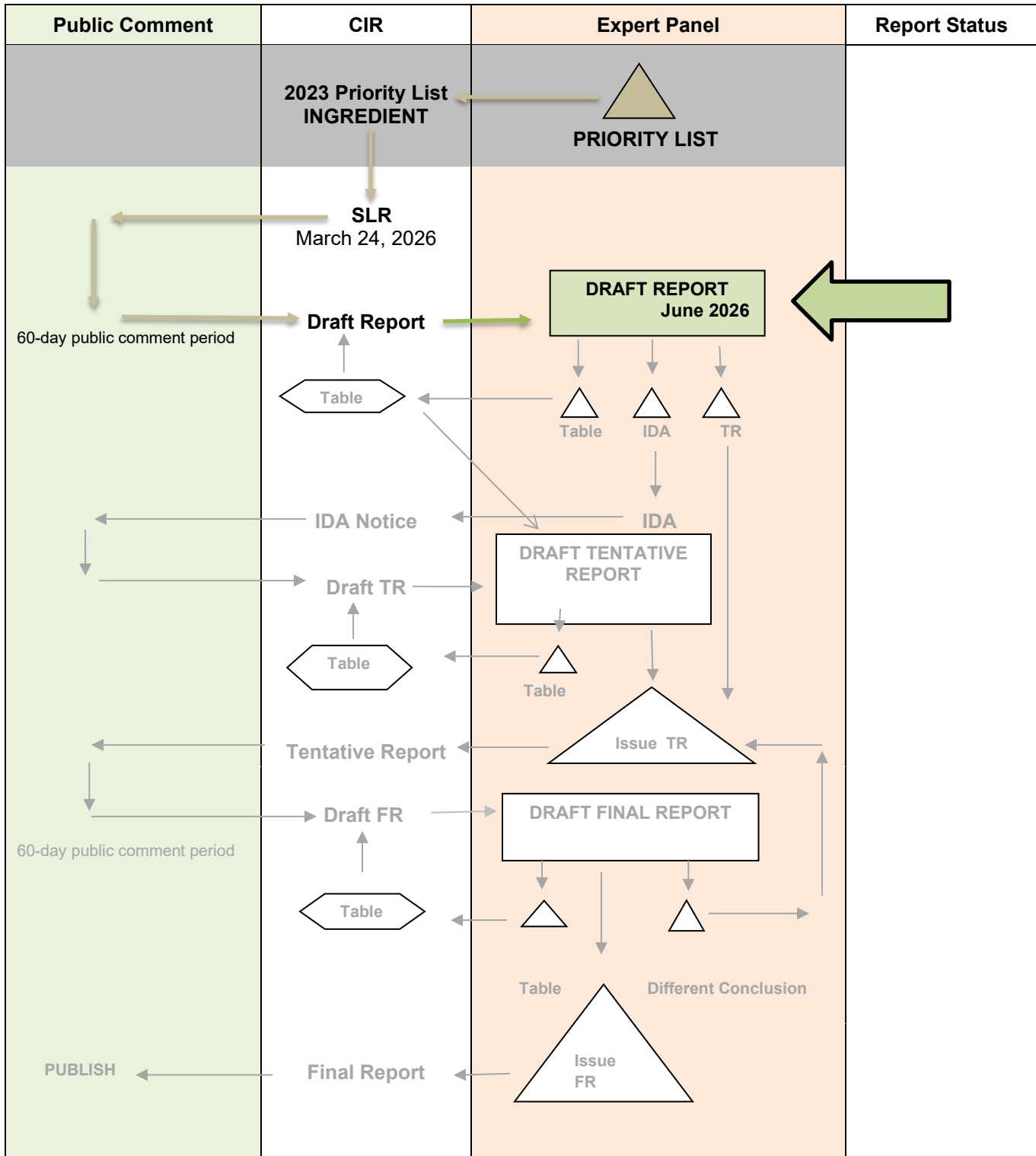

Safety Assessment of *Centaurea cyanus* Flower-Derived Ingredients as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: May 22, 2026
Earliest Possible Review: 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 Litta Paulson, M.P.H., Scientific Analyst/Writer, CIR.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Centauria cyanus Flower -Derived Ingredients
 MEETING June 2026





Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Litta Paulson, M.P.H., Scientific Analyst/Writer, CIR
Date: May 22, 2026
Subject: Safety Assessment of *Centaurea cyanus* Flower-Derived Ingredients

Enclosed is the Draft Report on the Safety Assessment of *Centaurea cyanus* Flower-Derived Ingredients as Used in Cosmetics. It is identified as *report_CentaureaCyanus_062026* in the pdf document. This is the first time the Panel is reviewing this report on the following 3 ingredients:

Centaurea Cyanus Flower
Centaurea Cyanus Flower Extract
Centaurea Cyanus Flower Water

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),” the product names were useful in determining the product type for some, but not all, formulations. Thereby, of the 2 products containing *Centaurea Cyanus* Flower reported as “other,” one is a body oil. However, for the other 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. Among 13 products containing *Centaurea Cyanus* Flower Extract categorized as “(17) Other preparations (i.e., those preparations that do not fit another category),” a few were co-categorized as “(06) Hair preparations (non-coloring)” or “(14) Skin care preparations (creams, lotions, powder, and sprays).” For the remaining products, the product names thereof were useful in determining the product type; they were hair removal creams, sheet masks, face serums, lip oils, a calming mask, a cleansing cloth, and a lip mask. The 3 products containing *Centaurea Cyanus* Flower Water, categorized as “(17) Other preparations (i.e., those preparations that do not fit another category)” were a make-up remover, an eye mask, and a face mask.

Results of a concentration of use survey using FDA cosmetic product categories under MoCRA were received and incorporated (*data1_CentaureaCyanus_062026*). In addition, since the issuing of the Scientific Literature Review (SLR), the following data were received, and have been incorporated into the report:

- Summary data on *Centaurea Cyanus* Flower Extract (*data2_CentaureaCyanus_062026*)
 - Chemical properties and method of manufacture
 - Composition and impurities
 - Acute oral toxicity
 - In vitro genotoxicity
 - Animal dermal irritation
 - Animal and human dermal sensitization
 - Animal ocular irritation
- Summary data on *Centaurea Cyanus* Flower Extract (*data3_CentaureaCyanus_062026*)
 - Composition and impurities
 - Human dermal irritation and sensitization
 - In vitro dermal sensitization
 - In vitro ocular irritation
 - In vitro phototoxicity
 - In vitro genotoxicity
- HRIPT data on a serum containing 0.0014% *Centaurea Cyanus* Flower Extract (*data4_CentaureaCyanus_062026*)

- Various data on Centaurea Cyanus Flower Extract (*data5_CentaureaCyanus_062026*)
 - Chemical properties and impurities
 - In vitro dermal irritation
 - In vitro ocular irritation
 - In chemico and in vitro dermal sensitization
 - In vitro phototoxicity
 - In vitro genotoxicity

Other information in this packet includes:

- comments on the Scientific Literature Review (SLR) from Council (*PCPCcomments_CentaureaCyanus_062026*)
- response to comments on the SLR (*response-PCPCcomments_CentaureaCyanus_062026*)
- flow chart (*flow_CentaureaCyanus_062026*)
- report history (*history_CentaureaCyanus_062026*)
- search strategy (*search_CentaureaCyanus_062026*)
- data profile (*datapofile_CentaureaCyanus_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.

***Centaurea cyanus* Flower-Derived Ingredients History**

March 2026

- SLR posted
- Comments on SLR from Council received

April 2026

- Summary data on *Centaurea Cyanus* Flower Extract received (chemical properties, method of manufacture, composition and impurities, acute oral toxicity, genotoxicity, dermal irritation and sensitization, ocular irritation)
- Summary data on *Centaurea Cyanus* Flower Extract received (composition and impurities, dermal irritation and sensitization, ocular irritation, phototoxicity, genotoxicity)
- Summary HRIPT data on a serum containing 0.0014% *Centaurea Cyanus* Flower Extract received
- Various data on *Centaurea Cyanus* Flower Extract received (chemical properties, impurities, dermal and ocular irritation, dermal sensitization, phototoxicity, genotoxicity)

June 2026

- Panel reviews Draft Report

Centaurea cyanus Flower-Derived Ingredients Data Profile* – June 2026 – Litta Paulson

					Toxico-kinetics	Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization					Ocular Irritation		Clinical Studies		
	Reported Use	GRAS	Method of Mfg	Constituents/Impurities	Dermal Penetration ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro/In Chemico	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports		
Centaurea Cyanus Flower	X			X																										
Centaurea Cyanus Flower Extract	X		X	X			X							X				X	X	X	X	X	X	X	X	X				
Centaurea Cyanus Flower Water	X																													

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

Centaurea cyanus Flower-Derived Ingredients

Ingredient	CAS #	PubMed	FDA	CompTox	ChemPort	NIOSH	NTIS	NTP	FEMA	EU	ECHA	SIDS	SCCS	AICIS	FAO	WHO	Web
Centaurea Cyanus Flower		X								X						X	X
Centaurea Cyanus Flower Extract	84012-18-0	X		X	X					X	X			X			X
Centaurea Cyanus Flower Water		X								X							X

“X” indicates data was found

Botanical and/or Fragrance Websites

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	AHPA	AGRICOLA	IFRA	RIFM
Centaurea Cyanus Flower		X	X	X					
Centaurea Cyanus Flower Extract	84012-18-0	X	X	X			X		
Centaurea Cyanus Flower Water		X	X	X					

“X” indicates data was found

Search Strategy

The following search string was used in PubMed:

((((centaurea cyanus) OR (centaurea cyanus flower)) OR (centaurea cyanus flower extract)) OR (centaurea cyanus flower water))
 [67 hits / 11 useful]

The following search terms were used in all links other than PubMed:

- Centaurea cyanus
- Centaurea cyanus flower
- Centaurea cyanus flower extract
- Centaurea cyanus flower water
- Cornflower
- 84012-18-0

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>
- 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/>

Botanical Websites, if applicable

- Dr. Duke's - <https://phytochem.nal.usda.gov/>
- Taxonomy database - <http://www.ncbi.nlm.nih.gov/taxonomy>
- GRIN (U.S. National Plant Germplasm System) - <https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx>
- Sigma Aldrich plant profiler- <http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html>
- American Herbal Products Association Botanical Safety Handbook (2nd Edition; 2013) - http://abc.herbalgram.org/site/DocServer/AHPABotanicalSafety_FMexcerpt.pdf?docID=4601
- National Agricultural Library NAL Catalog (AGRICOLA) <https://agricola.nal.usda.gov/>
- The Seasoning and Spice Association List of Culinary Herbs and Spices http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

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



Memorandum

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

FROM: Jaap Venema, Ph.D.
EVP and Chief Scientist – Personal Care Products Council
Industry Liaison to the CIR Expert Panel

DATE: March 30, 2026

SUBJECT: Scientific Literature Review: Safety Assessment of *Centaurea cyanus* Flower-Derived Ingredients as Used in Cosmetics (release date: March 24, 2026)

The Personal Care Products Council respectfully submits the following comments on the scientific literature review, Safety Assessment of *Centaurea cyanus* Flower-Derived Ingredients as Used in Cosmetics.

Definition and Plant Identification – It would be helpful to include the common names cornflower and bachelor’s button in this section.

In several places, the CIR report states that this plant is cultivated. As “weed” is a subjective word (any plant can be considered a weed if it is growing in the wrong place), please revise “flowering weed” to “flowering plant”.

Non-Cosmetic – Please indicate doses/concentrations and routes of exposure used in the studies included in the Non-Cosmetic section.

Please correct: “honey made from *Centaurea cyanus* pollen”. Bees make honey from nectar gathered from flowers, not pollen e.g., <https://www.sciencefocus.com/nature/how-do-bees-make-honey>

Summary – For someone that might just read the summary, please also include the common names of this plant in the Summary.

