Safety Assessment of Polyacrylate Crosspolymer-6 as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: May 22, 2026
Panel Meeting Date: June 15-16, 2026

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Bruce A. Brod, M.D., M.H.C.I., F.A.A.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Temima Nguyen, M.S., Scientific Analyst/Writer, CIR.

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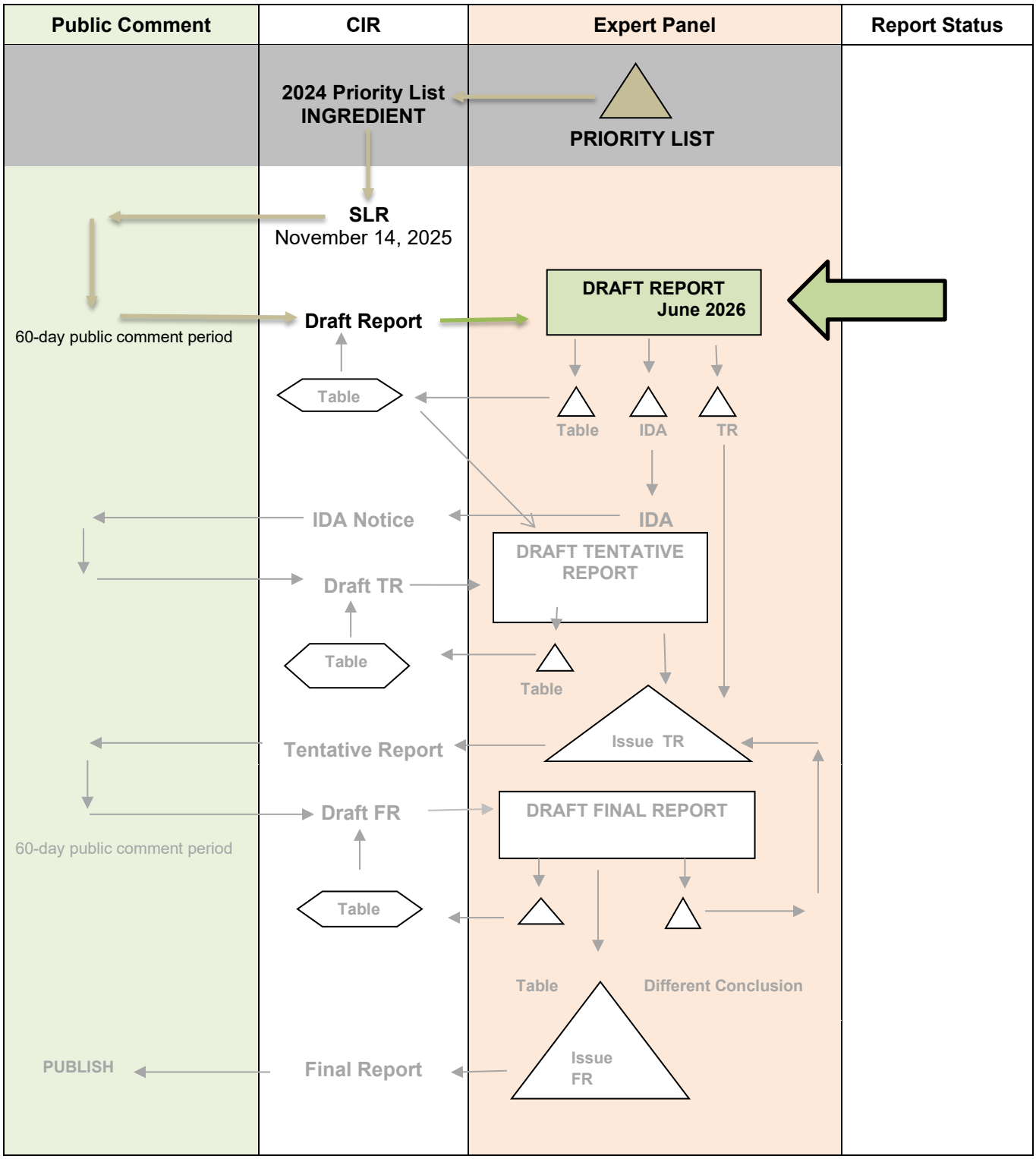
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Polyacrylate Crosspolymer-6
MEETING June 2026



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Temima Nguyen, M.S., Scientific Analyst/Writer, CIR
Date: May 22, 2026
Subject: Safety Assessment of Polyacrylate Crosspolymer-6 as Used in Cosmetics

Enclosed is the Draft Report on the Safety Assessment of Polyacrylate Crosspolymer-6 as Used in Cosmetics. (It is identified as *report_PolyacrylateCrosspolymer-6_062026* in the pdf document). This is the first time the Panel is seeing this safety assessment; the Scientific Literature Review (SLR) was issued by CIR on November 14, 2025.

In our analysis of each product reported in the RLD with a categorization of "(17) Other preparations (i.e., those preparations that do not fit another category)," 11 of the 23 products were either co-categorized as "(01) Baby products," "(06) Hair preparations (non-coloring)," or "(14) Skin care preparations, (creams, lotions, powder, and sprays)." According to the submitted names, 3 products are an eyelash product, body glitter makeup kit, and a hair spray. However, for the 9 remaining reported formulations exclusively categorized as "(17) Other preparations," neither the product type nor the area/route of exposure is obvious from the information submitted to the RLD.

The results of a concentration of use survey performed using MoCRA categories were submitted by industry and are included (*data1_PolyacrylateCrosspolymer-6_062026*). Also, since the issuing of the SLR, data on the method of manufacturing, impurities, and skin sensitization using a Sens-Is assay and human cell line activation test (h-CLAT) for Polyacrylate Crosspolymer-6 were submitted (*data2_PolyacrylateCrosspolymer-6_062026*). There was also a summary of a human-repeated-insult-patch-test (HRIPT) on a product containing 1.1% Polyacrylate-Crosspolymer-6 provided (*data3_PolyacrylateCrosspolymer-6_062026*). Additionally, studies on an eye cream containing 0.5% Polyacrylate Crosspolymer-6 evaluating dermal irritation and sensitization and ocular irritation potential through an HRIPT and EpiOcular™ screening assay, respectively, were included in this report (*data4_PolyacrylateCrosspolymer-6_062026*).

Other supporting items include:

- flow chart (*flow_PolyacrylateCrosspolymer-6_062026*)
- report history (*history_PolyacrylateCrosspolymer-6_062026*)
- search strategy (*search_PolyacrylateCrosspolymer-6_062026*)
- data profile (*dataprofile_PolyacrylateCrosspolymer-6_062026*)

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement.

Polyacrylate Crosspolymer-6 History

November 14th, 2025

SLR posted.

January 7th, 2026

A summary of data on Polyacrylate Crosspolymer-6 was provided which included method of manufacturing, impurities, a study testing skin sensitization using a Sens-Is assay, and a study testing skin sensitization using a human cell line activation test (h-CLAT).

March 11th, 2026

Studies on an eye cream containing 0.5% Polyacrylate Crosspolymer-6 were provided, which included a repeated insult patch test and EpiOcular™ screening assay for potential ocular irritation.

May 6th, 2026

A summary of a Human Repeat Insult Patch Test (HRIPT) on a product containing 1.1% Polyacrylate-Crosspolymer-6 was provided.

June 2026

Panel reviews Draft Report.

Polyacrylate Crosspolymer-6 Data Profile* – June 2026 – Writer, Temima Nguyen																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Polyacrylate Crosspolymer-6	X	X	X												X					X	X	X		X		X	X		

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

Polyacrylate Crosspolymer-6

Ingredient	CAS #	PubMed	FDA	CompTox	ChemPort	NIOSH	NTIS	NTP	FEMA	EU	ECHA	SIDS	SCCS	AICIS	FAO	WHO	Web
Polyacrylate Crosspolymer-6	N/A	NR	√	NR	NR	NR	NR	NR	NR	√*	NR	NR	NR	√	NR	NR	√

NR- not reported; √* - data is available, but is not new or relevant

Search Strategy

Pubmed

(Polyacrylate Crosspolymer-6) – 0 hits / 0 useful

Web

Polyacrylate Crosspolymer-6 toxicity – 22,500 hits/ 2 useful
Polyacrylate Crosspolymer-6 irritation – 485,000 hits/ 0 useful

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
- CompTox: https://comptox.epa.gov/dashboard/chemical/pubmed-abstract-sifter/DTXSID3039242; https://www.epa.gov/comptox-tools/downloadable-computational-toxicology-data#LM
- eChemPortal: https://www.echemportal.org/echemportal/
- DeepDyve: https://www.deepdyve.com/
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf; https://dps.fda.gov/omuf/monographsearch
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- 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/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees_en https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- 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
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- 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) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

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ABBREVIATIONS

CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
Dictionary	*International Cosmetic Ingredient Dictionary*
ET_{50}	exposure time required to reduce cell viability by 50%
FDA	Food and Drug Administration
h-CLAT	human cell line activation test
HET-CAM	hen's egg test on the chorioallantoic membrane
HRIPT	human repeated-insult patch test
l.o.	leave-on
MoCRA	Modernization of Cosmetics Regulation Act of 2022
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NR	not reported
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
RLD	Registration and Listing Data
r.o.	rinse-off
TG	test guideline
US	United States

INTRODUCTION

This assessment reviews the safety of Polyacrylate Crosspolymer-6 as used in cosmetic formulations. According to the *International Cosmetic Ingredient Dictionary (Dictionary)*, Polyacrylate Crosspolymer-6 is reported to function as an emulsion stabilizer and viscosity increasing agent - aqueous.[1]

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 extensive search of the world's literature; a search was last conducted May 2026. 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 Expert Panel for Cosmetic Ingredient Safety (Panel) typically evaluates, is provided on the Cosmetic Ingredient Review (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.

Much of the data included in this safety assessment was found on the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) website. (Please note that the NICNAS website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when NICNAS is cited.)

CHEMISTRY

Definition and Structure

According to the *Dictionary*, Polyacrylate Crosspolymer-6 is a vinyl-type copolymer comprising the ammonium salt of 2-acrylamido-2-methylpropane sulfonic acid (ammonium AMPS), dimethylacrylamide, lauryl methacrylate, and laureth-4 methacrylate monomer residues, and is crosslinked with trimethylolpropane triacrylate; this crosslinker is tridentate, linking 3 chains of the polymer per residue.[1] This ingredient has the following idealized chemical structure as shown in Figure 1.

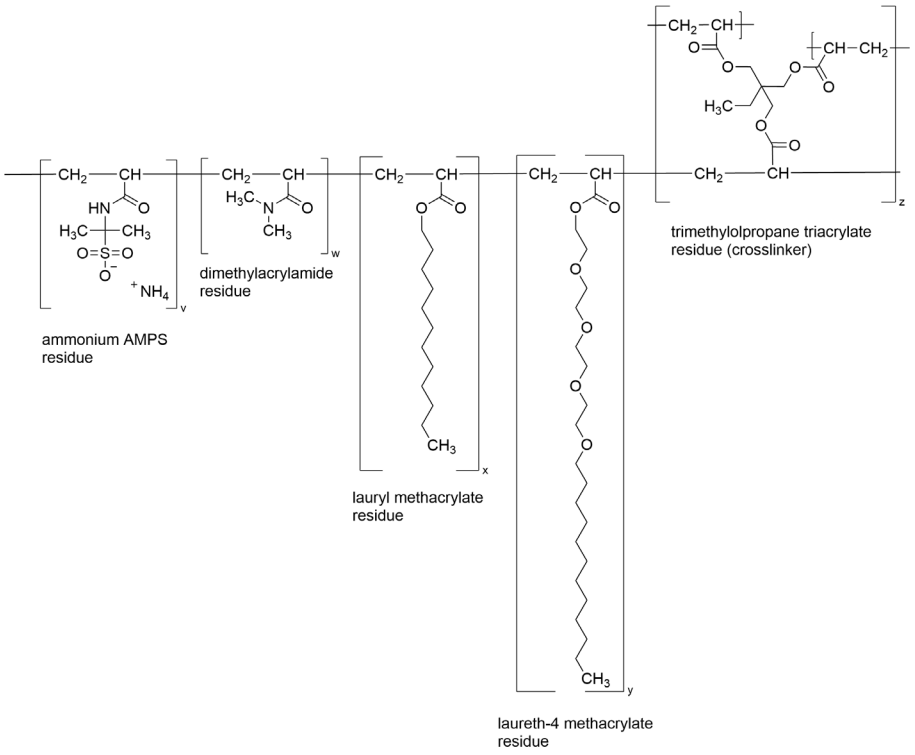

Figure 1. Polyacrylate Crosspolymer-6 (idealized structure; drawn as a block copolymer for convenience of depiction)[CIR Staff]

Chemical Properties

Polyacrylate Crosspolymer-6 is reported to be a white powder.[2] The number average formula weight of Polyacrylate Crosspolymer-6 is > 10,000 Da. Other chemical properties can be found in Table 1.

