, and an active S-9 preparation.

Evaluation criteria

For valid data, the test article was considered to be mutagenic if:

1. When assessed using Dunnett's test, an increase in revertant numbers gave a significant response ($p \leq 0.01$) which was concentration related.

2. The positive trend/effects described above were reproducible.

The test article was considered positive in this assay if all of the above criteria were met.

The test article was considered negative in this assay if none of the above criteria were met.

Results which only partially satisfied the above criteria were dealt with on a case-by-case basis. Biological relevance was taken into account, for example consistency of response within and between concentrations and (where applicable) between experiments.

Major computer systems

The major computer systems used on this study were as follows:

Table 6: Major Computer systems

Activity	Computer System
Scheduling	(CMS) Covance Management Systems
Formulations	Pristima
In-life data collection	Ames Study Manager/Sorcerer
Data generation and collection	Dunnett's Data Reporter
Report generation	Microsoft Office/Adobe Acrobat

Version numbers of the systems are held on file at Covance.

RESULTS

Toxicity, solubility and concentration selection

Details of all treatment solution concentrations and final E212966 concentrations are provided in the Test article section.

Experiment 1 treatments of all the tester strains were performed in the absence and in the presence of S-9, using final concentrations of E212966 at 5, 15.81, 50, 158.1, 500, 1581 and 5000 μg/plate, plus negative (vehicle) and positive controls. Following these treatments, evidence of toxicity in the form of a slight thinning of the background bacterial lawn with or without a concurrent reduction in revertant numbers, was observed in strain TA102 at 5000 μg/plate in the absence and presence of S-9.

Experiment 2 treatments of all the tester strains were performed in the absence and in the presence of S-9. The maximum test concentration of 5000 μg/plate was retained for all strains. Narrowed concentration intervals were employed covering the ranges 78.13 – 5000 μg/plate (strain TA102) or 156.3 – 5000 μg/plate (strains TA98, TA100, TA1535 and TA1537), in order to examine more closely those concentrations of E212966 approaching the maximum test concentration and considered therefore most likely to provide evidence of any mutagenic activity. In addition, all treatments in the presence of S-9 were further modified by the inclusion of a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected using this assay system. Following these treatments, evidence of toxicity in the form of a slight thinning of the background bacterial lawn was observed in strain TA102 at 5000 μg/plate in the absence and presence of S-9.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed.

Data acceptability and validity

The individual mutagenicity plate counts were averaged to give mean values, which are presented in Appendix 1 and Appendix 2. From the data it can be seen that mean vehicle control counts fell within, or slightly above, the normal historical ranges (Appendix 4, see minor deviation from protocol Appendix 8). The positive control chemicals all induced increases in revertant numbers of ≥2.0-fold (in strains TA98, TA100 or TA102) or ≥3.0-fold (in strains TA1535 or TA1537) the concurrent vehicle

control confirming discrimination between different strains, and an active S-9 preparation. The study therefore demonstrated correct strain and assay functioning and was accepted as valid.

Mutation

Following E212966 treatments of all the test strains in the absence and presence of S-9, no increases in revertant numbers were observed that were statistically significant when the data were analysed at the 1% level using Dunnett's test.

A small increase in revertant numbers attained significance when the data were analysed at the 5% level using Dunnett's test (Appendix 1; Table 11). This increase was small in magnitude, displayed no evidence of any concentration-relationship and was not reproduced in an independent experiment. This increase was attributed to chance occurrence and was not indicative of E212966-induced mutagenicity.

This study was considered therefore to have provided no evidence of any E212966 mutagenic activity in this assay system.

CONCLUSION

It was concluded that E212966 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 5000 µg/plate (the maximum recommended concentration according to current regulatory guidelines), in the absence and in the presence of a rat liver metabolic activation system (S-9).

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APPENDICES

Appendix 1
Raw plate counts and calculated mutagenicity data Experiment 1

Table 7: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA98 -S-9

Compound	Concentration (µg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		30, 19, 25, 25, 31 M B	26.0	5		4.8		
E212966	5	20, 17, 19	18.7	3	0.7	1.5	-1.89	NS
E212966	15.81	24, 27, 25	25.3	3	1.0	1.5	-0.12	NS
E212966	50	20, 21, 16	19.0	3	0.7	2.6	-1.80	NS
E212966	158.1	15, 15, 26	18.7	3	0.7	6.4	-1.98	NS
E212966	500	22, 19, 21	20.7	3	0.8	1.5	-1.33	NS
E212966	1581	29, 11, 25	21.7	3	0.8	9.5	-1.27	NS
E212966	5000	21, 29, 17	22.3	3	0.9	6.1	-0.95	NS
2NF	5	783, 704, 676	721.0	3	27.7	55.5		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 8: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA98 +S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		26, 27, 31, 34, 26	28.8	5		3.6		
E212966	5	36, 39, 35	36.7	3	1.3	2.1	2.20	NS
E212966	15.81	35, 30, 30	31.7	3	1.1	2.9	0.84	NS
E212966	50	29, 32, 25	28.7	3	1.0	3.5	-0.04	NS
E212966	158.1	30, 40, 29	33.0	3	1.1	6.1	1.17	NS
E212966	500	27, 37, 25	29.7	3	1.0	6.4	0.21	NS
E212966	1581	36, 25, 39	33.3	3	1.2	7.4	1.24	NS
E212966	5000	29, 20, 22	23.7	3	0.8	4.7	-1.61	NS
B[a]P	10	446, 474, 393	437.7	3	15.2	41.1		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 9: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA100 -S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		95, 95, 95, 96, 97	95.6	5		0.9		
E212966	5	97, 127, 106	110.0	3	1.2	15.4	2.37	NS
E212966	15.81	111, 106, 112	109.7	3	1.1	3.2	2.37	NS
E212966	50	75, 100, 88	87.7	3	0.9	12.5	-1.47	NS
E212966	158.1	87, 105, 98	96.7	3	1.0	9.1	0.16	NS
E212966	500	105, 102, 116	107.7	3	1.1	7.4	2.03	NS
E212966	1581	103, 101, 112	105.3	3	1.1	5.9	1.65	NS
E212966	5000	101, 105, 100	102.0	3	1.1	2.6	1.10	NS
NaN_3	2	698, 570, 619	629.0	3	6.6	64.6		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 10: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA100 +S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		106, 118, 98, 101, 101	104.8	5		7.9		
E212966	5	82, 95, 101	92.7	3	0.9	9.7	-1.43	NS
E212966	15.81	105, 91, 103	99.7	3	1.0	7.6	-0.59	NS
E212966	50	111, 111, 90	104.0	3	1.0	12.1	-0.11	NS
E212966	158.1	107, 117, 106	110.0	3	1.0	6.1	0.59	NS
E212966	500	145, 98, 110	117.7	3	1.1	24.4	1.34	NS
E212966	1581	84, 96, 112	97.3	3	0.9	14.0	-0.89	NS
E212966	5000	105, 87, 102	98.0	3	0.9	9.6	-0.79	NS
AAN	5	992, 1123, 965	1026.7	3	9.8	84.5		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 11: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA1535 -S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		20, 21, 12, 7, 12	14.4	5		5.9		
E212966	5	12, 16, 16	14.7	3	1.0	2.3	0.25	NS
E212966	15.81	20, 17, 20	19.0	3	1.3	1.7	1.66	NS
E212966	50	12, 20, 15	15.7	3	1.1	4.0	0.56	NS
E212966	158.1	10, 20, 20	16.7	3	1.2	5.8	0.82	NS
E212966	500	25, 20, 24	23.0	3	1.6	2.6	2.81	*
E212966	1581	16, 14, 16	15.3	3	1.1	1.2	0.50	NS
E212966	5000	15, 19, 16	16.7	3	1.2	2.1	0.93	NS
NaN_3	2	596, 657, 626	626.3	3	43.5	30.5		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 12: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA1535 +S-9

Compound	Concentration (µg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		12, 14, 14, 14, 15	13.8	5		1.1		
E212966	5	9, 19, 20	16.0	3	1.2	6.1	0.61	NS
E212966	15.81	14, 15, 16	15.0	3	1.1	1.0	0.42	NS
E212966	50	17, 14, 22	17.7	3	1.3	4.0	1.25	NS
E212966	158.1	15, 19, 27	20.3	3	1.5	6.1	2.02	NS
E212966	500	11, 17, 20	16.0	3	1.2	4.6	0.68	NS
E212966	1581	14, 22, 21	19.0	3	1.4	4.4	1.66	NS
E212966	5000	20, 12, 21	17.7	3	1.3	4.9	1.22	NS
AAN	5	275, 241, 259	258.3	3	18.7	17.0		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 13: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA1537 -S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		21, 16 M B, 19, 16, 19	18.2	5		2.2		
E212966	5	12, 12, 20	14.7	3	0.8	4.6	-0.76	NS
E212966	15.81	29, 19, 16	21.3	3	1.2	6.8	0.53	NS
E212966	50	32, 25, 5	20.7	3	1.1	14.0	0.06	NS
E212966	158.1	16, 21, 19	18.7	3	1.0	2.5	0.09	NS
E212966	500	30, 27, 11	22.7	3	1.2	10.2	0.66	NS
E212966	1581	21, 25, 24	23.3	3	1.3	2.1	0.93	NS
E212966	5000	11, 11, 20	14.0	3	0.8	5.2	-0.92	NS
AAC	50	241, 309, 299	283.0	3	15.5	36.7		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 14: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA1537 +S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		20, 24, 17, 20, 22	20.6	5		2.6		
E212966	5	19, 26, 15	20.0	3	1.0	5.6	-0.21	NS
E212966	15.81	16, 17, 15	16.0	3	0.8	1.0	-1.26	NS
E212966	50	20, 24, 24	22.7	3	1.1	2.3	0.53	NS
E212966	158.1	21, 30, 24	25.0	3	1.2	4.6	1.07	NS
E212966	500	16, 12, 6	11.3	3	0.6	5.0	-2.89	NS
E212966	1581	19, 37, 15	23.7	3	1.1	11.7	0.57	NS
E212966	5000	30, 21, 22 M B	24.3	3	1.2	4.9	0.91	NS
AAN	5	163, 126, 167	152.0	3	7.4	22.6		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 15: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA102 -S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		254, 278, 243, 238, 265	255.6	5		16.3		
E212966	5	303, 265, 253	273.7	3	1.1	26.1	0.94	NS
E212966	15.81	265, 233, 198	232.0	3	0.9	33.5	-1.32	NS
E212966	50	280, 232, 241	251.0	3	1.0	25.5	-0.26	NS
E212966	158.1	278, 216, 239	244.3	3	1.0	31.3	-0.63	NS
E212966	500	277, 232, 252	253.7	3	1.0	22.5	-0.11	NS
E212966	1581	186, 236, 193	205.0	3	0.8	27.1	-2.87	NS
E212966	5000	173 S, 145 S, 176 S	164.7	3	0.6	17.1	-5.39	NS
MMC	0.2	723, 846, 646	738.3	3	2.9	100.9		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 16: E212966 Raw plate counts and calculated mutagenicity data - Experiment 1, TA102 +S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		227, 224, 211, 218, 222	220.4	5		6.2		
E212966	5	221, 194, 227	214.0	3	1.0	17.6	-0.51	NS
E212966	15.81	219, 237, 214	223.3	3	1.0	12.1	0.22	NS
E212966	50	226, 234, 232	230.7	3	1.0	4.2	0.78	NS
E212966	158.1	204, 234, 285	241.0	3	1.1	41.0	1.47	NS
E212966	500	227, 214, 232	224.3	3	1.0	9.3	0.30	NS
E212966	1581	196, 191, 186	191.0	3	0.9	5.0	-2.34	NS
E212966	5000	203 S, 204 S, 163 S	190.0	3	0.9	23.4	-2.47	NS
AAN	20	1483, 1179, 1317	1326.3	3	6.0	152.2		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Appendix 2
Raw plate counts and calculated mutagenicity data Experiment 2

Table 17: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA98 -S-9

Compound	Concentration (µg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		31, 19, 16, 14, 9	17.8	5		8.2		
E212966	156.3	29, 11, 23	21.0	3	1.2	9.2	0.49	NS
E212966	312.5	26, 14, 43	27.7	3	1.6	14.6	1.33	NS
E212966	625	23, 49, 21	31.0	3	1.7	15.6	1.77	NS
E212966	1250	16, 28, 11	18.3	3	1.0	8.7	0.09	NS
E212966	2500	30, 23, 19	24.0	3	1.3	5.6	0.99	NS
E212966	5000	23, 15, 13	17.0	3	1.0	5.3	-0.06	NS
2NF	5	950, 872, 1144	988.7	3	55.5	140.1		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 18: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA98 +S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		28, 39, 21, 35, 38	32.2	5		7.6		
E212966	156.3	29, 34, 16	26.3	3	0.8	9.3	-1.14	NS
E212966	312.5	28, 24, 36	29.3	3	0.9	6.1	-0.49	NS
E212966	625	34, 34, 35	34.3	3	1.1	0.6	0.44	NS
E212966	1250	31, 33, 21	28.3	3	0.9	6.4	-0.69	NS
E212966	2500	28, 34, 49	37.0	3	1.1	10.8	0.80	NS
E212966	5000	21, 33, 30	28.0	3	0.9	6.2	-0.75	NS
B[a]P	10	455, 385, 441	427.0	3	13.3	37.0		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 19: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA100 -S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		90, 91, 94, 88, 91	90.8	5		2.2		
E212966	156.3	91, 100, 110	100.3	3	1.1	9.5	1.53	NS
E212966	312.5	84, 86, 88	86.0	3	0.9	2.0	-0.81	NS
E212966	625	88, 74, 91	84.3	3	0.9	9.1	-1.13	NS
E212966	1250	75, 80, 79	78.0	3	0.9	2.6	-2.23	NS
E212966	2500	99, 101, 71	90.3	3	1.0	16.8	-0.17	NS
E212966	5000	78, 85, 90	84.3	3	0.9	6.0	-1.11	NS
NaN_3	2	812, 739, 649	733.3	3	8.1	81.6		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 20: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA100 +S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		95, 108, 108, 104, 110	105.0	5		6.0		
E212966	156.3	128, 101, 99	109.3	3	1.0	16.2	0.56	NS
E212966	312.5	125, 103, 125	117.7	3	1.1	12.7	1.70	NS
E212966	625	127, 113, 130	123.3	3	1.2	9.1	2.45	NS
E212966	1250	118, 107, 118	114.3	3	1.1	6.4	1.28	NS
E212966	2500	96, 104, 114	104.7	3	1.0	9.0	-0.05	NS
E212966	5000	90, 105, 110	101.7	3	1.0	10.4	-0.48	NS
AAN	5	1507, 1467, 1387	1453.7	3	13.8	61.1		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 21: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA1535 -S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		24, 20, 16, 18, 19	19.4	5		3.0		
E212966	156.3	16, 10, 14	13.3	3	0.7	3.1	-3.20	NS
E212966	312.5	20, 20, 20	20.0	3	1.0	0.0	0.33	NS
E212966	625	15, 18, 16	16.3	3	0.8	1.5	-1.50	NS
E212966	1250	14, 15, 16	15.0	3	0.8	1.0	-2.20	NS
E212966	2500	18, 15, 18	17.0	3	0.9	1.7	-1.16	NS
E212966	5000	14, 24, 20	19.3	3	1.0	5.0	-0.10	NS
NaN_3	2	735, 604, 604	647.7	3	33.4	75.6		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 22: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA1535 +S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		19, 18, 23, 16, 16	18.4	5		2.9		
E212966	156.3	16, 19, 10	15.0	3	0.8	4.6	-0.93	NS
E212966	312.5	25, 18, 23	22.0	3	1.2	3.6	0.85	NS
E212966	625	14, 13, 20	15.7	3	0.9	3.8	-0.72	NS
E212966	1250	19, 4, 18	13.7	3	0.7	8.4	-1.59	NS
E212966	2500	19, 10, 19	16.0	3	0.9	5.2	-0.68	NS
E212966	5000	16, 18, 13	15.7	3	0.9	2.5	-0.70	NS
AAN	5	297, 237, 81	205.0	3	11.1	111.5		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 23: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA1537 -S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		20, 14, 11, 14, 20	15.8	5		4.0		
E212966	156.3	11, 24, 8	14.3	3	0.9	8.5	-0.51	NS
E212966	312.5	19, 5, 14	12.7	3	0.8	7.1	-0.96	NS
E212966	625	13, 14, 19	15.3	3	1.0	3.2	-0.09	NS
E212966	1250	15, 11, 16	14.0	3	0.9	2.6	-0.42	NS
E212966	2500	24, 10, 18	17.3	3	1.1	7.0	0.29	NS
E212966	5000	21, 15, 14	16.7	3	1.1	3.8	0.22	NS
AAC	50	455, 475, 328	419.3	3	26.5	79.7		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 24: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA1537 +S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		26, 31, 26, 30, 34	29.4	5		3.4		
E212966	156.3	25, 28, 41	31.3	3	1.1	8.5	0.35	NS
E212966	312.5	19, 40, 18	25.7	3	0.9	12.4	-1.02	NS
E212966	625	31, 29, 26	28.7	3	1.0	2.5	-0.15	NS
E212966	1250	26, 31, 24	27.0	3	0.9	3.6	-0.52	NS
E212966	2500	35, 24, 26	28.3	3	1.0	5.9	-0.26	NS
E212966	5000	30, 28, 38	32.0	3	1.1	5.3	0.53	NS
AAN	5	138, 135, 105	126.0	3	4.3	18.2		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 25: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA102 -S-9

Compound	Concentration (μg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		251, 195, 219, 221, 204	218.0	5		21.4		
E212966	78.13	219, 202, 217	212.7	3	1.0	9.3	-0.29	NS
E212966	156.3	224, 249, 238	237.0	3	1.1	12.5	1.07	NS
E212966	312.5	232, 185, 246	221.0	3	1.0	32.0	0.15	NS
E212966	625	249, 218, 238	235.0	3	1.1	15.7	0.96	NS
E212966	1250	251, 239, 202	230.7	3	1.1	25.5	0.71	NS
E212966	2500	219, 187, 175	193.7	3	0.9	22.7	-1.42	NS
E212966	5000	160 S, 213 S, 143 S	172.0	3	0.8	36.5	-2.82	NS
MMC	0.2	798, 788, 744	776.7	3	3.6	28.7		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Table 26: E212966 Raw plate counts and calculated mutagenicity data - Experiment 2, TA102 +S-9

Compound	Concentration (µg/plate)	Revertant numbers/plate	Mean	N	Fold Increase	Standard Deviation	Dunnett's t-value	Significance
DMSO		266, 258, 208, 286, 249	253.4	5		28.8		
E212966	78.13	238, 258, 283	259.7	3	1.0	22.5	0.36	NS
E212966	156.3	277, 278, 268	274.3	3	1.1	5.5	1.16	NS
E212966	312.5	274, 279, 286	279.7	3	1.1	6.0	1.44	NS
E212966	625	283, 268, 238	263.0	3	1.0	22.9	0.54	NS
E212966	1250	277, 218, 303	266.0	3	1.0	43.6	0.65	NS
E212966	2500	273, 274, 302	283.0	3	1.1	16.5	1.61	NS
E212966	5000	243 S, 216 S, 268 S	242.3	3	1.0	26.0	-0.60	NS
AAN	20	1318, 1265, 1115	1232.7	3	4.9	105.3		

For key to abbreviations, postfixes and significance values refer to Appendix 3.

Appendix 3
Key to abbreviations, postfixes and significance values

Dunnett's t-test significance values

NS	Not significant
*	$p \leq 0.05$
**	$p \leq 0.01$

Positive controls

2NF	2-Nitrofluorene
NaN_3	Sodium azide
AAC	9-Aminoacridine
MMC	Mitomycin C
B[a]P	Benzo[a]pyrene
AAN	2-Aminoanthracene

Table Postfixes

B	Bubbles or split in agar
M	Plate counted manually
S	Slight thinning of background bacterial lawn

Appendix 4
Historical negative (vehicle) control values for *S. typhimurium* strains

Table 27: Historical negative (vehicle) control values for *S. typhimurium* strains

				Revertant numbers for individual plates				
						99% confidence interval for group mean of:		
Strain	S-9	No. of studies	No. of plates	Mean	99% reference range[1]	4 values [2]	5 values [2]	6 values [2]
TA98	-	51	525	25	9.0-44.0	16.0-34.3	16.8-33.1	17.4-32.3
TA98	+	51	538	35	16.0-58.0	25.0-46.6	26.0-45.3	26.7-44.4
TA100	-	51	600	111	73.0-156.5	88.7-133.5	90.8-130.9	92.4-129.0
TA100	+	51	604	117	71.0-168.0	91.8-144.3	94.3-141.2	96.2-139.0
TA1535	-	50	520	17	5.0-30.0	10.8-24.8	11.4-23.9	11.8-23.3
TA1535	+	50	525	17	6.0-32.0	10.8-24.5	11.4-23.6	11.8-23.0
TA1537	-	51	530	13	3.0-30.0	6.3-20.1	6.9-19.2	7.3-18.5
TA1537	+	51	523	18	4.0-30.0	10.4-25.6	11.0-24.6	11.5-23.9
TA102	-	50	520	270	184.0-350.0	227.7-313.3	231.9-308.5	235.0-304.9
TA102	+	50	528	233	153.0-328.0	191.5-275.2	195.6-270.4	198.6-266.9

[1] Reference ranges are calculated from percentiles of the observed distributions.
[2] Calculated from square-root transformed data.

Ranges calculated in January 2011 by CLEH Statistics, using data selected without bias from studies# started during the periods given below:

S.typhimurium strains Feb 08 to Jul 09.

All studies had been audited prior to data collection.