<i>Centaurea cyanus</i>-Derived Ingredients – June 2026 – Litta Paulson	
Subject: Scientific Literature Review: Safety Assessment of <i>Centaurea cyanus</i> Flower-Derived Ingredients as Used in Cosmetics (release date: March 24, 2026)	
Comment Submitter: Personal Care Products Council	
Date of Submission: March 30, 2026	
Comment	Response/Action
Definition and Plant Identification – It would be helpful to include the common names cornflower and bachelor's button in this section.	addressed
In several places, the CIR report states that this plant is cultivated. As “weed” is a subjective word (any plant can be considered a weed if it is growing in the wrong place), please revise “flowering weed” to “flowering plant”.	addressed
Non-Cosmetic – Please indicate doses/concentrations and routes of exposure used in the studies included in the Non-Cosmetic section.	addressed
Please correct: “honey made from <i>Centaurea cyanus</i> pollen”. Bees make honey from nectar gathered from flowers, not pollen e.g., https://www.sciencefocus.com/nature/how-do-bees-make-honey	addressed
Summary – For someone that might just read the summary, please also include the common names of this plant in the Summary	addressed

Safety Assessment of *Centaurea cyanus* Flower-Derived Ingredients as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: May 22, 2026
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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 Litta Paulson, M.P.H., Scientific Analyst/Writer, CIR.

ABBREVIATIONS

A459	human lung adenocarcinoma epithelial cell
Caco-2	human colorectal adenocarcinoma epithelial cell
CFU	colony forming unit
CGE	cyanidin-3-glucoside equivalent
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
COX-2	cyclooxygenase-2
<i>Dictionary</i>	<i>International Cosmetic Ingredient Dictionary</i>
DMSO	dimethyl sulfoxide
DPBS	Dulbecco's phosphate-buffered saline
DPRA	direct peptide reactivity assay
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GAE	gallic acid equivalent
GC-MS	gas chromatography-mass spectrometry
GI ₅₀	half-maximal growth inhibition concentration
HeLa	human cervical carcinoma cell
HepG2	human hepatocellular carcinoma cell
HRIPT	human repeated-insult patch test
IC ₅₀	half-maximal inhibitory concentration
IL	interleukin
iNOS	nitric oxide synthase
INT	<i>p</i> -iodonitrotetrazolium chloride
LD ₅₀	median lethal dose
l.o.	leave-on
LPS	lipopolysaccharide
MCF-7	human breast adenocarcinoma cell
MIC	minimum inhibitory concentration
MoCRA	Modernization of Cosmetics Regulation Act of 2022
MTT	3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCI-H460	non-small cell lung cancer cell
NR	not reported
NRU	neutral red uptake
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PLP2	porcine liver primary cell
QE	quercetin equivalent
RAW 264.7	mouse macrophage cell
RLD	Registration and Listing Data
r.o.	rinse-off
RT-qPCR	reverse transcription quantitative polymerase chain reaction
SDS	sodium dodecyl sulfate
SRB	sulforhodamine B
TG	test guideline
TLC	thin-layer chromatography
TNF- α	tumor necrosis factor- α
US	United States
UV	ultraviolet
UVA	ultraviolet A

INTRODUCTION

This assessment reviews the safety of the following 3 *Centaurea cyanus* flower-derived ingredients as used in cosmetic formulations:

Centaurea Cyanus Flower
Centaurea Cyanus Flower Extract
Centaurea Cyanus Flower Water

According to the *International Cosmetic Ingredient Dictionary (Dictionary)*, *Centaurea Cyanus Flower Extract* and *Centaurea Cyanus Flower Water* are both reported to function in cosmetics as skin-conditioning agents; *Centaurea Cyanus Flower Extract* is also reported to function as a cosmetic astringent and skin protectant (Table 1).¹ The *Dictionary* does not report a cosmetic function for *Centaurea Cyanus Flower*.

Centaurea cyanus flowers reviewed in this safety assessment may be consumed as food, and daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. The primary focus of the safety assessment of these ingredients as used in cosmetics is on the potential for effects from topical exposure.

Botanicals, such as *Centaurea cyanus* flower-derived ingredients, may contain hundreds of constituents. In this assessment, the Expert Panel for Cosmetic Ingredient Safety (Panel) is evaluating the potential toxicity of each of the *Centaurea cyanus* flower-derived ingredients as a whole, complex substance; toxicity from single components may not predict the potential toxicity of botanical ingredients.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted in 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 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.

It should be noted that though the primary focus of this report is *Centaurea cyanus* flower-derived ingredients, a few studies testing the whole *Centaurea cyanus* plant were included. The whole plant presumably contains the flower, and therefore the data may be meaningful for the Panel's consideration.

The cosmetic ingredient names, according to the *Dictionary*, are written as listed above, without italics and without abbreviations. When referring to the plant from which these ingredients are derived, the standard scientific practice of using italics will be followed (i.e., *Centaurea cyanus*). Often in the published literature, the general name cornflower is used. If it is not known whether the substance being discussed is equivalent to the cosmetic ingredient, the test substance will be identified by the name used in the publication that is being cited (*Centaurea cyanus* flower). However, if it is known that the substance is a cosmetic ingredient, the *Dictionary* nomenclature (e.g., *Centaurea Cyanus Flower*) will be used.

CHEMISTRY

Definition and Plant Identification

The definitions of the ingredients included in this review are provided in Table 1. All the ingredients are obtained from the flowers of the *Centaurea cyanus* plant. The CAS no. given in the *Dictionary* for *Centaurea Cyanus Flower Extract* is 84012-18-0.¹

Centaurea cyanus, commonly known as cornflower or bachelor's button, is a flowering plant that belongs to the family Asteraceae.² It is native to Europe and Asia but is now cultivated worldwide, including in the United States (US). The plant has a slender, densely pubescent stem that is bright green in color and up to 3 mm wide. The lanceolate leaves are also pubescent and feature a strong central vein.^{2,3} *Centaurea cyanus* inflorescences are monopodial and blue in color; flowerheads are 1.5 - 3 cm wide with sterile, funnel-shaped ray florets surrounding a central cluster of disc florets with tubular, fused petals. Numerous bracts, bordered by brown-toothed fringes, enclose the flower head. The plant is considered to have a pleasant, aromatic odor.

Chemical Properties

Centaurea Cyanus Flower Extract

According to an industry submission, a tradename mixture comprising *Centaurea Cyanus Flower Extract* (20%) and butylene glycol is a light yellow liquid with a mild characteristic odor.⁴ Additional chemical properties are found in Table 2.

Method of Manufacture

Centaurea Cyanus Flower

In some cases, the definition of an ingredient, as given in the *Dictionary*, provides insight as to the method of manufacture. The definition of *Centaurea Cyanus Flower* states it is an aqueous solution of the steam distillate obtained from the flowers of *Centaurea cyanus*.¹

Centaurea Cyanus Flower Extract

An industry submission stated that *Centaurea Cyanus Flower Extract* was prepared by extracting dried raw material with 1,3-butylene glycolic solution.⁵ The solution was then filtered, matured, filtered, adjusted, and packaged.

Composition and Impurities

Centaurea cyanus comprises compounds such as flavonoids, polyphenols, aromatic acids, amino acids, sugars, and coumarins.² *Centaurea cyanus* has also been found to contain heavy metals such as lead, zinc, copper, and chromium.⁶

Centaurea Cyanus Flower

A gas chromatography-mass spectrometry (GC-MS) analysis of fresh *Centaurea cyanus* flowers found a total of 24 sesquiterpenoids (including 15 bicyclic sesquiterpenes; 89.52%), 1 oxygenated monoterpene (5.22%), and 5 unknown volatile compounds (5.26%).⁷ The total anthocyanin (the component that gives the flowers their blue color) content of a *Centaurea cyanus* flower sample was determined to be 1012.09 mg/kg fresh weight.⁸ Anthocyanin concentration differs based on flower color, with the highest concentration found in purple flowers (1.36 - 3.63%).²

Centaurea Cyanus Flower Extract

An industry submission stated that a 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* contains flavonoids, tannins, anthocyanins, and saponins.⁵ The heavy metal and arsenic content of this extract were not more than 20 and 2 ppm, respectively. According to another industry submission, an aqueous *Centaurea Cyanus Flower Extract* (flowers extracted in water at 1.7 - 2.7%) contains sugars (31%), minerals (26% by weight following pyrolysis), organic acids (15%), proteins (14%), and uronic acids (14%).⁹ The threshold value for heavy metals (antimony, arsenic, cadmium, chromium, cobalt, mercury, nickel, lead, and vanadium) is ≤ 0.5 ppm each. Another industry submission provided specifications for a tradename mixture comprising *Centaurea Cyanus Flower Extract* (20%) and butylene glycol for heavy metals (< 20 ppm), lead (< 10 ppm), arsenic (< 2 ppm), and cadmium (< 1 ppm).⁴ The microbial and yeast/mold content specification for this mixture is < 100 colony-forming units (CFU)/g each. The infrared spectrum of this mixture matched the reference standard, and the sample also passed the thin-layer chromatography (TLC) test.⁴

The major components of a 70% ethanol extract of *Centaurea cyanus* flower (determined by GC-MS) were catechol and α -myrin; total phytochemical composition values are provided in Table 3.¹⁰ Total phenolic content was determined to be 97.9 mg gallic acid equivalent (GAE)/l, and total flavonoid content was determined to be 118.52 mg quercetin equivalent (QE)/l. Quantified values of rutin, quercetin, and hesperidin were 13.2, 5.6, and 13.2 mg/l respectively. The total polyphenol content of a 70% hydromethanolic extract of *Centaurea cyanus* flower was determined by spectrophotometric method to be 12.49 mg GAE/g dried extract.¹¹

The composition of an aqueous extract of *Centaurea cyanus* flower is as follows: total phenolic compounds (393 - 485 mg GAE/100 g), total flavonoids (133 - 223 mg QE/100 g), and total anthocyanins (127 - 172 mg cyanidin-3-glucoside equivalent (CGE)/100 g).¹² Additional composition information may be found in Table 4.

The total values of phenolic acid derivatives, phenolic compounds, and flavonoids in a hydromethanolic extract of *Centaurea cyanus* flower were determined to be 4.52, 23.55, and 19.03 mg/g dry weight (dw), respectively.¹³ Total composition values of another hydromethanolic extract of *Centaurea cyanus* flower are as follows: organic acids and tocopherols (6.63 and 1.30 g/100 g dw, respectively), phenolic acids, phenolic compounds, anthocyanins, and non-anthocyanin flavonoids (0.134, 38, 27, and 10.7 mg/g extract, respectively).¹⁴

UV Absorption

Centaurea Cyanus Flower Extract

A tradename mixture comprising *Centaurea Cyanus Flower Extract* (20%) and butylene glycol (tested at 0.05% in water) absorbs ultraviolet (UV) light.¹⁵ It exhibits primary and secondary peaks at 256 and 211 nm, respectively.

USE

Cosmetic

The safety of the *Centaurea cyanus* flower-derived ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of *Centaurea cyanus* flower-derived ingredients 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.¹⁶ 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, *Centaurea Cyanus* Flower Extract has the highest frequency of use, and is reported to be used in 800 formulations.^{17,18} According to concentration of use data submitted in 2025 in response to the Council survey, *Centaurea Cyanus* Flower Water has the highest concentration of use, at up to 6.1% in eye makeup removers and at 5% in leave-on face and neck preparations (Table 5).¹⁹

Some of these ingredients may be incidentally ingested as they are used in products used near the mouth (e.g., *Centaurea Cyanus* Flower Water is used in lipstick and lip glosses; concentration not reported). In addition, these ingredients may be used near the eye area (e.g., *Centaurea Cyanus* Flower Water is used in eye makeup remover at up to 6.1%) or result in mucous membrane exposure (e.g., *Centaurea Cyanus* Flower Water is used in douches; concentration not reported). Lastly, these ingredients are reported to be used in baby products (e.g., *Centaurea Cyanus* Flower Extract is used in rinse-off “other” baby products at 0.003%).

Cosmetic products containing *Centaurea cyanus* flower-derived ingredients may be incidentally inhaled as they are used in spray (e.g., *Centaurea Cyanus* Flower Water is used in perfume; concentration not reported) and powder (e.g., *Centaurea Cyanus* Flower Extract is used in face powders; concentration not reported) formulations. 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. 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, a product containing a *Centaurea cyanus* flower-derived ingredient is marketed for use with airbrush delivery systems (i.e., *Centaurea Cyanus* Flower Extract is used in “other” makeup preparations, airbrush application). 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.