Method of Manufacture

Unpublished data submitted by industry stated Polyacrylate Crosspolymer-6 is precipitated out from a polymerization reaction.[3] No further details were provided.

Impurities

Specific impurities data were not found in the published literature. According to unpublished data submitted by industry, Polyacrylate Crosspolymer-6 contains < 20 ppm dimethyl acrylamide.[3] The NICNAS report stated that

Polyacrylate Crosspolymer-6 that was assessed contained the notified polymer at > 90% concentration, and that it contained only "low concern functional groups".[2]

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of Polyacrylate Crosspolymer-6 in cosmetics. Registration and Listing Data (RLD) obtained from the FDA report frequency of use, and responses to a survey conducted by the Personal Care Products Council (Council) indicate maximum reported concentrations of use; it is these values that define the present practices of use and concentration that are assessed by the Panel. Since 2024, as a result of the Modernization of Cosmetics Regulation Act of 2022 (MoCRA), manufacturers and processors are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-yr period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products, are not included in this exemption.[4] Another change resulting from MoCRA is the addition of tattoo preparations (permanent tattoo inks, temporary tattoo inks, and other tattoo products) to the product categories for which companies need to list their products with FDA. However, evaluating the safety of ingredients as used in tattoo preparations is not within the purview of the Panel; accordingly, such use is not included as part of the present practices of use that are assessed by the Panel.

According to RLD obtained from the FDA in 2025, Polyacrylate Crosspolymer-6 is reported to be used in 2771 formulations (Table 2).[5,6] In 2025, the concentration of use survey conducted by the Council reported the highest maximum concentration of use is 5% in mascaras.[7]

Polyacrylate Crosspolymer-6 is used in products that are applied near the eye (e.g., mascaras up to 5%), that can be incidentally ingested (e.g., lipsticks and lip glosses at up to 1.6%), and in products that result in mucous membrane exposure (e.g., bath soaps and body washes up to 0.7%; lipsticks and lip glosses at up to 1.6%). Additionally, Polyacrylate Crosspolymer-6 is used in sprays (e.g., cologne and toilet waters up to 0.89%; deodorants, concentration of use not reported) and could therefore be incidentally inhaled. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable. However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. 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 workplace.

With the advent of MoCRA and the current product categories outlined therein, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such for some, but not all, product categories in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available in some instances. Additionally, the concentration of use surveys are conducted based on the same product categories as identified in the RLD. Based on RLD, some products containing Polyacrylate Crosspolymer-6 are marketed for use with airbrush delivery systems. However, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

Polyacrylate Crosspolymer-6 is not restricted from use in any way under the rules governing cosmetic products in the European Union.[8]

Non-Cosmetic

Non-cosmetic uses were not found in the published literature, and unpublished data were not submitted.

TOXICOKINETIC STUDIES

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

TOXICOLOGICAL STUDIES

Neither acute nor repeated-dose toxicity studies were not found in the published literature, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental and reproductive toxicity studies were not found in the published literature, and unpublished data were not submitted.

GENOTOXICITY STUDIES

In Vitro

Polyacrylate Crosspolymer-6 did not induce gene mutations; results were negative in a bacterial reverse mutation test that was performed according to Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 471.[2] No additional information was provided.

CARCINOGENICITY STUDIES

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

DERMAL IRRITATION AND SENSITIZATION STUDIES

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

The dermal irritation of Polyacrylate Crosspolymer-6 was tested in rabbits according to OECD TG 404.[2] There were no effects observed, and the substance was considered non-irritating at > 90%. Polyacrylate Crosspolymer-6 was not predicted to be a skin sensitizer in a human cell line activation test (h-CLAT) nor a Sens-IS assay.[3] In human repeated-insult patch tests (HRIPT), an eye cream containing 0.5% Polyacrylate Crosspolymer-6 (102 subjects),[9] a leave-on face product containing 1.1% Polyacrylate Crosspolymer-6 (107 subjects),[10] and 5% Polyacrylate Crosspolymer-6 (number of subjects not stated)[2] were not irritants or sensitizers.

OCULAR IRRITATION STUDIES

Details of the ocular irritation studies are summarized below and described in Table 4.

The ocular irritation potential of an eye cream containing 0.5% Polyacrylate Crosspolymer-6 was predicted to be non-irritating in vitro in EpiOcular™ tissues when tested using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[11] The exposure time required to reduce cell viability by 50% (ET_{50}) of the eye cream was 23.1 h, indicating non/minimal irritation (ET_{50} > 60 min).[12] In an in vitro hen's egg test on the chorioallantoic membrane (HET-CAM) test, Polyacrylate Crosspolymer-6 at 2% was predicted to be non-irritating.[2] An ocular irritation study was performed according to OECD TG 404; Polyacrylate Crosspolymer-6 was tested at an unknown concentration in 3 rabbits.[2] Polyacrylate Crosspolymer-6 was slightly irritating to rabbit eyes.

SUMMARY

The safety of Polyacrylate Crosspolymer-6 as used in cosmetics is reviewed in this safety assessment. Polyacrylate Crosspolymer-6 is reported to function in cosmetics as an emulsion stabilizer and viscosity increasing agent - aqueous.

According to RLD obtained from the FDA in 2025, Polyacrylate Crosspolymer-6 is reported to be used in 2771 formulations. The concentration of use survey conducted in 2025 by the Council reported the highest maximum concentration of use is 5% in mascaras.

Polyacrylate Crosspolymer-6 tested negative in an Ames test at an unknown concentration. These results indicated that the substance was non-mutagenic.

The dermal irritation of Polyacrylate Crosspolymer-6 was tested in rabbits according to OECD TG 404. There were no effects observed, and the substance was considered non-irritating at > 90%. Polyacrylate Crosspolymer-6 was not predicted to be a skin sensitizer in a h-CLAT nor a Sens-IS assay. In HRIPTs, an eye cream containing 0.5% Polyacrylate Crosspolymer-6 (102 subjects), a leave-on face product containing 1.1% Polyacrylate Crosspolymer-6 (107 subjects), and 5% Polyacrylate Crosspolymer-6 (number of subjects not stated) were not irritants or sensitizers.

The ocular irritation potential of an eye cream containing 0.5% Polyacrylate Crosspolymer-6 was predicted to be non-irritating in vitro in EpiOcular™ tissues when tested using a MTT assay. The exposure time required to reduce the ET_{50} of the eye cream was 23.1 h, indicating non/minimal irritation (ET_{50} > 60 min). In an in vitro HET-CAM test, Polyacrylate Crosspolymer-6 at 2% was predicted to be non-irritating. An ocular irritation study was performed according to OECD TG 404; Polyacrylate Crosspolymer-6 was tested at an unknown concentration in 3 rabbits. Polyacrylate Crosspolymer-6 was slightly irritating to rabbit eyes.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties of Polyacrylate Crosspolymer-6

Property	Value		Reference
Physical Form (@ 20 ºC and 101.3 kPa)	Powder		[2]
Color	White		[2]
Formula Weight (number average; Da)	> 10,000		[2]
Density (@ 25 ºC; kg/m^3)	230		[2]
Glass Transition Temperature (ºC)	> 200 (decomp.)		[2]
Water Solubility (@ 20 ºC)	Fully soluble in water, forms a gel at high concentration.		[2]
Particle Size	Laser diffraction:	Sieve analysis:	[2]
	<371 µm 90%	<2000 µm 98%	
	<43 µm 50%	<150 µm 25%	
	<0.8 µm 10%	<80 µm 7%	

Table 2. Frequency and concentration of use of Polyacrylate Crosspolymer-6 according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use
	RLD (2025)[5,6]	% (2025)[7]
Totals*	**2771**	**0.008-5**
summarized by likely duration and exposure**		
Duration of Use		
Leave-On	*2669*	*0.008-5*
Rinse-Off	*474*	*0.7-2.9*
Diluted for (Bath) Use	*12*	*0.02*
Unknown	*23*	*NA*
Exposure Type		
Baby Products	7	1
Children's Makeup	NR	NR
Eye Area	153	0.49-5
Incidental Ingestion	225	0.34-1.6
Mucous Membrane	272	0.02-1.6
Incidental Inhalation-Spray	19; 777[a]; 1488[b]	0.39-0.89; 1[a]; 0.39-1.9[b]
Incidental Inhalation-Airbrush	1	NR
Incidental Inhalation-Powder	1; 1488[b]; 4[c]	0.39-1.9[b]; 0.4-1.6[c]
Dermal Contact	2735	0.008-2.9
Deodorant (underarm)	23 (not spray); 1 (spray)	NR
Hair - Non-Coloring	112	0.4
Hair-Coloring	22	NR
Nail	40	NR
Other Preparations (Unknown Exposure Type)	23	NR
as reported by product category		
Baby Products		
Baby Shampoos	1	NR
Baby Lotions/Oils/Powders/Creams	4	NR
Other Baby Products	2 (r.o.)	1 (r.o.)
Bath Preparations		
Bubble Baths	4	NR
Bath Capsules	NR	0.02
Other Bath Preparations	8	NR
Eye Makeup Preparations (other than children's eye makeup preparations)		
Eyebrow Pencil	2	NR
Eyeliner	16	NR
Eye Shadow	39	0.49
Eye Lotion	27	0.5-0.8
Eye Makeup Remover	1	NR
False Eyelashes	1	NR
Mascara	20	5
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)	22	NR
Eyelash Cleansers	3	NR
Other Eye Makeup Preparations	20	NR
Fragrance Preparations		
Cologne and Toilet Water	2	0.89
Perfumes	9	0.39
Other Fragrance Preparation	4	NR
Hair Preparations (non-coloring)		
Hair Conditioners	8 (l.o.); 1 (r.o)	0.4 (l.o.)
Hair Sprays (aerosol fixatives)	1	NR