Appendix 5
Historical positive control values for *S. typhimurium* strains

Table 28: Historical positive control values for *S. typhimurium* strains

					Induced numbers for individual plates	
					Reference ranges[1]	
Strain	S-9	No. of studies	No. of plates	Mean	95%	99%
TA98	-	51	315	824	383.8-1760.4	275.2-1923.4
TA98	+	51	324	321	164.4-532.6	99.6-647.2
TA100	-	51	360	660	339.2-1094.6	263.8-1171.6
TA100	+	51	363	1172	542.0-2068.4	407.4-2384.8
TA1535	-	50	312	601	325.4-876.2	271.4-989.4
TA1535	+	50	314	212	107.6-326.8	84.4-364.6
TA1537	-	51	316	108	40.6-281.4	32.2-576.2
TA1537	+	51	314	119	36.2-257.0	19.4-327.6
TA102	-	50	312	450	235.6-672.8	140.2-931.6
TA102	+	50	318	1370	450.2-2742.6	313.4-3045.4

[1] Reference ranges are calculated from percentiles of the observed distributions.

Ranges calculated in January 2011 by CLEH Statistics, using data selected without bias from studies# started during the periods given below:

S.typhimurium strains Feb 08 to Jul 09.

All studies had been audited prior to data collection.

Appendix 8
Minor deviation from protocol

Protocol section	Subject	Deviation
Analysis of results	Acceptance criteria	Following Experiment 2 treatments in strain TA1537 in the presence of S-9, the mean vehicle control revertant count was outside the 99% confidence interval and two of the individual revertant counts were outside the 99% reference range. As these counts were only marginally outside the historical control ranges and three of the five individual counts were within the 99% reference range, these data are considered to be characteristic of the strain and the data are accepted as valid.

Final Report

Study Title	Induction of micronuclei in cultured human peripheral blood lymphocytes
Test Article	E212966 mixture containing 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/ Hydroxyethylacrylate Copolymer
Author	[redacted]
Sponsor	
Study Monitor	
Test Facility	
Covance Client Identifier	
Covance Study Number	
Report Issued	April 2013
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[redacted]

[redacted]

STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

E212966: Induction of micronuclei in cultured human peripheral blood lymphocytes

I, the undersigned, hereby declare that the work was performed under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol and with [redacted] Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

The study was conducted in accordance with the following:

- The United Kingdom (GLP Monitoring Authority, Medicines and Healthcare products Regulatory Agency [MHRA]) Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No.3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations, 2004.

- The OECD Principles on Good Laboratory Practice ENV/MC/CHEM (98) 17 (Revised in 1997, Issued January 1998).

- Directive 2004/10/EC of the European Parliament and the Council of 11 February 2004 on the Harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L50 of 20.2.2004).

[redacted]

8 April 2013
Date

[redacted]

QUALITY ASSURANCE STATEMENT

E212966: Induction of micronuclei in cultured human peripheral blood lymphocytes

This study has been reviewed by the GLP Quality Assurance Unit [redacted] and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the Study Director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
11 Oct 2012	11 Oct 2012	Protocol Review	11 Oct 2012
03 Jan 2013	03 Jan 2013	Draft Report and Data Review	03 Jan 2013
08 Apr 2013	08 Apr 2013	Final Report Review	08 Apr 2013

Process

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
09 Oct 2012	09 Oct 2012	Slide Staining	09 Oct 2012
26 Oct 2012	26 Oct 2012	Dose Preparation	26 Oct 2012
26 Oct 2012	26 Oct 2012	S9 Quality Control Checks	30 Oct 2012

[redacted]

8 April 2013
Date

[redacted]

REVIEWING SCIENTIST'S STATEMENT

E212966: Induction of micronuclei in cultured human peripheral blood lymphocytes

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.

[redacted]

8 April 2013
Date

RESPONSIBLE PERSONNEL

E212966: Induction of micronuclei in cultured human peripheral blood lymphocytes

The following personnel were responsible for key elements of the study:

Study Director [redacted]
Laboratory Supervisor
Study Monitor [1]

[1] [redacted]

[redacted]

ARCHIVE STATEMENT

E212966: Induction of micronuclei in cultured human peripheral blood lymphocytes

The raw data, including documentation, study protocol, final report and study correspondence resulting from this study will be retained in the test facility archives for ten years from the date of report finalisation. After completion of this period, the Sponsor will be contacted in order to determine their requirements for further retention or disposition of the archived materials (excluding facility records, non-transferable electronic data and facility copies of protocol/final report, which will be retained by [redacted] in accordance with test facility SOPs). Where continued retention is requested, the archived materials may subsequently be transferred to alternative [redacted] Archive locations. In this event, the Sponsor will be informed, and documented chain of custody records will be maintained.

[redacted]

CONTENTS

[redacted]

SUMMARY

E212966 was tested in an *in vitro* micronucleus assay using duplicate human lymphocyte cultures prepared from the pooled blood of two female donors in a single experiment. Treatments covering a broad range of concentrations, separated by narrow intervals, were performed both in the absence and presence of metabolic activation (S-9) from Aroclor 1254-induced rats. The test article was formulated in anhydrous analytical grade dimethyl sulphoxide (DMSO). The highest concentrations used in the Micronucleus Experiment were limited by toxicity and were determined following a preliminary cytotoxicity Range-Finder Experiment.

Treatments were conducted (as detailed in the following summary table) 48 hours following mitogen stimulation by phytohaemagglutinin (PHA). The test article concentrations for micronucleus analysis were selected by evaluating the effect of E212966 on the replication index (RI). Micronuclei were analysed at three or four concentrations and a summary of the data is presented in Table 1.

Table 1: Micronucleus Experiment– Results summary

Treatment	Concentration (µg/mL)	Cytotoxicity (%) [$]	Mean MNBN cell frequency (%)	Historical Control Range (%) [#]	Statistical significance
3+21 hour -S-9	Vehicle [a]	-	0.60	0.10 – 1.00	-
	100.0	3	0.20		NS
	200.0	29	0.50		NS
	250.0	51	0.50		NS
	*MMC, 0.80	ND	7.40		$p \leq 0.001$
3+21 hour +S-9	Vehicle [a]	-	0.50	0.10 – 1.10	-
	100.0	14	0.45		NS
	200.0	38	0.30		NS
	250.0	47	0.50		NS
	300.0	65	0.30		NS
	*CPA, 12.50	ND	2.35		$p \leq 0.001$
24+0 hour -S-9	Vehicle [a]	-	0.50	0.10 – 1.40	-
	20.00	12	0.25		NS
	35.00	36	0.25		NS
	45.00	51	0.30		NS
	*VIN, 0.02	ND	32.23		$p \leq 0.001$

[a] Vehicle control was DMSO
* Positive control
[#] 95th percentile of the observed range
[$] Based on replication index
NS Not significant
ND Not determined

Appropriate negative (vehicle) control cultures were included in the test system under each treatment condition. The proportion of micronucleated binucleate cells (MNBN) in these cultures fell within current historical vehicle control (normal) ranges. Mitomycin C (MMC) and Vinblastine (VIN) were employed as clastogenic and aneugenic positive control chemicals respectively in the absence of rat liver S-9. Cyclophosphamide (CPA) was employed as a clastogenic positive control chemical in the presence of rat liver S-9. Cells receiving these were sampled in the Micronucleus Experiment at 24 hours after the start of treatment; all compounds induced statistically significant increases in the proportion of cells with micronuclei.

All acceptance criteria were considered met and the study was accepted as valid.

Treatment of cells with E212966 for 3+21 hours in the absence and presence of S-9 and for 24+0 hours in the absence of S-9 resulted in frequencies of MNBN cells that were similar to (and not significantly higher than) those observed in concurrent vehicle controls at all concentrations analysed under all three treatment conditions. The MNBN cell frequencies in all treated cultures fell within the normal ranges.

It is concluded that E212966 did not induce micronuclei in cultured human peripheral blood lymphocytes when tested for 3+21 hours in the absence and presence of a rat liver metabolic activation system (S-9) and for 24+0 hours in the absence of S-9. Under all treatment conditions, treatments were performed up to the limit of toxicity.

[redacted]

INTRODUCTION

Chromosome defects are recognised as the basis of a number of human genetic diseases (Mitelman, 1991). Assays for the detection of chromosome damage in mammalian cells *in vitro* are recommended in regulatory guidelines as a complement to Ames tests in a genotoxicity test battery. There is a large database on the use of chromosomal assays for screening purposes (Preston *et al.*, 1981; Fenech, 1998; Fenech *et al.*, 2003). The use of human peripheral blood lymphocytes is recommended because the cells are only used in short-term culture and maintain a stable karyotype (Evans & O'Riordan, 1975). Experiments with these cells can also be performed in conjunction with a rat liver metabolising system (S-9) since, for short incubation periods, no toxicity is induced by the liver homogenate itself.

An alternative to measuring structural aberrations in mitotic cells is to measure micronuclei. These are produced from whole chromosomes or acentric fragments that are unable to attach to the spindle at mitosis and appear during the next interphase as small darkly staining bodies adjacent to the main daughter nucleus. Cytochalasin B (Cyto-B), if added to cultures, inhibits cytokinesis (cell division) but not karyokinesis (nuclear division) resulting in the formation of binucleate cells (Fenech & Morley, 1985). If micronuclei are counted in binucleate cells, then a measurement of micronucleus induction resulting from cell division can be obtained.

Theoretical considerations, together with published data (Lorge *et al.*, 2006), indicate that most aneugens and clastogens will be detected by a short term treatment period of 3-6 hours in the presence and absence of S-9 followed by removal of the test article and a growth period of 1.5-2.0 cell cycles (Fenech & Morley, 1986).

The most efficient approach is to test lymphocytes 44-48 hours post-mitogen stimulation by PHA, when cycle synchronisation will have dissipated (Fenech, 2007).

The test article was added at 48 hours following culture initiation (stimulation by PHA). Cells were exposed to the test article for 3 hours in the absence and presence of S-9 (from rats induced with Aroclor 1254). In addition, a continuous 24 hour treatment (equivalent to approximately 1.5 to 2 times the average generation time of cultured lymphocytes from the panel of donors used in this laboratory) in the absence of S-9 was included. All cultures were sampled 24 hours after the beginning of treatment (i.e. 72 hours after culture initiation).

The objective of this study was to evaluate the clastogenic and aneugenic potential of E212966 by examining its effects on the frequency of micronuclei in cultured human peripheral blood lymphocytes treated in the absence and presence of S-9.

The test methodology is based on OECD guideline 487 (OECD, 2010) and accepted scientific/regulatory principles described in current guidelines for clastogenicity testing *in vitro* (Fenech, 1998; Fenech *et al.*, 2003; Rosefort *et al.*, 2004; Elhajouji *et al.*, 1998; Migliore & Nieri, 1991; Galloway *et al.*, 1994; Aardema *et al.*, 1998; Miller *et al.*, 1998; Fenech *et al.*, 1999; Thybaud *et al.*, 2007).

This study was performed according to the protocol.

The study was initiated on 9 October 2012. Experimental work started on 17 October 2012 and was completed on 15 November 2012. The study completion date is considered to be the date the Study Director signs the final report.

[redacted]

MATERIALS

Test article

E212966, batch number C2120726D, was a clear yellowish viscous liquid. It was received on 2 October 2012 and stored at 15-25°C protected from light in a tightly closed container. The major components were AMP-Acrylates/C1-18 Alkyl Acrylates/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate copolymer (38 parts), ethanol (60 parts). Purity was assumed 100% for testing purposes and the expiry date was given as 25 July 2015 under the prescribed storage conditions. The manufacturer's certificate of analysis, provided by the Sponsor, is presented in Appendix 5. The test article information and certificate of analysis provided by the Sponsor are considered an adequate description of the characterisation, purity and stability of the test article. Determinations of stability and characteristics of the test article were the responsibility of the Sponsor.

Preliminary solubility data indicated that E212966 was soluble in anhydrous analytical grade dimethyl sulphoxide (DMSO) at a concentration of at least 500 mg/mL. The solubility limit in culture medium was less than 2500 μg/mL, as indicated by visible precipitation at this concentration following an incubation period of approximately 20 hours. A maximum concentration of 3000 μg/mL was selected for the cytotoxicity Range-Finder Experiment, in order that treatments were performed up to a precipitating concentration (OECD, 2010). Concentrations selected for the Micronucleus Experiment were based on the results of this cytotoxicity Range-Finder Experiment.

Test article stock solutions were prepared by formulating E212966 under subdued lighting in DMSO, with the aid of vortex mixing, to give the maximum required treatment concentration. Subsequent dilutions were made using DMSO. The test article solutions were protected from light and used within approximately 3.5 hours of initial formulation as shown in Table 2.

Table 2: E212966 Concentration Ranges Tested

Experiment	Treatment	Concentration range (mg/mL)	Final concentration range (μg/mL)
Range-Finder	3+21, -S-9	1.088 to 300.0	10.88 to 3000
	3+21, +S-9	1.088 to 300.0	10.88 to 3000
	24+0, -S-9	1.088 to 300.0	10.88 to 3000
Micronucleus Experiment	3+21, -S-9	5.000 to 100.0	50.00 to 1000
	3+21, +S-9	5.000 to 100.0	50.00 to 1000
	24+0, -S-9	0.5000 to 10.00	5.000 to 100.0

[redacted]

Controls

DMSO was added to cultures designated as vehicle controls as described in the methods section of this report. The positive control chemicals were supplied and used as shown in Table 3.

Table 3: Positive Controls

Chemical	Stock concentration (mg/mL)*	Final concentration (μg/mL)	S-9
Mitomycin C	0.060	0.60	-
(MMC) **	0.080	0.80	-
Cyclophosphamide	0.625	6.25	+
(CPA) ***	1.250	12.50	+
Vinblastine	0.002	0.02	-
(VIN) **	0.003	0.03	-
	0.004	0.04	-

* In the Micronucleus Experiment, CPA was dissolved in anhydrous analytical grade dimethyl sulphoxide (DMSO), frozen (<-50°C) and thawed immediately prior to use. VIN and MMC were dissolved in purified water immediately prior to use.

** Obtained from Sigma-Aldrich Chemical Co, Poole, UK.

*** Obtained from Acros Organics, Loughborough, Leicestershire, UK.

For the 3+21 hour treatment in the absence of S-9, MMC was used as the positive control. For the 24+0 hour –S-9 treatment, VIN was used as the positive control.

Metabolic activation system

The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was obtained from Molecular Toxicology Incorporated, USA where it is prepared from male Sprague Dawley rats induced with Aroclor 1254. The batches of MolTox^{TM} S-9 were stored frozen in aliquots at <-50°C prior to use. Each batch was checked by the manufacturer for sterility, protein content, ability to convert known promutagens to bacterial mutagens and cytochrome P-450-catalyzed enzyme activities (alkoxyresorufin-O-dealkylase activities). The quality control statement, relating to the batch of S-9 preparation used, is included in Appendix 4 of this report. The S-9 mix was prepared in the following way:

Glucose-6-phosphate (G6P: 180 mg/mL), β-Nicotinamide adenine dinucleotide phosphate (NADP: 25 mg/mL), Potassium chloride (KCl: 150 mM) and rat liver S-9 were mixed in the ratio 1:1:1:2. For all cultures treated in the presence of S-9, an aliquot of the mix was added to each cell culture to achieve the required final concentration of test article in a total of 10 mL. The final concentration of the liver homogenate in the test system was 2%.

Cultures treated in the absence of S-9 received an equivalent volume of 150 mM KCl.

[redacted]

Blood cultures

Blood from two healthy, non-smoking female volunteers from a panel of donors at Covance was used for each experiment of this study:

Table 4: Blood Cultures

Experiment	Donor Sex	Donor Age (years)	Donor Identity
Range-Finder	Female	30, 33	9959, 9351
Micronucleus Experiment	Female	27, 25	9406, 8819

No donor was suspected of any virus infection or exposed to high levels of radiation or hazardous chemicals. All donors are non-smokers and are not heavy drinkers of alcohol. Donors were not taking any form of medication (contraceptive pill excluded). The measured cell cycle time of the donors used at Covance falls within the range 13 +/- 2 hours. For each experiment, an appropriate volume of whole blood was drawn from the peripheral circulation into heparinised tubes within one day of culture initiation. Blood was stored refrigerated and pooled using equal volumes from each donor prior to use.

Whole blood cultures were established in sterile disposable centrifuge tubes by placing 0.4 mL of pooled heparinised blood into 9.0 mL pre-warmed (in an incubator set to 37 ± 1°C) HEPES-buffered RPMI medium containing 10% (v/v) heat inactivated foetal calf serum and 0.52% penicillin/streptomycin, so that the final volume following addition of S-9 mix/KCl and the test article in its chosen vehicle was 10 mL. The mitogen Phytohaemagglutinin (PHA, reagent grade) was included in the culture medium at a concentration of approximately 2% of culture to stimulate the lymphocytes to divide. Blood cultures were incubated at 37 ± 1°C for approximately 48 hours and rocked continuously.

[redacted]

METHODS

The test system was suitably labelled (using a colour-coded procedure) to clearly identify the study number, assay type, experiment number, treatment time, sex of the donor, test article concentration (if applicable), positive and vehicle controls.

Cytotoxicity Range-Finder

Immediately prior to treatment, all 24+0 hour –S-9 (continuous) cultures had 0.1 mL culture medium removed to give a final pre-treatment volume of 9.3 mL.

S-9 mix or KCl (0.5 mL per culture) was added appropriately. Cultures were treated with the test article or vehicle control (0.1 mL per culture) as indicated in Table 5. Positive control treatments were not included.

Cyto-B, formulated in DMSO, was added directly (0.1 mL per culture) to all continuous cultures at the time of treatment to give a final concentration of 6 μg/mL per culture. The final culture volume was 10 mL. Cultures were incubated at 37 ± 1°C for the designated exposure time.

Micronucleus Experiment

Immediately prior to treatment, all continuous cultures had 0.1 mL culture medium removed to give a final pre-treatment volume of 9.3 mL.

S-9 mix or KCl (0.5 mL per culture) was added appropriately. Cultures were treated with the test article, vehicle, or positive controls (0.1 mL per culture) as indicated in Table 5. Cyto-B, formulated in DMSO, was added directly (0.1 mL per culture) to all continuous cultures at the time of treatment to give a final concentration of 6 μg/mL per culture.

The final culture volume was 10 mL. Cultures were incubated at 37 ± 1°C for the designated exposure time.

This scheme is illustrated in Table 5.

[redacted]

Table 5: Treatment Scheme

Treatment	S-9	Number of cultures			
		Cytotoxicity Range-Finder		Micronucleus Experiment	
		3+21*	24+0*	3+21*	24+0*
Vehicle control	-	2	2	4	4
	+	2		4	
Test article	-	1	1	2	2
	+	1		2	
Positive controls	-			2	2
	+			2	

* Hours treatment + hours recovery.

For removal of the test article, cells were pelleted (approximately 300 *g*, 10 minutes), washed twice with sterile saline (pre-warmed in an incubator set to 37 ± 1°C), and resuspended in fresh pre-warmed medium containing foetal calf serum and penicillin / streptomycin. At the appropriate times, Cyto-B (formulated in DMSO) was added to post wash-off culture medium to give a final concentration of 6 μg/mL per culture.

Table 6: Summary of treatment conditions

Duration of treatment (hours)	S-9	Hours after culture initiation			
		Addition of test article	Removal of test article	Addition of Cytochalasin B	Harvest time
3	-	48	51	51*	72
24	-	48	72	48	72
3	+	48	51	51*	72

* Approximate times.

Changes in osmolality of more than 50 mOsm/kg and fluctuations in pH of more than one unit may be responsible for an increase in chromosome aberrations (Scott *et al.*, 1991; Brusick, 1986). Osmolality and pH measurements on post-treatment incubation medium were taken in the cytotoxicity Range-Finder Experiment.

Harvesting

At the defined sampling time, cultures were centrifuged at approximately 300 *g* for 10 minutes, the supernatant removed and discarded and cells resuspended in 4 mL (hypotonic) 0.075 M KCl at 37 ± 1°C for 4 minutes to allow cell swelling to occur. Cells were fixed by dropping the KCl suspension into fresh, cold methanol/glacial acetic acid (3:1, v/v). The fixative was changed by centrifugation (approximately

300 *g*, 10 minutes) and resuspension. This procedure was repeated as necessary (centrifuging at approximately 1250 *g*, 2-3 minutes) until the cell pellets were clean.

Slide preparation

Lymphocytes were kept in fixative at 2-8°C prior to slide preparation for a minimum of 3 hours to ensure that cells were adequately fixed. Cells were centrifuged (approximately 1250 *g*, two to three minutes) and resuspended in a minimal amount of fresh fixative (if required) to give a milky suspension. Several drops of cell suspension were gently spread onto multiple clean, dry microscope slides. Slides were air-dried and stored protected from light at room temperature prior to staining. Slides were stained by immersion in 125 µg/mL Acridine Orange in phosphate buffered saline (PBS), pH 6.8 for approximately 10 seconds, washed with PBS (with agitation) for a few seconds before transfer and immersion in a second container of PBS for approximately 10 minutes. Slides were air-dried and stored protected from light at room temperature prior to analysis.

Selection of concentrations for the Micronucleus Experiment

Slides from the cytotoxicity Range-Finder Experiment were examined, uncoded, for proportions of mono-, bi- and multinucleate cells, to a minimum of 200 cells per concentration. From these data the replication index (RI) was determined.

RI, which indicates the relative number of nuclei compared to vehicle controls, was determined using the formulae below:

$$RI = \frac{\textit{number binucleate cells} + 2\ \textit{(number multinucleate cells)}}{\textit{total number of cells in treated cultures}}$$

Relative RI (expressed in terms of percentage) for each treated culture was calculated as follows:

$$\textit{Relative RI (\%)} = \frac{\textit{RI of treated cultures}}{\textit{RI of vehicle controls}}\ x100$$

Cytotoxicity (%) is expressed as (100 – Relative RI).

A selection of random fields was observed from enough treatments to determine whether chemically induced cell cycle delay or cytotoxicity had occurred.

A suitable range of concentrations was selected for the Micronucleus Experiment based on these toxicity data.

Selection of concentrations for micronucleus analysis (Micronucleus Experiment only)

Slides were examined, uncoded, for RI to a minimum of 500 cells per culture to determine whether chemically induced cell cycle delay or toxicity had occurred.

The highest concentration selected for micronucleus analysis under each treatment condition was, where possible, one at which approximately 55 ± 5% reduction in RI had occurred. The exception to this was the 3+21 hour +S-9 treatment, in which steep concentration related toxicity was observed between 250.0 and 300.0 μg/mL, giving 47% and 65% reduction in RI respectively, therefore both concentrations were analysed. Analysis of slides from highly toxic concentrations was otherwise avoided.