Centaurea cyanus flower-derived ingredients named in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.²⁰

Non-Cosmetic

Centaurea cyanus is commonly cultivated as a garden plant and has been used in traditional medicine, particularly for its anti-inflammatory properties.² The flowers have been used to treat eye diseases, nervous disorders, constipation, and mouth ulcers. The plant has been studied for its ameliorative effects; for example, a study reported that *Centaurea cyanus* flower fraction (doses of 100, 200, 400 and 800 µg/ear; applied topically) dose-dependently reduced croton oil-induced edema in mouse ears.²¹ In another study, oral administration of *Centaurea cyanus* (concentrations of 5 and 10%) improved liver structure, reduced hepatic degeneration, and showed hepatoprotective effects in rats that underwent paracetamol-induced liver toxicity.²²

Centaurea cyanus flowers are considered to be edible, and have been used in tea mixtures, soups, wine, and as a natural food coloring in Hungary.^{2,23} It is reported to be a nutritious plant because of its high dietary fiber content, and the honey made from *Centaurea cyanus* nectar is reported to have antibacterial properties.²⁴ A study found that yogurt made with the addition of *Centaurea cyanus* petals (1 g/100 ml of yogurt) had higher levels of protein, fiber, and antioxidant content, when compared to the control.

TOXICOKINETIC STUDIES

No relevant toxicokinetics studies on *Centaurea cyanus* flower-derived ingredients were found in the published literature, and unpublished data were not submitted. In general, toxicokinetics data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity

Oral

Centaurea Cyanus Flower Extract

An acute oral toxicity assay was conducted in mice (48 total; strain not stated) administered a 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* (concentration not stated), resulting in a median lethal dose (LD₅₀) of > 2000 mg/kg.⁵ No other details were provided.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No relevant developmental and reproductive toxicity studies on *Centaurea cyanus* flower-derived ingredients were found in the published literature, and unpublished data were not submitted.

GENOTOXICITY STUDIES

Details regarding the genotoxicity studies summarized herein may be found in Table 6. *Centaurea Cyanus Flower Extract* did not produce genetic mutations. An Ames assay conducted with a 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* (5000 µg/plate) gave non-mutagenic results.⁵ A bacterial reverse mutation assay performed using a tradename mixture comprising *Centaurea Cyanus Flower Extract* (20%) and butylene glycol (1.5 - 5000 µg/plate), with and without metabolic activation, gave non-mutagenic results.²⁵ A tradename mixture containing *Centaurea Cyanus Flower Extract* (extracted in water at 1.7 - 2.7%; tested at 100%) was found to be non-mutagenic in a bacterial reverse mutation assay.⁹ *Centaurea Cyanus Flower Extract* also did not result in chromosomal damage. A chromosomal aberration assay performed in CHL/IU cells treated with a 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* (1250 - 5000 µg/ml, short treatment; 1000 - 4000 µg/ml, continuous treatment) gave non-mutagenic results.⁵

CARCINOGENICITY STUDIES

No relevant carcinogenicity studies on *Centaurea cyanus* flower-derived ingredients were found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Cytotoxicity and Effects on Cell Proliferation

Centaurea Cyanus Flower Extract

Several studies were conducted on porcine liver primary cells (PLP2) treated with hydromethanolic extracts of *Centaurea cyanus* flower (concentrations not stated).^{13,14} The half-maximal growth inhibition concentration (GI₅₀) was found to be > 0.4 mg/ml. Sulforhodamine B (SRB) assays performed on breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), cervical carcinoma (HeLa), and hepatocellular carcinoma (HepG2) cell lines treated with hydromethanolic extracts of *Centaurea cyanus* flower (exact concentrations not stated; stock solution of 4 mg/ml) did not result in any antiproliferative activity.^{13,14}

A trypan blue assay conducted on human acute T leukemia cells (J-45.01) treated with an ethanolic extract of *Centaurea cyanus* flower (0.04 - 1.0 mg/ml) found the half-maximal inhibitory concentration (IC₅₀) of the test substance to be 0.77 mg/ml.¹¹ An annexin V assay performed on J-45.01 cells treated with an ethanolic extract of *Centaurea cyanus* flower (concentration close to IC₅₀ (0.77 mg/ml); exact concentration not stated), resulted in time-dependent apoptosis. An 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay testing the cytotoxic effects of an aqueous extract of *Centaurea cyanus* flower (0.1 - 0.9 mg/ml) in human colorectal adenocarcinoma epithelial (Caco-2), human lung adenocarcinoma epithelial (A459), and HepG2 cells resulted in IC₅₀ and GI₅₀ values of > 0.9 mg/ml for all treated cell lines.¹²

Centaurea Cyanus Flower Water

No cytotoxicity was found in a cell culture assay performed on PLP2 cells treated with an aqueous infusion of *Centaurea cyanus* flower (concentrations not stated; GI₅₀ = > 0.4 mg/ml).¹³ An SRB assay performed on MCF-7, NCI-H460, HeLa, and HepG2 cell lines treated with an aqueous infusion of *Centaurea cyanus* flower (exact concentrations not stated; stock solution of 4mg/ml) did not result in any antiproliferative activity.

Centaurea cyanus extract

Mouse macrophages (RAW 264.7) were treated with a methanolic extract of the aerial parts of *Centaurea cyanus* (0.125 - 1 mg/ml) in an MTT assay.²⁶ No cytotoxicity was observed at any concentration of the test substance.

Anti-Microbial Activity

Centaurea Cyanus Flower Extract

Using microdilution method and rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assays, the anti-bacterial effects of several hydromethanolic extracts of a *Centaurea cyanus* flower (exact concentrations not stated; stock solution of 100 mg/ml) were tested with gram-negative and gram-positive bacteria.^{13,14} The minimum inhibitory concentrations (MIC) ranged from 2.5 - 10 mg/ml for gram-positive and 5 - 20 mg/ml for gram-negative bacteria. No growth inhibition was

observed in an antimicrobial activity assay conducted with several strains of gram-negative/positive bacteria and fungi, treated with an aqueous extract of *Centaurea cyanus* flower (0.01 - 1.25 mg/ml).¹²

Centaurea Cyanus Flower Water

Using microdilution method and rapid INT colorimetric assay, the anti-bacterial effect of an aqueous infusion of *Centaurea cyanus* flower (exact concentrations not stated; stock solution of 100 mg/ml) was tested with gram-negative and gram-positive bacteria.¹³ The test substances was found to be more active against gram-positive bacteria, with the MIC ranging from 5 to 20 mg/ml for gram-positive and 20 to >20 mg/ml for gram-negative bacteria.

Centaurea cyanus extract

Agar well diffusion and disk diffusion methods were used to test the antibacterial effect of an ethanolic extract of *Centaurea cyanus* (whole plant extract; concentration not stated) on strains of *Escherichia coli* and *Staphylococcus aureus* (ampicillin used as control).²⁷ The test substance had higher antibacterial effect against *S. aureus* when compared to the control, indicating its effectiveness against gram-positive bacteria.

Anti-Inflammatory Effects

Centaurea cyanus extract

A nitric oxide assay conducted on lipopolysaccharide (LPS)-stimulated RAW 264.7 cells found that treatment with a methanolic extract of the aerial parts of *Centaurea cyanus* (0 - 1 mg/ml) significantly reduced nitric oxide production ($IC_{50} = 0.2836$ mg/ml), when compared to the control.²⁶ An enzyme-linked immunosorbent assay (ELISA) and reverse transcription quantitative polymerase chain reaction (RT-qPCR) were used to test the suppressive effects of a methanolic extract of the aerial parts of *Centaurea cyanus* (0 - 1 mg/ml) on the expression of nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin (IL)-6, IL-1 β , and tumor necrosis factor- α (TNF- α) in LPS-stimulated RAW 264.7 cells. Following treatment with the test substance, the expression of all the listed enzymes and cytokines were significantly reduced in a dose-dependent manner, when compared to the controls.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation and Sensitization

Details regarding the dermal irritation and sensitization studies summarized herein may be found in Table 7.

A tradename mixture comprising *Centaurea Cyanus* Flower Extract (20%) and butylene glycol (tested undiluted) was predicted to be non-irritating in an in vitro EpiDerm™ assay.²⁸ A primary and a cumulative skin irritation assay performed in 3 and 4 rabbits (strain not stated), respectively, treated with a 1,3-butylene glycolic *Centaurea Cyanus* Flower Extract (tested at 10 and 100% concentrations) each gave non-irritating results.⁵ A tradename mixture containing *Centaurea Cyanus* Flower Extract (extracted in water at 1.7 - 2.7%; tested at 15%) was found to be non-irritating in a 48-h occlusive bandage assay performed on human subjects (no other details provided).⁹ In a closed-patch assay performed on 20 human subjects, a 1,3-butylene glycolic *Centaurea Cyanus* Flower Extract (tested at 50%) was determined to be safe.⁵

A tradename mixture containing *Centaurea Cyanus* Flower Extract (extracted in water at 1.7 - 2.7%; tested at 100%) assessed using a KeratinoSens™ assay was found to have no sensitizing potential.⁹ A tradename mixture comprising *Centaurea Cyanus* Flower Extract (20%) and butylene glycol was not predicted to be sensitizing in vitro in a KeratinoSens™ assay (test concentrations of ≤ 2000 μ M)²⁹ and in chemico in a direct peptide reactivity assay (DPRA).³⁰ A skin sensitization assay performed on 20 guinea pigs (strain not stated) treated with a 1,3-butylene glycolic *Centaurea Cyanus* Flower Extract (50%, 1st induction; 20%, 2nd induction; 4 and 20%, challenge) gave negative results.⁵ A Marzulli–Maibach sensitization assay conducted in 120 adult subjects with normal skin using a tradename mixture containing *Centaurea Cyanus* Flower Extract (extracted in water at 1.7 - 2.7%; tested at 15%) found the test substance to be non-sensitizing.⁹ A human repeated-insult patch test (HRIPT) performed using a serum containing 0.0014% *Centaurea Cyanus* Flower Extract (about 0.1 – 0.15 g of product applied) in 52 subjects gave non-irritating and non-sensitizing results.³¹

Phototoxicity

Details regarding the phototoxicity studies summarized herein may be found in Table 7. A tradename mixture containing *Centaurea Cyanus* Flower Extract (extracted in water at 1.7 - 2.7%; tested at 100%) was found to be non-phototoxic in a 3T3 neutral red uptake (NRU) phototoxicity assay.⁹ In an EpiDerm™ phototoxicity assay, a tradename mixture comprising *Centaurea Cyanus* Flower Extract (20%) and butylene glycol (tested at 0.5 - 10%; with and without 60 min of 6 J/cm² ultraviolet A (UVA)-irradiation) was found to be non-phototoxic.³²

OCULAR IRRITATION STUDIES

Details regarding the ocular irritation studies summarized herein may be found in Table 8. In reconstructed human cornea-like epithelium assays, a tradename mixture containing *Centaurea Cyanus* Flower Extract (extracted in water at 1.7 - 2.7%)⁹ and a tradename mixture comprising *Centaurea Cyanus* Flower Extract (20%) and butylene glycol were both predicted to be non-irritating to eyes when tested at 100%.²⁸ An eye irritation assay was performed in 3 rabbits (strain not stated) treated with a 1,3-butylene glycolic *Centaurea Cyanus* Flower Extract (tested at 100%).⁵ The results were non-irritating.

SUMMARY

The safety of 3 *Centaurea cyanus* flower-derived ingredients is reviewed in this safety assessment. According to the *Dictionary*, two of these ingredients are reported to function in cosmetics as skin-conditioning agents; additional functions are also reported. The *Dictionary* does not report a function for *Centaurea Cyanus Flower*. *Centaurea cyanus* is also commonly known as cornflower or bachelor's button.

According to RLD obtained from the US FDA in 2025, *Centaurea Cyanus Flower Extract* has the highest frequency of use and is reported to be used in 800 formulations. Concentration of use data submitted in 2025 indicates that *Centaurea Cyanus Flower Water* has the highest concentration of use, at up to 6.1% in eye makeup removers and 5% in leave-on face and neck preparations.