Table 2. Frequency and concentration of use of Polyacrylate Crosspolymer-6 according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use
	RLD (2025)[5,6]	% (2025)[7]
Hair Straighteners	1	NR
Permanent Waves	1	NR
Shampoos (non-coloring)	11 (r.o.)	NR
Tonics, Dressings, and Other Hair Grooming Aids	27	NR
Other Hair Preparations	53 (l.o.); 8 (r.o.)	NR
Hair Coloring Preparations		
Hair Dyes and Colors (all types requiring caution statements and patch tests)	1	NR
Hair Lighteners with Color	15	NR
Eyelash and Eyebrow Dyes	2	NR
Other Hair Coloring Preparation	4	NR
Makeup Preparations (not eye; not children's)		
Blushers and Rouges (all types)	23	NR
Face Powders	1	NR
Foundations	30 (traditional application); 1 (airbrush application)	0.5 (traditional application)
Leg and Body Paints	8 (traditional application)	NR
Lipsticks and Lip Glosses	225	0.34-1.6
Makeup Bases	32 (traditional application)	0.008-0.05 (traditional application)
Makeup Fixatives	7	NR
Other Makeup Preparations	36	NR
Manicuring Preparations		
Basecoats and Undercoats	1	NR
Nail Extenders	2	NR
Nail Polish and Enamel	17	NR
Nail Polish and Enamel Removers	10	NR
Other Manicuring Preparations	10	NR
Personal Cleanliness		
Bath Soaps and Body Washes	24	0.7
Deodorants (underarm)	23 (not spray); 1 (spray)	NR
Shaving Preparations		
Aftershave Lotions	6	NR
Beard Softeners	2	NR
Pre-shave Lotions (all types)	2	NR
Shaving Creams (aerosol, brushless, and lather)	3	NR
Skin Care Preparations (creams, lotions, powder, and sprays)		
Cleansing (cold creams, cleansing lotions, liquids, and pads)	135	1-2
Depilatories	2	NR
Face and Neck (excluding shaving preparations)	1117 (l.o.), 140 (r.o.)	not spray: 0.4-1.6 (l.o.), 0.8-1.4 (r.o.)
Body and Hand (excluding shaving preparations)	139 (l.o.), 11 (r.o.)	not spray: 0.7 (l.o), 2.4 (r.o.)
Foot Powders and Sprays	1	NR
Moisturizing	389	0.85 (not spray)
Night	92	0.5 (not spray)
Paste Masks (mud packs)	40	1.2-2.9
Skin Fresheners	28	NR
Other Skin Care Preparations	151 (l.o.); 47 (r.o.)	0.39-1.9 (l.o.); 1 (r.o.)
Suntan Preparations		
Suntan Gels, Creams, and Liquids	54	0.5 (not spray)
Indoor Tanning Preparations	9 (traditional application); 3 (spray)	NR
Other Suntan Preparations	4	NR
Other Preparations (i.e., those preparations that do not fit another category)	23	NR

NR – not reported
l.o. – leave-on; r.o. – rinse-off
* The sum of the counts given for duration of use and by exposure type, and the sum of the frequency reported by product category, may not equal the sum of total uses because each ingredient may be used in cosmetic formulations that are reported under more than one product category.
**Likely duration and exposure are derived from survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)
[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.

Table 3. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
IRRITATION						
ANIMAL						
Polyacrylate Crosspolymer-6	NR	>90% (no further information given)	Rabbits (no further information given)	OECD TG 404; no further information given	No effects observed. Substance was considered to non-irritating.	[2]
SENSITIZATION						
IN VITRO						
Polyacrylate Crosspolymer-6	NR	5000 µg/ml (no further information given)	NR	Human cell line activation test (h-CLAT); OECD TG 442E; no further information given	CD86 and CD54 were not overexpressed. Substance did not induce dendritic cell activation.	[3]
Polyacrylate Crosspolymer-6	DMSO	100% (no further information given)	NR	Sens-IS assay; no further information given	Substance considered to be a non-sensitizer.	[3]
HUMAN						
Eye cream containing 0.5% Polyacrylate Crosspolymer-6	none	0.2 g of test material	102 subjects	HRIPT using 0.6 in^2 occlusive patches. Induction consisted of 9 applications (3 times per week for 3 wk). Challenge patch applied after a 10-d non-treatment period; observations were made on days 1 and 3 post- application.	Not an irritant or a sensitizer.	[9]
Leave-on face product containing 1.1% Polyacrylate Crosspolymer-6	none	tested neat 0.2 ml/mg of test material applied 50 mg/cm^2 dose density of product applied	107 subjects	HRIPT using 1 cm^2 24-h occlusive patches. Induction phase lasted 3 wk with 9 total induction patches. Challenge patch was applied after a 2-wk non-treatment period; observations were made 24, 48, 72, and 96 h post-application.	Non-sensitizing. The participants experienced either no visible reaction or some erythema with only 2 subjects having edema.	[10]
Polyacrylate Crosspolymer-6	NR	~5% (no further information given)	NR	HRIPT was completed using Marzulli-Maibach Method; no further information given	No effects observed. Substance considered non-sensitizing and non-irritating	[2]

DMSO – dimethyl sulfoxide; h-CLAT - human cell line activation test; HRIPT - human repeated-insult patch test; NR – not reported; OECD – Organisation for Economic Co-operation and Development; TG – test guideline

Table 4. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Protocol	Results	Reference
IN VITRO						
Eye cream containing 0.5% Polyacrylate Crosspolymer-6	none	applied neat in a 100 µl application	EpiOcular™ tissues	MTT assay to measure ET_{50}; cells treated for 4, 8, 16, and 24 h; positive control was 0.3% Triton X-100 solution while negative control was sterile, deionized water	ET_{50} was 23.1 h while positive control was 22 min. Since $ET_{50} > 60$ min, the ocular irritation potential was non-irritating.	[11,12]
Polyacrylate Crosspolymer-6	NR	2% (no further information given)	NR	HET-CAM test was completed; no further information given	The substance was predicted to be non-irritating.	[2]
ANIMAL						
Polyacrylate Crosspolymer-6	NR	NR	3 rabbits	OECD TG 405; no further information given	There was redness of the conjunctiva, slight discharge, and chemosis in all rabbits. The substance was slightly irritating to the eye.	[2]

ET_{50} - exposure time required to reduce cell viability by 50%; HET-CAM - hen's egg test on the chorioallantoic membrane; MTT - (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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4. U.S. Food and Drug Administration. Federal Food, Drug, and Cosmetic Act Section 612 Title 21.
5. US Food and Drug, Administration (FDA) Office of Colors & Cosmetics (OCAC). 2025. Data from: Registration and Listing of Cosmetic Product Facilities and Products. College Park, MD [Obtained under the Freedom of Information Act].
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8. EUR-Lex. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast) (Text with EEA relevance). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009R1223&qid=1682529291932.
9. Anonymous. 2023. Repeated Insult Patch Test of an eye cream containing 0.5% Polyacrylate Crosspolymer-6. [Unpublished data submitted by Personal Care Products Council on March 11, 2026].
10. Anonymous. 2026. Summary and data tables of an HRIPT of a product containing 1.1% Polyacrylate Crosspolymer 6. [Unpublished data submitted to Cosmetic Ingredient Review on May 6, 2026].
11. Anonymous. 2023. Topical application ocular irritation screening assay using the Epiocular™ human cell construct of an eye cream containing 0.5% Polyacrylate Crosspolymer-6. [Unpublished data submitted by Personal Care Products Council on March 11, 2026].
12. Stern M, Klausner M, Alvarado R, Renskers K, Dickens M. Evaluation of the EpiOcular((TM)) tissue model as an alternative to the draize eye irritation test. *Toxicology in Vitro*. 1998;12(4):455–461.

Concentration of Use by FDA Product Category[1]

Polyacrylate Crosspolymer-6

Product Category	Maximum Concentration of Use
Other baby products Rinse-off	 1%
Bath capsules	0.02%
Eye shadows	0.49%
Eye lotions	0.5-0.8%
Mascaras	5%
Colognes and toilet waters	0.89%
Perfumes	0.39%
Hair conditioners Leave-on	 0.4%
Foundations Traditional	 0.5%
Lipstick	0.34-1.6%
Makeup bases Traditional	 0.008-0.05%
Bath soaps and body washes	0.7%
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	1-2%
Face and neck products (not spray) Leave-on Rinse-off	 0.4-1.6% 0.8-1.4%
Body and hand products (not spray) Leave-on Rinse-off	 0.7% 2.4%
Moisturizing products (not spray)	0.85%
Night products (not spray)	0.5%
Paste masks and mud packs	1.2-2.9%
Other skin care preparations Leave-on Rinse-off	 0.39-1.9% 1%
Suntan products (not spray)	0.5%

Information collected in 2025

Table prepared: March 31, 2025

[1] The new FDA cosmetic product categories under MoCRA were used for this survey.

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: January 7, 2026

SUBJECT: Polyacrylate Crosspolymer-6

Anonymous. 2026. Summary information – Polyacrylate Crosspolymer-6.

January 2026

Summary Information Polyacrylate Crosspolymer-6

Method of manufacturing data (pertaining to cosmetic formulations): Polyacrylate Crosspolymer-6 is precipitated out from a polymerization reaction.

Impurities data: Dimethyl acrylamide < 20 ppm.

Dermal irritation and sensitization data (at maximum concentration of use):

Sens-Is: The skin sensitization potential of Polyacrylate Crosspolymer-6 was evaluated in a Sens-Is assay (draft OECD method: https://www.oecd.org/content/dam/oecd/en/events/public-consultations/2024/7/draft-test-guidelines-sens-is-assay.pdf). The test substance was concluded to be a non-sensitizer when tested up to pure in DMSO.

h-CLAT: The skin sensitization potential of Polyacrylate Crosspolymer-6 was evaluated according to the human Cell Line Activation Test (h-CLAT, OECD 442E). The test substance did not induce dendritic cell activation (CD86 and CD54 were not overexpressed) when tested up to the maximum recommended dose of 5000 µg/mL.

Personal Care Products Council

Memorandum

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

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

DATE: May 6, 2026

SUBJECT: Summary of an HRIPT on a product containing 1.1% Polyacrylate Crosspolymer-6

Anonymous. 2026. Summary and data tables of an HRIPT of a product containing 1.1% Polyacrylate Crosspolymer 6.

Product Number	% Polyacrylate Crosspolymer 6	Product Type	HRIPT Test Yes/No	Occlusivity	Completed Subjects	Did formula induce an allergic response
1	1.1	Leave-on	YES	occlusive	107	NO

Product Number 1

Calculation of Amount of Polyacrylate Crosspolymer-6 mg/cm2	
Concentration of Polyacrylate Crosspolymer-6 in %	1.1
Amount of Product applied to Skin during HRIPT in ml/mg	0.2
Patch Size cm^2	1
Dose density of product applied to patched skin in mg/cm2	50
Dose Density of Disodium Lauroamphodiacetate applied to patch skin in mg/cm2	0.55000000
Was the product diluted or undiluted? Undiluted	

ICDRG Reading scale	
0	No Visible Reaction
±	Faint Minimal Erythema
1	Erythema
2	Intense Erythema, Induration
3	Intense Erythema, Induration, Vesicles
4	Severe reaction with Erythema, Induration, Vesicles (may be weeping)

E	Edema
–	No reading

Details of Test methodolgy and Results	
0	panelist discontinued due to test material reactions
24 hrs	patch duration
9	induction patches
3	weeks induction
2	week rest period
virgin site	challenge
24, 48, 72, 96 hrs post patching	Challenge readings

Grading Scale interpretation	
Low Level Reactions	1
High Level Reaction	2 and above

[redacted]

polyacrylate crosspolymer 6 1.1%
Leave-On Face product

[redacted]

[redacted]

TABLE I: SUMMARY OF REACTIONS

TOTAL NUMBER OF SUBJECTS ENROLLED: 118
TOTAL NUMBER OF SUBJECTS COMPLETED: 107

Reaction	Induction Reading									Challenge Reading			
Grade	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**1**	**2**	**3**	**4**
0	115	108	102	98	97	91	92	89	95	100	103	102	107
±		1	7	8	11	17	16	18	11	7	4	5	
1		1	2	4	2	1		2	3				
1E		1					1						
2													
2E													
3E													
4E													
-													
N9R													
Total	115	111	111	110	110	109	109	109	109	107	107	107	107

SCORING SYSTEM:
0 = No visible reaction
± = Faint, minimal erythema
1 = Erythema
2 = Intense erythema, induration
3 = Intense erythema, induration, vesicles
4 = Severe reaction with erythema, induration, vesicles, pustules (may be weeping)
E = Edema
- = No reading
N9R = No 9th reading