Slides from the highest selected concentration and at least two lower concentrations were taken for microscopic analysis, such that a range of cytotoxicity from maximum to little was covered

Slide analysis

For each treatment regime, two vehicle control cultures were analysed for micronuclei. Slides from the CPA, MMC and VIN positive control treatments were checked to ensure that the system was operating satisfactorily. One concentration from each positive control, which gave satisfactory responses in terms of quality and quantity of binucleated cells and numbers of micronuclei, was analysed.

All slides for analysis were coded, using randomly generated letters, by an individual not connected with the scoring of the slides. Labels with only the study number, assay type, experiment number, the sex of the donor and the code were used to cover treatment details on the slides.

Immediately prior to analysis 1-2 drops of PBS were added to the slides before mounting with glass coverslips. Where possible, one thousand binucleate cells from each culture (2000 per concentration) were analysed for micronuclei. The number of cells containing micronuclei and the number of micronuclei per cell on each slide was noted. Observations were recorded on raw data sheets. The microscope stage co-ordinates of the first six micronucleated cells were recorded.

Binucleate cells were only included in the analysis if all of the following criteria were met:

1. The cytoplasm remained essentially intact, and

2. The daughter nuclei were of approximately equal size.

A micronucleus was only recorded if it met the following criteria:

1. The micronucleus had the same staining characteristics and a similar morphology to the main nuclei, and

2. Any micronucleus present was separate in the cytoplasm or only just touching a main nucleus, and

3. Micronuclei were smooth edged and smaller than approximately one third the diameter of the main nuclei.

Micronucleus analysis was not conducted on slides generated from the Range-Finder treatments.

Slide analysis was performed by competent analysts trained in the applicable Covance Laboratories Harrogate (CLEH) standard operating procedures. The analysts were physically located remote from the CLEH facility, but were subject to CLEH management and GLP control systems (including QA inspection). All slides and raw data generated by the remote analysts were returned to CLEH for archiving on completion of analysis.

Analysis of results

Treatment of data

After completion of scoring and decoding of slides, the numbers of binucleate cells with micronuclei (MNBN cells) in each culture were obtained.

The proportions of MNBN cells in each replicate were used to establish acceptable heterogeneity between replicates by means of a binomial dispersion test (Richardson *et al.*, 1989).

The proportions of MNBN cells for each treatment condition were compared with the proportion in vehicle controls by using Fisher's exact test (Richardson *et al.*, 1989). Probability values of $p \leq 0.05$ were accepted as significant. Additionally, the number of micronuclei per binucleate cell were obtained and recorded.

Acceptance criteria
The assay was to be considered valid if the following criteria were met:

1. The binomial dispersion test demonstrated acceptable heterogeneity (in terms of MNBN cell frequency) between replicate cultures, particularly where no positive responses were seen.

2. The frequency of MNBN cells in vehicle controls fell within the current historical vehicle control (normal) ranges.

3. The positive control chemicals induced statistically significant increases in the proportion of cells with micronuclei. Both replicate cultures at the positive control concentration analysed under each treatment condition demonstrated MNBN cell frequencies that clearly exceeded the current historical vehicle control ranges.

4. A minimum of 50% of cells had gone through at least one cell division (as measured by binucleate + multinucleate cell counts) in vehicle control cultures at the time of harvest.

Evaluation criteria
For valid data, the test article was considered to induce clastogenic and/or aneugenic events if:

1. A statistically significant increase in the frequency of MNBN cells at one or more concentrations was observed.

2. An incidence of MNBN cells at such a concentration that exceeded the normal range in both replicates was observed.

3. A concentration-related increase in the proportion of MNBN cells was observed.

The test article was considered positive in this assay if all of the above criteria were met.

The test article was considered negative in this assay if none of the above criteria were met.

Results which only partially satisfied the above criteria were dealt with on a case-by-case basis. Evidence of a concentration-related effect was considered useful but not essential in the evaluation of a positive result (Scott *et al.*, 1990).

Major computer systems

The major computer systems used on this study were as follows:

Table 7: Major computer systems

Activity	Computer system
Scheduling	CMS (Covance Management System)
Formulations	Pristima
Slide coding, data generation and collation	Vitroabs, CBPI, Vitronuc
Report generation	Microsoft Office / Adobe Acrobat

Version numbers of the systems are held on file at Covance.

[redacted]

RESULTS

Selection of concentrations for micronucleus analysis

The results of the RI determinations from the cytotoxicity Range-Finder Experiment were as follows:

Table 8: Data for 3+21 hour treatments -S-9, Range-Finder - female donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total Number of Cells	RI	Cytotoxicity (%)
Vehicle	A	34	156	10	200	0.88	-
	B	43	150	7	200	0.82	
10.88	A	NS					-
18.14	A	33	155	12	200	0.90	0
30.23	A	46	143	11	200	0.83	3
50.39	A	45	150	5	200	0.80	6
83.98	A	51	149	0	200	0.75	12
140.0	A	80	120	0	200	0.60	29
233.3	A	96	103	1	200	0.53	38
388.8	A	146	54	0	200	0.27	68
648.0	A	151	49	0	200	0.25	71
1080	A	189	11	0	200	0.06	94
1800	A	188	12	0	200	0.06	93
3000	A	183	17	0	200	0.09	90

NS = Not scored
Mono = Mononucleate
Bi = Binucleate
Multi = Multinucleate
RI = Replication index

Table 9: Data for 3+21 hour treatments +S-9, Range-Finder - female donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total Number of Cells	RI	Cytotoxicity (%)
Vehicle	A	32	159	9	200	0.89	-
	B	30	162	8	200	0.89	
10.88	A	NS					-
18.14	A	NS					-
30.23	A	NS					-
50.39	A	32	159	9	200	0.89	0
83.98	A	34	156	10	200	0.88	1
140.0	A	48	144	8	200	0.80	10
233.3	A	71	128	1	200	0.65	27
388.8	A	77	123	0	200	0.62	31
648.0	A	120	80	0	200	0.40	55
1080	A	176	24	0	200	0.12	86
1800	A	183	17	0	200	0.09	90
3000	A	190	10	0	200	0.05	94

Table 10: Data for 24+0 hour treatments -S-9, Range-Finder - female donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total Number of Cells	RI	Cytotoxicity (%)
Vehicle	A	38	151	11	200	0.87	-
	B	24	169	7	200	0.92	
10.88	A	42	149	9	200	0.84	6
18.14	A	56	135	9	200	0.77	14
30.23	A	96	104	0	200	0.52	42
50.39	A	149	51	0	200	0.26	71
83.98	A	172	28	0	200	0.14	84
140.0	A	186	14	0	200	0.07	92
233.3	A	191	9	0	200	0.05	95
388.8	A	185	15	0	200	0.08	92
648.0	A	188	12	0	200	0.06	93
1080	A	195	5	0	200	0.03	97
1800	A	NE					- H
3000	A	NE					- H

NS = Not scored
NE = Not evaluated - no scoreable cells
H = Precipitation observed at harvest
Mono = Mononucleate
Bi = Binucleate
Multi = Multinucleate
RI = Replication index

No marked changes in osmolality or pH were observed at the highest concentration tested in the Range-Finder (3000 µg/mL), compared to the concurrent vehicle controls (individual data not reported).

The results of the cytotoxicity Range-Finder Experiment were used to select suitable maximum concentrations for the Micronucleus Experiment.

The results of the RI determinations from the Micronucleus Experiment were as follows:

Table 11: Data for 3+21 hour treatments -S-9, Micronucleus Experiment - female donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total Number of Cells	RI	Cytotoxicity (%)
Vehicle	A	139	351	10	500	0.74	-
	B	137	363	10	510	0.75	
	C	122	370	8	500	0.77	
	D	203	293	4	500	0.60	
50.00	A	106	386	8	500	0.80	0
	B	109	379	12	500	0.81	
100.0	**A**	**173**	**327**	**0**	**500**	**0.65**	**3 #**
	B	**134**	**362**	**4**	**500**	**0.74**	
200.0	**A**	**234**	**265**	**1**	**500**	**0.53**	**29 #**
	B	**258**	**240**	**2**	**500**	**0.49**	
250.0	**A**	**310**	**188**	**2**	**500**	**0.38**	**51 #**
	B	**339**	**161**	**0**	**500**	**0.32**	
300.0	A	326	174	0	500	0.35	50
	B	317	183	0	500	0.37	
350.0	A	370	130	0	500	0.26	63
	B	363	137	0	500	0.27	
400.0	A	368	132	0	500	0.26	61
	B	353	147	0	500	0.29	
450.0	A	381	119	0	500	0.24	64
	B	360	140	0	500	0.28	
500.0	A	363	137	0	500	0.27	67
	B	404	96	0	500	0.19	
550.0	A	396	104	0	500	0.21	67
	B	370	130	0	500	0.26	
600.0	A	422	78	0	500	0.16	77
	B	416	84	0	500	0.17	
700.0	A	431	69	0	500	0.14	82
	B	437	63	0	500	0.13	
800.0	A	450	50	0	500	0.10	87
	B	460	40	0	500	0.08	
900.0	A	463	37	0	500	0.07	89
	B	459	41	0	500	0.08	
1000	A	476	24	0	500	0.05	94
	B	479	21	0	500	0.04	

Mono = Mononucleate
Bi = Binucleate
Multi = Multinucleate
RI = Replication index

Highlighted concentrations selected for analysis.

[redacted]

Table 12: Data for 3+21 hour treatments +S-9, Micronucleus Experiment - female donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total Number of Cells	RI	Cytotoxicity (%)
Vehicle	A	60	429	11	500	0.90	-
	B	76	414	10	500	0.87	
	C	65	415	20	500	0.91	
	D	59	418	23	500	0.93	
50.00	A	92	401	7	500	0.83	4
	B	77	394	29	500	0.90	
100.0	**A**	**120**	**378**	**2**	**500**	**0.76**	**14 #**
	B	**110**	**384**	**6**	**500**	**0.79**	
200.0	**A**	**228**	**272**	**0**	**500**	**0.54**	**38 #**
	B	**217**	**283**	**0**	**500**	**0.57**	
250.0	**A**	**251**	**249**	**0**	**500**	**0.50**	**47 #**
	B	**272**	**228**	**0**	**500**	**0.46**	
300.0	**A**	**335**	**165**	**0**	**500**	**0.33**	**65 #**
	B	**346**	**154**	**0**	**500**	**0.31**	
350.0	A	358	142	0	500	0.28	62
	B	302	198	0	500	0.40	
400.0	A	330	170	0	500	0.34	60
	B	312	188	0	500	0.38	
450.0	A	324	175	1	500	0.35	65
	B	364	136	0	500	0.27	
500.0	A	356	144	0	500	0.29	68
	B	357	143	0	500	0.29	
550.0	A	380	120	0	500	0.24	73
	B	377	123	0	500	0.25	
600.0	A	372	128	0	500	0.26	73
	B	383	117	0	500	0.23	
700.0	A	435	65	0	500	0.13	83
	B	409	91	0	500	0.18	
800.0	A	389	111	0	500	0.22	82
	B	448	52	0	500	0.10	
900.0	A	437	63	0	500	0.13	85
	B	428	71	1	500	0.15	
1000	A	442	58	0	500	0.12	88
	B	451	49	0	500	0.10	

Mono = Mononucleate
Bi = Binucleate
Multi = Multinucleate
RI = Replication index

Highlighted concentrations selected for analysis.

[redacted]

Table 13: Data for 24+0 hour treatments -S-9, Micronucleus Experiment - female donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total Number of Cells	RI	Cytotoxicity (%)
Vehicle	A	75	393	32	500	0.91	-
	B	75	403	22	500	0.89	
	C	73	410	17	500	0.89	
	D	61	399	40	500	0.96	
5.000	A	70	401	29	500	0.92	2
	B	84	393	23	500	0.88	
10.00	A	80	396	24	500	0.89	2
	B	72	401	27	500	0.91	
20.00	**A**	**113**	**377**	**10**	**500**	**0.79**	**12 #**
	B	**107**	**380**	**13**	**500**	**0.81**	
25.00	A	146	354	0	500	0.71	20
	B	133	361	6	500	0.75	
30.00	A	196	300	4	500	0.62	30
	B	171	328	1	500	0.66	
35.00	**A**	**193**	**302**	**5**	**500**	**0.62**	**36 #**
	B	**228**	**270**	**2**	**500**	**0.55**	
40.00	A	232	268	0	500	0.54	43
	B	249	251	0	500	0.50	
45.00	**A**	**293**	**207**	**0**	**500**	**0.41**	**51 #**
	B	**264**	**235**	**1**	**500**	**0.47**	
50.00	A	307	193	0	500	0.39	55
	B	278	222	0	500	0.44	
55.00	A	371	129	0	500	0.26	68
	B	334	166	0	500	0.33	
60.00	A	367	133	0	500	0.27	73
	B	382	118	0	500	0.24	
70.00	A	406	94	0	500	0.19	77
	B	380	120	0	500	0.24	
80.00	A	419	81	0	500	0.16	83
	B	426	74	0	500	0.15	
90.00	A	417	83	0	500	0.17	82
	B	419	81	0	500	0.16	
100.0	A	391	109	0	500	0.22	75
	B	380	120	0	500	0.24	

Mono = Mononucleate
Bi = Binucleate
Multi = Multinucleate
RI = Replication index

Highlighted concentrations selected for analysis.

[redacted]

Micronucleus analysis

Raw data

The raw data for the observations on the test article plus positive and vehicle controls are retained by Covance Laboratories Limited. A summary of the number of cells containing micronuclei is given in Appendix 1.

Validity of study

The data in Appendix 1, Appendix 2, Appendix 3 and Table 11 to Table 13 confirm that:

1. The binomial dispersion test demonstrated acceptable heterogeneity (in terms of MNBN cell frequency) between replicate cultures (Appendix 2).

2. The frequency of MNBN cells in vehicle controls fell within the normal ranges (Appendix 3).

3. The positive control chemicals induced statistically significant increases in the proportion of cells with micronuclei. Both replicate cultures at the positive control concentration analysed under each treatment condition demonstrated MNBN cell frequencies that clearly exceeded the current historical vehicle control ranges (Appendix 1).

4. A minimum of 50% of cells had gone through at least one cell division (as measured by binucleate + multinucleate cell counts) in vehicle control cultures at the time of harvest (Table 11 to Table 13).

Analysis of data

Treatment of cells with E212966 for 3+21 hours in the absence and presence of S-9 and for 24+0 hours in the absence of S-9 resulted in frequencies of MNBN cells that were similar to (and not significantly higher than) those observed in concurrent vehicle controls (Appendix 1 and Appendix 2) at all concentrations analysed under all three treatment conditions. The MNBN cell frequencies in all treated cultures fell within the normal ranges (Appendix 3).

[redacted]

CONCLUSION

It is concluded that E212966 did not induce micronuclei in cultured human peripheral blood lymphocytes when tested for 3+21 hours in the absence and presence of a rat liver metabolic activation system (S-9) and for 24+0 hours in the absence of S-9. Under all treatment conditions, treatments were performed up to the limit of toxicity.

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APPENDICES

Appendix 1
Binucleate cells with micronuclei

Table 14: E212966, 3+21 hour treatments in the absence of S-9
Micronucleus Experiment - female donors

Treatment (µg/mL)	Replicate	Total BN Cells Scored	Total MNBN Cells Scored	Frequency of MNBN Cells/ Cells Scored (%)	Significance § (% Toxicity)
Vehicle	A	1000	4	0.40	
	B	1000	8	0.80	
	Total	2000	12	0.60	-
100.0	A	1000	2	0.20	
	B	1000	2	0.20	
	Total	2000	4	0.20	NS (3)
200.0	A	1000	4	0.40	
	B	1000	6	0.60	
	Total	2000	10	0.50	NS (29)
250.0	A	1000	6	0.60	
	B	1000	4	0.40	
	Total	2000	10	0.50	NS (51)
MMC, 0.80	A	1000	75	**7.50 #**	
	B	1000	73	**7.30 #**	
	Total	2000	148	7.40	$p \leq 0.001$

MNBN = Micronucleated Binucleate
§ Statistical significance (Appendix 2)
NS = Not significant

= Numbers highlighted exceed historical vehicle control range (Appendix 3).

[redacted]

Table 15: E212966, 3+21 hour treatments in the presence of S-9
Micronucleus Experiment - female donors

Treatment (µg/mL)	Replicate	Total BN Cells Scored	Total MNBN Cells Scored	Frequency of MNBN Cells/ Cells Scored (%)	Significance § (% Toxicity)
Vehicle	A	1000	7	0.70	
	B	1000	3	0.30	
	Total	2000	10	0.50	-
100.0	A	1000	7	0.70	
	B	1000	2	0.20	
	Total	2000	9	0.45	NS (14)
200.0	A	1000	5	0.50	
	B	1000	1	0.10	
	Total	2000	6	0.30	NS (38)
250.0	A	1000	4	0.40	
	B	1000	6	0.60	
	Total	2000	10	0.50	NS (47)
300.0	A	1000	2	0.20	
	B	1000	4	0.40	
	Total	2000	6	0.30	NS (65)
CPA, 12.50	A	1000	22	**2.20 #**	
	B	1000	25	**2.50 #**	
	Total	2000	47	2.35	$p \leq 0.001$

MNBN = Micronucleated Binucleate
§ Statistical significance (Appendix 2)
NS = Not significant

= Numbers highlighted exceed historical vehicle control range (Appendix 3).

[redacted]

Table 16: E212966, 24+0 hour treatments in the absence of S-9 Micronucleus Experiment - female donors

Treatment (µg/mL)	Replicate	Total BN Cells Scored	Total MNBN Cells Scored	Frequency of MNBN Cells/ Cells Scored (%)	Significance § (% Toxicity)
Vehicle	A	1000	6	0.60	
	B	1000	4	0.40	
	Total	2000	10	0.50	-
20.00	A	1000	1	0.10	
	B	1000	4	0.40	
	Total	2000	5	0.25	NS (12)
35.00	A	1000	3	0.30	
	B	1000	2	0.20	
	Total	2000	5	0.25	NS (36)
45.00	A	1000	3	0.30	
	B	1000	3	0.30	
	Total	2000	6	0.30	NS (51)
VIN, 0.02	A	372	101	**27.15 #**	
	B	413	152	**36.80 #**	
	Total	785	253	32.23	$p \leq 0.001$

MNBN = Micronucleated Binucleate
§ Statistical significance (Appendix 2)
NS = Not significant

= Numbers highlighted exceed historical vehicle control range (Appendix 3).

[redacted]

Appendix 2
Statistical analysis of test article data

Table 17: E212966, 3+21 hour treatments in the absence of S-9
Micronucleus Experiment - female donors

Binomial Dispersion Test $\chi^2 = 2.15$ DF: 4
Significance: NS

Treatment (µg/mL)	Total BN Cells	BN Cells with micronuclei	Proportion	Fisher's exact test	Significance
Vehicle	2000	12	0.006	-	-
100.0	2000	4	0.002	0.976	NS
200.0	2000	10	0.005	0.662	NS
250.0	2000	10	0.005	0.662	NS
MMC, 0.80	2000	148	0.074	0.000	$p \leq 0.001$

Table 18: E212966, 3+21 hour treatments in the presence of S-9
Micronucleus Experiment - female donors

Binomial Dispersion Test $\chi^2 = 8.14$ DF: 5
Significance: NS

Treatment (µg/mL)	Total BN Cells	BN Cells with micronuclei	Proportion	Fisher's exact test	Significance
Vehicle	2000	10	0.005	-	-
100.0	2000	9	0.005	0.588	NS
200.0	2000	6	0.003	0.834	NS
250.0	2000	10	0.005	0.500	NS
300.0	2000	6	0.003	0.834	NS
CPA, 12.50	2000	47	0.024	0.000	$p \leq 0.001$

Table 19: E212966, 24+0 hour treatment in the absence of S-9
Micronucleus Experiment - female donors

Binomial Dispersion Test $\chi^2 = 2.41$ DF: 4
Significance: NS

Treatment (µg/mL)	Total BN Cells	BN Cells with micronuclei	Proportion	Fisher's exact test	Significance
Vehicle	2000	10	0.005	-	-
20.00	2000	5	0.003	0.895	NS
35.00	2000	5	0.003	0.895	NS
45.00	2000	6	0.003	0.834	NS
VIN, 0.02	785	253	0.322	0.000	$p \leq 0.001$

NS = Not significant DF = Degrees of freedom BN = Binucleate

[redacted]

Appendix 3
Historical vehicle control ranges for the human peripheral blood lymphocyte micronucleus assay

Table 20: Historical vehicle control range – Female donors

		Frequency of MNBN cells/cells scored (%)
		Female donors
3+21-S9	Number of studies	7
	Number of cultures	54
	Median	0.40
	Mean	0.45
	SD	0.366
	Observed range	**0.10 – 2.40**
	95% reference range	**0.10 – 1.00**
3+21+S9	Number of studies	7
	Number of cultures	67
	Median	0.40
	Mean	0.41
	SD	0.232
	Observed range	**0.00 – 1.20**
	95% reference range	**0.10 – 1.10**
24+0-S9	Number of studies	5
	Number of cultures	70
	Median	0.50
	Mean	0.55
	SD	0.347
	Observed range	**0.10 – 1.80**
	95% reference range	**0.10 – 1.40**

Reference ranges are calculated from percentiles of the observed distributions.

Calculated in January 2012 by CLEH Statistics, for studies started between March 2011 and September 2011.