An acute oral toxicity study performed in mice treated with a 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* reported an LD₅₀ of > 2000 mg/kg. No other details were provided.

Multiple bacterial reverse mutation assays gave non-mutagenic results when tested with a 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* (5000 µg/plate), a tradename mixture comprising *Centaurea Cyanus Flower Extract* (20%) and butylene glycol (1.5 – 5000 µg/plate; with and without metabolic activation), and a tradename mixture containing *Centaurea Cyanus Flower Extract* (extracted in water at 1.7 - 2.7%; tested at 100%). A chromosomal aberration assay performed in CHL/IU cells using a 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* (1250 - 5000 µg/ml, short treatment; 1000 - 4000 µg/ml, continuous treatment) also gave non-mutagenic results.

Several studies found that hydromethanolic extracts of *Centaurea cyanus* flower did not exhibit cytotoxic or antiproliferative activity in PLP2 cells and various human cancer cells lines (MCF-7, NCI-H460, HeLa, and HepG2). However, treatment with an ethanolic extract of *Centaurea cyanus* flower resulted in IC₅₀ value of 0.77 mg/ml and time-dependent apoptosis in J-45.01 cells. An aqueous extract of *Centaurea cyanus* flower was found to be non-cytotoxic to several human cancer cell lines (Caco-2, A549, and HepG2).

An aqueous infusion of *Centaurea cyanus* flower was found to be non-cytotoxic to PLP2 cells. The same aqueous infusion was tested with various human cancer cells lines (MCF-7, NCI-H460, HeLa, and HepG2), and the test substance did not inhibit cell proliferation. RAW 264.7 cells were treated with a methanolic extract of the aerial parts of *Centaurea cyanus*. The assay did not result in any cytotoxic effects.

Hydromethanolic extracts of *Centaurea cyanus* flower demonstrated antibacterial activity, with MIC values ranging from 2.5 - 10 mg/ml against gram-positive bacteria and 5 - 20 mg/ml against gram-negative bacteria. In contrast, an aqueous extract of *Centaurea cyanus* flower did not show antimicrobial activity against the gram-positive, gram-negative, or fungal strains evaluated. The antibacterial effect of an aqueous infusion of *Centaurea cyanus* flower was tested with gram-negative and gram-positive bacteria. The test substance was found to have increased antibacterial activity against gram-positive bacteria (MIC = 5 - 20 mg/ml). The antibacterial effect of an ethanolic extract of *Centaurea cyanus* (whole plant extract) was tested with strains of *S. aureus* and *E. coli*. The test substance demonstrated strong inhibition of *S. aureus*, when compared to the ampicillin control.

A methanolic extract of the aerial parts of *Centaurea cyanus* significantly reduced nitric oxide production in LPS-stimulated RAW 264.7 cells (IC₅₀ = 0.2836 mg/ml), indicating anti-inflammatory activity. ELISA and RT-qPCR analyses further showed that the test substance suppressed the expression of iNOS, COX-2, IL-6, IL-1β, and TNF-α in a significant and dose-dependent manner.

An EpiDerm™ assay gave non-irritating results when tested with a tradename mixture comprising *Centaurea Cyanus Flower Extract* (20%) and butylene glycol (tested undiluted). Primary and cumulative skin irritation assays conducted in rabbits treated with a 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* (tested at 10 and 100% concentration) also gave non-irritating results. No irritation was observed in a 48-h occlusive bandage assay performed on humans, testing a tradename mixture containing *Centaurea Cyanus Flower Extract* (extracted in water at 1.7 - 2.7%; tested at 15%). A 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* (tested at 50%) was determined to be safe in a closed-patch assay performed on human subjects.

Multiple KeratinoSens™ assays gave non-sensitizing results when assessing a tradename mixture containing *Centaurea Cyanus Flower Extract* (extracted in water at 1.7 - 2.7%; tested at 100%) and a tradename mixture comprising *Centaurea Cyanus Flower Extract* (20%) and butylene glycol (test concentrations of ≤ 2000 µM). In a DPRA, a tradename mixture comprising *Centaurea Cyanus Flower Extract* (20%) and butylene glycol was not predicted to be a sensitizer. A 1,3-butylene glycolic *Centaurea Cyanus Flower Extract* was found to be non-sensitizing in a guinea pig assay (50%, 1st induction; 20%, 2nd induction; 4 and 20%, challenge). A tradename mixture containing *Centaurea Cyanus Flower Extract* (extracted in water at 1.7 - 2.7%; tested at 15%) was non-sensitizing in a Marzulli–Maibach assay performed on adults. No irritation or sensitization was observed in an HRIPT testing a serum containing 0.0014% *Centaurea Cyanus Flower Extract* (about 0.1 – 0.15 g of product applied).

A 3T3 NRU phototoxicity assay found that a tradename mixture containing *Centaurea Cyanus Flower Extract* (extracted in water at 1.7 - 2.7%; tested at 100%) was non-phototoxic. A tradename mixture comprising *Centaurea Cyanus Flower*

Extract (20%) and butylene glycol (tested at up to 10%) was also found to be non-phototoxic in an EpiDerm™ phototoxicity assay.

A tradename mixture containing Centaurea Cyanus Flower Extract (extracted in water at 1.7 - 2.7%; tested at 100%) and a tradename mixture comprising Centaurea Cyanus Flower Extract (20%) and butylene glycol (tested undiluted) were non-irritating in reconstructed human cornea-like epithelium assays. A 1,3-butylene glycolic Centaurea Cyanus Flower Extract (tested at 100%) was non-irritating in a rabbit eye irritation assay.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES**Table 1. Definitions and reported functions¹**

Ingredient/CAS No.	Definition	Function(s)
Centaurea Cyanus Flower	Centaurea Cyanus Flower are the flowers of <i>Centaurea cyanus</i> .	None Reported
Centaurea Cyanus Flower Extract 84012-18-0	Centaurea Cyanus Flower Extract is the extract of the flowers of the cornflower, <i>Centaurea cyanus</i> .	Cosmetic Astringent Skin Protectant Skin-Conditioning Agent - Miscellaneous
Centaurea Cyanus Flower Water	Centaurea Cyanus Flower Water is an aqueous solution of the steam distillate obtained from the flowers of <i>Centaurea cyanus</i> .	Skin-Conditioning Agent - Miscellaneous

Table 2. Chemical properties of a tradename mixture comprising Centaurea Cyanus Flower Extract (20%) and butylene glycol

Property	Value	Reference
Physical Form	Liquid	4
Color	Light yellow	4
Odor	Mild characteristic	4
Specific Gravity (@ 25 °C)	1.000 – 1.050	4
pH (@25 °C)	5.0 – 7.0	4
UV Absorption (λ) (nm)	256, 211	15

Table 3. Phytochemical composition of a 70% ethanol extract of *Centaurea cyanus* flower¹⁰

Compound (type)	% of the total composition
catechol (phenol)	17.79
2-hydroxy-5-methylbenzaldehyde (aldehyde)	9.91
4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (phenol)	1.37
neophytadiene (diterpene)	1.71
14-methyl pentadecanoic acid methyl ester (ester)	0.37
palmitic acid (fatty acid)	8.76
phytol (diterpene alcohol)	4.15
linolenic acid (fatty acid)	8.02
2-monopalmitin (alcohol)	4.49
1-monolinolein (glyceride)	4.34
stigmasterol (sterol)	4.87
γ -sitosterol (sterol)	8.64
β -amyirin (triterpene)	9.33
α -amyirin (triterpene)	12.86
vitamin E acetate	3.39

Table 4. Composition of an aqueous extract of *Centaurea cyanus* flower¹²

Compound	Content (mg/100g)
chlorogenic acid	3897
caffeic acid	1.6
isoquercitrin	116
<i>p</i> -coumaric acid	1.3
ferulic acid	1.4
coumarin	1.5

Table 5. Frequency and concentration of use according to likely duration and exposure and by product category¹⁷⁻¹⁹

	Centaurea Cyanus Flower		Centaurea Cyanus Flower Extract		Centaurea Cyanus Flower Water	
	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use
	RLD (2025)	% (2025)	RLD (2025)	% (2025)	RLD (2025)	% (2025)
Totals*	198	0.001	800	0.00001 – 0.075	494	0.5 – 6.1
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	173	NR	692	0.00001 – 0.075	439	0.5 - 5
Rinse-Off	97	0.001	258	0.001 – 0.01	102	3.6 – 6.1
Diluted for (Bath) Use	13	NR	5	NR	NR	NR
Unknown	2	NR	13	NR	3	NR
Exposure Type						
Baby Products	1	NR	5	0.003	4	NR
Children's Makeup	NR	NR	NR	NR	NR	NR
Eye Area	4	NR	75	0.00001 – 0.075	258	0.5 – 6.1
Incidental Ingestion	3	NR	NR	NR	15	NR
Mucous Membrane	30	NR	28	NR	37	NR
Incidental Inhalation-Spray	1; 76 ^a ; 95 ^b	NR	1; 294 ^a ; 337 ^b	0.001 ^a	6; 42 ^a ; 97 ^b	NR
Incidental Inhalation-Airbrush	NR	NR	1	NR	NR	NR
Incidental Inhalation-Powder	95 ^b ; 1 ^c	NR	6; 337 ^b ; 2 ^c	0.0068 ^c	97 ^b ; 2 ^c	NR
Dermal Contact	258	NR	828	0.00001 – 0.075	488	0.5 – 6.1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	14	0.001	103	NR	18	NR
Hair-Coloring	NR	NR	12	0.0011	2	NR
Nail	7	NR	3	NR	NR	NR
Other Preparations (Unknown Exposure Type)	2	NR	13	NR	3	NR
as reported by product category						
Baby Products						
Baby Shampoos					1	NR
Baby Lotions/Oils/Powders/Creams	1	NR	2	NR	2	NR
Other Baby Products			3 (r.o.)	0.003 (r.o.)	1 (r.o.)	NR
Bath Preparations (diluted for use)						
Bath Oils, Tablets, and Salts	7	NR	2	NR		
Bubble Baths			1	NR		
Other Bath Preparations	6	NR	2	NR		
Eye Makeup Preparations (not children's)						
Eyeliner			5	0.075	1	0.5
Eye Shadow			23	NR	209	0.5
Eye Lotion	1	NR	8	0.00001 – 0.0014	2	NR
Eye Makeup Remover	1	NR	15	0.01	22	5 – 6.1
Mascara	1	NR	9	0.015	17	1
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)			4	NR	2	NR
Other Eye Makeup Preparations	1	NR	11	NR	5	0.5
Fragrance Preparations						
Perfumes					3	NR
Other Fragrance Preparation	1	NR	1	NR	3	NR
Hair Preparations (non-coloring)						
Hair Conditioners	1 (l.o.); 3 (r.o.)	NR	11 (l.o.); 13 (r.o.)	NR	2 (l.o.); 3 (r.o.)	NR
Hair Straighteners			3	NR		
Rinses (non-coloring)			3	NR		

Table 5. Frequency and concentration of use according to likely duration and exposure and by product category¹⁷⁻¹⁹