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 11)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
1		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	0	0	0	0	0	0	0	0	0	0	0	0
4		0	0	0	0	0	0	0	0	0	0	0	0	0
5		0	0	0	0	0	0	0	0	0	0	0	0	0
6		0	0	0	0	0	0	0	0	0	0	0	0	0
7		0	0	0	0	0	0	0	0	0	0	0	0	0
8		0	0	0	0	0	0	0	0	0	0	0	0	0
9		0	0	0	0	±	0^	0^	0^	0^	0	0	0	0
10		0	0	0	0	0	0	0	0	0	0	0	0	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
13		0	0	0	0	0	0	0	0	0	0	0	0	0
14		0	0	0	0	0	0	0	0	0	0	0	0	0
15		0	X	X	X	X	X	X	X	X	X	X	X	X
16		0	0	±	0	0	±	±	±	0	0	0	0	0
17		0	0	0	0	0	0	0	0	0	0	0	0	0
18		0	0	0	0	0	0	0	0	0	0	0	0	0
19		0	0	0	X	X	X	X	X	X	X	X	X	X
20		0	0	0	0	0	0	0	0	0	0	0	0	0
21		0	0	0	0	0	0	0	0	0	0	0	0	0
22		0	0	0	0	0	0	0	0	0	X	X	X	X
23		0	X	X	X	X	X	X	X	X	X	X	X	X
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

[redacted]

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 11)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
26		0	0	0	0	0	0	0	0	0	±	0	0	0
27		0	0	0	0	0	0	0	±	±	0	0	0	0
28		0	0	0	0	0	0	0	0	0	0	0	0	0
29		0	0	0	0	0	0	0	0	0	0	0	0	0
30		0	X	X	X	X	X	X	X	X	X	X	X	X
31		0	X	X	X	X	X	X	X	X	X	X	X	X
32		0	0	0	0	0	0	0	0	0	0	0	0	0
33		0	1	1	1	±	±	±	±	1	0	0	0	0
34		0	0	±	±	±	±	±	±	±	0	0	0	0
35		0	0	±	1	1	±	±	1	1	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
38		0	0	0	0	0	0	0	0	0	0	0	0	0
39		0	0	0	0	0	0	0	0	0	0	0	0	0
40		0	±	±	±	±	±	±	±	±	0	0	0	0
41		0	0	0	0	0	0	0	0	0	0	0	0	0
42		0	0	0	0	0	0	0	0	0	0	0	0	0
43		0	1EC	0	0	0	±	±	±	0	0	0	0	0
44		0	0	0	0	0	0	0	0	0	0	0	0	0
45		0	0	0	0	0	0	0	0	0	±	±	0	0
46		0	0	0	0	0	0	0	0	0	0	0	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
50		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 11)

Sub	[redacted]	Age	Induction Reading 1	2	3	4	5	6	7	8	9	Challenge Reading 1	2	3	4
51		64	0	0	±	±	±	±	±	±	±	0	0	0	0
52		66	0	0	0	0	0	0	0	±	0	0	0	0	0
53		40	0	0	0	0	0	0	0	0	0	0	0	0	0
54		68	0	0	0	0	0	0	0	0	0	0	0	0	0
55		36	0	0	0	0	0	0	0	0	0	0	0	0	0
56		42	0	0	0	0	0	0	0	0	0	0	0	0	0
57		51	X	X	X	X	X	X	X	X	X	X	X	X	X
58		26	0	0	0	0	0	0	±	±	±	0	0	0	0
59		24	0	0	0	0	0	0	0	0	0	0	0	0	0
60		66	0	0	0	0	0	0	0	0	0	0	0	0	0
61		67	0	0	0	0	0	0	0	0	0	0	0	0	0
62		29	0	0	0	0	0	0	0	0	0	±	0	0	0
63		52	0	0	0	0	0	0	0	0	0	0	0	0	0
64		69	0	0	1	1	0^	±^	±^	±^	±^	0	0	0	0
65		42	0	0	0	0	0	0	0	0	0	0	0	0	0
66		35	0	0	0	0	0	0	0	0	0	0	0	0	0
67		64	0	0	0	±	±DR	±	1EC	0	0	0	±	±	0
68		29	0	0	0	0	0	0	0	0	0	0	0	0	0
69		66	0	0	0	0	0	0	0	0	0	0	0	0	0
70		60	0	0	0	0	0	±	±	±	1	0	0	0	0
71		34	0	0	0	0	0	0	0	0	0	0	0	0	0
72		45	0	0	0	0	0	0	0	0	0	0	0	0	0
73		58	0	0	0	0	0	0	0	0	0	0	0	0	0
74		57	0	0	0	0	0	0	0	0	0	0	0	0	0
75		22	0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 11)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
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		0	0	0	0	0	0	0	0	0	0	0	0	0
79		0	0	0	0	0	0	0	0	0	±	0	0	0
80		0	0	0	0	0	0	0	±	0	0	0	0	0
81		0	0	0	0	0	0	0	0	0	0	±	±	0
82		0	0	0	0	0	0	0	0	0	0	0	±	0
83		0	0	0	0	±	±	±	1	±	0	0	0	0
84		0	0	0	±DR	±	±	0	0	0	0	0	0	0
85		0	0	0	0	0	0	0	0	0	0	0	0	0
86		0	0	0	0	0	0	0	0	0	0	0	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	X	X	X	X
89		0	0	0	0	0	0	0	0	0	0	0	0	0
90		0	0	0	0	0	±	0	0	0	0	0	0	0
91		0	0	0	0	0	0	0	0	0	±	0	0	0
92		0	0	0	0	0	0	0	0	0	0	±	±	0
93		0	0	0	0	0	0	±	0	0	0	0	0	0
94		0	0	0	±	0	0	0	0	0	0	0	0	0
95		0	0	0	0	0	0	0	0	0	0	0	0	0
96		0	0	±	±	±	±	±	±	±	0	0	0	0
97		0	0	0	0	0	0	0	0	0	0	0	0	0
98		0	0	0	0	±	±	±	±	±	0	0	0	0
99		0	0	0	0	0	0	0	0	0	±	0	0	0
100		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 11)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
101		0	0	0	0	0	0	0	0	0	0	0	0	0
102		0	0	0	0	0	0	0	0	0	0	0	0	0
103		0	0	0	0	0	0	0	0	0	0	0	0	0
104		0	0	0	0	0	0	0	0	0	0	0	0	0
105		0	0	0	0	0	0	0	0	0	0	0	0	0
106		0	0	0	0	0	0	0	0	0	0	0	0	0
107		0	0DR	0DR	0	0	0	0	±	0	0	0	0	0
108		0	0	0	0	0	0	0	0	0	0	0	0	0
109		0	0	0	0	0	0	0	0	0	0	0	0	0
110		0	0	0	1P	1	1P	±	±	±	0	0	0	0
111		0	0	0	0	0	0	0	±	0	±	0	0	0
112		X	X	X	X	X	X	X	X	X	X	X	X	X
113		0	0	0	0	0	0	0	0	0	0	0	0	0
114		0	0	0	0	0	0	0	0	0	0	0	0	0
115		0	0	0	0	0	0	0	0	0	0	0	±	0
116		0	0	0	0	0	X	X	X	X	X	X	X	X
117		0	0	0	0	0	0	0	0	0	0	0	0	0
118		X	X	X	X	X	X	X	X	X	X	X	X	X

[redacted]

[redacted]

SCORING SYSTEM*:

0	=	No visible reaction
±	=	Faint, minimal erythema
1	=	Erythema
2	=	Intense erythema
3	=	Intense erythema, induration, vesicles
4	=	Severe reaction with erythema, induration, vesicles, pustules (may be weeping)
E	=	Edema
DR	=	Dryness
P	=	Peeling
S	=	Staining
^	=	Hyperpigmentation / Hypopigmentation
TR	=	Tape Reaction
C	=	Change of test site
N9R	=	No 9th reading
-	=	No reading
X	=	Discontinued

*International Contact Dermatitis Research Group System: Fisher, Alexander A., *Contact Dermatitis*, Lea & Febiger, Philadelphia, 2008: p 27. (Modified)

[redacted]

Personal Care Products Council

Memorandum

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

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

DATE: March 11, 2026

SUBJECT: Studies on an eye cream containing 0.5% Polyacrylate Crosspolymer-6

Anonymous. 2023. Repeated Insult Patch Test of an eye cream containing 0.5% Polyacrylate Crosspolymer-6.

Anonymous. 2023. TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT of an eye cream containing 0.5% Polyacrylate Crosspolymer-6.

Stern M, Klausner M, Alvarado R, et al. 1998. Evaluation of the EpiOcular™ Tissue Model as an Alternative to the Draize Eye Irritation Test. Toxicology in Vitro 12: 455-461.

[redacted]

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
[redacted]
Protocol Date: 03/30/23

TEST MATERIAL: 4304-2.01 Eye Cream
containing 0.5% Polyacrylate Crosspolymer-6

STUDY NUMBER: [redacted]

Reviewed by: [redacted]
Board Certified Dermatologist

Approved by: [redacted]

[redacted]

[redacted]

Clinical Summary

One hundred two (102) subjects completed the Repeated Insult Patch Test. Observations remained negative throughout the test phases. Test material, 4304-2.01 Eye Cream, did not induce irritation nor any evidence of induced allergic contact dermatitis in human subjects.

[redacted]

[redacted]

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[redacted]

[redacted]

QUALITY ASSURANCE UNIT STATEMENT

Study Number: [redacted]

[redacted]

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

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

All records and documents pertaining to the conduct of this trial shall be retained in the [redacted] for a minimum of five (5) years. At any time prior to the completion of the fifth archival year, a Sponsor may submit a written request to the [redacted] to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period with no further notice in a manner that renders them useless.

[redacted]

[redacted]

[redacted]

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

Subjects: One hundred fifteen (115) qualified subjects, male and female, ranging in age from 18 to 70 years, were selected for this evaluation. One hundred two (102) subjects completed this clinical trial. The remaining subjects discontinued their participation for various reasons unrelated to the application of the test material.

Inclusion Criteria:

1. Subjects who had read, signed, and dated an Informed Consent Form that included a HIPAA statement;
2. Subjects who were male or female, aged 18 to 70 years, inclusive; and
3. Subjects who were considered reliable and capable of understanding and following directions.

Exclusion Criteria:

1. Subjects who were in ill health, as determined by the Principal Investigator;
2. Subjects who were taking medication, other than birth control, that, in the opinion of Investigator, might have influenced the purpose, integrity, or outcome of the trial;
3. Subjects who had used any prescribed or OTC anti-inflammatory, antihistamine, corticosteroid, immunosuppressant, or antibiotic drug for at least 7 days prior to the initiation of the trial, or during their participation on this trial;
4. Female subjects who were pregnant, planning to become pregnant, or lactating during the course of the trial;
5. Subjects with any visible disease, or sunburn, scars, excessive tattoos, etc., that might have been confused with a skin reaction to the test material or, in the opinion of the Principal Investigator, interfere with the evaluation;
6. Subjects who had a history of adverse reactions to cosmetics, adhesive tapes, OTC drugs, or other personal care products; or
7. Subjects who introduced the use of any new cosmetic, toiletry, or personal care products during the trial.

Test Material: 4304-2.01 Eye Cream
Description: Eye Cream

Trial Schedule:

Panel #	Initiation Date	Completion Date
20230079	April 12, 2023	June 8, 2023

[redacted]

[redacted]

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 g of the test material, or an amount sufficient to cover the contact surface, was applied to the 0.6 in^2 absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

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

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

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

Challenge Phase:

After at least 10 days following the final Induction Phase patch removal, a Challenge Phase patch was applied to a virgin test site adjacent to the original Induction Phase patch site, following the same procedure described for Induction. The patch was removed and the test site evaluated at the clinic on Day 1 and Day 3 post-application. The scale below was used to score the reactions during the Induction and Challenge phases.