FIANL REPORT

STUDY TITLE

28-DAY REPEATED DOSE TOXICITY STUDY OF E212966 IN WISTAR RATS BY DERMAL ROUTE

test material contains 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide / Hydroxyethylacrylate Copolymer

STUDY No.: [redacted]

STUDY DIRECTOR: [redacted]

STUDY COMPLETED ON: 28 June 2013

SPONSOR

[redacted]

TEST FACILITY

[redacted]

[redacted]

[redacted]

STATEMENT OF GLP COMPLIANCE

The Study No.: [redacted] was performed in accordance with the OECD Principles of Good Laboratory Practice [C (97) 186/Final] and in accordance with the Standard Operating Procedures and the mutually agreed Study Plan and Amendments. The Study Plan and Amendments were signed by Study Director and Monitoring Scientist on the following dates:

Details	Dates of signature	
	Study Director	Monitoring Scientist
Study Plan	30 November 2012	04 December 2012
Amendment No.1	24 January 2013	31 January 2013
Amendment No.2	23 February 2013	26 February 2013
Amendment No.3	14 May 2013	17 May 2013

The analytical determination of stability, homogeneity and concentration of the test solutions in the vehicle at experimental conditions were not performed for this study. This is an exception to GLP compliance and is the responsibility of the Sponsor.

DECLARATION

The Study Director hereby declares that the work was performed under his supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

The Study Director accepts overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation, reporting of the results and archiving of related data.

[redacted]

28 June 2013
Date:

QUALITY ASSURANCE STATEMENT

The Study No.: [redacted] entitled "28-Day Repeated Dose Toxicity Study of E212966 in Wistar Rats by Dermal Route" has been inspected in accordance with the Principles of Good Laboratory Practice OECD[C (97) 186/Final].

This study was inspected and findings reported to the Management and Study Director on the dates shown below:

INSPECTION DATE	STUDY PHASE	REPORTING DATE
	Initiation Phase	
29 November 2012	Study plan review	29 November 2012
17 January 2013	Review of Amendment No. 1 to study plan	17 January 2013
22 February 2013	Review of Amendment No. 2 to study plan	22 February 2013
10 May 2013	Review of Amendment No. 3 to study plan	10 May 2013
	In-Life Phase	
10 December 2012	Dose formulation preparation and administration (application) – Day 1	17 December 2012
24 December 2012	Detailed clinical examination – Day 15	31 December 2012
31 December 2012	Body weights, feed output and input measurement, bed and cage change – Week 4	07 January 2013
07 January 2013	Necropsy and analysis of blood samples for hematological parameters	15 January 2013
	Reporting Phase	
26 March 2013 to 27 March 2013	Draft report review (Pathology)	27 March 2013
27 March 2013 to 29 March 2013	Draft report review (Toxicology)	29 March 2013
16 May 2103 to 17 May 2013	Final Report Review	17 May 2013
26 June 2103	Final Report Review II	26 June 2013

Inspections were performed according to the Standard Operating Procedures of the test facility's Quality Assurance Unit. The report was inspected against the approved study plan and pertinent raw data and accurately reflects the raw data.

Date: 28 June, 2013.

[redacted]

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[redacted]

LIST OF ABBREVIATIONS AND SYMBOLS USED IN THE REPORT

%	Percent
µmol	Micromole
°C	Degree Celsius
µ	Microns
Bwt/bwt	Body weight
F/f	Female
fl	Femtolitre
G	Giga
g	Gram
GGT	Gamma Glutamyl Transpeptidase
K	Potassium
K_2EDTA	Ethylene diamine tetra acetic acid di potassium salt
kg	Kilogram
M/m	Male
MPCE	Micronucleated Polychromatic Erythrocytes
mEq	Milliequivalent
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
mmol	Millimole
NA	Not Applicable
NAD	No Abnormality Detected
No.	Number
PCE	Polychromatic Erythrocytes
pg	Picogram
Pi	Inorganic phosphorus
rpm	revolutions per minute
s	Seconds
SD	Standard Deviation
U	Units

1. STUDY DETAILS

Study Title	:	28-Day Repeated Dose Toxicity Study Of E212966 In Wistar Rats By Dermal Route
Test Item	:	E212966
Study No.	:	[redacted]
Sponsor	:	
Monitoring Scientist	:	
Sponsor's Representative	:	
Test Facility	:	

Study Schedule

Study Initiation		:	30 November 2012
Experimental Start		:	04 December 2012
Acclimatization	Start	:	05 December 2012
	End	:	09 December 2012
Treatment	Start	:	10 December 2012
	End	:	06 January 2013
Sacrifice		:	07 January 2013
Experimental Completion		:	08 March 2013
Submission of Draft Report		:	29 March 2013
Study Completion		:	28 June 2013

[redacted]

2. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name, Responsibility, Section / Department	Function	Signature with date
[redacted]		

3. SUMMARY

The purpose of this repeated dose toxicity study was to assess the systemic toxicity of the test item **E212966** in male and female Wistar rats after repeated dermal exposure for a period of 28 consecutive days. This study was designed to provide information on major systemic toxic effects, target organs, and an estimate of a No Observed Adverse Effect Level (NOAEL).

Methods:

A total of 20 male and 20 female rats were randomly allocated to four groups as described thereafter:

Group number	Dose (mg/kg/day)	Animal number	Dose volume (mL/kg)	Terminal sacrifice
G1	Vehicle*	5M + 5F	2	Day 29
G2	100	5M + 5F	2	Day 29
G3	300	5M + 5F	2	Day 29
G4	1000	5M + 5F	2	Day 29

* The vehicle was Milli-Q water
M : Males F: Females

Rats were observed for clinical signs, skin reactions (erythema and edema), morbidity, mortality, eye abnormalities, and changes in body weight and food consumption. Blood and urine samples were collected from all toxicity group rats at the end of the treatment period (on Day 29) for clinical pathology investigations. All rats were subjected to detailed necropsy at terminal sacrifice and specified organs were weighed. Histopathological examination was carried out on the preserved organs and tissues of the high dose and vehicle control groups and gross lesions from all rats in the study.

Results:

No test item related clinical signs, skin changes or mortalities/moribundity were observed during the course of the study at all the tested dose levels, except slight erythema in two females at 1000 mg/kg Bwt/day from Days 26 to 29.

No significant changes were observed in mean body weight, mean net weight gain, mean food consumption and in hematology, coagulation, clinical chemistry and urinalysis parameters, terminal fasting weights, organ weights and organ to body weight ratios of any treated groups, when compared to the vehicle control group.

No test item-related microscopic lesions were detected. Microscopic examination of the application site did not reveal any test item-related changes at any dose level.

Conclusion:

Repeated dermal administration of the test item **E212966** to male and female Wistar rats at dose levels of 100, 300 and 1000 mg/kg/day for 28 consecutive days did not induce any relevant adverse effects with respect to the application site, clinical signs, body weight and food consumption. No relevant adverse test item-related changes were observed in haematology, coagulation, clinical chemistry, urinalysis, terminal fasting body weights and anatomic pathology (organ weights, gross and histopathology) parameters.

Under the conditions of this study, the "No Observed Adverse Effect Level" (NOAEL) for **E212966** was defined at 1000 mg/kg Bwt/day in Wistar rats after repeated dermal application for a period of 28-days .

[redacted]

4. GUIDELINES

OECD Guideline for the Testing of Chemicals 410 "Repeated Dose Dermal Toxicity : 21/28-day Study" adopted on 12th May, 1981.

5. OBJECTIVE

The purpose of this repeated dose toxicity study was to assess the systemic toxicity of the test item E212966 in male and female Wistar rats after repeated dermal exposure for a period of 28 consecutive days. This study is designed to provide information on major systemic toxic effects, target organs, and an estimate of a No Observed Adverse Effect Level (NOAEL).

6. MATERIALS AND METHODS

6.1 Materials

6.1.1 Test Item Information

(as furnished by the Sponsor)

Test Substance/Item	:	E212966
Common Name	:	Not Applicable
Batch No. /Lot No.	:	[redacted]
Batch supplied by (Name and Address)	:	
Batch Manufactured by	:	
Manufactured (date)	:	Not Applicable
Retest Date	:	25/07/2015
Purity as per Certificate of Analysis	:	39.8%
Physical appearance	:	Clear, yellowish viscous liquid
Storage condition	:	Ambient (+18 to + 36°C)

Date of receipt of E212966 at test facility: 05.10.2012

Test Item Code by Test Facility: [redacted]

The identity of the test item was provided by the study Sponsor by a Certificate of Analysis (refer Appendix 9). The correct identity and purity are the responsibility of the Sponsor. The test item was not authenticated at the test facility.

6.1.2 Vehicle Information and Justification for Selection

Solubility of the test item was assessed using Milli Q-water.

The test item was freely soluble in Milli-Q- water at the dose volume of 2 mL/kg bw/day; hence Milli-Q-water was selected as the vehicle for test item formulation for dermal application.

6.2 Stability and Homogeneity of Dose Preparations

Due to the chemical nature of the test item (polymer), the stability, homogeneity and concentration of the Test Solutions in vehicle at the experimental conditions was not performed for this study and was considered as exception to GLP compliance.

Since no chemical analysis of the Test Solutions was carried out, particular care was implemented during the Test Solutions preparation process (calibration, chronology of steps, traceability of weights) in order to ensure the accurate concentration of the Test Solutions. A sample (1mL) of each concentration of the Test Solution used at the beginning and at the end of the treatment was kept and stored at -62°C to -86°C. The frozen test solution (prepared formulation samples) were discarded.

6.2.1 Test System

Species	:	Rats
Strain	:	Wistar Han rats
Source	:	[redacted]
Justification for the selection of species	:	Rat is one of the standard rodent species used for toxicity assessment and also recommended by various regulatory authorities for toxicity assessment.
Justification for route		The dermal route is chosen because it is the

[redacted]

of administration	:	route of exposure for humans.
No. of groups	:	4 Control Vehicle Group (G1) Low Dose Group (G2) Mid Dose Group (G3) High Dose Group (G4)
No. of rats/group	:	10 (5 males + 5 females) Total number of rats: 20 males + 20 females
Age at Treatment		11 - 12 weeks
Mean Body weight range at the start of the treatment	:	At the commencement of treatment, the weight variation of rats used did not exceed ± 20 % of the mean body weight in each sex and group.

	Males (g)	**Females (g)**
G1	339.38 ± 23.78	212.79 ± 9.86
G2	336.77 ± 20.23	218.17 ± 9.16
G3	341.93 ± 23.58	216.94 ± 10.05
G4	336.57 ± 23.88	216.87 ± 5.05

Identification	:	Temporary: All animals were identified using cage card and crystal violet solution. Permanent: All animals were identified using rat accession number, cage card and tail tattooing.
Acclimatization	:	After clinical examination for good health and suitability for the study, the rats were acclimatized for five days, before the start of the treatment. Only nulliparous and non-pregnant females were used in the study.

6.3 Methods

6.3.1 Husbandry

6.3.1.1 Environmental Conditions

Rats were housed under standard laboratory conditions. The temperature maintained during the experiment was between 21 to 24 °C and relative

humidity was between 65 to 68 percent. The photoperiod was 12 hours light and 12 hours dark cycle. Adequate range of fresh air supply (12 - 15 air changes/hour) was maintained in the experimental room.

The maximum and minimum temperature in the experimental room was recorded once daily. The relative humidity in the experimental room was calculated daily from dry and wet bulb temperature recordings.

6.3.1.2 Housing

Rats were housed individually in sterilized suspended polysulfone cages with solid floor (size: Length 425 x Breadth 266 x Height 175 mm) with stainless steel top grill having facilities for providing pelletted food and drinking water in polycarbonate bottle with stainless steel sipper tubes. During the experimental period, rats were housed in a single experimental room of Barrier Area (Room No.: SC-14).

6.3.1.3 Bedding

Steam sterilized clean corn cob was used as bedding and changed along with the cage once a week.

6.3.1.4 Diet and water

Teklad Certified (2014C) Global 14 % Protein Rodent Maintenance Diet - Pellet (Certified) manufactured by Harlan Laboratories B.V. Maasheseweg 87c PO Box 553, 5800, AN Venray, The Netherlands was provided *ad libitum* to the animals.

Deep bore-well water passed through activated charcoal filter and exposed to UV rays in 'Aquaguard' on-line water filter-cum-purifier manufactured by Eureka Forbes Ltd., Mumbai 400 001, India was provided *ad libitum* to rats in polycarbonate bottles with stainless steel sipper tubes.

The food and water provided to the animals were tested for contaminants. There were no known contaminants in the bedding/food/water at levels that would have interfered with the experimental results obtained. Analysis and contaminant analysis reports of bedding, food and water are included as Annexures 1 to 4.

6.3.2 Dose Levels and Dose Justification

Based on "7-Day Repeated Dose Toxicity Study of E212966 in Wistar Rats by Dermal Route" (Study No.: G8500), the following dose levels have been

selected for the current study along with a concurrent vehicle control group in consultation with the Sponsor:

- Low dose: 100 mg/kg body weight/day
- Mid dose: 300 mg/kg body weight/day
- High dose: 1000 mg/kg body weight/day

6.3.3 Randomization

Animals were randomly distributed to different groups by body weight stratification method using Provantis™ software. The grouping was done two days prior to initiation of treatment.

6.3.4 Experimental Design, Group Allocation and Number of Rats

The selected male and female rats were assigned to the vehicle and treatment groups as shown below:

Group No.	Group	Colour of cage card	Dose (mg/kg Bwt/day)	Dose Concen-tration (mg/mL)	No. of rats	Sex	Rat Numbers	
							From	To
G1	Vehicle control	White	0	0	5	M	Ro 4401	Ro 4405
					5	F	Ro 4406	Ro 4410
G2	Low dose	Yellow	100	50	5	M	Ro 4411	Ro 4415
					5	F	Ro 4416	Ro 4420
G3	Mid dose	Green	300	150	5	M	Ro 4421	Ro 4425
					5	F	Ro 4426	Ro 4430
G4	High dose	Pink	1000	500	5	M	Ro 4431	Ro 4435
					5	F	Ro 4436	Ro 4440

M: Male F: Female

6.4 Dose Formulation Preparation

Fresh Test solutions was prepared daily prior to application at the Test Facility and was administered within two hour after preparation.

The appropriate amount of Test Item was weighed in a dry labeled beaker. A small aliquot of vehicle (Milli-Q Water) was first added to the beaker and the mixture was stirred well with a glass rod. Further vehicle was added up to the desired final volume and concentration.

The above prepared test item doses was applied to all the treatment groups at an equivolume dose of 2 mL/kg.

The animals in the vehicle control group were treated with Milli-Q-water (vehicle) at the dose volume of 2 mL/kg.

The dose formulation preparation was done as below:

Group No. ►	**G2**	**G3**	**G4**
Dose (mg/kg bw/day)	100	300	1000
Dose volume (mL/kg)	2	2	2
Test item weight (mg)	500	1500	5000
Volume to be made up with Vehicle (mL)	10	10	10
Concentration (mg/mL)	50	150	500

The left over dose formulation sample were discarded as per the procedure mentioned in the SOP.

6.4.1 Preparation of Test Animals

Approximately 24 hours before test item application (first application), the hair on the dorsolateral thoracic region of the rats were clipped (approximately 8 X 10 cm square) using an electric clipper. During clipping, care was taken to avoid skin abrasions. Hair was clipped repeatedly once in 3 or 4 days on all the animals at the same time (16-18 hours prior to next application). Nails were clipped within 24 hours before the first application.

6.5 Area of Application

The area of application (approximately 3.5 X 5 cm^2) was represented approximately 10 % of the total body surface area or the maximum possible area that the test item could be applied.

6.6 Test Item Administration

The Test item was applied dermally on a daily basis to a clipped skin area representing about 10% of the total body surface area, under semi occlusive conditions for 6 hours.

Rats were administered dermally (at the volume of 2 mL/kg) to the clipped skin area 0 (vehicle), 100, 300 and 1000 mg/kg Bwt /day of the test item under semi-occlusive conditions for 6 hours. At the end of the 6 hour exposition period, the bandage was removed. The skin was then washed with water and dried with absorbent paper.

This administration was repeated at the same time (± 2 hours) for 28 days. The dose volume was adjusted to the most recent individual rat body weight.

The dosage form was applied using a syringe fitted with a canula and spread with a gentle massage. The application area was then covered with a cotton gauze (size: Males: 9 x 6 cm; Females 8 x 5 cm of 6 ply), and the patches were

held in place by a crepe elastic bandage (semi-occlusive dressing) and safety pins. The canula used for spreading was autoclaved daily before using.

7. OBSERVATIONS

7.1 General Clinical Signs and Mortality

Each rat was observed twice daily i.e., once in the morning and once in the afternoon for mortality and morbidity. Routine cage side observations for checking general clinical signs were performed once prior to application of the test item and once after washing. On the days of scheduled weekly detailed clinical examinations (as described in section 7.2), daily clinical signs were included as a part of the detailed clinical examinations.

The treated skin areas were examined twice daily (prior to application and approximately 30 minutes after washing) and skin reactions were assessed according to the numerical scoring system of Draize *et. al.* 1944.

1. ERYTHEMA AND ESCHAR FORMATION

	Score
No erythema……	0
Very slight erythema (barely perceptible)……	1
Well-defined erythema …….....	2
Moderate to severe erythema……	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)…….	4

Maximum Possible Score – 4

2. EDEMA FORMATION

	Score
No edema……...	0
Very slight edema (barely perceptible)……..	1
Slight edema (edges of area well defined by definite raising)……...	2
Moderate edema (raised approximately 1 millimetre)……...	3
Severe edema (raised more than 1 millimetre and extending beyond area of exposure)…….....	4

Maximum Possible Score – 4

7.2 Detailed Clinical Examinations

Detailed clinical examination was done prior to test item administration on Day 1 and once a week thereafter (-1 day) during the treatment period for all rats.

During detailed clinical examination, all rats were observed for changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern), changes in gait, posture and response to handling as well as the presence of clonic/tonic movements, stereotypies (e.g., excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards).

7.3 Ophthalmological Examination

Ophthalmological examination of all animals were performed with an ophthalmoscope (Direct ophthalmoscope; Make: WelchAllyn) by a Veterinarian prior to the start of treatment and at the end of the treatment period. Before examination, mydriasis was induced using a 1% solution of Tropicamide.

7.4 Body Weights

Individual body weights (g) were recorded prior to test item application for all animals on Day 1 and at weekly intervals (- 1 day) for all surviving animals. Also, body weight was measured on Day 29 following overnight fasting.

7.5 Food Intake

The food consumption (g) was measured at weekly intervals (- 1 day) for each animal. Food spillage was taken into consideration for calculation of food consumption.

7.6 Clinical Pathology Investigations

7.6.1 Blood Collection

At the end of the treatment period, all rats were fasted overnight (water allowed) and blood was collected by retro-orbital sinus puncture with a fine capillary tube under isoflurane anaesthesia. An aliquot of blood was collected in tubes containing 3.2 % sodium citrate solution for determination of coagulation parameters and the remaining blood was collected in K_2EDTA and heparinized tubes for haematology and clinical chemistry respectively.

After analysis, the left over coagulation analysis, haematology and clinical chemistry samples were discarded within two days of sampling after data review by the analyst.

7.6.2 Haematology

The following haematological parameters were determined using ADVIA 2120 haematology system (Bayer Health Care LLC, USA):

Serial No.	Parameter	Abbreviation	Units
1	Haematocrit	HCT	L/L
2	Haemoglobin	HGB	g/L
3	Mean Corpuscular Haemoglobin	MCH	pg
4	Mean Corpuscular Haemoglobin Concentration	MCHC	g/L
5	Mean Corpuscular Volume	MCV	fL
6	Mean Platelet Volume	MPV	fL
7	Platelets	Plat	G/L
8	Red Blood Corpuscles	RBC	T/L
9	Reticulocytes	Retic	T/L and %
10	White Blood Corpuscles	WBC	G/L
11	Differential leukocyte count (absolute)[1]	DLC	G/L

[1]: Differential Leukocyte parameters and their respective abbreviations are: Neutrophils (Neut), Lymphocytes (Lymph), Monocytes (Mono), Eosinophils (Eosi) and Basophils (Baso).

Additionally, blood smears were prepared from the hematology (K_2EDTA tube) samples. All smears were stained with Wright's stain (solution) and the morphological evaluation was not done by conventional microscopy. The smears were discarded.

7.6.3 Coagulation

Blood samples collected for coagulation analysis were centrifuged at 2500 gravity for 10 minutes for separation of plasma. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) analysis was carried out using STart-4 coagulation analyzer (Diagnostica stago, 92600 Asnieres, France).

7.6.4 Clinical Chemistry

Plasma was separated by centrifugation of the whole blood samples at 4ºC, 5000 rpm for 10 minutes and analyzed using Dimension RxL MaX Clinical Chemistry System (Dade Behring Inc. Newark, DE 19714, USA) for the following parameters:

Serial No.	Parameters	Abbreviations	Units	References
1	Alanine aminotransferase	ALT	U/L	1
2	Albumin	Alb	g/L	3
3	Abumin/Globulin ratio	A/G	-	5

Serial No.	Parameters	Abbreviations	Units	References
	(calculated values)			
4	Alkaline phosphatase	ALP	U/L	2
5	Aspartate aminotransferase	AST	U/L	6
6	Blood urea nitrogen	BUN	mmol/L	7
7	Calcium	Ca	mmol/L	19
8	Chloride	Cl	mEq/L	8
9	Creatine kinase	CK	U/L	
10	Creatinine	Creat	µmol/L	9
11	Gamma glutamyl transpeptidase	GGT	U/L	10
12	Globulin (calculated value)	Glb	g/L	4
13	Glucose	Glu	mmol/L	11
14	Inorganic phosphorous	Pi	mmol/L	12
15	Lactate dehydrogenase	LDH	U/L	
16	Potassium	K	mEq/L	13
17	Sodium	Na	mEq/L	14
18	Total bilirubin	T.Bil	µmol/L	17
19	Total cholesterol	T.Chol	mmol/L	15
20	Total plasma protein	T.Pro	g/L	16
21	Triglycerides	Trig	mmol/L	18
Note: Direct and indirect bilirubin were not determined as all the total bilirubin values were below 10 µmol/L				

7.6.5 Urinalysis

At the end of the treatment period, urine was collected from all surviving rats prior to sacrifice using urine collection tubes. For urine collection, each rat was placed in specially fabricated cages overnight (water allowed) and the next morning the collected urine was sent for analysis.