	Centaurea Cyanus Flower		Centaurea Cyanus Flower Extract		Centaurea Cyanus Flower Water	
	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use
	RLD (2025)	% (2025)	RLD (2025)	% (2025)	RLD (2025)	% (2025)
Shampoos (non-coloring)	3 (r.o.)	0.001	25 (r.o.)	NR	4 (r.o.)	NR
Tonics, Dressings, Other Hair Grooming Aids	5	NR	21	NR	6	NR
Wave Sets					1	NR
Other Hair Preparations	1 (l.o.); 1 (r.o.)	NR	17 (l.o.); 10 (r.o.)	NR	1 (l.o.)	NR
<i>Hair Coloring Preparations</i>						
Hair Dyes and Colors (all types requiring caution statements and patch tests)			11		2	NR
Hair Rinses (coloring)			NR	0.0011 (r.o.)		
Hair Shampoos (coloring)			1 (r.o.)	0.0011 (r.o.)		
<i>Makeup Preparations (not eye or children's)</i>						
Blushers and Rouges (all types)			1	NR	15	NR
Face Powders			6	NR		
Foundations	4 (traditional application)	NR	22 (traditional application)	NR	2 (traditional application)	NR
Lipsticks and Lip Glosses	3	NR			15	NR
Makeup Bases	2 (traditional application)	NR	10 (traditional application)	0.0075 (traditional application)	4 (traditional application)	NR
Makeup Fixatives	2	NR	7	NR		
Other Makeup Preparations	6 (traditional application)	NR	10 (traditional application); 1 (airbrush)	NR	28 (traditional application)	NR
<i>Manicuring Preparations</i>						
Nail Creams and Lotions	6	NR	3	NR		
Other Manicuring Preparations	1	NR				
<i>Personal Cleanliness</i>						
Bath Soaps and Body Washes	11	NR	15	NR	18	NR
Douches					1	NR
Disposable Wipes	1 (l.o.)	NR	3 (l.o.)	NR		
Other Personal Cleanliness Products	2 (r.o.)	NR	3 (l.o.); 2 (r.o.)	NR	3 (r.o.)	NR
<i>Shaving Preparations</i>						
Aftershave Lotions	1	NR	1	NR	2	NR
Pre-shave Lotions (all types)					2	NR
Shaving Cream (aerosol, brushless, lather)			3	NR		
<i>Skin Care Preparations</i>						
Cleansing	27	NR	77	0.01	29	3.6 – 5.8
Depilatories	1	NR	2	NR		
Face and Neck (excluding shaving preps)	58 (l.o.); 22 (r.o.)	NR	190 (l.o.); 47 (r.o.)	0.0068 (l.o.; not spray)	63 (l.o.); 3 (r.o.)	5 (l.o.; not spray); 3.6 (r.o.; not spray)
Body and Hand (excluding shaving preps)	10 (l.o.); 3 (r.o.)	NR	74 (l.o.); 13 (r.o.)	0.0068 (l.o.; not spray)	10 (l.o.); 2 (r.o.)	NR
Foot Powders and Sprays					2	NR
Moisturizing	29	NR	197	0.0014 (not spray)	28	NR
Night	1	NR	9	NR	1	NR
Paste Masks (mud packs)	19	NR	5	0.0014	3	NR
Skin Fresheners	16	NR	8	NR	1	NR
Other Skin Care Preparations	21 (l.o.); 4 (r.o.)	NR	35 (l.o.); 10 (r.o.)	0.001 (r.o.)	15 (l.o.); 7 (r.o.)	NR
<i>Other Preparations (i.e., those that do not fit another category)</i>	2	NR	13	NR	3	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 6. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
IN VITRO						
Gene Mutation						
Centaurea Cyanus Flower Extract (1,3-butylene glycol)	NR	5000 µg/plate	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, and <i>Escherichia coli</i> WP2uvrA	Ames assay; no other details provided	Non-mutagenic	5
tradename mixture comprising Centaurea Cyanus Flower Extract (20%) and butylene glycol	sterile deionized water	1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, and <i>E. coli</i> WP2uvrA	OECD TG 471; bacterial reverse mutation assay; with and without metabolic activation; appropriate positive and negative controls used	Non-mutagenic; controls gave expected results	25
tradename mixture containing Centaurea Cyanus Flower Extract (extracted in water at 1.7 - 2.7%)	NR	100%	NR	OECD TG 471; bacterial reverse mutation assay	Non-mutagenic	9
Chromosomal Damage						
Centaurea Cyanus Flower Extract (1,3-butylene glycol)	NR	1250, 2500, and 5000 µg/ml (short treatment); 1000, 2000, and 4000 µg/ml (continuous treatment)	CHL/IU cells	Chromosomal aberration assay using cultured mammalian cell; short and continuous treatment times	Negative; non-mutagenic	5

ISO = International Organization for Standardization; NR = not reported; OECD = Organisation for Economic Co-operation and Development; TG = test guideline

Table 7. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
IRRITATION						
IN VITRO						
tradename mixture comprising Centaurea Cyanus Flower Extract (20%) and butylene glycol	NR	100%; treatment amounts of 30 µl (liquid) and 25 mg (solid)	reconstructed human epidermis (3 tissues)	EpiDerm™ reconstructed human epidermal model assay; DPBS as negative control; 5% SDS solution as positive control; incubated for 60 min; tissue viability evaluated with MTT assay	Predicted to be non-irritating; tissue viability was not ≤ 50% indicating negative results; controls gave expected results	28
ANIMAL						
Centaurea Cyanus Flower Extract (1,3-butylene glycol)	NR	10 and 100%	rabbits (3 total; strain not stated)	Primary skin irritation assay (details not provided)	Non-irritant	5
Centaurea Cyanus Flower Extract (1,3-butylene glycol)	NR	10 and 100%	rabbits (4 total; strain not stated)	Cumulative skin irritation assay (details not provided)	Non-irritant	5
HUMAN						
tradename mixture containing Centaurea Cyanus Flower Extract (extracted in water at 1.7 - 2.7%)	NR	15%	human subjects (no other details stated)	single 48-h occlusive bandage assay	Non-irritant	9
Centaurea Cyanus Flower Extract (1,3-butylene glycol)	NR	50%	human subjects (20 total)	Human patch test; closed patch (details not provided)	Test substance determined to be safe (no other details provided)	5
SENSITIZATION						
IN VITRO/IN CHEMICO						
tradename mixture containing Centaurea Cyanus Flower Extract (extracted in water at 1.7 - 2.7%)	NR	100%	NR	OECD TG 442D; KeratinoSens™ in vitro skin sensitization assay	No sensitizing potential	9
tradename mixture comprising Centaurea Cyanus Flower Extract (20%) and butylene glycol	DMSO	0.98, 1.95, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µM	immortalized human skin keratinocytes (3 replicates/group; 2 experiments)	OECD TG 442D; KeratinoSens™ in vitro ARE-Nrf2 luciferase assay; cinnamic aldehyde as positive control; DMSO as negative control	Not predicted to be sensitizing; no significant increase in luciferase expression; I _{max} = 0.33; IC ₅₀ = > 1000 µM; EC _{1.5} = no induction; controls gave expected results	29
tradename mixture comprising Centaurea Cyanus Flower Extract (20%) and butylene glycol	acetonitrile	5 mM for cysteine peptide assay; 25 mM for lysine peptide assay	cysteine and lysine peptides	OECD TG 442C; in chemico skin sensitization DPRA; appropriate positive and negative controls used	Not predicted to be a sensitizer; not predicted to cause allergic contact dermatitis; mean percent depletion of cysteine and lysine was 3.06% causing minimal reactivity; controls gave expected results	30
ANIMAL						
Centaurea Cyanus Flower Extract (1,3-butylene glycol)	NR	50% (1 st induction); 20% (2 nd induction); 4 and 20% (challenge)	guinea pigs (20 total; strain not stated)	Skin sensitization assay	Negative	5
HUMAN						
tradename mixture containing Centaurea Cyanus Flower Extract (extracted in water at 1.7 - 2.7%)	NR	15%	adult subjects with normal skin (120 total)	Marzulli-Maibach assay; evaluated the sensitizing power of the test substance	Non-sensitizing	9
serum containing 0.0014% Centaurea Cyanus Flower Extract	NR	about 0.1 – 0.15 g	52 subjects (10 males, 45 females)	HRIPT	Non-irritating and non-sensitizing; no dermal reactions observed	31

Table 7. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
PHOTOXICITY						
IN VITRO						
tradenam mixture containing Centaurea Cyanus Flower Extract (extracted in water at 1.7 - 2.7%)	NR	100%	NR	OECD 432; 3T3 NRU phototoxicity assay	Non-phototoxic	9
tradenam mixture comprising Centaurea Cyanus Flower Extract (20%) and butylene glycol	NR	0.5, 1.5, 5, and 10%	reconstructed human epidermis (2 tissues)	EpiDerm™ phototoxicity assay; with and without 60 min of 6 J/cm ² UVA-irradiation; sterile deionized water as negative control; chlorpromazine as positive control; tissue viability evaluated with MTT assay	Non-phototoxic; tissue viability was not ≤ 20% indicating negative results; controls gave expected results	32

DMSO = dimethyl sulfoxide; DPBS = Dulbecco's phosphate-buffered saline; DPRA = direct peptide reactivity assay; HRIPT = human repeated-insult patch test; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NR = not reported; NRU = neutral red uptake; OECD = Organisation for Economic Co-operation and Development; SDS = sodium dodecyl sulfate; TG = test guideline; UVA = ultraviolet A

Table 8. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Protocol	Results	Reference
IN VITRO						
tradenam mixture containing Centaurea Cyanus Flower Extract (extracted in water at 1.7 - 2.7%)	NR	100%	reconstructed human cornea-like epithelium	OECD TG 492; reconstructed human cornea-like epithelium assay	Predicted to be non-irritating	9
tradenam mixture comprising Centaurea Cyanus Flower Extract (20%) and butylene glycol	NR	100%; treatment amounts of 50 µl (liquid) and 50 mg (solid)	reconstructed human cornea-like epithelium (2 tissues)	EpiOcular™ reconstructed human cornea epithelial model assay; sterile deionized water as negative control; 5% methyl acetate as positive control; incubated for 90 min; tissue viability evaluated with MTT assay	Predicted to be non-irritating; tissue viability was not ≤ 60% indicating negative results; controls gave expected results	28
ANIMAL						
Centaurea Cyanus Flower Extract (1,3-butylene glycol)	NR	100%	rabbits (3 total; strain not stated)	eye irritation assay; no other details provided	Non-irritating	5

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NR = not reported; OECD = Organisation for Economic Co-operation and Development; TG = test guideline

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Concentration of Use by FDA Product Category¹ - *Centaurea cyanus*-Derived Ingredients*

Centaurea Cyanus Callus Lysate Extract
 Centaurea Cyanus Flower
 Centaurea Cyanus Flower Extract

Centaurea Cyanus Flower Water
 Centaurea Cyanus Seed

Ingredient	Product Category	Maximum Concentration of Use
Centaurea Cyanus Flower	Shampoos (noncoloring) Rinse-off	0.001%
Centaurea Cyanus Flower Extract	Other baby products Rinse-off	0.003%
Centaurea Cyanus Flower Extract	Eyeliners	0.075%
Centaurea Cyanus Flower Extract	Eye lotions	0.00001-0.0014%
Centaurea Cyanus Flower Extract	Eye makeup removers	0.01%
Centaurea Cyanus Flower Extract	Mascaras	0.015%
Centaurea Cyanus Flower Extract	Hair rinses (coloring) Rinse-off	0.0011%
Centaurea Cyanus Flower Extract	Hair shampoos (coloring) Rinse-off	0.0011%
Centaurea Cyanus Flower Extract	Makeup bases Traditional	0.0075%
Centaurea Cyanus Flower Extract	Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	0.01%
Centaurea Cyanus Flower Extract	Face and neck products (not spray) Leave-on	0.0068%
Centaurea Cyanus Flower Extract	Body and hand products (not spray) Leave-on	0.0068%
Centaurea Cyanus Flower Extract	Moisturizing products (not spray)	0.0014%
Centaurea Cyanus Flower Extract	Paste masks and mud packs	0.0014%
Centaurea Cyanus Flower Extract	Other skin care preparations Rinse-off	0.001%
Centaurea Cyanus Flower Water	Eyeliners	0.5%
Centaurea Cyanus Flower Water	Eye shadows	0.5%
Centaurea Cyanus Flower Water	Eye makeup removers	5-6.1%
Centaurea Cyanus Flower Water	Mascaras	1%
Centaurea Cyanus Flower Water	Other eye makeup preparations	0.5%
Centaurea Cyanus Flower Water	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	3.6-5.8%

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

Centaurea Cyanus Flower Water	Face and neck products (not spray)	
	Leave-on	5%
	Rinse-off	3.6%

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

Information collected in 2025
Table prepared: October 21, 2025



Memorandum

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

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

DATE: April 10, 2026

SUBJECT: Centaurea Cyanus Flower Extract

Anonymous. 2026. Summary Information Centaurea Cyanus Flower Extract.