Evaluation (Erythemal Scoring Scale (ESS) and Additional Reaction Scoring)

Erythema at the test site was evaluated according to the ESS shown below.

[redacted]

[redacted]

Methodology (continued):

Score	Description
0	No visible erythema
0.5	Slight, barely perceptible erythema
1	Mild erythema
2	Moderate erythema
3	Marked erythema
4	Severe erythema

If present, each additional reaction sequela was evaluated according to the following numerical scale to indicate severity and letter scale as shown below.

Severity Score	Description
1	Mild
2	Moderate
3	Marked
4	Severe

Letter	Description
B	Bullae
D	Dryness
E	Edema
P	Papules
S	Staining
Sp	Spreading
U	Ulceration
V	Vesicles

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

[redacted]

[redacted]

[redacted]

[redacted]

Results:

A Summary of Observations is presented in Table 1. The results of each participant are appended (Table 2). Table 3, Attrition Form, lists each subject, time of discontinuation and reason.

Observations remained negative throughout the test interval.

Subject demographics are presented in Table 4.

Summary:

Under the conditions of this clinical trial, test material, 4304-2.01 Eye Cream, did not indicate a potential for dermal irritation or allergic contact sensitization.

[redacted]

[redacted]

Table 1

Summary Observations

4304-2.01 Eye Cream

Reaction Scores	Induction Readings										Challenge Readings	
	Day 1	1	2	3	4	5	6	7	8	9	Day 1	Day 3
0	113	108	108	108	107	107	106	105	105	105	105	102
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0

Reaction Scores:

Score	Description
0	No visible erythema
0.5	Slight, barely perceptible erythema
1	Mild erythema
2	Moderate erythema
3	Marked erythema
4	Severe erythema

[redacted]

FINAL REPORT

TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT

cwc apc_k am l r_g l g l e .,3# Nmjw_apwj_rc Apmqqnmjw k cp+4

Laboratory Study Number:

23AD56-AD59.015001

Sponsor Study Number:

[redacted] 4304-1, [redacted]

Study Completion Date:

5 July 2023

Authors:

[redacted]

Sponsor

[redacted]

Performing Laboratory:

[redacted]

Laboratory Project Number:

12943

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Initiation Date:	9 May 2023
Laboratory Start Date:	15 May 2023
Laboratory Completion Date:	18 May 2023
Completion Date:	5 July 2023

[redacted]

[redacted]

[redacted]

[redacted]

TEST ARTICLE RECEIPT

[redacted] Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
[redacted]				
23AD58	4304-1.01 Eye Cream	white cream	21 April 2023	15 to 30 °C (Room Temp)
[redacted]				

* - Protected from exposure to light

INTRODUCTION

The EpiOcular™ Human Cell Construct was used to assess the potential ocular irritation of the test articles. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular metabolism after exposure to each test article for various exposure times[1]. The duration of exposure resulting in a 50% decrease in MTT conversion in test article-treated EpiOcular™ human cell constructs, relative to control cultures, was determined (ET_{50}).

MATERIALS AND METHODS

The assay procedures were performed as outlined in the study protocol (See Appendix A).

DEVIATIONS

There were no deviations from the study protocol during the conduct of this study.

[1] Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

RESULTS AND DISCUSSION

Test Article Preparation

The test articles, [redacted] 4304-1.01 Eye Cream, and [redacted] were administered to the test system without dilution (neat). [redacted]

Due to its viscosity, dosing devices (i.e., flat-headed cylinders of slightly less diameter than the inner diameter of the tissue insert) were placed over the dose of the test article to ensure a more even spreading over the surface of the tissues for the test article, 4304-1.01 Eye Cream.

Direct MTT Reduction Test

The test articles, [redacted], were not observed to directly reduce MTT in the absence of viable tissue. Therefore, a killed control experiment was not performed.

The test article, 4304-1.01 Eye Cream, was observed to directly reduce MTT in the absence of viable tissue. Therefore, a killed control experiment was performed. The results of the killed control experiment showed that there was little or no direct MTT reduction in the test article-treated killed control compared to the negative control-treated killed controls and the MTT reduction in the test article-treated viable tissue was ascribed to the viable cells.

Test Article & Control Exposures

One hundred microliters of the test articles, [redacted], 4304-1.01 Eye Cream, [redacted], were tested in duplicate EpiOcular™ tissues at 4 exposure times of 4, 8, 16, and 24 hours. Similarly, the test article, [redacted] was tested at 4 exposure times of 1, 2, 4, and 8 hours. The positive control, 100 µL of 0.3% Triton X-100 Solution, was exposed in duplicate tissues for 5, 20, and 60 minutes. The negative control, 100 µL of sterile, deionized water, was treated in duplicate tissues for 1, 8, and 24 hours.

Residual Test Article

The test article, 4304-1.01 Eye Cream, could not be completely removed from the exposed viable tissues following the rinsing and soaking process after the 4, 8, and 16 hour exposure times and from all killed control tissues. In addition, residual test article was also noted on the second 24-hour viable tissue treated with the test article and minimal residual test article was observed on the first 24-hour viable tissue.

For the test article, 4304-1.01 Eye Cream, the residual test article may have influenced the toxic effect, lowering the ET_{50} result.

Evaluation of Test Results

Table 1 summarizes the ET_{50} for each of the test article and positive control exposures. The ET_{50} values for the test articles, [redacted] and 4304-1.01 Eye Cream, were calculated by interpolation of two exposure times, one that showed less than 50% relative survival and one that showed greater than 50% survival. For the test articles, [redacted] as all of the exposure times showed greater than 50% survival, the ET_{50} value was presented as greater than the maximum exposure time.

Criteria for a Valid Test

The assay results were accepted when: 1) The ET_{50} value of the positive control fell within the acceptance criteria range of 10.4 – 45.0 minutes, and 2) The OD_{570} value for the minimum negative control exposure time of 60 minutes was greater than 1.100.

Table 1
Summary Results of the EpiOcular™ Screening Assay
Assay Date: 17 May 2023

IIVS Test Article Number	Sponsor's Designation	Conc. (w/v)	ET_{50} (Hours)	pH
[redacted]				
23AD58	4304-1.01 Eye Cream	100%	23.1	5.0
[redacted]				
Positive Control	0.3% Triton™ X-100 Solution	100%	22.0 minutes	NA

NA – Not Applicable

Based on Table 2 of Stern et al. 1998, the result of an ET50 of 23.1 hours means the product is non-irritating.
Table 2 of Stern et al. indicates that an ET50> 60 minutes indicates non/minimal irritation.

APPENDIX A (Protocol & Protocol Attachment 1)

TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT

1.0 PURPOSE

The purpose of this study is to evaluate the potential ocular irritation of the test article by measuring 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) dye conversion by the EpiOcular™ tissue construct after topical exposure to the test article.

2.0 [redacted]

3.0 IDENTIFICATION OF TEST ARTICLE(S) AND ASSAY CONTROLS

3.1	Test Article(s):	See Protocol Attachment 1	
3.2	Assay Controls:	Positive:	0.3% Triton-X-100 Solution (prepared in sterile, deionized water) by MatTek Corporation (or equivalent)
		Negative:	Sterile deionized water or other solvent as appropriate

[redacted]

4.0 [redacted]

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 15 May 2023

5.2 Proposed Experimental Completion Date: 2 June 2023

5.3 Proposed Report Date: 14 July 2023

6.0 TEST SYSTEM

The EpiOcular™ human cell construct, provided by the MatTek Corporation, will be used in this study. The use of EpiOcular™ cultures offers features appropriate for a model for ocular irritation. First, the model is composed of stratified human keratinocytes in a three-dimensional structure. Secondly, test materials can be applied topically to the model so that water-insoluble materials may be tested. Prior to use, each plate (6-, 12-, 24-, and 96-well) will be uniquely identified with a number written in permanent marker on the plate and its cover, the test article number, and the exposure time.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The experimental design of this study consists of a determination of the direct MTT reduction potential and the pH of the pipettable test article if possible (and/or dosing solution as appropriate) and a single definitive assay. The toxicity of the test article will be evaluated by the exposure time required to reduce tissue viability to 50% of the negative control (ET_{50} value). Viability will be determined by the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, by the succinate dehydrogenase reduction of MTT) in control and test article-treated cultures (Berridge, et al., 1996). Data will be presented in the form of relative survival (relative MTT conversion) versus test article exposure time.

One of two exposure time ranges may be used. The short exposure study extends up to 4 hours. A long exposure study might be used for extremely mild materials, such as those that might be applied around or in the eyes. For the long exposure study, exposure times of up to 24 hours could be used. Alternative exposure times may be used at the study director's discretion.

7.1 Media and Reagents:

7.1.1 EpiOcular™ Assay Medium: OCL-200-MM supplied by MatTek Corporation

7.1.2 EpiOcular™ Tissue: OCL-200_2.0 supplied by MatTek Corporation

7.1.3 Dulbecco's Modified Eagle's Medium (DMEM) containing 2 mM L-glutamine by Quality Biological (or equivalent) (MTT Addition Medium)

7.1.4 Sterile deionized water by Quality Biological (or equivalent)

7.1.5 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) 10X stock solution: 10 mg/mL MTT in Phosphate Buffered Saline (PBS)

7.1.6 Sterile Ca^{++} and Mg^{++} Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS)

7.1.7 Extraction Solvent: Isopropanol

7.2 Environmental Conditions

Throughout this protocol, ranges for test material and test system exposure or incubation conditions (e.g., temperature, humidity, CO_2) are presented. These ranges describe the equipment performance specifications under static conditions (i.e., in the absence of frequent opening of equipment doors, accessing chambers, changing loads, etc.), as presented in the relevant equipment SOPs.

7.3 Preparation and Delivery of Test Article

Test articles will generally be tested neat. End-use concentrations or other forms may be used as directed by the Sponsor. Alternate volumes of the test article may be used as specified by the Sponsor. Alternate volumes or concentrations will be specified in Protocol Attachment 1.

One hundred microliters of pipettable substances, such as liquids, gels, creams, and foams, will be applied directly onto the tissue to cover the upper surface. To aid in filling the pipette for pipettable materials that are viscous, the test article may first be transferred to a syringe. The pipette tip of the positive displacement pipette will be inserted into the dispensing tip of the syringe so that the material can be loaded into the displacement tip under pressure. Simultaneously, the syringe plunger is depressed as the pipette piston is drawn upwards. If air bubbles appear in the pipette tip, the test article should be removed (expelled) and the process repeated until the tip is filled without air bubbles. This method should be used for any materials that cannot be easily drawn into the pipette such as gels (e.g., toothpastes, mascaras, and face creams) and solid test articles that are creamed like lipsticks and antiperspirants/deodorant sticks. A dosing device (a flat-headed cylinder of slightly less diameter than the inner diameter of the tissue insert) may be placed over the test article to assure even spreading if required.

Materials that are too viscous to spread over the tissue will first be spread onto the flat end of a dosing device. When the test article must first be applied to a dosing device, approximately 30 µL or 30 ± 1 mg of material will be applied to the dosing device to cover the dosing surface. The sample should be spread to form a relatively smooth even layer on the surface of the dosing device to maximize uniform tissue contact. The dosing device

will then be placed into the cell culture insert to bring the test article in contact with the tissue.

Solids such as lipsticks or antiperspirant/deodorant sticks can be pre-softened by creaming a portion in a weigh boat. The softened portion can be transferred to a syringe affixed with a three-way stopcock attached to a second syringe. The sample is pushed from syringe to syringe until it is of a consistency that can be pipetted.

Dry powders may be ground with a mortar and pestle and passed through a #40 copper sieve if needed. Powders will be placed directly onto the culture at approximately 30 ± 1 mg/culture.