Urine samples were analyzed for the following parameters:

Serial No.	Parameters
1	Specific gravity[2]
2	Nitrite[3]
3	pH[3]
4	Proteins[3]
5	Glucose[3]
6	Ketone bodies[3]
7	Urobilinogen[3]
8	Bilirubin[3]
9	Creatinine[5]
10	Urea[5]

[redacted]

Serial No.	Parameters
11	Appearance (colour and clarity)[4]
12	Volume [4] (approximate)

[2]: Measured by refractometry
[3]: Analyzed with Clinitek status analyzer using Multistix 10 SG strips (Bayer Healthcare LLC, UK
[4]: Recorded manually
[5]: Analyzed using Roche/Hitachi 902 (Hitachi High Technologies Corporation, Tokyo, Japan

Urine was also be subjected to microscopic examination for sediments such as crystals, epithelial cells, erythrocytes, leukocytes and casts.

7.7 Anatomic Pathology

7.7.1 Necropsy

All rats were subjected to detailed necropsy by Veterinary Pathologist and findings were recorded. Rats (terminal sacrifice) were euthanized by exsanguination under isoflurane anesthesia. At terminal sacrifice, all surviving rats in the study were fasted overnight (water allowed) and weighed as per the random numbers generated before necropsy.

7.7.2 Tissue Collection and Organ Weights

On completion of the gross pathology examination the tissues and organs noted below were collected from all rats and preserved in the 10% Neutral Buffered Formalin except for the testes. The listed tissues were weighed from all rats. Organ to the body weight ratio (%) was calculated by using the fasting body weight (weight of animals on the day of necropsy i.e., on Day 29) and was presented in the report. The combined weight was taken for the paired organs.

Serial No.	Tissues/Organ	Organ weigh-ing	Collection and Preservation	Microscopic Examination
1	Adrenal glands	X	X	X
2	Aorta		X	X
3	Bone marrow smear[6]		X	X
4	Brain including medulla/pons, cerebellum and cerebrum	X	X	X
5	Cecum		X	X
6	Colon		X	X
7	Diaphragm		X	X
8	Duodenum		X	X
9	Epididymides	X	X	X
10	Esophagus		X	X

Serial No.	Tissues/Organ	Organ weigh-ing	Collection and Preservation	Microscopic Examination
11	Eyes with optic nerve[7]		X	X
12	Femoral muscles (Skeletal Muscle)		X	X
13	Femur Bone with joint[8]		X	X
14	Gross lesions (if any)		X	X
15	Heart	X	X	X
16	Ileum with peyer's patch		X	X
17	Jejunum		X	X
18	Kidneys	X	X	X
19	Liver	X	X	X
20	Lungs [9]	X	X	X
21	Mammary gland		X	X
22	Mandibular lymph nodes		X	X
23	Mesenteric lymph nodes		X	X
24	Ovaries	X	X	X
25	Pancreas		X	X
26	Pituitary[10]	X	X	X
27	Prostate[11]	X	X	X
28	Preputial gland		X	X
29	Rectum		X	X
30	Salivary glands		X	X
31	Sciatic nerves		X	X
32	Seminal vesicles and coagulating glands[11]	X	X	X
33	Skin (treated and untreated)		X	X
34	Spinal cord at 3 levels - Cervical, mid-thoracic and Lumbar		X	X
35	Spleen	X	X	X
36	Sternum with marrow[8]		X	X
37	Stomach		X	X
38	Thyroid and Parathyroid[10]	X	X	X
39	Testes[12]	X	X	X
40	Thymus	X	X	X
41	Trachea		X	X
42	Urinary bladder		X	X
43	Uterus with cervix	X	X	X
44	Vagina		X	X

X: Activity carried out.
[6]: Stained with Giemsa stain
[7]: Collected in Davidson's fluid.
[8]: Decalcified prior to sectioning
[9]: Inflated with 10%NBF before immersion in the fixative.
[10]: Weighed after formalin fixation

[redacted]

Serial No.	Tissues/Organ	Organ weigh-ing	Collection and Preservation	Microscopic Examination
[11]: The combined weight for prostate, seminal vesicles and coagulating glands were taken initially. The prostate was separated and weighed. The derived weight was presented for seminal vesicles and coagulating glands [12]: Collected in modified Davidson's fluid.				

7.7.3 Histopathology

The tissues mentioned in the table above were processed from all the animals of control group (G1) and the high dose groups (G4). Histopathological examination was carried out on the tissues from all the animals of vehicle control and high dose group. Gross lesions from all the animals were subjected to histopathological examination. Microscopic evaluated tissues in the high dose group has not showed any treatment related changes, hence histopathological evaluation was not done in the lower dose groups (G2 and G3).

The tissues were processed for routine paraffin embedding and 5 micron sections were stained with Mayer's Haematoxylin and Eosin stain. Representative tissues/organs from all rats will be archived.

8. STATISTICAL ANALYSES

The Data captured using Provantis™ for the parameters namely body weights, food consumption and organ weights were analyzed using built-in statistical tests. Derived data like net body weight change and organ weight ratios was also analyzed using above mentioned methods.

Data captured outside of Provantis™: The statistical analysis of the experimental data was carried out using the validated package in Excel and also using licensed copies of SYSTAT Statistical package Ver.12.0. All quantitative variables like laboratory investigations (haematology and clinical chemistry) was tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) within the group before performing a one-factor ANOVA modeling by treatment groups. Non-optimal (non-normal or heteroschedastic) data will be transformed, before ANOVA is performed. Comparison of means between treatment groups and vehicle control group was done using Dunnett's test when the overall treatment, 'F' test when found significant.

All analyses and comparisons were evaluated at the 5% ($P \leq 0.05$) level. Statistically significant differences ($P \leq 0.05$), indicated by the aforementioned tests were designated by the superscripts throughout the report as stated below:

+/-: Significantly higher / lower than the vehicle control group

9. ARCHIVING

Advinus will archive at the test facility the following for 10 years after completion of the study: study plan, raw data, draft and final reports. The data captured through Provantis™ Software will be archived in data base server and also raw data available in Provantis™ will be copied on to CD/DVD in PDF format (Readable without Provantis™ Software) and archived at archives of test facility. A representative sample of test item will be sent from the test item stores to the Archives in the test facility before the first dispensing of test item to studies. The sample shall be stored for a period of 2 years from the date of this final report or until the next GLP inspection, whichever is later, however not beyond 5 years. All tissue specimens, blocks and slides (if histopathology performed) will be archived for 10 years after which these will be handed over to the Sponsor or preserved longer at the cost of the Sponsor. Archived study materials cannot be discarded without any written authorization from the Sponsor.

10. REPORT DISTRIBUTION

Test Facility : One signed final report in original (Copy No. 1/2).

Sponsor : One signed final report in original (Copy No. 2/2) and an electronic copy in PDF format.

[redacted]

11. RESULTS AND DISCUSSION

Details of Experimental Design, Treatment Schedule, Clinical Pathology Investigations and Sacrifice Schedule are as provided in the Table 1.

11.1 In-Life Data

11.1.1 Clinical Signs, Skin changes and Mortality

Refer to: Tables 2 and 3, Appendices 1 and 2

No relevant clinical signs or mortality were observed in any of the treatment groups when compared to the control.

Skin reactions: A very slight erythema (Grade 1 - barely perceptible) was observed between Days 26 to 29 in two females (animals No Ro4436 and Ro4438) at 1000 mg/kg/day. These findings were considered as incidental as they were without histopathological correlate. No other skin reactions were observed in any other group.

11.1.2 Ophthalmological Examination

No ophthalmological changes were observed in any of the treatment groups when compared to the control.

11.1.3 Body Weights and Body Weight Gains

Refer to: Tables 4 and 5, Appendices 3 and 4

Mean body weight and mean body weight gains were not affected by the treatment.
A transient statistically significantincrease of net body weight gain during days 1-8 was observed at 300 mg/kg/day in females. This finding was considered incidental.

11.1.4 Food Intake

Refer to: Table 6, Appendix 5

The food intake remained unaffected by treatment at all dose levels.

11.2 Clinical Pathology Investigation

Refer to: Appendix 8

Summary: Repeated dermal administration of the test item **E212966** to male and female Wistar rats at the dose levels of 100, 300 and 1000 mg/kg/day for 28 consecutive days did not induce relevant adverse changes in haematology, coagulation, clinical chemistry, and urinalysis parameters.

11.3 Anatomic Pathology

Refer to: Appendix 8

Summary: Repeated dermal administration of the test item **E212966** to male and female Wistar rats at the dose levels of 100, 300 and 1000 mg/kg/day for 28 consecutive days did not induce relevant adverse changes in terminal fasting body weights and anatomic pathology (organ weights, gross and histopathology) parameters.

12. CONCLUSION

Repeated dermal administration of the test item E212966 to male and female Wistar rats at the dose levels of 100, 300 and 1000 mg/kg/day for 28 consecutive days did not cause relevant adverse changes with respect to the application site, clinical signs, body weight and food consumption. No relevant adverse test item-related changes were observed in haematology, coagulation, clinical chemistry, urinalysis, terminal fasting body weights and anatomic pathology (organ weights, gross and histopathology) parameters.

Under the conditions of this study, the “No Observed Adverse Effect Level” (NOAEL) for **E212966** was defined at 1000 mg/kg Bwt/day in Wistar rats after repeated dermal application for a period of 28-days .

13. REFERENCES

1. Alanine Amino Transferase (ALT) U/L:
   i. Greilling H, Gresssener AM, eds.Lehrbuch der Klinischen Chemie und Pathonichemie, 3rd ed. Stuttgart/ New York: Schattauer verlag; 1995.
   ii. Wroblewski F, LaDue JS. Ann Intern Med 1956; 45:801.

2. Alkaline phosphatase (ALP) U/L: Kinetic method: Bowers G N. Jr; Mc Comb RB: A continuous spectrophotometric method for measuring the activity of serum Alk phosphatase; clinchem . 12:70 1966.

3. Albumin (Alb) g/l:
   i. Grant GH, Silverman LM, Christenson RH. Amino acids and protein. In: Tietz NW. Fundamentals of clinical chemistry, 3rd edition Philadelphia, Pa: WB Saunders 1987:328-330.
   ii. Marshall WJ, ed. Illustrated textbook of clinical chemistry, 3rd edition London: Gower medical publishing, 1989:207-218.

4. Globulin (Glob) g/L:

Calculated from Albumin and Total Protein values by formula.

Globulin = Total Protein - Albumin

5. Albumin/Globulin ratio (A/G) (calculated):

Calculated from Albumin and Globulin values

6. Aspartate Amino Transferase (AST) U/L:
   i. Greilling H, Gresssener AM, eds.Lehrbuch der Klinischen Chemie und Pathonichemie, 3rd ed. Stuttgart/ New York: Schattauer verlag; 1995.
   ii. Schmidt FW. Ref Med Ges, Marburg/Lahn, December 1959.

7. Blood Urea Nitrogen (BUN) mmol/L:
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   ii. Marshall EK Jr. J Biol Chem. 1913;15:487.

8. Chloride (Cl) mmol/L: Tietz NW. Clinical guide to Laboratory Tests. Philadelpian Pa: WB Saunders Co; 1983 : 110, 398, 446. Kaplan L, Pesce

A.Clinical Chemistry theory, analysis and correlation. St. Louis, Mo:CV Mosby Co; 1984:1061, 1077.

9. Creatinine (Creat) µmol/L:
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    ii. Marshall WJ, ed. Illustrated textbook of clinical chemistry, 3rd edition London: Gower medical publishing, 1989:207-218.
10. Gamma Glutamyl Transpeptidase (GGT) U/L:
    i. Thomas L, ed. Labor and diagnose, 4th ed. Marburg: Die medizinische verlagsgesellschaft, 1992
    ii. Shaw LM. Keeping pace with a popular enzyme GGT. Diagnostic Medicine 1982; May/june:1-8.
11. Glucose (Glu) mmol/L:
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    ii. Thomas L, ed. Labor and diagnose, 4th ed. Marburg: Die medizinische verlagsgesellschaft, 1992.
12. Inorganic Phosphorus (Pi) mmol/L:
    i. Tietz NW. Fundamentals of clinical chemistry. Philadelphia…….PA: WB Saunders Co, 1976:901.
    ii. Fiske CH, Subbarow Y. the colorimetric determination of phosphorus. J Biol chem. 1925;66:375-400.
13. Potassium (K): mEq/L.

    Using selective electrode principle (indirect potentiometry).
14. Sodium (Na): mEq/L

    Using ion selective electrode principle (indirect potentiometry).

[redacted]

15. Total Cholesterol (T.Chol) mmol/L:
    i. Greilling H, Gresssener AM, eds.Lehrbuch der Klinischen Chemie und Pathonichemie, 3rd ed. Stuttgart/ New York: Schattauer verlag ;1995
    ii. Liebermann C. Ber Dtsch chem. Ges 1885;18:1803.

16. Total Plasma Protein (T.Pro.) g/l:
    i. Brobeck JR, ed. Physiological basis of medical practice, 9th ed. Baltimore, MD: Wilkins and Wilkins, 1973:4-7>
    ii. Weichselbaum TE. Amer J Clin path 1946;16:40.

17. Total Bilirubin (T. Bil) mmol/L:
    i. Ehrlich P. Charite Ann 1883; 8:140
    ii. Malloy HT, Evelyn KA. The determination of Bilirubin with the photoelectric colorimeter. J Biol chem. 1937; 119:481-490.

18. Triglycerides (Trig) mmol/L: GPO-PAP method: Schettler G and Nussel E., Arb. Med. Soz. med. Prav. med; 10(1975)25.

19. Calcium (Ca) mmol/L: Arsenazo III method: Michaylova V, Ilkova P; Photometric determination of microamounts of calcium with Arsenazo III; Anal Chem Acta., 53:194, 1971.

20. Draize, J. H., Woodard, G., and Calvery, H. O. (*1944*). Methods for the study of irritation and toxicity of substances applied to the skin and mucous membranes. *J. Pharmacol. Exp. Ther. 82, 377*–390.

21. OECD Principles of Good Laboratory Practice [C(97) 186/Final].

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23. Snedecor, G.W. and Cochran, W.G. (1987): Statistical Methods, 7th ed.(Reprint) Iowa State University Press, Ames, IA.

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[redacted]

FINAL REPORT

STUDY TITLE

PRENATAL DEVELOPMENTAL TOXICITY STUDY OF E212966 IN WISTAR RATS BY DERMAL ROUTE

TEST ITEM: E212966

STUDY No.: [redacted]

test material contains 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/ Hydroxyethylacrylate Copolymer

STUDY DIRECTOR: [redacted]

STUDY COMPLETED ON

23 JANUARY 2014

SPONSOR

[redacted]

TEST FACILITY

[redacted]

[redacted]

[redacted]

STATEMENT OF GLP COMPLIANCE

The Study No. [redacted] was performed in compliance with the OECD Principles of Good Laboratory Practice [C(97) 186/Final] and in accordance with the standard operating procedures and the mutually agreed study plan signed by Study Director on 12 December 2012 and by Monitoring Scientist on 17 December 2012.The study plan was amended once. Amendment No.1 to study plan was signed by Study Director on 10 January 2014 and by Monitoring Scientist on 22 January 2014.

Due to the chemical nature of the test item (polymer), the stability, homogeneity and concentration of the Test Solutions in the vehicle at experimental conditions were not performed for this study.This was considered as an exception to GLP compliance.

DECLARATION

The Study Director hereby declares that the work was performed under her supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

The Study Director accepts overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

[redacted]

23 January 2014
Date

[redacted]

QUALITY ASSURANCE STATEMENT

The Study No.: [redacted] entitled "Prenatal Developmental Toxicity Study of E212966 in Wistar Rats by Dermal Route" has been inspected in accordance with the OECD Principles of Good Laboratory Practice [C(97) 186/Final].

This study was inspected and findings reported to Test Facility Management and to the Study Director on the dates shown below:

INSPECTIONS DATE	PHASE	REPORTING DATE
	Initiation Phase	
06 December 2012	Study plan review	06 December 2012
	In-life Phase	
24 December 2012	Body weights,feed input and output measurement and dose formulation preparation and application	31 December 2012
02 January 2013	Observation for general clinical signs and scoring of treated skin area	03 January 2013
08 January 2013	Cesarean section	15 January 2013
	Reporting Phase	
24 September 2013 to 17 October 2013	Draft report review	17 October 2013
22 January 2014	Final report review	22 January 2014

Inspections were performed according to the Standard Operating Procedures of the test facility's Quality Assurance Unit. The report was inspected against the approved study plan and pertinent raw data and accurately reflects the raw data.

Date: 23 JANUARY, 2014.

[redacted]

[redacted]

LIST OF ABBREVIATIONS AND SYMBOLS USED IN THE REPORT

Acc.	Accessory
Ann.	Annexure
ANOVA	Analysis of Variance
App.	Appendix/ Appendices
Antr.	Anterior
Asy	Asymmetrical
B	Both
Bwt	Body weight
CdV	Caudal vertebra
Contd.	Continued
CV	Cervical vertebra
Cm	Centimeter
DB	Dumble bell
Diln	Dilation/dilatation
DSO	Delayed skeletal ossification
Dt. Phal.	Distal phalange
Exam.	Examined
F	Female
F/H Limb	Fore/Hind Limb
Fet.	Fetuses
Fwt	Food weight
G/Gr	Group
g	gram
GD	Gestation day
Hypo.	Hypoplastic
INO/PO	Incomplete/Poor ossification
KOH	Potassium Hydroxide
L	litre
Lt.	Left
LV	Lumbar vertebra
M	Male
Malform.	Malformation
Mean ± SD	Mean ± Standard Deviation
Metacarp.	Metacarpals
Metatar.	Metatarsals
Md. phal	Middle phalange
mg/kg	milligram/kilogram
mm	millimeter
mL	millilitre
NAD	No abnormality detected
No./#	Number
NP	Non pregnant
NOEL	No observed effect level
NOAEL	No observed adverse effect level
Ossi.	Ossification
OECD	Organization for Economic Co-operation and Development
P	Pregnant
Pa	Parietal
Pr. Phal	Proximal phalange
$p \leq$	Probability (less than or equal to)
Rt.	Right
Sl.	Serial
Stern	Sternebra
Supraocci.	Supraoccipital
SV	Sacral vertebra
TV	Thoracic vertebra
Wt.	Weight
%	Per cent
+	Slight
++	Moderate

[redacted]

TABLE OF CONTENTS

[redacted]

[redacted]

1. STUDY DETAILS

Monitoring Scientist : [redacted]

Study Schedule

Study Initiation Date	:	12 December 2012
Experiment Start Date	:	12 December 2012
Acclimatization	:	13 December 2012 to 17 December 2012
Mating	:	18 December 2012 to 21 December 2012
Treatment	:	24 December 2012 to 10 January 2013
Sacrifice	:	08 January 2013 to 11 January 2013
Experiment Completion Date (in life phase)	:	11 January 2013
Experiment Completion Date	:	21 May 2013
Draft Report	:	30 May 2013
Study Completion Date	:	23 January 2014

2. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name, Responsibility, Section / Department	Function	Signature with date
[redacted]		

3. SUMMARY

The objective of this study was to determine the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of the test item **E212966** after dermal administration to pregnant Wistar rats during the gestation period (Gestation Days 5 to 19).

Ninety six presumed pregnant female rats were assigned to four groups by body weight stratification as described thereafter:

Group No.	Dose (mg/kg/day)	Number of Animals	Dose volume (mL/kg)	Terminal sacrifice
G1	Vehicle*	24F	2	GD 20
G2	100	24F	2	GD 20
G3	300	24F	2	GD 20
G4	1000	24F	2	GD 20

* Vehicle was Milli-Q water F: Female GD: gestation day

The test item **E212966** was administered dermally to the clipped skin area under semi-occlusive conditions for at least 6 hours. At the end of the 6 hour exposure period, the bandage was removed. The skin was washed with water and dried with absorbent paper. Treatment period was from gestation day (GD) 5 to 19.

Dams were observed for clinical signs, local skin reactions, mortality, body weight and net body weight gain and food consumption. The cesarean section was performed on all rats on GD 20 and uterine contents were assessed. Fetuses were examined for external, and visceral or skeletal alterations.

Results

- There was no mortality at any of the doses tested.
- No clinical signs and local skin reactions were observed at any of the doses tested.
- Mean maternal body weight, maternal body weight gain and food consumption were unaffected by the treatment with the test item E212966 up to 1000 mg/kg/day.
- The maternal and litter data parameters were unaffected by the treatment with the test item E212966 up to 1000 mg/kg/day.
- Following external, visceral and skeletal examination, there were no developmental effects attributed to treatment with E212966 up to 1000 mg/kg/day.

Conclusion

Daily dermal administration of the test item **E212966** to pregnant Wistar rats at the dose levels of 100, 300 and 1000 mg/kg/day during the gestation period (GD 5 to 19) did not result in any adverse test item-related findings.

The No Observed Adverse Effect Level (NOAEL) for both maternal and fetal toxicity of the test item **E212966** was defined at 1000 mg/kg/day.

Thus, the results of this study indicate that the test item **E212966** is not a developmental toxicant after daily dermal administration of up to 1000 mg/kg/day.

4. OBJECTIVES

The objective of this study was to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of the test item E212966 after dermal administration to pregnant Wistar rats from Gestation Days (GD) 5 to 19. This study provides a rational basis for risk assessment in humans and an estimate of a No Observed Adverse Effect Level (NOAEL)/ No Observed Effect Level (NOEL).

5. GUIDELINE

This study was conducted in compliance with the following guideline:

- OECD Guideline for the Testing of Chemicals, No. 414, "Prenatal developmental toxicity study", adopted on January 22, 2001.

6. MATERIALS AND METHODS

6.1 Materials

6.1.1 Test Item Information

(as provided by the Sponsor)

Test Item	:	E212966
Common Name	:	Not Applicable
Lot No.(as per COA)	:	[redacted]
Batch supplied by (Name and Address)	:	
Batch Manufactured by	:	
Manufactured (date)	:	Not Applicable
Retest Date	:	25/07/2015
Purity as per Certificate of Analysis	:	39.8%

[redacted]

Physical appearance : Clear, yellowish viscous liquid

Storage condition : Ambient (+18 to + 36°C)

Note: Test item code by test facility: [redacted]
Date of receipt at test facility: 05 October 2012

The identity of the test item was provided by the study Sponsor via an Analytical Certificate and is included as Appendix 1 in this study report. The correct identity and purity are within the responsibility of the Sponsor. The test item was not authenticated at the test facility.