April 2026

Summary Information Centaurea Cyanus Flower Extract

1. Chemical properties and method of manufacturing data

The chemical properties	<Composition> Flavonoids , Tannins , Anthocyanins and Saponins
The method of manufacture	Dried raw material ⇒ extraction with 1,3-butylene glycolic solution ⇒ filtration ⇒ maturation ⇒ filtration ⇒ adjustment ⇒ packaging

2. Composition and impurities data

Composition	Please refer to the answer 1.
Impurities	Heavy metals : not more than 20 ppm Arsenic : not more than 2 ppm

3. Acute toxicity data

Test Item	Result	Method
Acute oral toxicity	LD ₅₀ >2000mg/kg	48 Mouses

4. Genotoxicity data

Test Item	Concentration of test solution	Result	Method
Mutagenicity (Reverse mutation testing using microorganisms)	5000 µg/plate	Negative	Ames test (TA98, TA100, TA1535, TA1537, WP2uvrA)
Mutagenicity (Chromosomal aberration testing using cultured mammalian cell)	Short time treatment method:1250, 2500, 5000 µg/mL Continuous treatment method:1000, 2000, 4000µg/mL	Negative	CHL/IU cell

5. Dermal irritation and sensitization data

Test Item	Concentration of test solution	Result	Method
Primary skin irritation	100%, 10%	Non irritant	3 Rabbits
Cumulative skin irritation	100%, 10%	Non irritant	4 Rabbits
Skin sensitization	1 st induction : 50% 2 nd induction : 20% Challenge : 20%, 4%	Negative	20 Guinea pigs
Human patch test	50%	Safe product	Closed patch, 20 subjects

6. Eye Irritation

Test Item	Concentration of test solution	Result	Method
Eye irritation	100%	Non irritant	3 Rabbits



Memorandum

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

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

DATE: April 15, 2026

SUBJECT: Centaurea Cyanus Flower Extract

Anonymous. 2026. Summary Information Centaurea Cyanus Flower Extract (aqueous extract).

April 2026

Summary Information Centaurea Cyanus Flower Extract

The composition of Centaurea Cyanus Flower Extract (flowers extracted in water) at 1.7%-2.7% in water is the following:

- Sugars : 31%
- Mineral ashes: 26%
- Organic acids : 15%
- Proteins: 14%
- Uronic acids: 14%

Impurities:

Heavy metals	N°CAS	Threshold (ppm)
Antimony	7440-36-0	≤0.5
Arsenic	7440-38-2	≤0.5
Cadmium	7440-43-9	≤0.5
Chromium	7440-47-3	≤0.5
Cobalt	7440-48-4	≤0.5
Mercury	7439-97-6	≤0.5
Nickel	7440-02-0	≤0.5
Lead	7439-92-1	≤0.5
Vanadium	7440-62-2	≤0.5

Toxicological studies completed in 2024 on an ingredient containing Centaurea Cyanus Flower Extract at 1.7%- 2.7% in water:

- Evaluation of the skin compatibility of a cosmetic ingredient after a single application of an occlusive bandage for 48 hours (tested dose : 15%; n=human volunteers) : Non-irritant
- In vitro sensitization test: KeratinoSens (OECD 442D) (tested dose : 100%): no sensitizing potential
- Evaluation of the sensitizing power on adult volunteers with normal skin (Marzulli-Maibach; n=120) (tested dose : 15%) : Non-sensitizing
- Evaluation of ocular irritant potential (OECD 492 Reconstructed Human Cornea-like Epithelium Test) (tested dose : 100%): Non-irritant
- Phototoxicity (OECD 432 In Vitro 3T3 NRU Phototoxicity Test) (tested dose : 100%): not phototoxic
- Test of mutagenicity (OECD 471 Bacterial Reverse Mutation Test) (tested dose : 100%) : Non-mutagenic



Memorandum

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

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

DATE: April 23, 2026

SUBJECT: Centaurea Cyanus Flower Extract

Anonymous. 2026. Summary Information HRIPT data for the serum containing 0.0014% Centaurea Cyanus Flower Extract.

Summary Information

HRIPT data for the serum containing 0.0014% Centaurea Cyanus Flower Extract

Test procedure: Human repeated insult patch test (HRIPT)
 Date of final report: August 30, 2024
 Test article: Serum containing 0.0014% Centaurea Cyanus Flower Extract
 Amount of product applied: Approximately 0.1 – 0.15 g
 Test subjects : 55 subjects (10 males and 45 females)

Results: Three subjects discontinued the study for personal reasons unrelated to the test article. All remaining 52 subjects completed the study and did not exhibit any dermal reactions throughout the study period; all scores were “0”.

Induction Phase Summary

Test Article	Induction Scores (Number of Responses)						Evidence of Irritation
	0.5	1	2	3	4	Other	
██████████	0	0	0	0	0	0	No

Challenge Phase Summary

Test Article	Challenge Scores (Number of Responses)						Evidence of Sensitization
	0.5	1	2	3	4	Other	
██████████	0	0	0	0	0	0	No

The following dermal scoring system was used:

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

Conclusion: The test article did not demonstrate the potential to elicit dermal irritation or induce sensitization under the conditions of this study.



Memorandum

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

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

DATE: April 28, 2026

SUBJECT: Centaurea Cyanus Flower Extract

Active Concepts. 2023. Product Specification ABS Cornflower Extract (contains 20% Centaurea Cyanus Flower Extract).

Active Concepts. 2018. Dermal and ocular irritation tests ABS Cornflower Extract (tested undiluted).

Active Concepts. 2018. OECD TG 442C: In Chemico Skin Sensitization (direct peptide reactivity) ABS Cornflower Extract.

Active Concepts. 2018. OECD TG 442D: In Vitro Skin Sensitization (ARE-Nrf2 Luciferase Test Method) ABS Cornflower Extract.

Active Concepts. 2018. Phototoxicity Assay Analysis ABS Cornflower Extract.

Active Concepts. 2019. ABS Corn Flower Extract UV Spectrum.

Active Concepts. 2019. Bacterial Reverse Mutation Test ABS Cornflower Extract.



Product Specification

107 Technology Drive, Lincolnton, NC 28092 USA

info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101

Product Name: ABS Corn Flower Extract
Code Number: 10109REV
CAS #'s: 107-88-0 & 84012-18-0
EINECS #'s: 203-529-7 & 281-664-0
INCI Name: Butylene Glycol & Centaurea Cyanus Flower Extract
Status: Approved

(contains 20%
Centaurea Cyanus
Flower Extract)

Specification	Parameter
Appearance	Light Yellow Liquid
Odor	Mild Characteristic
pH (as is @ 25°C)	5.0 – 7.0
Specific Gravity (25°C)	1.000 – 1.050
Infrared Spectrum	To Match Standard
TLC	To Pass Test
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm
Microbial Content	< 100 CFU/g; No pathogens
Yeast & Mold	< 100 CFU/g
Gram Negative Bacteria	0 CFU/g

May Sediment upon Standing; Mix Well Prior to Use

****Note:** Product may change appearance if exposed to cold temperatures during shipment or storage. If this happens, please gently warm to 45-50°C and mix until normal appearance is restored



Dermal and Ocular Irritation Tests

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Tradename: ABS Corn Flower Extract (the trade name mixture as listed on the specification sheet was tested undiluted in both assays)

Code: 10109REV

CAS #: 107-88-0 & 84012-18-0

Test Request Form #: 4929

Lot #: 59950P

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

Study Director: Maureen Danaher

Principle Investigator: Jennifer Goodman

Test Performed:

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

EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **ABS Corn Flower Extract** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be **non-irritating**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO₂, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(3-4,5-dimethyl thiazole 2-y)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-irritant**. The negative and positive controls performed as anticipated.



Dermal and Ocular Irritation Tests

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I. Introduction

A. Purpose

In vitro dermal and ocular irritation studies were conducted to evaluate whether a test article would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays. MatTek Corporation's reconstructed human epidermal and human ocular models are becoming a standard in determining the irritancy potential of test substances. They are able to discriminate between irritants and non-irritants. The EpiDerm™ assay has accuracy for the prediction of UN GHS R38 skin irritating and no-label (non-skin irritating) test substances. The EpiOcular™ assay can differentiate chemicals that have been classified as R36 or R41 from the EU classifications based on Dangerous Substances Directive (DSD) or between the UN GHS Cat 1 and Cat 2 classifications.

II. Materials

- A. Incubation Conditions:** 37°C at 5% CO₂ and 95% relative humidity
- B. Equipment:** Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
- C. Media/Buffers:** DMEM based medium; DPBS; sterile deionized H₂O
- D. Preparation:** Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
- E. Tissue Culture Plates:** Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
- F. Reagents:** MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
- G. Other:** Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™, and cornea epithelial model, EpiOcular™, consist of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis and cornea epithelium. These models consist of organized basal, spinous, and granular layers, and the EpiDerm™ systems also contains a multilayer stratum corneum containing intercellular lamellar lipid layers that the EpiOcular™ system is lacking. Both the EpiDerm™ and EpiOcular™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile DPBS and sterile deionized water are used as negative controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

C. Positive Control

Known dermal and eye irritants, 5% SDS solution and Methyl Acetate, were used as positive controls for the EpiDerm™ and EpiOcular™ assays, respectfully.



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D. Data Interpretation Procedure

a. EpiDerm™

An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls and a non-irritant's viability is > 50%.

b. EpiOcular™

An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls and a non-irritant's viability is > 40%.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO₂ and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO₂ and 95% relative humidity for an additional 18 to 21 hours.

B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO₂, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO₂, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

All tissue inserts are washed with DPBS, dried with cotton tipped swab, and transferred to fresh media and culture plates. After 24 hours the inserts are again transferred into fresh media and culture plates for an additional 18 to 20 hours.

b. EpiOcular™

Tissue inserts are washed with DPBS and immediately transferred into 5mL of assay medium for 12 to 14 minutes. After this soak the inserts are transferred into fresh media and tissue culture plates for 120 minutes for liquid substances and 18 hours for solid substances.

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO₂, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD₅₇₀) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).



Dermal and Ocular Irritation Tests

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B. Positive Control

a. EpiDerm™

The assay meets the acceptance criterion if the mean viability of positive control tissues expressed as a % of the negative control is $\leq 20\%$.

b. EpiOcular™

The assay meets the acceptance criterion if the mean viability of positive control tissues is $< 60\%$ of control viability.

C. Standard Deviation

Since each irritancy potential is predicted from the mean viability of 3 tissues for EpiDerm™ and 2 tissues for EpiOcular™, the variability of the replicates should be $< 18\%$ for EpiDerm™ and $< 20\%$ EpiOcular™.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ and EpiOcular™ assay kits were in good condition, intact, and viable.

B. Tissue Viability Assay

The results are summarized in Figure 1. In no case was the tissue viability $\leq 50\%$ for EpiDerm™ or $\leq 60\%$ for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

C. Test Validity

The data obtained from this study met criteria for a valid assay.