The exact exposure conditions used for other test article forms will be determined after consultation with the Sponsor and/or the Study Director. All exposure conditions will be documented in the study workbook.

7.4 Route of Administration

The test article(s) will be administered by topical application to the construct.

7.5 pH Determination

The pH of the pipettable test article (and/or dosing solution as appropriate) will be determined, if possible. The pH will be determined using pH paper (for example, with a pH range of 0 to 14 to estimate, and/or a pH range of 5 to 10 to determine a more precise value). The typical increments on the pH paper used to report the pH are approximately 0.3 to 0.5 pH units. The maximum increment on the pH paper is 1.0 pH units.

7.6 Controls

Generally, at least 1 negative control exposure time will be used and treated with 100 µL of sterile, deionized water. One negative control exposure time will be selected to fit the range of the shortest test article or positive control exposure times (typically 60 minutes). On occasion, additional negative control exposure times may be selected to fit the longest test article exposure time of a test article run concurrently, but from an independent study. For the long exposure assay (exposures of greater than 4 hours), multiple negative control exposure times may be selected to fit the range of test article exposure times (e.g., 8 and 24 hours). Additional negative control exposure times may be selected at the discretion of the Study Director. Positive control cultures are treated with 100 µL of 0.3% Triton-X-100 Solution prepared in sterile deionized water and are exposed for 5, 20, and 60 minutes. At least 2 cultures will be used for each negative and positive control exposure time.

7.7 Assessment of Direct Test Article Reduction of MTT

It is necessary to assess the ability of each test article to directly reduce MTT. A 1.0 mg/mL MTT solution will be prepared in warm MTT Addition Medium as described in § 7.9. The approximate appropriate dosing volume as outlined in § 7.3 will be added to 1 mL of the

MTT solution, and the mixture incubated in the dark at 37 ± 1 °C in a humidified atmosphere of 5 ± 1% CO_2 in air (standard culture conditions) for 1 to 3 hours. The negative control (100 µL of sterile, deionized water) will be run concurrently. If the MTT solution color turns blue/purple, the test article is presumed to have reduced the MTT and a killed control experiment will be performed as described in § 7.10. If a test article is dark colored and potential MTT reduction cannot be observed, a killed control experiment will be performed. Water-insoluble test materials may show direct reduction (darkening) only at the interface between the test article and the medium.

The MTT direct reduction test for the test article(s) may have been previously performed in an independent study. In such cases, the results of the MTT direct reduction test may be used for this specific study and the initial study will be referenced.

7.8 Receipt of the EpiOcular™ model

Upon receipt of the EpiOcular™ assay materials, the solutions will be stored as indicated by the manufacturer. The tissue will be stored at 2 to 8 °C until used. Tissues should generally be used within 2 days of receipt from the manufacturer.

On the day of dosing, EpiOcular™ Assay Medium will be warmed to approximately 37 °C. Nine-tenths (0.9) milliliters of Assay Medium will be aliquoted into the appropriate wells of labeled 6-well plates. The 6-well plates will be labeled with the test article(s) and exposure time(s). Each tissue will be inspected for air bubbles between the agarose gel and cell culture insert before opening the sealed package. Cultures with air bubbles covering greater than 50% of the cell culture insert area will not be used. Each 24-well shipping container will be removed from its plastic bag and its surface disinfected by wiping with 70% ethanol-soaked tissue paper. An appropriate number of tissues will be transferred aseptically from the 24-well shipping containers into the 6-well plates. The EpiOcular™ tissues will be incubated at standard culture conditions for at least 1 hour. The medium will be aspirated, and 0.9 mL of fresh, warm (37 ± 1 °C) Assay Medium will be aliquoted into each assay well below the tissue prior to dosing.

Upon opening the bag, any unused tissues remaining on the shipping agar at the time of tissue transfer will be briefly gassed with an atmosphere of 5% CO_2/95% air, and the bag will be sealed and stored at 2 to 8 °C for subsequent use.

7.9 Definitive MTT Assay

Generally, 4 to 5 exposure times will be tested for each test article. Three exposure times may be tested in the long-term exposure assay if there is sufficient evidence that the ET_{50} value will exceed 24 hours. In the short-term exposure assay, if the expected range of toxic response is unknown, a 20-minute exposure time may be performed first to determine the remaining exposure durations. The maximum exposure time will be 240 minutes unless otherwise directed by the Sponsor.

Each test article and control exposure time will be tested by treating 2 tissues. The dosing procedure will be determined as indicated in § 7.3 and § 7.6. Generally, exposure times

of 10 minutes or less will be incubated at room temperature and exposure times of greater than 10 minutes will be incubated at standard culture conditions.

At the end of the treatment time, the test article and assay controls will be removed by extensively rinsing both sides of the culture with sterile, room temperature CMF-DPBS. The process will be performed until the culture appears free from any test article. or controls.

After rinsing, the tissue will be decanted onto absorbent paper and transferred to approximately 5 mL of Assay Medium (warmed to approximately 37 °C) for a 10 to 20-minute soak at room temperature. This rinse is intended to remove any test article absorbed into the tissue.

A 10X stock of MTT prepared in PBS (filtered at time of batch preparation) will be thawed and diluted in warm MTT Addition Medium to produce the 1.0 mg/mL solution that will be used within 2 hours of preparation. Alternatively, a 1.0 mg/mL MTT solution will be prepared in warm MTT Addition Medium and filtered through a 0.45 µm filter to remove undissolved crystals. Three hundred microliters of the MTT solution will be added to each designated well of a labeled 24-well plate. At the end of the 10 to 20-minute soak, the tissues will be transferred to the appropriate wells of the 24-well MTT plate after rinsing with sterile, room temperature CMF-DPBS and decanting onto absorbent paper. The plates incubated for 3 ± 0.1 hours under standard culture conditions. If it is not possible to remove all of the visible test material after this rinse it will be noted in the workbook.

After 3 ± 0.1 hours, the bottom of the EpiOcular™ tissue constructs will be blotted on absorbent paper, cleared of excess liquid, and transferred to a labeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates will be sealed with parafilm and stored in the refrigerator (2 to 8 °C) until the last exposure time is harvested. If necessary, plates may be stored overnight (or up to 20 hours after the last exposure time is harvested) in the refrigerator prior to extracting the MTT. The plates will then be shaken for 2 to 3 hours at room temperature. At the end of the extraction period, the liquid within each cell culture insert will be decanted into the well from which it was taken. The extract solution will be mixed and 200 µL transferred to the appropriate wells of a labeled 96-well plate(s). Two hundred microliters of isopropanol will be added to the wells designated as blanks. The absorbance at 570 nm (OD_{570}) of each well will be measured with a Molecular Devices VersaMax plate reader with the "shake" function selected. If the extraction solution is cloudy and/or precipitate is noted after extraction, the sample may be transferred into centrifuge tubes and centrifuged (in a centrifuge with settings up to 15,000 rpm, for up to 5 minutes at room temperature) prior to transfer to the 96-well plates for the absorbance determination.

7.10 Killed Controls for Assessment of Residual Test Article Reduction of MTT

In cases where the test article is shown to reduce MTT, or is dark colored and MTT reduction could not be determined, test articles that remain bound to the tissue after rinsing, resulting in a false MTT reduction signal, present a problem. To demonstrate that

any residual test article is not acting to directly reduce the MTT, a functional check is performed in the definitive assay to show that the test material is not binding to the tissue and leading to a false MTT reduction signal.

To determine whether any residual test article is acting to directly reduce the MTT, a freeze-killed control tissue is used. Freeze-killed tissues are prepared in accordance with facility procedures or may be received already prepared from MatTek Corporation. To test for residual test article MTT reduction, freeze-killed tissues (killed controls) are treated with the test article in the same fashion as for the viable tissues. Generally, each test article will be evaluated for at least the shortest and longest exposure times (or longest exposure time if all exposures are 1 hour or less) in single freeze-killed tissues. All assay procedures for killed controls will be performed as for the viable tissues. At least 1 killed control treated with sterile, deionized water (negative killed control) will be tested in parallel since a small amount of MTT reduction is expected from the residual NADH and associated enzymes within the freeze-killed tissue. If any test articles treated in the long exposure time assay require killed controls, then a long term negative control killed control may also be tested in parallel.

If little or no MTT reduction is observed in the test article-treated killed control, the MTT reduction observed in the test article-treated viable tissue may be ascribed to the viable cells. If there is appreciable MTT reduction in the treated killed control, additional steps must be taken to account for the chemical reduction, or the test article may be considered untestable in this system. The OD_{570} values from the killed controls will be analyzed as described in § 7.11.

7.11 Presentation of Data

The raw absorbance values will be captured, and the following calculations made:

The mean OD_{570} value of the blank wells will be calculated. The blank corrected mean OD_{570} value of the negative control(s) will be determined by subtracting the mean OD_{570} value of the blank wells from the mean negative control OD_{570} values for each exposure time. The blank corrected OD_{570} value of the individual test article or positive control tissues for each exposure time will be determined by subtracting the mean OD_{570} value of the blank wells from their respective OD_{570} values. Generally, all calculations will be performed using Microsoft Excel.

$$Blank\ Corrected\ OD_{570} = Raw\ Tissue\ OD_{570} - Blank\ Mean\ OD_{570}$$

If killed controls (KC) are used, the following additional calculations will be performed to correct for the amount of MTT reduced directly by test article residues. The raw OD_{570} value for the appropriate negative control killed control will be subtracted from the raw OD_{570} values for each of the test article-treated killed controls (at each exposure time), to determine the net OD_{570} values of the test article-treated killed controls.

$$Net\ KC\ OD_{570} = Raw\ KC\ OD_{570} - Raw\ \ Negative\ Control\ KC\ OD_{570}$$

The net KC OD_{570} values represent the amount of reduced MTT due to direct reduction by test article residues at specific exposure times. In general, if the net KC OD_{570} value is greater than 0.150, the net amount of MTT reduction will be subtracted from the blank corrected OD_{570} values of the viable treated tissues, at each corresponding exposure time, to obtain a final corrected OD_{570} value. If killed controls are not used or the net KC OD_{570} value is minimal (≤ 0.150), then no other adjustments are necessary and the final corrected OD_{570} value for each test article tissue at each exposure time will be the blank corrected OD_{570} value. These final corrected OD_{570} values will be used to determine the % of Control viabilities at each exposure time for each tissue.

$$Final\ Corrected\ OD_{570} = Blank\ Corrected\ \text{Tissue}\ OD_{570}\ (viable) - Net\ KC\ OD_{570}\ )$$

Next, the following % of Control calculations will be made for each test article or positive control tissue at each exposure time:

$$\%\ of\ Control\ = \frac{Final\ Corrected\ OD_{570}}{Corrected\ Mean\ OD_{570}\ of\ Negative\ Control} \times 100$$

Viability calculations for test articles treated in the long exposure time assay may be performed by comparing the final corrected OD_{570} values of each test article tissue at each exposure time to the appropriate negative control(s). Finally, the individual % of Control values are then averaged to calculate the mean % of Control per exposure time.

Exposure time response curves may be plotted with the % of control on the ordinate and the test article or positive control exposure time on the abscissa. Other plot forms may be used as requested by the Sponsor. The ET_{50} value will be interpolated from each plot. To determine the ET_{50} value, two adjacent points will be selected, one that shows greater than 50% survival and one that shows less than 50% survival. The two selected points will be used to determine the slope and the y-intercept for the equation y = m(x) + b. Finally, to determine the ET_{50} value, the equation will be solved for y = 50. If all of the exposure time points show greater than 50% survival, the ET_{50} value will be listed as greater than the longest exposure time. If all of the exposure times show less than 50% survival, the ET_{50} value may be presented as less than the shortest exposure time; or a theoretical exposure time of 0 with a viability of 100% may be added to calculate the ET_{50} value. At the Study Director's option, additional assays may be performed to produce the final ET_{50} value.