6.1.2 Vehicle Control and Justification for the Selection of Vehicle

Based on the solubility and suspensibility test carried out at Advinus Therapeutics Limited under Study [redacted] the test item E212966 was freely soluble in Milli-Q-water at the dose volume of 2 mL/kg. Hence, Milli-Q-water was selected as vehicle for the preparation of the dose formulations for the dermal application.

6.1.3 Chemicals

The details of chemicals used are as follows:

Name	Batch No.	Manufactured by
Potassium ferricyanide	J068B10	RFCL Limited, New Delhi 110020
Ammonium sulphide solution	1368	Rolex Chemical Industries Mumbai 400002
Thymol crystals	K10Z/1810/0211/08	s.d. fine chemicals limited, Mumbai 400030
Hydrochloric acid	F09A/0709/1505/13	
Potassium hydroxide pellets	H11Z/1311/1303/08	
Alizarin Red S stain	1283485	Fluka (Sigma Aldrich Chemie GmbH)
Glycerol	S020F12	Ranbaxy Fine chemicals Limited, New Delhi 110020.
Alcohol	-	My Sugar Company Limited, Mandya

6.1.4 Test System

Species	:	Rat
Strain	:	Wistar rats - HsdHan: WIST rats Conventionally bred (In-house random bred) young adult males and virgin females
Source	:	[redacted]
Justification for selection of species	:	Rat is a standard laboratory rodent species used for prenatal developmental toxicity assessment and also recommended by various regulatory authorities for toxicity assessment. The Wistar rat was selected due to the large amount of background knowledge accumulated for this strain.
No. of groups	:	4 G1 - Vehicle control: 0 mg/kg/day G2 - Low dose: 100 mg/kg/day G3 - Mid dose: 300 mg/kg/day G4 - High dose: 1000 mg/kg/day
No. of mated females / group	:	24 Total = 96 females
Date of Birth	:	27 September 2012 to 30 September 2012
Age at the start of treatment	:	12 - 13 weeks
Body weight of pregnant rats on "Day 0" (g)	:	G1 : 229.09 ± 20.31 G2 : 228.42 ± 19.73 G3 : 229.87 ± 18.19 G4 : 229.70 ± 19.15

Identification	:	Before mating, the rats were allotted by temporary identification numbers and were identified by crystal violet colour body marking and after mating, the rats were identified by the last three digits of the permanent accession number written on the tail. The permanent accession number was included on the cage cards.
Acclimatization	:	After physical examination, for good health and suitability for the study, rats were acclimatized for five days before the initiation of mating. Only nulliparous and non-pregnant females were acclimatized.

6.1.5 Route of Administration and Justification of Choice

The dermal route was chosen because it is the route of exposure in humans.

6.2 Methods

6.2.1 Husbandry

6.2.1.1 Environmental Conditions

During the experiment, animals were housed in Room No. SC-43

Rats were housed under standard laboratory conditions, air conditioned with adequate fresh air supply (12-15 air changes/hour). Environment: with temperature 20 to 22 °C, relative humidity 65 – 66 %, with 12 hours light and 12 hours dark cycle.

The maximum and minimum temperature in the experimental room was recorded once daily. The relative humidity in the experimental room was calculated daily from dry and wet bulb temperature recordings.

6.2.1.2 Housing

Rats were housed in standard polysulfone rat cages (size: Length 425 mm x Breadth 266 mm x Height 175 mm) with stainless steel top grill having facilities for pellet food and drinking water in polycarbonate bottle with stainless steel sipper tube.

i. Pre mating / Acclimatization: Two rats of the same sex per cage were housed.

[redacted]

ii. Mating: Female rats were cohabited with males in a 1:1 ratio in the same cage.
iii. Post-mating / Treatment: After mating confirmation, females were housed individually.

NOTE: The males were euthanized after the mating procedure.

6.2.1.3 Bedding

Steam sterilized corn cob was used as bedding and was changed along with the cage at least twice a week.

Contaminant analysis report of bedding, (most recent available at test facility) is included in the report as Annexure 1.

6.2.1.4 Diet and Water

Teklad Certified (2014C) Global 14 % Protein Rodent Maintenance Diet - Pellet (Certified) manufactured by Harlan Laboratories B.V. Maasheseweg 87c PO Box 553, 5800, AN Venray, The Netherlands, was provided *ad libitum* to the animals.

Deep bore-well water passed through activated charcoal filter and exposed to UV rays in 'Aquaguard' on-line water filter-cum-purifier manufactured by Eureka Forbes Ltd., Mumbai 400 001, India was provided *ad libitum* to rats in polycarbonate bottles with stainless steel sipper tubes.

The food and water provided to the animals were tested for contaminants.There were no known contaminants in the food or water at levels that would have interfered with the experimental results obtained

Analysis and contaminant analysis reports of food and water (close to the experimental period and /or most recent available at test facility) are included as Annexures 2 to 4.

6.2.2 Selection of Dose Levels and Dose Justification

Based on a [redacted] the following dose levels were selected for the current study in consultation with the sponsor:

- Low dose: 100 mg/kg/day
- Mid dose: 300 mg/kg/day
- High dose: 1000 mg/kg/day

A concurrent vehicle control group (G1: 0 mg/kg/ day) was included.

[redacted]

6.2.3 Allocation to Groups

During the mating period, female rats were cohabited with males in a 1:1 ratio. The day of detection of sperm positive vaginal smear/vaginal plug, was considered as Day 0 of gestation. The mated female rats obtained each day were assigned to the treatment groups and vehicle control groups by body weight stratification. This procedure was continued till the required numbers of Day 0 pregnant rats (mated females) were obtained (24 per group).

The selected female rats were assigned to vehicle control and treatment groups as shown below:

Group Nos.	Groups	Colour of cage card	Dose (mg/kg /day)	Dosage volume (mL/kg)	Concentration (mg/mL)	No. of Day 0 mated rats	Rat numbers	
							From	To
G1	Vehicle control	White	0	2	0	24	Rj7411	Rj7434
G2	Low dose	Yellow	100	2	50	24	Rj7441	Rj7464
G3	Mid dose	Green	300	2	150	24	Rj7471	Rj7494
G4	High dose	Pink	1000	2	500	24	Rj7501	Rj7524

6.3 Stability and Homogeneity of Dose Preparations

Due to the chemical nature of the test item (polymer), the stability, homogeneity and concentration of the Test Solutions in the vehicle in experimental conditions was not performed for this study and was considered as an exception to GLP compliance.

Since no chemical analysis of the Test Solutions was carried out, particular care was implemented during the Test Solutions preparation process (calibration, chronology of steps, traceability of weights) in order to ensure the accurate concentration of the Test Solutions. A sample (1mL) of each concentration of the Test Solution used at the beginning of the treatment and at the end of the treatment was kept and stored at -62°C to -86°C. The frozen test solution (prepared formulation samples) were discarded before final report submission.

6.3.1 Dose formulation Preparation

Fresh Test solutions were prepared daily prior to application and were applied within 2 hours after preparation.

The appropriate amount of Test Item was weighed in a dry labeled beaker. A small aliquot of vehicle (Milli-Q Water) was first added to the beaker and the

mixture was stirred with a glass rod. The resultant solution was transferred to a measuring cylinder and further vehicle was added up to the desired final volume to get the required concentration.

The above prepared test item solutions was applied to all the treatment groups at an equivolume dose of 2 mL/kg.

The animals in the vehicle control group were treated with Milli-Q-water (vehicle) at the dose volume of 2 mL/kg.

An example of dose formulation preparation for 30 mL was as below:

Group No. ►	**G2**	**G3**	**G4**
Dose (mg/kg/day)	100	300	1000
Dose volume (mL/kg)	2	2	2
Test item weight (mg)	1002.6	3002.6	10003.1
Volume to be made up with Vehicle (mL)	20	20	20
Concentration (mg/mL)	50	150	500

The weight of the test item and volume prepared varied depending on the requirement. The left over dose formulation samples were discarded.

6.3.2 Preparation of Test Animals

Approximately 24 hours before test item application (first application), the hair on the dorsolateral thoracic region of the rats was clipped (approximately 6-8 X 10-12 cm) using an electric clipper. During clipping, care was taken to avoid skin abrasions. Hair was clipped repeatedly once in 3 or 4 days as per the requirement (16-18 hours prior to next application).

Nails were clipped 24 hours before the first application.

6.4 Area of Application

The area of application (approximately 3.5 X 5 cm^2) was representing approximately 10 % of the total body surface area that the test item was applied.

6.5 Test Item Application

The Test item was applied dermally to rats on a daily basis to clipped skin area representing about 10 % of the total body surface area, under semi occlusive conditions for 6 hours.

The dermal application of test item was done (at the volume of 2 mL/kg) to the clipped skin area of rats at 0 (vehicle), 100, 300 and 1000 mg/kg/day under semi-occlusive conditions and the contact period was for at least 6 hours. At the end of the 6 hour exposure period, the bandage was removed. The skin was then washed with water and dried with absorbent paper.

This application was approximately at the same time each day (varying by ± 2 hours) to rats of specific groups once daily from GD 5 to GD 19 of presumed gestation. The dose volume was adjusted to the most recent individual rat body weight.

The dosage form was applied using a syringe fitted with a canula and spread with a gentle massage. The application area was then covered with a cotton gauze (size: 8 x 5 cm of 6 ply), and the patches were held in place by a crepe elastic bandage (semi-occlusive dressing) and safety pins. The canula used for spreading was autoclaved daily before using.

6.6 Observations

6.6.1 Physical / Veterinary Examination

The rats were subjected to physical examination after mating i.e., on Day '0', of gestation and at weekly intervals during the presumed gestation period and findings were recorded.

6.6.2 General Clinical Signs, Mortality and Morbidity

Each rat was observed at least twice daily i.e., once in the morning and once in the afternoon for mortality and morbidity. Routine cage side observations for checking general clinical signs were performed once prior to application of the test item and once after washing. Based on the assessment as there were no toxic signs of concern the observations for mortality and morbidity were carried out once during weekends and public holidays.

The treated skin areas were examined twice daily (prior to application and approximately 30 minutes after washing). From 24 December 2012 to 01 January 2013, the actual time of observation of treated skin areas was not recorded in raw data.The skin reactions were assessed according to the numerical scoring system of Draize et. al. 1944.

1. ERYTHEMA AND ESCHAR FORMATION

No erythema…….	0
Very slight erythema (barely perceptible)……	1
Well-defined erythema …….....	2

[redacted]

Moderate to severe erythema……	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)…….	4

Maximum Possible Score – 4

2. EDEMA FORMATION

No edema……...	0
Very slight edema (barely perceptible)……..	1
Slight edema (edges of area well defined by definite raising)……....	2
Moderate edema (raised approximately 1 millimetre)……...	3
Severe edema (raised more than 1 millimetre and extending beyond area of exposure)…….....	4

Maximum Possible Score – 4

6.6.3 Body Weight

All females included in the study were weighed on gestation days 0, 3, 5, 8, 11, 14, 17 and 20.

From this, intermittent body weight gain i.e., 0 - 3, 3 - 5, 5 - 8, 8 - 11, 11 - 14, 14 - 17 and 17 - 20 was calculated. Further the body weight gain for pre-treatment period (0 - 5), treatment period (5 - 20) and for throughout gestation period (0 - 20) was derived.

The corrected body weight gain was calculated by subtracting carcass weight from body weight on Day 5 of gestation. The carcass weight was obtained by subtracting terminal body weight (body weight on Day 20 of gestation) from unopened uterine weight.

6.6.4 Food Intake

A known weight of food (food input) was provided on Day 0. The food output was recorded and replenished to a known weight on Days 3, 5, 8, 11, 14 and 17 and food output on Day 20 of presumed gestation.

Food intake is expressed as grams of food consumed daily per rat. Intermittent food intake i.e., 0 - 3, 3 - 5, 5 - 8, 8 - 11, 11 - 14, 14 - 17 and 17 - 20 was calculated. Further food intake for pre-treatment (0 - 5), treatment (5 - 20) and for throughout gestation period (0 - 20) was derived.

6.7 Necropsy

On gestation Day 20, all the rats were sacrificed (Cesarean section) under isoflurane anaesthesia and gross pathological changes in the visceral organs of the dams were recorded.

Prior to caesarean section, random numbers were generated for coding to avoid bias during caesarean section and subsequent fetal evaluation. The animal code was written on the tail by striking out the permanent accession number.

6.8 Dams Examination

Gross pathological changes, which involved an external observation and examination of thoracic and abdomen viscera and an examination of uterine contents, was performed on all animals sacrificed at term.

Cesarean section: The gravid uterus along with the ovaries were excised, weighed and immediately examined.

The following maternal data was recorded.
a. Pregnancy status
b. Gravid uterine weight
c. No. of corpora lutea
d. No. of implantation sites
e. No. of early resorptions
f. No. of late resorptions

Uteri that appeared non-gravid were subjected to ammonium sulphide staining to confirm the non-pregnant status.

Pre-implantation and post-implantation loss rates were calculated for each pregnant animal (see Appendix 16).

6.9 Fetal Examination

On Day 20, the individual fetuses were delivered by hysterectomy and removed sequentially. All the live fetuses were identified by serial numbers. The fetuses were euthanised using isoflurane anaesthesia.

The following litter data were recorded:
a. Total number of fetuses
b. No. of viable fetuses
c. Individual fetal body weight (g)
d. Fetus sex (external determination based on anogenital distance)

[redacted]

e. Sex Ratio

All the fetuses were examined for external malformations and the observations were classified as normal variants, minor anomalies and major malformations as per Appendix 13.

Fetus fixation: Approximately half the number of fetuses from each litter was preserved in 70 % alcohol for visceral organ evaluation and the remaining half was skinned and preserved in 70 % alcohol for skeletal evaluation in individual bottles. The bottles were labeled with study number, code number and fetal number among total number of fetuses.

Fetal Visceral Evaluation: Approximately half the number of fetuses from each dam was prepared for visceral organ evaluation by modified Wilson's Razor Blade Sectioning Technique and assessed as per Appendix 14.

a) Head: Using scalpel blade the head was sectioned at:

- the nasal region - to reveal nasal cavities
- the region of the eye - to observe gross abnormalities of the eye and fore part of the brain
- across the central part of the head and brain for mid brain, lateral and third ventricles
- across the ears - to observe the cerebellum

b) Trunk: Thorax and abdomen was opened to expose the organs in the thoracic and abdominal regions without disturbing their position. After the evaluation, tissues of each fetus were preserved dam-wise in bottles containing 10 % buffered neutral formalin. The bottles were labeled with study number, code number and the number of fetuses among the total number of fetuses.

Fetal Skeletal Evaluation: The remaining half of the fetuses of a litter was prepared for skeletal evaluation by wet skinning followed by evisceration and staining. Fetuses were fixed in 70 % alcohol, eviscerated and dehydrated in 95 % alcohol; macerated in 1.5 % KOH and stained with saturated, aqueous Alizarin red S in Mall's solution. The excess stain was removed in Mall's solution and the fetuses were cleared by passing through grades of glycerol with thymol crystals and assessed as per Appendix 15.

The fetuses were pooled dam-wise in bottles containing 100 % glycerol with crystals of thymol to prevent fungal growth. The bottles were labeled with study number, code number and number of fetuses among total number of fetuses.

[redacted]

The indices for the derivation of maternal data and litter data are enclosed as Appendix 16. All findings recorded were presented in the report as per the standard reporting format.

The results of the study were discussed taking into consideration the historical data of this lab.

7. STATISTICAL ANALYSES

The following statistical tests were used.

The data on maternal body weight, body weight change, maternal food consumption, gravid uterus weight, number of corpora lutea, number of implantations, litter size, litter weight, male and female fetus number and fetal body weight were analyzed using ANOVA model, after testing for homogeneity for intra group variance using Levene's test. When the intra group variances were heterogeneous, ANOVA was performed after suitable transformation of data. Dunnett's pairwise comparison of the treated group means with the control group mean was performed if the group differences are found significant.

Incidence of pre-implantation loss, post implantation loss, Number of early, late resorptions was analyzed using Kruskal Wallis.

Overall percentage of minor external, visceral and skeletal malformations, Sex ratio and number of dams with any resorptions were analyzed using 2 X 2 Contingency Table.

Statistically significant differences ($p \leq 0.05$), indicated by the aforementioned tests are designated by the superscripts throughout the report as stated below:

+/-: Significantly higher (+)/lower (-) than the vehicle control group

8. ARCHIVING

Advinus will archive at the Archives of the test facility the following for 10 years after completion of the study: Study Plan, raw data, draft and final reports. A sample of the test item has been sent from the test item stores to the Archives before the first dispensing of test item. This sample shall be stored for a period of 2 years from the date of this final report. Specimens fixed in alcohol or formalin such as ovaries, resorptions, fetuses subjected to visceral organ evaluation will be preserved for 10 years and fetuses subjected to skeletal evaluation (preserved in glycerol) will be archived for 10 years

after which they will be handed over to the Sponsor or retained longer in consultation with of the Sponsor. Archived study materials will not be discarded without written authorization from the Sponsor.

9. REPORT DISTRIBUTION

The final report (original copies) will be distributed as follows:

Test Facility: One signed final report in original (Copy No. 1/2)
Sponsor: One signed final report in original (Copy No. 2/2) and an electronic copy of the signed final report in PDF format.

10. RESULTS AND DISCUSSION

10.1 In Life Data

10.1.1 Details of the Experimental Design, Treatment Schedule, Maternal and Litter Data

The details are presented in Table 1.

10.1.2 Clinical Signs, Local Skin Reation and Mortality

Refer to: Table 2**,** Appendix 2

No mortality occurred and no relevant clinical signs and local skin reactions were observed at any of the doses tested compared to the control group.

10.1.3 Maternal Body Weight and Weight Gain

Refer to: Tables 3A, 3B**,** 3C, Appendix 3

No relevant test item-related effect was observed on body weight and body weight gain of treated dams when compared to the control group.

Mean body weight gain was statistically significantly lower during GD 5-8 at doses ≥ 300 mg/kg/day and during GD 8-11 at 1000 mg/kg/day, when compared to the control group. However, these changes were not considered toxicologically relevant becauser they were only transient and since there was no clear dose-response.

10.1.4 Food Intake

Refer to: Table 4**,** Appendix 4

No relevant test item-related effect was observed on food consumption in treated dams when compared to the control group.

The statistically significant lower food consumption at 1000 mg/kg/day between GD 8-11 was not considered toxicologically relevant because it was only transient.

10.2 Maternal Data

Refer to: Table 5, Appendix 5

No relevant test item-related effect was observed on maternal parameters such as mean gravid uterine weights, mean number of corpora lutea, implantations, early and late resorptions, pre and post-implantation loss rates when compared to the control group.

10.3 Litter Data

Refer to: Table 6, Appendix 6

No relevant test item-related effect was observed on litter parameters such as total number of fetuses, mean litter size, number of live fetuses, mean fetus weights and sex ratio when compared to the control group.

10.4 Gross Pathological (Necropsy) Findings of Dams

Refer to: Table 7, Appendix 7

Necropsy revealed no gross findings in the control and treatment groups.

10.5 External Observations

Refer to: Table 8, Appendix 8

Minor Anomalies

No relevant test item-related minor anomalies were observed during the external examination of the fetuses of the treated groups when compared to the control group.

Major Malformations

No relevant test item-related major malformations were observed during the external examination of the fetuses of the treated groups when compared to the control group.

10.6 Visceral Observations

Refer to: Table 9, Appendix 9

Minor Anomalies

No relevant test item-related minor anomalies were observed during the visceral examination of the fetuses of the treated groups when compared to the control group. The slightly increased incidence in both fused lung lobes at 300 mg/kg/day and moderate dilation of kidney renal pelvis at 1000 mg/kg/day was considered not test item-related due to the low incidence (0.67 % and 0.68 % respectively) and due to the lack of concomitant findings.

Major Malformations

No relevant test item-related major visceral malformations were observed during the visceral examination of the fetuses of the treated groups when compared to the control group.

10.7 Skeletal Observations

Refer to: Table 10, Appendix 10

Minor Anomalies

No relevant test item-related minor visceral anomalies were observed during the visceral examination of the fetuses of the treated groups when compared to the control group. The few statistically significant changes were not considered test item-related either due to lack of dose-response and/or being within the range of the historical control data.

Major Malformations

There were no major skeletal malformations in the control and the treatment groups.

11. CONCLUSION

Daily dermal administration of the test item **E212966** to pregnant Wistar rats at the dose levels of 100, 300 and 1000 mg/kg/day during the gestation period (GD 5 to 19) did not result in any adverse test item-related findings. The No Observed Adverse Effect Level (NOAEL) for both maternal and fetal toxicity of the test item **E212966** was defined at 1000 mg/kg/day.

Thus, the results of this study indicate that the test item **E212966** is not a developmental toxicant after daily dermal administration of up to 1000 mg/kg/day.

12. REFERENCES

1. The OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C(97) 186/Final] Legislation.
2. OECD Guideline for the Testing of Chemicals, No. 414, "Prenatal developmental toxicity study", adopted on January 22, 2001
3. Snedecor, G.W. and Cochran, W.G. (1987): Statistical Methods, 7th ed.(Reprint) Iowa State University Press, Ames, IA.
4. Rochelle W.T. and Melissa C.M. (2006). Developmental Toxicity Testing -Methodology, Developmental and Reproductive Toxicology (2nd Edition), Eds., Ronald D.H. p. 201-256

[redacted]

[redacted]

[redacted]

Study Title

Evaluation of skin sensitization potential in the mouse of **E212966** using the Local Lymph Node Assay (LLNA)

Methodology Reference

Organization for Economic Cooperation and Development (OECD)
Guideline for Testing of Chemicals Nº 429
"Skin Sensitization: Local Lymph Node Assay"
(July 22nd, 2010).

Study Director

[redacted]

test material contains 38% AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/ Hydroxyethylacrylate Copolymer

Study Concluded

02/Aug/2013

Performing Laboratory

[redacted]

Sponsor

[redacted]

Study nº

[redacted]

[redacted]

[redacted]

Study Compliance Statement

[redacted]

This report represents an accurate and true recording of the results obtained.