VII. Conclusion

Under the conditions of this assay, the test article substance was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

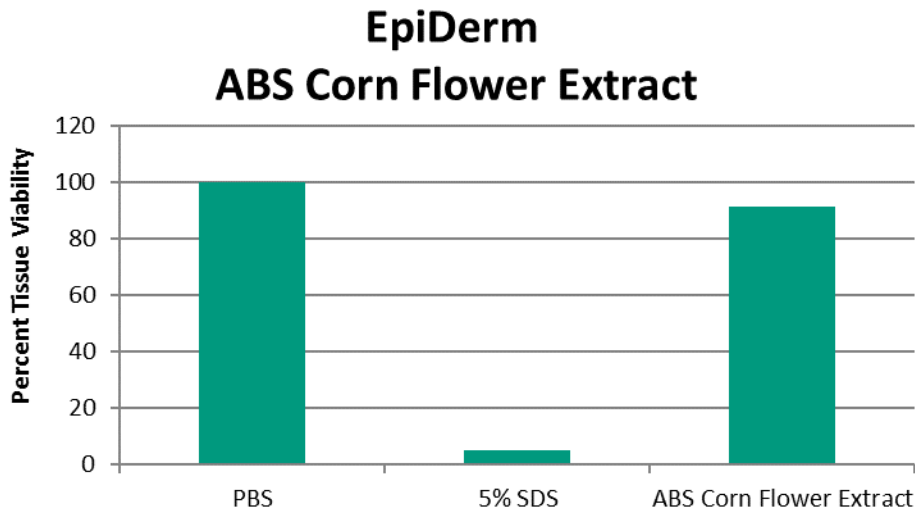


Figure 1: EpiDerm tissue viability

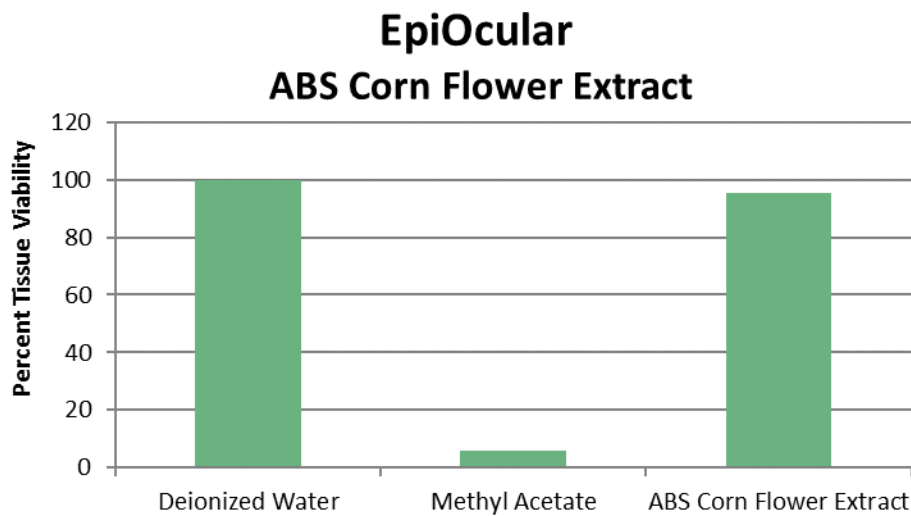


Figure 2: EpiOcular tissue viability

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Tradename: ABS Corn Flower Extract

Code: 10109REV

CAS #: 107-88-0 & 84012-18-0

Test Request Form #: 4931

Lot #: 59950P

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

Study Director: Maureen Danaher

Principle Investigator: Jennifer Goodman

Test Performed:

OECD TG 442C: *In Chemico* Skin Sensitization

Direct Peptide Reactivity Assay (DPRA)

Introduction

A skin sensitizer is a substance that will lead to an allergic response following skin contact¹. Haptenation is the covalent binding of a hapten, or low-molecular weight substance or chemical, to proteins in the skin. This is considered the prominent mechanism which defines a chemical as a sensitizer. Haptenation is described as a "molecular initiating event" in the OECD Adverse Outcome Pathway (AOP) for skin sensitization which summarizes the key events known to be involved in chemically-induced allergic contact dermatitis². The direct peptide reactivity assay (DPRA) is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine. The DPRA is able to distinguish sensitizers from non-sensitizer with 82% accuracy (sensitivity of 76%; specificity of 92%)³.

This assay was conducted to determine skin sensitization hazard of **ABS Corn Flower Extract** in accordance with European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and OECD Test Guideline 442C.

Assay Principle

The DPRA is an *in chemico* method which addresses peptide reactivity by measuring depletion of synthetic heptapeptides containing either cysteine or lysine following 24 hours incubation with the test substance. The peptide is a custom material containing phenylalanine to aid in detection. Depletion of the peptide in the reaction mixture is measured by HPLC with gradient elution and UV detection at 220 nm. Cysteine and lysine peptide percent depletion values are then calculated and used in a prediction model which allows assigning the test chemical to one of four reactivity classes used to support the discrimination between sensitizers and non-sensitizers.

1. United Nations Economic Commission (UNECE) (2013) Global Harmonized System of Classification and Labelling of Chemicals (GHS) 5th Revised Edition
2. OECD (2012). The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence. Series on Testing and Assessment No. 168
3. EC EURL ECVAM (2012) Direct peptide reactivity assay (DPRA) validation study report; pp 1 -74.

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Materials

- A. Equipment:** HPLC-UV (Waters Breeze - Waters 2998 Photodiode Array Detector); Pipettes; Analytical balance
- B. HPLC/Guard Columns:** Agilent Zorbax SB-C18 2.1mm x 100mm x 3.5µm; Phenomenex Security Guard C18 4mm x 2mm
- C. Chemicals:** Trifluoroacetic acid; Ammonium acetate; Ammonium hydroxide; Acetonitrile; Cysteine peptide (Ac-RFAACAA-COOH); Lysine peptide (Ac-RFAAKAA-COOH); Cinnamic aldehyde
- D. Reagents/Buffers:** Sodium phosphate buffer (100mM); Ammonium acetate buffer (100mM)
- E. Other:** Sterile disposable pipette tips

Methods

Solution Preparation:

- 0.667mM Cysteine Peptide in 100mM Phosphate Buffer (pH 7.5)
- 0.667mM Lysine Peptide in 100mM Ammonium Acetate Buffer (pH 10.2)
- 100mM Cinnamic Aldehyde in Acetonitrile
- 100mM* **ABS Corn Flower Extract** in Acetonitrile

*For mixtures and multi-constituent substances of known composition such as **ABS Corn Flower Extract**, a single purity should be determined by the sum of the proportion of its constituents (excluding water), and a single apparent molecular weight determined by considering the individual molecular weights of each component in the mixture (excluding water) and their individual proportions. The resulting purity and apparent molecular weight can then be used to calculate the weight of test chemical necessary to prepare a 100 mM solution.

Reference Controls:

- Reference Control A: For calibration curve accuracy
- Reference Control B: For peptide stability over analysis time of experiment
- Reference Control C: For verification that the solvent does not impact percent peptide depletion

Sample, Reference Control, and Co-Elution Control Preparation:

- Once these solutions have been made they should be incubated at room temperature, protected from light, for 24±2 hours before running HPLC analysis.
- Each chemical should be analyzed in triplicate.

1:10 Ratio, Cysteine Peptide 0.5mM Peptide, 5mM Test Chemical	1:50 Ratio, Lysine Peptide 0.5mM Peptide, 25mM Test Chemical
<ul style="list-style-type: none"> • 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls) • 200µL Acetonitrile • 50µL Test Chemical Solution (or Acetonitrile for Reference Controls) 	<ul style="list-style-type: none"> • 750µL Lysine Peptide Solution (or 100mM Ammonium Acetate Buffer, pH 10.2, for Co-Elution Controls) • 250µL Test Chemical Solution (or Acetonitrile for Reference Controls)

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Calibration Curve:

- Standards are prepared in a solution of 20% Acetonitrile:Buffer
 - For the Cysteine peptide using the phosphate buffer, pH 7.5
 - For the Lysine peptide using the ammonium acetate buffer, pH 10.2

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
mM Peptide	0.534	0.267	0.1335	0.0667	0.0334	0.0167	0.000

HPLC Analysis:

- HPLC-UV system should be equilibrated at 30 °C with 50% Mobile Phase A (0.1% (v/v) trifluoroacetic acid in water) and 50% Mobile Phase B (0.085% (v/v) trifluoroacetic acid in acetonitrile) for 2 hours
- Absorbance is measured at 220nm
- Flow Conditions:

Time	Flow	%A	%B
0 minutes	0.35 mL/min	90	10
10 minutes	0.35 mL/min	75	25
11 minutes	0.35 mL/min	10	90
13 minutes	0.35 mL/min	10	90
13.5 minutes	0.35 mL/min	90	10
20 minutes	End Run		

Data and Reporting

Acceptance Criteria:

- The following criteria must be met for a run to be considered valid:
 - Standard calibration curve should have an $r^2 > 0.99$.
 - Mean percent peptide depletion values of three replicates for the positive control cinnamic aldehyde should be between 60.8% and 100% for the cysteine peptide and between 40.2% and 69% for the lysine peptide and the maximum standard deviation should be <14.9 for the percent cysteine depletion and <11.6 for the percent lysine depletion.
 - Mean peptide concentration of reference controls A should be 0.50 ± 0.05 mM and the coefficient of variable of the peptide peak areas for reference B and C in acetonitrile should be <15.0%.
- The following criteria must be met for a test chemical's results to be considered valid:
 - Maximum standard deviation should be <14.9 for percent cysteine depletion and <11.6 for percent lysine depletion.
 - Mean peptide concentration of the three reference control C should be 0.50 ± 0.05 mM.



Prediction Model:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	Reactivity Class	Prediction
0% < Mean % Depletion < 6.38%	Minimal Reactivity	Non-sensitizer
6.38% < Mean % Depletion < 22.62%	Low Reactivity	Sensitizer
22.62% < Mean % Depletion < 42.47%	Moderate Reactivity	Sensitizer
42.47% < Mean % Depletion < 100%	High Reactivity	Sensitizer

If co-elution occurs with the lysine peptide, than the cysteine 1:10 prediction model can be used:

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	Reactivity Class	Prediction
0% < Cys % Depletion < 13.89%	Minimal Reactivity	Non-sensitizer
13.89% < Cys % Depletion < 23.09%	Low Reactivity	Sensitizer
23.09% < Cys % Depletion < 98.24%	Moderate Reactivity	Sensitizer
98.24% < Cys % Depletion < 100%	High Reactivity	Sensitizer

Therefore the measured values of % depletion in the three separated runs for each peptide depletion assay include:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	Reactivity Class	Prediction
3.02	Minimal Reactivity	Non-sensitizer
3.01	Minimal Reactivity	Non-sensitizer
3.03	Minimal Reactivity	Non-sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	Reactivity Class	Prediction
3.12	Minimal Reactivity	Non-sensitizer
3.06	Minimal Reactivity	Non-sensitizer
3.09	Minimal Reactivity	Non-sensitizer

Results and Discussion

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

Percent peptide depletion is determined by the following equation:

$$\text{Percent Peptide Depletion} = \left[1 - \left(\frac{\text{Peptide Peak Area in Replicate Injection}}{\text{Mean Peptide Peak Area in Reference Controls C}} \right) \right] \times 100$$

Based on HPLC-UV analysis of **ABS Corn Flower Extract (10109REV)** we can determine this product is not classified as a sensitizer and is not predicted to cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 3.06% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.



OECD TG 442D: *In Vitro* Skin Sensitization

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Tradename: ABS Corn Flower Extract

Code: 10109REV

CAS #: 107-88-0 & 84012-18-0

Test Request Form #: 4930

Lot #: 59950P

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

Study Director: Maureen Danaher

Principle Investigator: Jennifer Goodman

Test Performed:

OECD TG 442D: In Vitro Skin Sensitization *ARE-Nrf2 Luciferase Test Method*

Introduction

Skin sensitization refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals¹. Substances are classified as skin sensitizers if there is evidence in humans that the substance can lead to sensitization by skin contact or positive results from appropriate tests, both *in vivo* and *in vitro*. Utilization of the KeratinoSens™ cell line allows for valid *in vitro* testing for skin sensitization.

This assay was conducted to determine skin sensitization potential of **ABS Corn Flower Extract** in accordance with the UN GHS.

Assay Principle

The ARE-Nrf2 luciferase test method addresses the induction of genes that are regulated by antioxidant response elements (ARE) by skin sensitizers. The Keap1-Nrf2-ARE pathways have been shown to be major regulator of cytoprotective responses to oxidative stress or electrophilic compounds. These pathways are also known to be involved in the cellular processes in skin sensitization. Small electrophilic substances such as skin sensitizers can act on the sensor protein Keap1 (Kelch-like ECH-associated protein 1), by covalent modification of its cysteine residue, resulting in its dissociation from the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2). The dissociated Nrf2 can then activate ARE-dependent genes such as those coding for phase II detoxifying enzymes.

The skin sensitization assay utilizes the KeratinoSens™ method which uses an immortalized adherent human keratinocyte cell line (HaCaT cell line) that has been transfected with a selectable plasmid to quantify luciferase gene induction as a measure of activation of Keap1-Nrf2-antioxidant/electrophile response element (ARE). This test method has been validated by independent peer review by the EURL-ECVAM. The addition of a luciferin containing reagent to the cells will react with the luciferase produced in the cell resulting in luminescence which can be quantified with a luminometer.