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The assay will be accepted if the positive control, 0.3% Triton-X-100 Solution, causes a ET_{50} value within 2.0 standard deviations of the historical mean as established by the testing facility. The range will be updated every 3 months. Additionally, the blank corrected mean OD_{570} value for the minimum negative control exposure time (e.g., 60 minutes) must be greater than 1.100.

9.0 EVALUATION OF TEST RESULTS

The significance of the ET_{50} value is dependent on the class of materials tested. The Sponsor should refer to existing information in the literature to determine the significance of the ET_{50} value of this test article. If necessary, the Irritancy Classifications of Draize and EpiOcular™ Data Ranges may be used to determine an irritancy classification based on the ET_{50} value (Stern, *et al.*, 1998).

10.0 REPORT

A report of this study will be prepared by the Testing Laboratory and will accurately describe all methods used for generation and analysis of the data. A summary will be presented for each treatment group. The report will also include a discussion of the results. A copy of the protocol used for the study and any significant deviation(s) from the protocol will appear as a part of the final report.

11.0 RECORDS AND ARCHIVES

A separate working notebook will be used to record the materials and procedures used to perform this study. Upon completion of the final report, all raw data, reports, and specimens will be retained in the archives for a period of either a) 5 years, b) the length of time specified in the contract terms and conditions, or c) as long as the quality of the preparation affords evaluation, whichever is applicable.

12.0 TEST MATERIAL RETENTION

Unless indicated otherwise, all test articles provided by the Sponsor will be retained for 6 months after completion of the final report. These test articles may be disposed of after this 6-month retention period according to IIVS' procedure. Unless indicated otherwise, dose dilutions used for testing or analysis before or during the course of the assay will be discarded after testing.

13.0 PROTOCOL AMENDMENTS

When it becomes necessary to change the approved protocol for a specific study, the change and the reason for it should be put in writing and signed by the Study Director as soon as practical. When the change may impact the study design and/or execution, a verbal agreement to make this change should be made between the Study Director and Sponsor. This document is then provided to the Sponsor and is attached to the protocol as an amendment.

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15.0 APPROVAL

[redacted]

Pergamon

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Toxicology in Vitro

Evaluation of the EpiOcular™ Tissue Model as an Alternative to the Draize Eye Irritation Test

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Abstract—Cosmetic ingredients were tested to determine the ability of the EpiOcular™ tissue model to predict eye irritation potential. *In vitro* results were compared with historical Draize eye irritation records. Forty-three samples, consisting of 40 cosmetic raw ingredients of different type and physical form (i.e. liquids, powders, gels) were evaluated. Using the MTT cytotoxicity assay, an ET_{50} value (effective time of exposure to reduce tissue viability to 50%) was determined for each sample. ET_{50} values were categorized into four irritation groups: (a) non-irritating/minimal; (b) mild; (c) moderate; or (d) severe/extreme. Comparison of *in vitro* EpiOcular™ and *in vivo* Draize classifications showed that 63% (27 of 43 samples) were classified identically. Assay performance improved to 95% (41 of 43 samples) with the addition of samples overpredicted by a single irritation class. This evaluative exercise represents a conservative safety assessment. There were no underpredictions of eye irritation for any material in this study. Based on these results, use of the EpiOcular™ tissue model shows promise as an *in vitro* assay to assess the ocular irritation potential of cosmetic ingredients.

Abbreviations: DMEM = Dulbecco's modified Eagle's medium; DPBS = Dulbecco's phosphate buffered saline; MAS = maximum average score; OD = optical density; ET_{50} = time for 50% viability.

INTRODUCTION

In order to replace *in vivo* eye irritation testing (Draize *et al.*, 1944), many different *in vitro* alternatives are being evaluated, undergoing redesign or are in further development. At the Second World Congress on Alternatives and Animal Use in the Life Sciences, no fewer than 21 *in vitro* alternatives to the Draize rabbit eye irritation test were presented (Van der Valk *et al.*, 1996). Based on presentations at this meeting (Brantom *et al.*, 1996), one of the more promising *in vitro* assays was the Tissue Equivalent Assay or TEA (Osborne *et al.*, 1995). Other studies have also demonstrated that tissue equivalent models have promise in predicting ocular irritancy potential (Fisher *et al.*, 1995; Ghassmei *et al.*, 1997; Rachui *et al.*, 1994a,b; Swanson *et al.*, 1995). The present study investigated whether past successes, seen with other tissue equivalent assays, could also be achieved with the EpiOcular™ (OCL-200) model system (MatTek Corporation, Ashland, MA, USA).

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MATERIALS AND METHODS

Chemicals

Dulbecco's modified Eagle's medium (DMEM)-based assay medium, Triton X-100 (positive control), extraction medium (isopropyl alcohol), MTT concentrate and diluent were obtained from MatTek Corporation (Ashland, MA, USA). Dulbecco's phosphate buffered saline (DPBS) with calcium and magnesium chloride was obtained from Sigma Chemical Co. (St Louis, MO, USA) or Gibco BRL (Grand Island, NY, USA). Chemicals used for the evaluation (Table 1) were obtained from the Standards Department, Avon Products Inc. (Suffern, NY, USA). The test chemicals represented a range of ocular irritancies (i.e. non, mild, moderate, severe), physical/chemical form (i.e. solid, liquid, gel, range of aqueous solubility) and chemical type (i.e. surfactant, preservative, emollient, colourant, etc.).

Cell cultures

EpiOcular™ tissue was purchased from MatTek Corporation as tissue inserts (Millicells™). MatTek describes its EpiOcular™ tissue model as consisting

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PII: S0887-2333(98)00017-4

Table 1. Evaluation of the MatTek EpiOcular™ assay: comparison of Draize data with ET_{50}

Chemical	Draize test		Epi	Epi	Test	Ingredient	Physical
	Score*	Class+	ET_{50}**	class+	concn %++	function	nature
Cloisonne Red	0	A	> 240	A/B	100	Colourant	Powder
C12–15 Alcohols benzoate	0	A	> 240	A/B	100	Emollient	Liquid
Zinc phenolsulfonate	0	A	123.9	A/B	5	Biocide	Powder
Diazolidinyl urea	0	A	147.6	A/B	5	Preservative	Powder
Methylchloroisothiazolinone and other ingredients	0	A	> 240	A/B	0.3	Preservative	Liquid
2-Phenoxyethanol	0	A	60.3	A/B	2	Preservative	Liquid
PEG-120 methyl glucose dioleate	0	A	> 60	A/B	25	Surf. Nonionic	Solid
Polyquaternium-11 (50%)	0	A	> 60	A/B	100	Conditioning Agent	Liquid
Salicylamide	0	A	> 60	A/B	5	Chemical Additive	Powder
Cocamide DEA	0	A	> 240	A/B	10	Surf. Nonionic	Liquid
Cationic emulsion (35%)	1	B	41.7	C	10	Surf. Cationic	Liquid
Imidazolidinyl urea	1	B	155.5	A/B	100	Preservative	Powder
Isopropyl palmitate	1	B	> 240	A/B	100	Emollient	Liquid
Methylparaben	1	B	> 60	A/B	100	Preservative	Powder
Polyquaternium-2	1	B	178.9	A/B	100	Surf. Cationic	Liquid
Isopropyl myristate	1	B	> 240	A/B	100	Emollient	Liquid
Polysorbate 60	1	B	> 240	A/B	100	Emulsifier	Liquid
Octyl dimethyl PABA	2	B	> 240	A/B	100	UV Absorber	Liquid
Palm oil	3	B	> 240	A/B	100	Conditioning Agent	Paste
Nonionic/anionic amphoteric blend II	3	B	48.5	C	20	Surf. Amphoteric	Liquid
Benzalkonium chloride (50%)	6	B	> 240	A/B	2	Surf. Cationic	Liquid
Octyl salicylate	6	B	> 240	A/B	100	UV Absorber	Liquid
Triethanol amine cocoyl glutamate (30%)	9	B	51.5	C	10	Surf. Nonionic	Liquid
Cocamidopropyl betaine	18	C	< 3	E/F	25	Surf. Amphoteric	Liquid
Pareth-25-12	20	C	17.2	D	100	Surf. Nonionic	Paste
Nonoxynol-12	20	C	32.0	C	50	Surf. Nonionic	Liquid
Nonoxynol-12	20	C	11.3	D	50	Surf. Nonionic	Liquid
Sodium laureth sulfate (30%) preserved	23	C	19.5	D	10	Surf. Anionic	Liquid
Sodium methyl cocoyl taurate	24	C	9.9	D	10	Surf. Anionic	Powder
Sodium lauramphoacetate	25	C	22.1	D	20	Surf. Amphoteric	Liquid
Menthoxypropanediol	25	C	< 3	E/F	20	Flavouring Agent	Liquid
Sodium C14–16 olefin sulfonate (90%)	26	D	15.0	D	100	Surf. Anionic	Powder
Stearalkonium chloride (25%)	26	D	28.3	D	8	Surf. Cationic	Paste
Miranol 2MCA mod (38%)	31	D	18.3	D	100	Surf. Amphoteric	Liquid
TEA lauryl sulfate (40%)	33	D	7.1	D	100	Surf. Anionic	Liquid
Sodium cetearyl sulfate	35	D	23.2	D	100	Surf. Anionic	Powder
2-Phenoxyethanol	34	D	< 2	E/F	100	Preservative	Liquid
Zinc phenolsulfonate	36	D	4.4	D	100	Biocide	Powder
Disodium cocoamphodiacetate (50%)	38	D	< 2	E/F	100	Surf. Amphoteric	Liquid
Sodium lauryl sulfate	38	D	2.7	E/F	10	Surf. Anionic	Powder
Lauryl monophosphate	41	D	2.7	E/F	25	Surf. Anionic	Paste
Benzyl alcohol	42	D	< 2	E/F	100	Preservative	Liquid
Chlorohexidine digluconate (20%)	87	F	< 2	E/F	100	Biocide	Liquid

*Draize score was based on the 24-hr maximum average score.
**ET_{50} = Time point when cell viability drops to 50% as determined by MTT assay.
+ Irritancy Classes as described in Table 2.
++ Equivalent concentrations were used for EpiOcular™ and Draize testing. liquids - vol./vol., solids - wt./vol.

of normal, human-derived epidermal keratinocytes cultured to form a stratified squamous epithelium similar to that found in the human cornea. A histological cross-section of the EpiOcular™ tissue is shown in Plate 1. Each batch of EpiOcular™ tissue (OCL-200) is tested with the negative control, ultrapure (18 Mohm) water, and the positive control, 0.3% Triton X-100. For calendar year 1996 (N = 47 batches), the negative control averaged 1.274 ± 0.138 OD units and the ET_{50} scores (effective time of exposure to reduce tissue viability to 50%) for the positive control averaged 24.9 ± 6.3 min.

Tissue preparation

Tissues were stored in the dark at 4°C and used within 48 hr of receipt. Using sterile technique, 1.0 ml warmed assay medium (37°C) was added to each well of a six-well plate. One Millicell™ containing the EpiOcular™ tissue was placed into each well of the six-well plate with the basal surface in contact with the medium. In this way nutrients could be supplied through the underside of the Millicell™ while simultaneously allowing topical application of test material. Tissues were incubated at 37°C in 5% CO_2/95% air in a humidifying incubator for 45 min. Following this equilibration period, the assay medium was removed by aspiration and replaced with 1.0 ml fresh, warmed assay medium.

Sample preparation and application

Liquid sample concentrations were prepared according to historical *in vivo* Draize test records. Deionized water was used for dilution. Solids and powders were applied neat, or dissolved/dispersed in water. Pastes were applied to just cover the flat end of a dosing device (a push pin with the point cut off). The pin was then placed onto the tissue, sample side down. Except for pastes, test materials were applied to the tissue surface at either 100 μl (liquid/suspension) or 100 mg (solid/powder) in duplicate. Test concentrations were chosen to match those used for *in vivo* testing.