Study plan, original raw data, copy of the Final Report, and observations referent to this study are archived at [redacted]

[redacted]

23 / Aug / 2013
dd mmm yyyy

[redacted]

[redacted]

Final Report Approval

[redacted]

[redacted]

02 / Aug / 2013
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[redacted]

[redacted]

Study n°: [redacted]
Study Title: Evaluation of the skin sensitization potential in the mouse of **E212966** using the Local Lymph Node Assay (LLNA)

Quality Assurance Statement

This Final Report has been inspected by the Quality Assurance (QA) [redacted] The dates and phases of the inspections in the study are given below:

Inspection		Information Date	
Date	**Phase**	**Study Director**	**Test Facility Manager**
10/Dec/2012	Study Plan	10/Dec/2012	10/Dec/2012
12/Dec/2012	Solubility Test	12/Dec/2012	12/Dec/2012
21/Jan/2013	Pre-screen Test	21/Jan/2013	21/Jan/2013
21/Feb/2013	Main Test	21/Feb/2013	21/Feb/2013
04/Feb/2013	RAS 0282/12 – related to the following phases: sample weighing for the solubility test; animals randomization and weighing; weighing of the test item; preparation of the test solutions for application; anesthesia; ear thickness measurements; preparation and injection of the radiolabeled thymidine solution; necropsy; LSC analyses	05/Feb/2013	06/Feb/2013
10/Jun/2013	Draft Report	10/Jun/2013	10/Jun/2013
02/Aug/2013	Final Report	02/Aug/2013	02/Aug/2013

The most recent process inspection prior to the completion of the laboratory phase of this class of study was performed between December 11st, 2012 and February 04th, 2013. This inspection has been recorded in the internal document identified as RAS 0282/12. The names of the Study Director and Test Facility Manager were reported and the dates are described in the table above.

The results and observations presented in this Final Report are an accurate representation of the raw data generated during this study. All the raw data, study plan amendment, and study plan deviation generated during the conduction of this study were inspected.

[redacted]

02 / Aug / 2013
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[redacted]

[redacted]

Summary

The present study was designed to evaluate the skin sensitization potential of the test item E212966 in the mouse using the Local Lymph Node Assay (LLNA).

Test concentrations used in the Main Study were selected in accordance with the Sponsor on the basis of the results of a Solubility Assay followed by a Pre-screen Test, in which 75% w/v of E212966 in the vehicle *N,N*-Dimethylformamide (DMF) was considered as the maximum suitable test concentration for dermal application.

In the Main Study, four CBA/J female mice per group were treated with Test Solutions at the concentrations of 5%, 10%, 25%, 50% and 75% (w/v) in DMF for three consecutive days by topical application on the dorsum of both ears. The negative control group received the vehicle alone (DMF). Since a positive control group was treated with alpha-hexylcinnamaldehyde (HCA) at the concentration of 25% (w/v) in Acetone: Olive Oil (acetone/olive oil, 4:1 v/v, AOO), an additional vehicle control group treated with AOO alone was added in the study.

Three days after the last exposure, all animals were injected with ^{3}H-methyl thymidine and after five hours, the draining auricular lymph nodes were excised and pooled per group to assess the proliferation of lymph node cells by radioactivity measurement. Lymphoproliferative responses of treated groups were expressed as the number of disintegrations per minute (dpm) and a Stimulation Index (SI) was subsequently calculated for each group related to vehicle control lymphoproliferation.

The results of this study are summarized in the following table:

Substance	Conc. (% w/v)	Number of lymph nodes per group	Lymphoproliferative response			Local ear skin irritation		Lymph Nodes Cellular Parameters	
			Net dpm		Stimulation Index	Day 6 % ear thickness increase	Irritation category	Cell viability (%)	Cellularity Index
			Per group	Per node					
Negative Control (DMF)	100	8	8,294	1,037	1.0	-2.9	no local irritation	76	1.0
E212966	5	8	6,298	787	0.8	-3.3	no local irritation	87	1.0
	10	8	7,348	918	0.9	0.6	no local irritation	78	0.6
	25	8	8,821	1,103	1.1	3.8	no local irritation	80	0.9
	50	8	12,572	1,572	1.5	7.5	no local irritation	80	1.1
	75	8	7,009	876	0.8	0.6	no local irritation	84	1.0
Positive Control (HCA)	25*	8	65,827	8,228	11.4	-	-	79	1.5
Vehicle of the Positive Control (AOO)	100	8	5,768	721	1.0	-	-	76	1.0

EC_3 Value: Not calculable%

* - 25% of HCA in AOO on a volume to volume basis. Stimulation Index = (E212966 dpm/node) / (DMF dpm/node). % w/v = percentage concentration on a weight to volume basis. Net dpm = Raw counting rate – BG counting rate, both in dpm. BG = background 97.80 dpm. DMF = *N,N*-Dimethylformamide. AOO = acetone/olive oil (4:1. v/v). HCA = α-hexylcinnamaldehyde. EC_3 = Estimated concentration of a test item needed to produce a stimulation index of three.

Since all the acceptance criteria were met (in particular HCA positive control yielding an appropriate lymphoproliferative response), the study was considered as valid.

[redacted]

[redacted]

No mortality or significant body weight losses (related to controls) were observed in any of the treated groups.

In the groups treated at 25%, 50% and 75% (w/v) in DMF, some residual test item (inducing a white coloration of the treated ear skin) was observed, but did not prevent erythema grading.

No local skin irritation (erythema or ear thickness increase) was induced by test item application.

Local alopecia was induced by the test item at 25%, 50% and 75% (w/v) in DMF.

Cell viability indices were considered satisfactory in all treated groups as well as in control groups.

Stimulation Indices (SI values) calculated for test item-treated groups were found to be 0.8, 0.9, 1.1, 1.5 and 0.8 for the test concentrations of 5%, 10%, 25%, 50% and 75% (w/v) in DMF, respectively, and SI value for the HCA positive control group was 11.4.

Since none of the tested concentrations of **E212966** gave a stimulation index (SI value) higher than 3 in any group receiving the test item up to 75% (w/v) in DMF, it was concluded that, under these experimental conditions, this test item did not induce contact sensitization to mice in the Local Lymph Node Assay.

Based on these results, no EC3 value was calculated.

[redacted]

[redacted]

1. General Information

Study Initiation Date:	10/Dec/2012
Experimental Starting Date:	11/Dec/2012
Experimental Completion Date:	20/Feb/2013
Draft Report Date:	10/Jun/2013
Final Report:	02/Aug/2013

2. Technical Staff

Study Director:
Researchers:

Laboratory Assistant:

[redacted]

3. Definitions and Abbreviations

Lymph Node: lymph nodes are small round or bean-shaped peripheral lymphoid organs of the immune system, linked by lymphatic vessels and distributed in the lymphatic system throughout the body (including superficial locations like auricular/cervical area or axilla as well as deep locations like gastro-intestinal tract). Lymph nodes act as filters or traps for foreign particles (bacteria, neoplasic cells or chemical sensitizers).

Lymphocytes: Lymphocytes are white blood cells of the immune system spread all over the body and involved in defending the organism against foreigner compounds (xenobiotics) or infectious particles.

LLNA: Local Lymph Node Assay

LNC: Lymph Node Cells

LSC: Liquid Scintillation Counting

TCA: Trichloroacetic Acid

4. Objective

The objective of this study was to evaluate the skin sensitization potential of **E212966** in the mouse using the Local Lymph Node Assay (LLNA).

5. Material and Methods

5.1. Test Item Information

Test item:	**E212966**
Batch number:	C2120726D [(1)]
Received on:	05-Dec-2012

[redacted]

[redacted]

[redacted]

Storage conditions:	At room temperature and protected from light. Keep container well closed when not in use (1)
Expiry date:	25-July-2015 (1)

(1) Information provided by the Sponsor

Data related to characterization of the test item are documented in a Characterization Assay Element sheet provided by the Sponsor (see Annex 4) and available in [redacted] archives along with the study raw data. The correct identity and purity were of the responsibility of the Sponsor.

5.2. Reference Substance for Positive Control

Reference substance:	α-Hexylcinnamaldehyde
Batch number:	MKBJ8846V
CAS number:	101-86-0
Declared Concentration (Manufacturer):	97.8%
Manufacture date:	24-Jan-2012
Expiry date:	22-Feb-2017
Physical state:	Liquid
Supplier:	Sigma Aldrich

5.3. Test Solution Preparation

Fresh Test Solutions were prepared daily, prior to each application. The bottles were appropriately identified with adhesive tags.

Four different concentrations of test item were used in each Pre-Screen test and five concentrations were used for the Main test.

Maximum achievable test concentrations were determined in a Solubility test prior to starting the main assay.

5.4. Test System

5.4.1. Description

Young adult female CBA/JRj mice (nulliparous and non-pregnant) supplied by Janvier SAS (France) were used in this study. All mice were inspected soon after arriving at the test facility. Animals were 9 week-old in the Pre-Screen and in the 8 week-old in the main test at the beginning of treatment.

5.4.2. Justification of the Test System Selection

Female CBA/J mice are one of the test systems recommended by the OECD Test Guideline Nº 429 (2010) to perform the Local Lymph Node Assay because it demonstrated appropriate sensitivity to detect agents that cause skin sensitization in humans.

5.4.3. Acclimation

A larger number of animals than necessary for the study were ordered in order to permit the selection and/or replacement of individuals before the start of treatment. Upon receipt from the supplier, the animals were placed into cages, examined and acclimated for a period of 5 days. During this period, the animals

[redacted]

[redacted]

5.4.4. Grouping

Allocation to groups was performed just before the beginning of treatment. Once randomized, the animals were weighed. The required number of animals was selected according to their health status and body weight (± 20% of the mean body weight) and was randomly assigned to the test groups among the animals acclimatized.

5.4.5. Housing

For acclimation purposes, animals were housed five per cage in polypropylene cages covered with a metallic suspended grid.

During the in-life study period, the animals were housed individually.

Sterile sawdust was used for bedding. Hygiene/cleaning conditions and sanitation practices were in accordance with the standard operational procedures for mice.

The cages were identified by tags with study number, test item code and concentration levels. Animals were individually identified by tail marks using a permanent paint pen.

5.4.6. Environmental Conditions

The animals' rooms were maintained in controlled and monitored environmental conditions during the study period for temperature (22 ± 3°C), relative humidity (30 to 70%), illumination intensity and cycle (between 5 and 60 Lux, with 12 hours light / 12 hours dark through an automatic time-switch) and ventilation (continuous). Temperature and relative humidity data were recorded twice daily, except on weekend.

5.4.7. Feeding

The animals were fed with a commercial balanced pelletized diet (Commercial name: Presence, brand: Evialis) and water *ad libitum*. The compositional analysis of the feeding was provided by the supplier. Microbiological, mycotoxicological and pesticides residues parameters are periodically evaluated according to standard operational procedures and the results are archived. In the present study, all relevant parameters fell within the expected ranges.

Water used in this study fulfilled the quality standards for human consumption.

5.4.8. Animal Number

In the Pre-screen test, two female mice were used per test concentration (4 tested concentrations plus the negative control).

In the Main Study, thirty-two female mice were used (4 animals per group, five groups with different test item concentrations, plus two negative and one positive control groups).

[redacted]

[redacted]

5.4.9. Animal Protection Regulations

The study was conducted in compliance with the Animal Protection Regulations (Guidance Document on the Recognition, Assessment, and Use of Clinical Sings as Human Endpoints for Experimental Animals Used in Safety Evaluation, OECD Environmental Health and Safety Publications, Nº 19).

5.4.10. Animal Welfare Compliance Statement

This study was conducted according to applicable rules for animal welfare, cares and use of laboratory animals. Whenever possible, the used procedures were designed to avoid or minimize discomfort, distress and pain to the animals, in accordance to the principles of the Guide for the Care and Use of Laboratory Animals (Manual sobre Cuidados e Usos de Animais de Laboratório - National Research Council – Portuguese edition, 2003, 162 p.).

5.5. Experimental Procedure

5.5.1. Solubility Test

In order to select the highest test item concentration that maximizes exposure, a Solubility Test was performed in Acetone/Olive Oil (AOO, 4:1 v/v) and *N,N*-Dimethylformamide (DMF) at 10% (w/v), then at the concentrations of 50% and 75% (w/v) in DMF.

5.5.2. Pre-Screen Test

In the absence of information on skin irritation potential or dermal toxicity of the test item, a Pre-screen Test was conducted to select the appropriate highest concentration to be tested in the main assay, avoiding systemic toxicity and/or excessive local skin irritation.

Two mice per group received 25 µL of the vehicle alone (DMF) or the Test Solutions at 10%, 25%, 50% and 75% (w/v) in DMF, on the dorsal surface of both ears, once a day for 3 consecutive days.

All mice were weighed on Day 1, just before the test item application and on Day 6, just before sacrifice. Body weight was recorded and used as a parameter to identify possible toxic effects.

All mice were observed daily for any clinical signs of systemic toxicity or local irritation at the application site.

Both ears of each mouse were observed for erythema and scored as follows:

[redacted]

[redacted]

Clinical Observations	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to scar formation preventing grading of erythema	4

Ear thickness was recorded using a thickness gauge before each test item application on Days 1 and 3, under slight anesthesia by intraperitoneal injection of mix xylazine and ketamine, and on Day 6 just after euthanasia.

The sedation was employed not only to avoid licking of test substance/vehicle, but also to ensure an optimized application and precise ear measurements.

The percentage of ear thickness increase on Day 6 compared to day 1 was used to categorize the local irritation induced by the test item, as follows:

% Increase in ear thickness	Irritation Level	Interpretation
≤10%	I	No significant local irritation
>10 – <25%	II	Slight local irritation
≥ 25%	III	Excessive local irritation

Excessive local skin irritation is indicated by an erythema score ≥ 3 and/or an increase in ear thickness ≥ 25% on any day of measurement compared to Day 1.

After treatment, each animal was put back into its cage, which was kept on an electrical heating blanket to avoid hypothermia of sedated mice (up to they recover normal behavior).

5.5.3. Main Study

5.5.3.1. Test Item Application

Four mice per group received 25 µL of the test item onto the dorsal surface of both ears on Days 1, 2 and 3 at the concentrations of 5%, 10%, 25%, 50% and 75% (w/v) in DMF, whereas the concurrent negative control group received the vehicle alone (DMF). The positive control group received HCA at 25% (v/v) in AOO, whereas its concurrent vehicle control group received AOO alone.

5.5.3.2. Clinical Examinations

As in the Pre-screen Test, all treated animals (with test item or vehicle) were observed at least once a day for possible mortality and clinical signs. They were weighed before treatment on Day 1 (baseline)

and on the day of euthanasia (Day 6). Ear thickness was measured before each topical application on days 1, 3 (prior application) and 6 (just after euthanasia) as described for Pre-screen test.

5.5.3.3. Auricular Lymph Node Cell Proliferation

On Day 6, after weighing, each mouse was put into a restraining tube to facilitate the intravenous injection of [^{3}H]-thymidine. The tail was cleaned with alcohol and 250 µL of a [^{3}H] TdR-methyl-thymidine solution in PBS was injected through the lateral tail vein. Animals were left resting into their cages in the test room, with food and water *ad libitum*. Approximately 5 hours after the thymidine injection, the animals were sacrificed under CO_2/O_2 inhalation. Auricular lymph nodes were excised and pooled per group (8 nodes corresponding to 4 animals per group) in pre-identified Petri plates (containing 1 mL of PBS solution). The pooled lymph nodes in the PBS solution were transferred onto a stainless steel sieve with 150 µm (100 mesh) aperture (A Bronzinox – Telas Metálicas e Sintéticas Ltda., Brasil). The nodes were disaggregated under gentle manual scoring with a rubber-tipped plunger over the sieve and cells suspensions were collected in centrifuge tubes.

Cell suspensions from all groups were centrifuged at 100 g for 20 minutes. Then, 9 mL of the centrifuged supernatant were reserved in identified tubes. The rest of suspension (1mL) was carefully homogenized by hand.

90 µL of the homogenate were transferred to another identified tube and reserved for the cell viability test (Trypan blue exclusion test), as described below.

The 9 mL supernatants were transferred back to their respective original tubes, the volumes were equalized to 10 mL with PBS and the tubes were centrifuged again at 2,500 rpm for 20 minutes. The supernatant was discarded and 10 mL of PBS were added to each tube, which was gently shaken by hand and centrifuged at 2,500 rpm for 20 minutes. The remaining pellet of lymph nodes cells was treated with 1 mL of trichloroacetic acid solution (TCA, at a concentration 5% w/v) in the respective tubes, which were left incubated for 18 hours at approximately 4 °C.

After incubation, cell pellets will be re-suspended in 1 mL TCA and transferred to scintillation vials containing 10 mL fluid for 3H-TdR counting (LSC Analysis), as described below.

5.5.3.4. Cell Viability Test

90 µL of lymph node cells suspension was mixed with 10 µL of 0.4% (w/v) Trypan Blue solution. The tube was gently shaken by hand and left resting for 3 minutes prior counting the colored cells in a Neubauer chamber under the microscope with a 400x magnification. The number of viable (slightly colored membranes) and unviable cells (broken membranes and distinctly blue to purple cells) was counted in at least five quadrants.

The cell viability was established in a percentage basis, taking into account the total number of cells (viable and unviable) counted in the chamber, as shown in **Appendix 1**.

5.5.3.5. LSC Analysis

To avoid loss of material, each centrifuge tube was rinsed with 2 mL of the Solvable®, twice with 5 mL of scintillation cocktail Ultima Gold®, mixing it thoroughly with a vortex. The rinses were collected into the respective scintillation vial and 2 mL of scintillation cocktail home made were added to each vial to break the quenching. The samples were counted by 90 minutes in a low level liquid scintillation counter (LS 6500, Beckman Coulter). A vial with 1 mL of TCA solution, 2 mL of Solvable®, 2 mL of the homemade scintillation cocktail and 10 mL of Ultima Gold® scintillation cocktail, was also counted as background. The scintillation counter was set up for counting exclusively tritium.

Stimulation indices were calculated on the basis of recorded LSC results. The results were expressed as disintegrations per minute (dpm) per group and dpm per node.

5.5.3.6. Calculation of Results

Results were expressed in the form of data tables using Microsoft Excel® software and all formula used in the analysis of results and an example of calculations are presented in Appendix 1.

Stimulation Index (SI) for each test group (8 nodes corresponding to 4 animals per group) was calculated according to the following formula:

$$SI = \frac{\text{dpm per node of the treated group}}{\text{dpm per node of the negative control group}}$$

Cellularity and viability of enumerated cells were also determined in each group. Cell viability was expressed in % of viable cells per group. The cellularity index (CI) was calculated as follows.

$$CI = \frac{\text{Nb of viable cells/number of nodes in the treated group}}{\text{Nb of viable cells/number of nodes in the negative control group}}$$

5.5.3.7 Acceptance Criteria

The study was considered valid if all the following acceptance criteria were met:

- the counting measurement (dpm) in the vehicle control group was at least 2-fold higher than the control blank,
- the SI for the positive control item was ≥ 3,
- the cell viability in the vehicle group was higher than 70%.

[redacted]

[redacted]

5.5.3.8 Evaluation of Results

The test item should be regarded as a skin sensitizer when the SI for a dose group is ≥ 3 together with consideration of a dose-response relationship, or as a non-sensitizer if these conditions are not met. Other relevant criteria such as the consistency of the vehicle and positive control responses, the observation of a concurrent local skin irritation and (when appropriate) the statistical significance of the inflammatory reaction in the test group compared to the control group, as well as cellular parameters (cell viability, cellularity index) can also be taken into account to evaluate the data.

The EC3 value (theoretical concentration resulting in a SI value of 3) was determined by linear interpolation of points on the dose-response curve, immediately above and below the 3-fold threshold. The equation used for calculation of EC3 was:

$$EC_3 = c + [(3 - d)/(b - d)] \times (a - c)$$

Where:

a = the lowest concentration giving stimulation index > 3;

b = the actual stimulation index caused by a;

c = the highest concentration failing to produce a stimulation index of 3;

d = the actual stimulation index caused by c.

Categorization of contact allergens on the basis of relative skin sensitizing potency of the test item is used on the basis of the work of KIMBER et al. (2003), which proposed the following categories according to the EC3 value:

EC_3 Value	Category
< 0.1%	Extreme sensitizer
≥ 0.1 - < 1%	Strong sensitizer
≥ 1 - < 10%	Moderate sensitizer
≥ 10 - ≤ 100%	Weak sensitizer

6. Deviations to Study Plan

Deviation 1: related to Section 12. Proposed dates

Change from:

Experimental Completion Date: 21-Jan-2013;

To:

Experimental Conclusion Date: 20-Feb-2013;

Deviation 2: related to Section 5.5 Animals Number

Change from:

In the Main Study, five female mice will be used per test concentration. Thirty-five to forty female mice will be used (5 tested concentrations, plus

[redacted]

[redacted]

is not soluble in the same vehicle than the positive control) and one positive control).

To:

In the Main Study, Thirty-two female mice were used (5 tested concentrations, plus two negative controls and one positive control).

Deviation 3: related to Section 6.3.5 Main Study

Change from:

Forty (40) or thirty-five (35) animals will be allocated in 6 or 7 groups of five animals each (5 tested concentrations, plus 1 or 2 negative controls (2 in case the vehicle selected for the test item is different from AOO) and one positive control).

To:

Thirty-two (32) animals were allocated in 8 groups of four animals each (5 tested concentrations, plus two negative controls and one positive control).

Deviation 4: related to Section Table 3. Description of the experimental procedure of the Main Study for the 5 test item's.

Change from:

Number of animals: 5

To:

Number of animals: 4

Impact of the deviations

The aforementioned deviations did not impact the outcome of the study or the interpretation of its results.

7. Results and Discussion

7.1. Solubility Test Results

The results of the Solubility assay of the test item in the vehicle AOO and DMF are presented in the **Table 1**.

Inhomogeneous suspension was obtained with the test item at 10% (w/v) in AOO.