Following sample application, tissues were incubated for specific time intervals at 37°C in 5% CO_2/95% air. Tissues treated with Triton X-100 (positive control) were incubated for 20 min and 60 min. Positive controls were consistent and varied within an acceptable range of ET_{50} scores, predicting mild to moderate irritation. In the initial phase of the evaluation, tissues treated with samples classified *in vivo* as "minimal" irritants were incubated for 60, 120 or 240 min; samples classified as "mild" irritants were incubated for 5, 20 or 60 min; while samples classified as "moderate–severe" irritants were incubated for 2, 10 or 30 min. In later stages of this evaluation, tissues for all samples were incubated for 3 min, 30 min and 60 min. Deionized water (negative control) was incubated at the longest time period (i.e. 60 or 240 min).

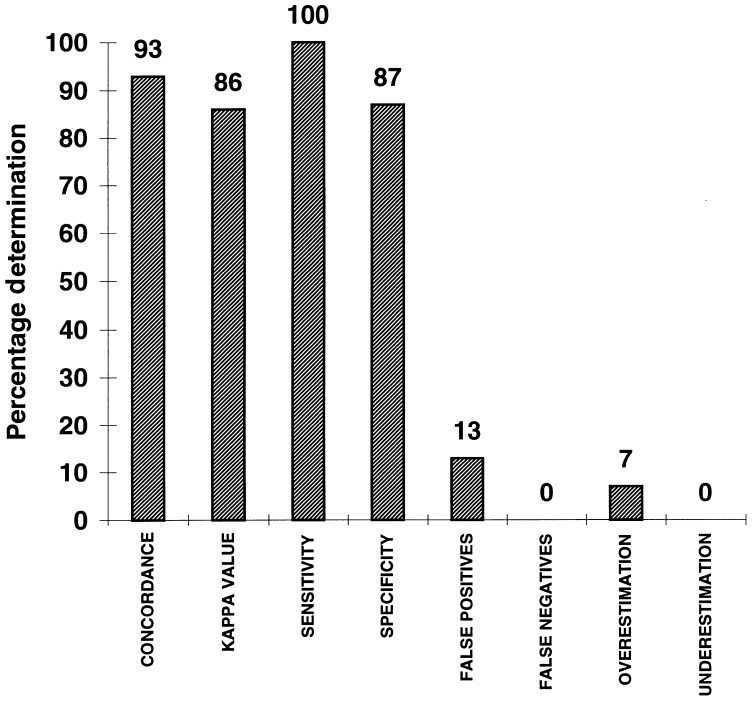

Fig. 1. Overall statistical comparison between EpiOcular™ and Draize test data.

MTT assay

Following incubation with a raw ingredient, tissues were removed from the six-well plates, rinsed thoroughly with DPBS, and placed into 12-well plates containing 5 ml assay medium per well. Tissues remained in medium for at least 10, but not more than 20 min, rinsed with DPBS and transferred to 24-well plates containing 0.3 ml of diluted MTT solution/well. These MTT plates were further incubated for 3 hr at 37°C in 5% CO_2. After incubation, the tissues were again rinsed with DPBS. MTT dye was extracted from the tissues by adding 2.0 ml extraction medium to each well at room temperature, or storing the plates overnight, in 2.0 ml extraction medium, at 4°C in sealed plastic bags. In either case, plates were then gently rocked (IKA-Shuttler MTS 4 plate rocker) at room temperature for 2 hr to extract the dye. Following extraction, 200 μl extraction solution for each sample was transferred to a 96-well plate. The plate was immediately read at 540 nm in a 7520 Microplate Reader (Cambridge Technology, Inc., Watertown, MA, USA). Optical densities (OD) were converted to % viability using the following formula:

$$\% \text{ viability} = 100 \times [\text{OD (sample)}/\text{OD (negative control)}]$$

The time for 50% viability (ET_{50}) was determined by linear interpolation between two points, one which exceeded 50% viability, the other less than 50% viability.

Historical Draize eye irritation data

For each ingredient, a 24-hr Draize maximum average score (MAS) was obtained from Avon's historical data bank (prior to 1989). The scores ranged from 0 to 87 (maximum possible score = 110) (Draize *et al.*, 1944).

Statistics

Analysis of the results involved the following parameters (Fleiss, 1973; Scala, 1987):

Concordance—Number of *in vitro* judgments which agreed with the *in vivo* judgments divided by the total number of samples tested

Kappa Value—Agreement existing beyond that expected by chance (1.0 = perfect agreement)

Sensitivity—Number of irritants correctly judged *in vitro* divided by the number of eye irritants tested

Specificity—Number of non-irritants correctly judged *in vitro* divided by the number of non-irritants tested

False Positives—Number of non-irritants assessed *in vitro* as irritants divided by the number of eye irritants tested

False Negatives—Number of irritants assessed *in vitro* as non-irritants divided by the number of non-irritants tested

Overestimation—Number of non-irritants assessed *in vitro* as irritants divided by the total number of samples tested

Underestimation—Number or eye irritants assessed *in vitro* as non-irritants divided by total number of samples tested.

RESULTS

A total of 40 raw ingredients (43 samples) were evaluated using the EpiOcular™ Tissue Model to determine the ability of this *in vitro* test to assess eye irritation potential. Raw ingredients included several chemical classes: 21 surfactants (five amphoteric, seven anionic, four cationic, and five nonionic), eight biocides/preservatives, three emollients, two conditioners, two UV absorbers, and one each of flavouring agent, emulsifying agent, colourant and chemical additive. The ingredients represented a full range of *in vivo* ocular irritancy, from non-irritating to extremely irritating. The physical nature of the ingredients was diverse, including liquids with a range of viscosities, powders and gels. As previously described, viabilities of two different time points were used to determine an ET_{50}. Each viability was calculated using the average of individual optical density readings from duplicate cultures. EpiOcular™ ET_{50} values and 24-hr Draize scores were converted to irritancy classifications as shown in Table 2. EpiOcular™ assay results and Draize classifications for each compound tested are presented in summary form to facilitate comparison (Table 1).

Results of statistical evaluations are described in Material and Methods, Statistics are shown in Fig. 1. Ocular irritancy predictions provided by the EpiOcular™ tissue model correlated well with

Table 2. Irritancy classifications of Draize and EpicOcular™ data ranges

Draize:			EpiOcular:		
Classification		Score range	Classification		ET_{50} range (min of exposure)
Non	(A)	0.0	Non/minimal	(AB)	> 60
Minimal	(B)	0.1–15.0			
Mild	(C)	15.1–25.0	Mild	(C)	31–60
Moderate	(D)	25.1–50.0	Moderate	(D)	3–30
Severe	(E)	50.1–80.0	Severe/Extreme	(EF)	< 3
Extreme	(F)	80.1–110.0			

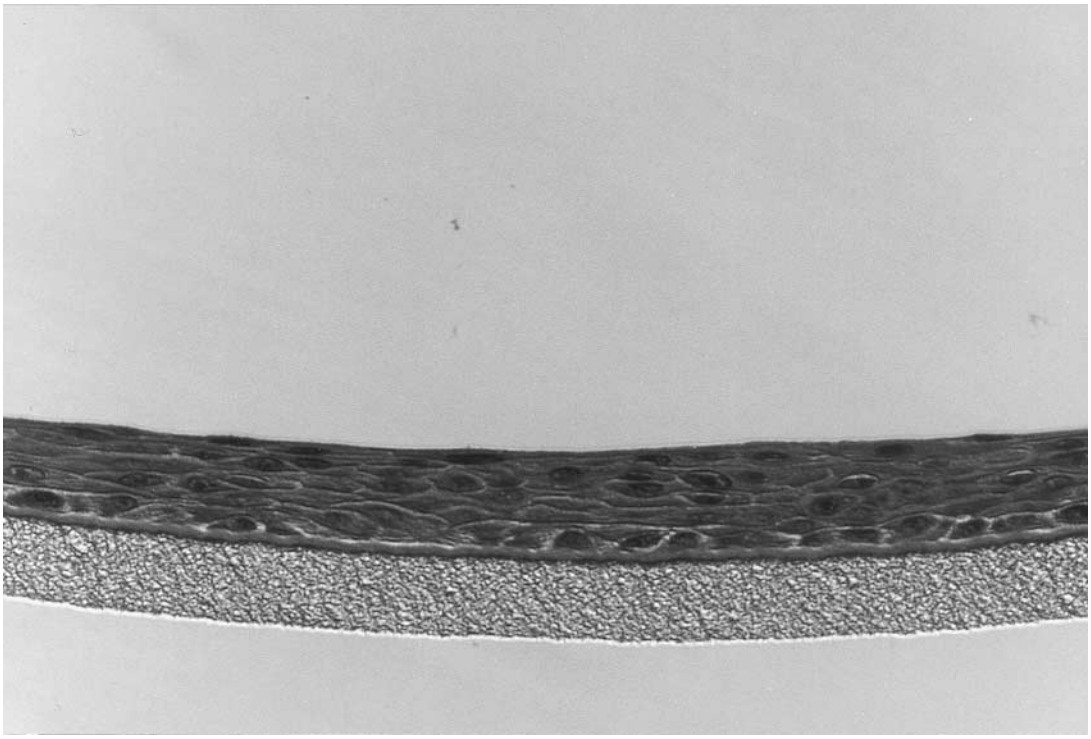

Plate 1. H & E stained histological cross-section of EpiOcular™ tissue. Final magnification ×430.

Draize judgments. For 28 compounds (65.1%), classification by EpiOcular™ was virtually identical to their Draize irritancy classification. For another 13 compounds (30.2%), EpiOcular™ and Draize classifications differed by one irritant class (e.g. moderate *v*. mild). In each of these instances the result with the EpiOcular™ tissue model overestimated the Draize judgment. The EpiOcular™ classification of two compounds overestimated Draize judgements by two classes (cocamidopropyl betaine, and menthoxypropanediol, severe/extreme *v*. mild). No samples were underestimated by EpiOcular™ evaluation.

DISCUSSION

The MatTek EpiOcular™ system was evaluated for its ability to predict the ocular irritancy classification of a set of chemicals commonly used in cosmetic products.

Several authors have noted variability in Draize eye irritancy testing (Balls *et al*., 1995; Lovell, 1996; Weil and Scale, 1971). Consequently, direct comparisons of the numerical scores from Draize eye irritancy testing with alternative assays have found only limited utility. Evaluation of the EpiOcular™ assay system relied on statistical comparison of EpiOcular™ irritancy classifications and 24-hr Draize judgments utilizing the irritancy classification as described in Table 2. The use of an irritancy classification scheme corrects for the inherent variability in Draize scoring. This evaluation thus provided identification of four EpiOcular™ classifications: non/minimal, mild, moderate, and severe/extreme, which are directly equivalent to their corresponding Draize classifications.

Figure 1 demonstrates the ability of the EpiOcular™ assay system to predict the irritation potential of cosmetic ingredients. Only 3 (13%) compounds predicted to be irritants by the EpiOcular™ assay were judged non-irritant in the Draize assay (false positive). More importantly, no compounds indicated to be irritants by *in vivo* analysis were predicted to be non-irritants by EpiOcular™ evaluation (false negative).

This study demonstrates that the EpiOcular™ model system is a viable alternative for the analysis of eye irritation potential. The method was able to quantify the effects of a variety of chemicals with different physical forms and ranges of ocular irritancy. Although the database for this method is small, additional studies will add to its viability and utility. Such studies could include chemicals with irritancy potentials greater than cosmetic ingredients and involve shorter time points of incubation. At a minimum, the EpiOcular™ model may be used to augment other alternative methods in determining the safety of cosmetic products and their ingredients.

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