Homogeneous solutions of the test item in DMF were noted at 10%, 50% and 75% (w/v). Due to its high viscosity, the test item could not be used undiluted (100%) for dermal application.

Accordingly, the maximum achievable test concentration chosen for the Pre-screen Test was 75% (w/v) in the vehicle DMF.

[redacted]

[redacted]

7.2. Pre-screen Test Results

The results of the Pre-Screen Test are described in **Tables 2** to **6**.

No mortality or relevant body weight changes (related to controls) were observed in any of the treated groups (mortality and clinical signs **Table 2**, body weight **Table 3**).

Local alopecia was noted on the ears of animals treated with the test item at 25%, 50% and 75% (w/v) in DMF (**Table 2**).

Despite a white coloration induced on ear skin by the test item at 25%, 50% and 75% (w/v) in DMF, erythema grading could be performed.
No local skin erythema was induced by the test item application (**Table 4**).

Additionally, no significant ear thickness increase was induced by the test item. Individual ear thickness measurements in millimeters on days 1, 3 and day 6 are shown in **Table 5**.

At 10%, 25%, 50% and 75% (w/v) in DMF, mean ear thickness increases were +4.90%, +9.43%, +9.80% and +5.88%, respectively, on day 6 compared to day 1(**Table 6**).
Overall, no skin local irritation (irritation or ear thickness increase) was induced by the test item on ears of animals treated up to 75% (w/v) in DMF.

Accordingly, the Main Study was conducted with the following concentrations of test item: 5%, 10%, 25%, 50% and 75% (w/v) in DMF.

7.3. Main Study Results

Since all the acceptance criteria were met (in particular HCA positive control yielding an appropriate lymphoproliferative response), the study was considered as valid.

No mortality or clinical signs of toxicity were observed in any of the treated groups (mortality and clinical signs, **Table 7**).

Local alopecia was noted on the ears of animals treated at 25%, 50% and 75% (w/v) in DMF (**Table 7**).

Mean body weight of animals from the positive control and treated groups were comparable to the mean body weight of the vehicle control groups during the study (**Table 8**).

In the groups treated at 25%, 50% and 75% (w/v) in DMF, residual test item (inducing a white coloration of the treated ear skin) was observed, but did not prevent erythema grading. No local skin erythema was induced by the test item application (**Table 9**).

Additionally, no significant ear thickness increase was induced by the test item. Individual ear thickness measurements are shown in **Table 10**. Individual ear thickness increases on days 3 and 6 compared to day 1 are shown in **Table 11**. Mean ear thickness increases (in mm and in %) per group in the Main Study are shown in **Table 12**.

Stimulation Indices (SI values) were found to be 0.8, 0.9, 1.1, 1.5 and 0.8 in the groups treated by the test item at 5%, 10%, 25%, 50% and 75% (w/v) in DMF, respectively, and the SI value for the HCA positive control group was 11.4 (**Table 13**).

Cell viability indices (**Table 14**) were considered satisfactory in all groups.

Since none of the tested concentrations of **E212966** gave a stimulation index (SI value) higher than 3 in any group receiving the test item up to 75% (w/v) in DMF, it was concluded that, under these experimental conditions, this test item did not induce contact sensitization to mice in the Local Lymph Node Assay.

Based on these results, no EC3 value was calculated.

8. Conclusions

Under the experimental conditions of the present assay, the test item E212966 was not a skin sensitizer in the murine Local Lymph Node Assay.

[redacted]

[redacted]

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Google Translate translations of Studies on Acrylamide/Ammonium Acrylate Copolymer (32%) and Acrylates/t-Butylacrylamide Copolymer (100%) (information originally submitted to CIR on August 23, 2021)

Study of Cytotoxicity on Reconstructed Human Epidermis

PURPOSE: knowledge

summary and conclusions for the raw material

Headings	**53845 32% Acrylamide/Ammonium Acrylate Copolymer**	**100% Acrylates/t-Butylacryamide Copolymer**
characteristics of the raw material studied		
code of the first studied material	53845	71596
physical appearance and color	translucent milk	white powder
No of the lot	114270	01240229U0
Nature	Polymer	Polymer
MTT interaction test	Negative	Negative
Protocol		
experimental protocol reference	P61/007-V01	P61/007-V01
concentration tested	As is	As is
quantity deposited	10 µL	10 mg
application time	15 min ± 0.5 min	15 min ± 0.5 min
incubation time	42 h ± 1 h	42 h ± 1 h
special conditions	none	None
Dates		
Beginning of experiments	02/27/2007	10/17/2006
End of experiments	08/02/2007	01/22/2007
Acceptability	Valid test (based on acceptability values)	Valid test (based on acceptability values)
OD of negative witness	≥ 0.600	≥ 0.600
viability of sodium dodecyl sulfate (5%)	≤ 35%	≤ 35%
standard deviation % viability	< 18%	< 18%

results: % viability and IL-1α concentration		
% Viability	Trial 1 84.7% Trial 2 85.2% Trial 3 100.0%* Mean 90.0% Standard deviation 8.7%	Trial 1 100.0 Trial 2 100.0 Mean 100.- Standard deviation /
IL-1α (pg/ml)	Trial 1 88.2 Trial 2 7.1 Trial 3 0.0** Mean 31.8 Standard deviation 49.0	Trial 1 0.0** Trial 2 0.0** Mean 0.0** Standard deviation /**
Footnotes	* The viability was reduced to 100%, with a viability greater than 100% not indicating a biological response other than equal to 100% in the MTT test. ** The result was reduced to 0.0 because a negative value is not different from 0.	The results were reduced to 100% viability greater than 100% not indicating a biological response different from a viability equal to 100% in the MTT test. There is therefore no need to calculate the standard deviation. ** The result was reduced to 0.0 because a negative value is not different from 0. There is therefore no need to calculate the standard deviation.
Conclusion	According to the experimental conditions adopted, the study aimed at evaluating the primary skin tolerance on a reconstructed human epidermis model suggests that the raw material 53845 is potentially non-irritating.	According to the experimental conditions adopted, the study aimed at evaluating the primary skin tolerance on a reconstructed human epidermis model suggests that the raw material 71596 is potentially non-irritating.
Remark	The study of MP on reconstructed epidermis is based on the predictive in vitro model established during preliminary studies to optimize the protocol for skin irritation from chemicals (Cotovio et al 2005) and the ECVAM study. This method is awaiting regulatory validation.	The study of MP on reconstructed epidermis is based on the predictive in vitro model established during preliminary studies to optimize the protocol for skin irritation from chemicals (Cotovio et al 2005) and the ECVAM study. This method is awaiting regulatory validation.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: August 12, 2021

SUBJECT: Acrylates/Octylacrylamide Copolymer

Anonymous. 2021. Toxicology Studies (summaries) for Acrylates/Octylacrylamide Copolymer.

Toxicology Studies for Acrylates/Octylacrylamide Copolymer

Acute Oral Toxicity

A 15% solids aqueous Acrylates/Octylacrylamide Copolymer neutralized solution was given orally to two male and two female Charles River albino rats per group at 1.0, 1.5 and 2.3 g solids/kg body weight. Minor clinical signs (ruffed fur, hypoactivity) were observed 2 hours after dosing but were not present after 48 hours. No animal deaths were observed. The oral LD_{50} was greater than 2.3 g/kg.

Acute Dermal Toxicity

2.0 g/kg of Acrylates/Octylacrylamide Copolymer was applied to the skin of 10 albino rabbits. All animals survived the 2.0 g/kg dermal application. Animals were examined daily for clinical signs. Diarrhea, few feces and wetness or soiling of the anogenital area were noted during the observation period. Body weight changes were normal in 9/10 animals. One animal lost weight during the study. Dermal reactions, absent to slight (one animal) at 24 hours, were absent on days 7 and 14. The LD_{50} was greater than 2.0 g/kg of body weight.

Acute Inhalation Toxicity Study in Albino Rats

Ten Sprague Dawley rats (5 male and 5 female) were exposed in whole body chambers to an average gravimetric exposure concentration of 3.4 mg polymer/L of 10% solids solution of Acrylates/Octylacrylamide Copolymer in water. This was considered the maximum attainable exposure level. The particle size was 5.5 μm (84% of the aerosol was less than 10 microns in size). Animals were observed routinely for clinical signs and body weight changes. All animals survived the exposure and 14-day observation period. Signs of treatment were minimal both during the exposure and the 14-day observation period. A minimal, transient decrease in body weight was produced by treatment. Gross postmortem observations were generally considered unremarkable. The LC_{50} of Acrylates/Octylacrylamide Copolymer was greater than 3.4 mg/L.

Eye Irritation In Rabbits

0.1 ml of Acrylates/Octylacrylamide Copolymer was placed in the eyes of six New Zealand White rabbits. Animal eyes were examined for irritation of the cornea, iris and conjunctiva on days 1, 2, and 3. There was no conjunctival irritation, corneal opacity, ulceration or iritis noted in any eye at any observation period. Under the conditions of the study, Acrylates/Octylacrylamide Copolymer is considered to be non-irritating to the rabbit eye.

Primary Eye Irritation

0.1 milliliters of a neutralized 15% solids Acrylates/Octylacrylamide Copolymer aqueous solution was placed in the eye of six New Zealand White rabbits. There was no corneal opacity noted at any observation period. Iritis, noted in 3/6 eyes, cleared by 24 hours. Mild conjunctival irritation, noted in 6/6 eyes, cleared by 24 hours. Acrylates/Octylacrylamide Copolymer is considered mildly irritating to the rabbit eye.

Primary Dermal Irritation

0.5 milliliters of a 15% solids aqueous Acrylates/Octylacrylamide Copolymer neutralized was applied to occluded intact and abraded sites on six New Zealand White rabbits. Erythema was observed at 24 and 72 hours and was similar in both intact and abraded sites. The primary irritation score was 2.9 and Acrylates/Octylacrylamide Copolymer was concluded to be mildly irritating.

Guinea Pig Maximization Test (Magnusson-Kligman)

Twenty female guinea pigs were dosed with Acrylates/Octylacrylamide Copolymer and twenty females were dosed with distilled water (control). The induction phase consisted of two applications. First, animals were injected with 0.1 ml of a 5% solids solution of Acrylates/Octylacrylamide Copolymer with and without Freund's Complete Adjuvant. Second, an 8 cm^2 patch saturated with moistened Acrylates/Octylacrylamide Copolymer powder was applied topically for 48 hours. The challenge phase consisted of two saturated 4 cm^2 occlusive applications of a 100 % solids and 50 % solids aqueous solution of Acrylates/Octylacrylamide Copolymer for 24 hours with application site evaluations 24, 48 and 72 hours after patch removal. Slight, barely perceptible erythema was observed in two animals exposed to the test article at 24 hours. These dermal reactions were considered similar to those observed in the control animals. Under the described test conditions, Acrylates/Octylacrylamide Copolymer did not cause a sensitization reaction in guinea pigs.

Thirteen Week Inhalation Toxicity Study In Rats

Twenty Sprague Dawley (Charles River) rats (ten male and ten female) were exposed in whole body chambers to 0.0 (air), 199, 491 or 828 ug/m^3 Acrylates/Octylacrylamide Copolymer in ethanol for four hours per day, seven days a week for 13 weeks. The particle mean aerodynamic diameter was 1.9 microns. All animals were observed routinely for clinical signs and body weight changes. Hematological, serum biochemical and histopathological evaluations were conducted. Although some minor differences between exposed and control animals were observed, these differences were not considered related to exposure conditions. Exposure of rats to concentrations approximately 100 times simulated-human-use-conditions did not produce any adverse effects over a thirteen-week exposure period.

Human Repeated Insult Patch Test

A neutralized 15% solids Acrylates/Octylacrylamide Copolymer aqueous solution was sprayed onto patches and applied to the skin of a non-exclusive panel of 50 people (25 females and 25 male) for nine induction and one challenge exposures. The patch was in contact with the skin for 24 hours and the site was evaluated. Thirty subjects reacted one or more times to the application of the test material. The majority of the reactions (49) were classified as very slight erythema with six being classified as mild erythema. There was no evidence of skin sensitization with any of the subjects. Reactions to the challenge application were classified as transient irritation. Based on the incidence and severity of the reactions, Acrylates/Octylacrylamide Copolymer was not classified as a primary skin irritant and did not cause sensitization type reactions in human skin.

Personal Care Products Council
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Memorandum

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

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

DATE: August 25, 2021

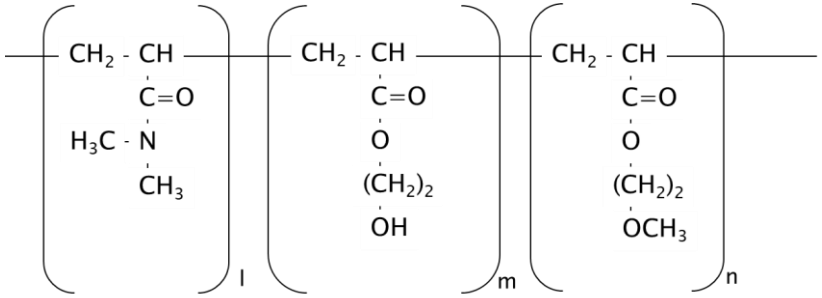
SUBJECT: Acrylamide/Acrylate Copolymers

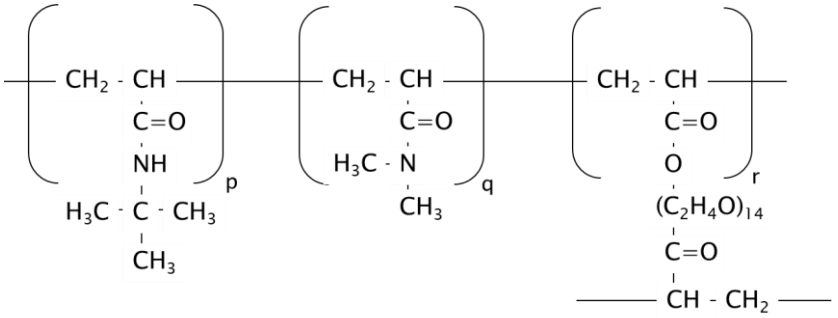
Anonymous. 2021. Summary information Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer.

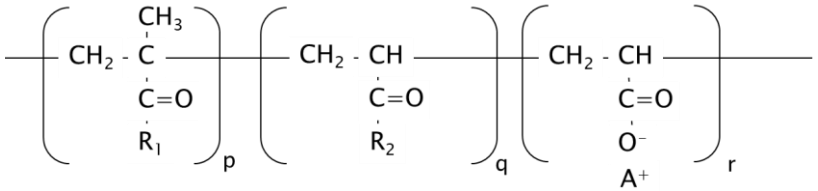
Anonymous. 2021. Summary information t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer.

Anonymous. 2021. Summary information AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer.

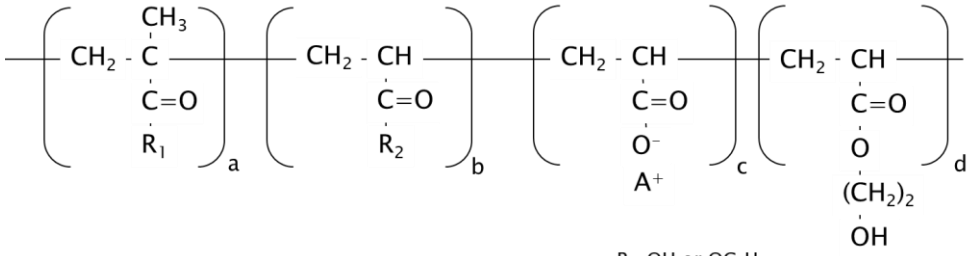
Anonymous. 2021. Summary information AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer.

INCI name	Dimethyl Acrylamide/ Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer						
Structural formula							
Manufacturing method	The starting monomers are polymerized in ethanol. This copolymer solution is refined.						
Molecular weight(Mw)	approx. 10,000						
Percentage of molecular weight less than 500 daltons	0.0124%						
Residual monomer	Total	＜200ppm					
Impurities	Acrylamide	Not detected					
Safety test	Single dose toxicity		70% ethanol solution	＞2,000mg/kg	Rat	Drug toxicity testing guidelines H5/8/10	March 2008
	Ocular irritation		10% aqueous solution	Non-irritating	Rabbit	Draize method	March 2008
	Primary skin irritation		10% aqueous solution	PII=0, Mild irritants	Rabbit	Draize method	March 2008
	Cumulative skin irritation		10% aqueous solution	Negative	Guinea pig	Guidabce for the safety evaluation of cosmetics 2001	March 2008
	Skin sensitization		70% ethanol solution	Negative	Guinea pig	Guinea Pig Maximization Test	August 2008
	Genotoxicity		70% ethanol solution	Negative		Ames test	March 2008
	Human patch		5% aqueous solution	Negative		40 people	March 2012
	Alternative methods for ocular irritation testing		50% aqueous solution	Non-irritating		SkinEthic™ HCE method	March 2013
	Alternative methods for skin sensitization testing		50% aqueous solution	Non-irritating		EpiSkin method	June 2013

INCI name	t-Butylacrylamide/ Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer	
Structural formula		
Manufacturing method	The starting monomers are polymerized in ethanol. This copolymer solution is refined.	
Molecular weight(Mw)	approx. 5000	
Percentage of molecular weight less than 500 daltons	＜0.0005%	
Residual monomer	Total	No data
Impurities	Acrylamide	No data
Safety test	No data	

INCI name	AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer					
Structural formula	 R_1 : OH or OC_nH_{2n+1} R_2 : OH or OC_nH_{2n+1} or NHC_nH_{2n+1} A : 2-amino-2-methyl -1-propanol					
Manufacturing method	The starting monomers are polymerized in ethanol. This copolymer solution is refined and neutralized with 2-Amino-2-methyl-1-propanol.					
Molecular weight(Mw)	approx. 24,000					
Percentage of molecular weight less than 500 daltons	0.0001%					
Residual monomer	Total	＜2,000ppm				
Impurities	Acrylamide	Not detected				
Safety test	Single dose toxicity	40% ethanol solution	＞2,000mg/kg	Rat	Drug toxicity testing guidelines H5/8/10	March 1996
	Ocular irritation	40% ethanol solution	Slightly irritant	Rabbit	Draize method	March 2006
	Primary skin irritation	40% ethanol solution	PII=0, Mild irritants	Rabbit	Draize method	March 2006
	Skin sensitization	40% ethanol solution	Negative	Guinea pig	Guinea Pig Maximization Test	January 2008
	Genotoxicity	40% ethanol solution	Negative		Ames test	March 2008
	Alternative methods for ocular irritation testing	Polymer	Non-irritating		SkinEthic™ HCE method	March 2013
	Alternative methods for skin sensitization testing	Polymer	Non-irritating		EpiSkin method	June 2013

August 2021

INCI name	AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer					
Structural formula						
Manufacturing method	The starting monomers are polymerized in ethanol. This copolymer solution is refined and neutralized with 2-Amino-2-methyl-1-propanol.					
Molecular weight(Mw)	approx.250,000					
Percentage of molecular weight less than 500 daltons	0%					
Residual monomer	Total	＜3,000ppm				
Impurities	Acrylamide	Not detected				
Safety test	Single dose toxicity	40% ethanol solution	＞2,000mg/kg	Rat	Drug toxicity testing guidelines H5/8/10	September 2003
	Ocular irritation	40% ethanol solution	Slightly irritant	Rabbit	Draize method	March 2006
	Primary skin irritation	40% ethanol solution	PII=0, Mild irritants	Rabbit	Draize method	March 2006

Acrylamide/Acrylate Copolymer 2021 FDA VCRP Data

Acrylamide/Ammonium Acrylate Copolymer

Moisturizing	1

Total: 1 use

Acrylamide/Sodium Acrylate Copolymer

Shampoos (non-coloring)	1
Tonics, Dressings, and Other Hair Grooming Aids	1
Lipstick	2
Face and Neck (exc shave)	4
Body and Hand (exc shave)	1
Moisturizing	4
Suntan Gels, Creams, and Liquids	1

Total: 14 uses

Acrylates/Acrylamide Copolymer

Bath Soaps and Detergents	3
Face and Neck (exc shave)	2
Other Skin Care Preps	2

Total: 7 uses

Acrylates/*t*-Butylacrylamide Copolymer

Hair Spray (aerosol fixatives)	1
Other Hair Preparations	3

Total: 4 uses

Acrylates/Methacrylamide Copolymer

Shampoos (non-coloring)	2

Total: 2 uses

Acrylates/Octylacrylamide Copolymer

Eyeliner	10
Mascara	7
Cologne and Toilet waters	67
Perfumes	42
Other Fragrance Preparation	4

Hair Spray (aerosol fixatives)	10
Tonics, Dressings, and Other Hair Grooming Aids	4
Other Hair Preparations	1
Blushers (all types)	4
Face Powders	3
Other Makeup Preparations	1
Basecoats and Undercoats	1
Nail Polish and Enamel	1
Aftershave Lotion	3
Other Suntan Preparations	2

Total: 160 uses

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide Copolymer

Hair Spray (aerosol fixatives)	2
Makeup Fixatives	2

Total: 4 uses

Corn Starch/Acrylamide/Sodium Acrylate Copolymer

Other Bath Preparations	4
Face and Neck (exc shave)	1

Total: 5 uses

Dimethyl Acrylamide/Hydroxyethyl Acrylate/Methoxyethyl Acrylate Copolymer

Hair Spray (aerosol fixatives)	2
Other Hair Preparations	3
Nail Polish and Enamel	1
Other Manicuring Preparations	1

Total: 7 uses

Ingredients not reported to be in use:

AMP-Acrylates/C1-18 Alkyl Acrylate/C1-8 Alkyl Acrylamide/Hydroxyethylacrylate Copolymer

t-Butylacrylamide/Dimethylacrylamide/PEG-14 Diacrylate Crosspolymer

Butyl Acrylate/Isopropylacrylamide/PEG-18 Diemthacrylate Crosspolymer

Dimethylacrylamide/Lauryl Methacrylate Copolymer (concentration reported)

Potassium Acrylates/Acrylamide Copolymer

Sodium Acrylates/Hydroxyethyl Acrylamide Copolymer

Starch Acrylates/Acrylamide Copolymer