1. United Nations (UN) (2013). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth revised edition, UN New York and Geneva, 2013

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OECD TG 442D: *In Vitro* Skin Sensitization

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Materials

- A. Incubation Conditions:** 37 °C at 5% CO₂ and 95% relative humidity (RH)
- B. Equipment:** Humidified incubator; Biosafety laminar flow hood; Microplate Reader; Pipettes
- C. Cell Line:** KeratinoSens™ by Givaudan Schweiz AG
- D. Media/Buffers:** Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Geneticin
- E. Culture Plate:** Flat bottom 96-well tissue culture treated plates
- F. Reagents:** Dimethyl Sulfoxide (DMSO); Cinnamic Aldehyde; ONE-Glo Reagent; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); sodium lauryl sulfate (SLS)
- G. Other:** Sterile disposable pipette tips; wash bottles

Methods

KeratinoSens™ were into seeded four 96-well tissue culture plates and allowed to grow to 80 – 90% confluency in DMEM containing 10% FBS and 500µg/mL G418 geneticin. Twelve test concentrations of **ABS Corn Flower Extract** were prepared in DMSO with a concentration range from 0.98 - 2000 µM. These 12 concentrations were assayed in triplicate in 2 independently performed experiments. The positive control was cinnamic aldehyde for which a series of 5 concentrations prepared in DMSO had final test concentrations of 4 – 64 µM. The negative control was a 1% test concentration of DMSO.

24 hour post KeratinoSens™ seeding, the culture media was removed and replaced with fresh media containing 10% FBS without G418 geneticin. 50 µL of the above described test concentrations was added to the appropriate wells. The treated plates were then incubated for 48 hours at 37 °C in the presence of 5% CO₂ and 95% relative humidity. After treatment incubation was complete the media was removed and the wells were washed with PBS 3 times.

One of the four plates was used for a cytotoxicity endpoint, where MTT was added to the wells and incubated for 4 hours at 37 °C in the presence of 5% CO₂. SLS was then added to the wells and incubated overnight at room temperature. A spectrometer measured the absorbance at 570 nm. The absorbance values (optical density) were then used to determine the viability of each well by comparing the optical density of each test material treated well to that of the solvent control wells to determine the IC₅₀ and IC₃₀ values.

The remaining 3 plates were used in the luciferase induction endpoint of the assay. 100 µL of Promega's ONE-Glo Reagent was added to 100 µL of fresh media containing 10% FBS without geneticin. Cells were incubated for 5 minutes to induce cell lysis and release luciferin into the media. Plates were read with a luminometer and EC_{1.5} and maximum response (I_{max}) values were obtained.



OECD TG 442D: *In Vitro* Skin Sensitization

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Data and Reporting

Acceptance Criteria:

1. Gene induction obtained with the positive control, cinnamic aldehyde, should be statistically significant above the threshold of 1.5 in at least one of the tested concentrations (from 4 to 64 μM).
2. The EC_{1.5} value should be within two standard deviations of the historical mean and the average induction in the three replicates for cinnamic aldehyde at 64 μM should be between 2 and 8.
3. The average coefficient of variability of the luminescence reading for the negative (solvent) control DMSO should be below 20% in each experiment.

A KeratinoSens™ prediction is considered positive if the following conditions are met:

1. The I_{max} is higher than 1.5-fold and statistically significantly higher as compared to the solvent (negative) control
2. The cellular viability is higher than 70% at the lowest concentration with a gene induction above 1.5 fold (i.e., at the EC_{1.5} determining concentration)
3. The EC_{1.5} value is less than 1000 μM (or < 200 $\mu\text{g/ml}$ for test chemicals with no defined MW)
4. There is an apparent overall dose-response for luciferase induction

Results

Compound	Classification	EC _{1.5} (μM)	IC ₅₀	I _{max}
Cinnamic aldehyde	Sensitizer	19	289.19 μM	30.86
DMSO	Non-Sensitizer	No Induction	243.24 μM	0.19
ABS Corn Flower Extract	Non-Sensitizer	No Induction	> 1000 μM	0.33

Table 1: Overview of KeratinoSens™ Assay Results (I_{max} equals the average induction values Fig.1)

KeratiNoSens™ Assay ABS Corn Flower Extract

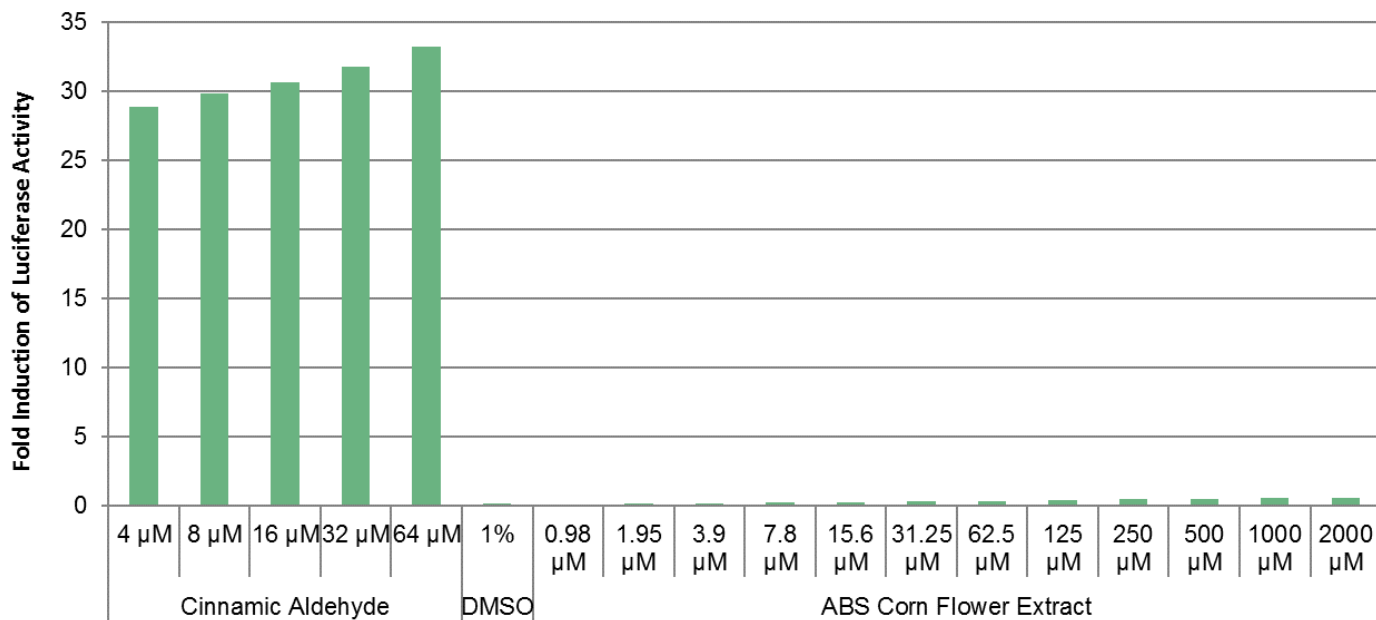


Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **ABS Corn Flower Extract (10109REV)** was not predicted to be a skin sensitizer based on the KeratiNoSens™ ARE-Nrf2 Luciferase Test Method as there was not a significant increase in luciferase expression. It can be concluded that **ABS Corn Flower Extract** can be safely used in cosmetics and personal care products at typical use levels.



Phototoxicity Assay Analysis

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Tradename: ABS Corn Flower Extract

Code: 10109REV

CAS #: 107-88-0 & 84012-18-0

Test Request Form #: 4933

Lot #: 59950P

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

Study Director: Maureen Danaher

Principle Investigator: Jennifer Goodman

Test Performed:

In Vitro EpiDerm™ Model (EPI-200-SIT) Phototoxicity

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **ABS Corn Flower Extract** would induce phototoxic irritation in the EpiDerm™ model assay.

The product was tested according to the manufacturer's protocol. The test article solution was found to be a non-photoirritant at concentrations of 0.5%, 1.5%, 5.0% and 10.0%. Reconstructed human epidermis was incubated in growth media for one hour to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substance was applied to the tissue inserts in four varying concentrations and incubated overnight at 37°C, 5% CO₂, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm² (=6 J/cm²). After substance incubation, irradiation, and washing was completed, the cell viability test was conducted. Cell viability was measured by dehydrogenase conversion of MTT [(3-4,5-dimethyl thiazole 2-yl)], present in the cell mitochondria, into blue formazan salt that was measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues.

Under the conditions of this assay, the test article was considered to be non-phototoxic at concentrations of 0.5%, 1.5%, 5.0%, and 10.0%. The negative and positive controls performed as anticipated.

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Phototoxicity Assay Analysis

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I. Introduction

A. Purpose

In vitro dermal phototoxicity study was conducted to evaluate whether a test article would induce photoirritation in the EpiDerm™ model assay. MatTek Corporation's reconstructed human epidermal model is becoming a standard in determining the phototoxicity potential of a test substance. This assay is able to discriminate between photoirritants and non-photoirritants at varying concentrations.

II. Materials

- | | |
|----------------------------------|---|
| A. Incubation Conditions: | 37°C at 5% CO ₂ and 95% relative humidity |
| B. Equipment: | Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; UVA-vis Irradiation Equipment; UVA meter; Pipettes |
| C. Media/Buffers: | Dulbecco's Modified Eagle Medium (DMEM) based medium; Dulbecco's Phosphate-Buffered Saline (DPBS); sterile deionized H ₂ O |
| D. Preparation: | Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components |
| E. Tissue Culture Plates: | Falcon flat bottom 96-well, 24-well, and 6-well tissue culture plates |
| F. Reagents: | MTT (3-4,5-dimethyl thiazole 2-yl) (1.0mg/mL); Extraction Solution (Isopropanol); Chlorpromazine; Triton X-100 (1%) |
| G. Other: | Wash bottle; sterile disposable pipette tips; Parafilm; forceps |

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™ consists of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis. This model consists of organized basal, spinous, and granular layers, and contains a multilayer stratum corneum containing intercellular lamellar lipid layers. The EpiDerm™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile deionized water is used as the negative controls for the EpiDerm™ Phototoxicity assay.

C. Positive Control

Concentrations of chlorpromazine, ranging from 0.001% to 0.1%, were used as positive controls for the EpiDerm™ Phototoxicity assay.

D. Data Interpretation Procedure

A photoirritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance and 60 minutes of 6 J/cm² is reduced by 20% compared to the non-irradiated control tissues.

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Phototoxicity Assay Analysis

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IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO₂ and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and tissue insert dosing begins.

B. Test Substance Exposure

50µL of the diluted test substance in their respective concentrations are applied to 2 tissue inserts and allowed to incubate for overnight in a humidified incubator (37°C, 5% CO₂, 95% RH).

C. Tissue Irradiation

Tissue inserts in their 6-well plates are UVA-irradiated for 60 minutes with 6 J/cm² at room temperature. The non-irradiated tissue inserts are incubated at room temperature in the dark.

D. Tissue Washing and Post Incubation

After UVA-irradiation and dark incubation is complete the tissue inserts are washed using sterile DPBS and transferred to fresh 6-well plates and media for overnight incubation at 37°C, 5% CO₂, 95% RH.

E. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO₂, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD₅₇₀) is ≥ 0.8.

B. Positive Control

The assay meets the acceptance criterion if a dose dependent reduction in cell viability in the UVA-irradiated tissues is between 0.00316% and 0.0316%.

C. Standard Deviation

Since the phototoxicity potential is predicted from the mean viability of 2 tissues for the EpiDerm™ Phototoxicity Protocol, the variability of the replicates should not exceed 30%.

Phototoxicity Assay Analysis

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VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ assay kit were in good condition, intact, and viable.

B. Tissue Viability Assay

The results are summarized in Figure 1. Cell viability is calculated for each tissue as a percentage of the corresponding vehicle control either irradiated or non-irradiated. Tissue viability was not reduced by 20% in the presence of the test substance and UVA-irradiation at concentrations of 0.5%, 1.5%, and 5.0%. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited dose dependent loss of tissue viability and cell death.

C. Test Validity

The data obtained from this study met criteria for a valid assay. The negative and positive controls performed as anticipated.

VII. Conclusion

Phototoxicity (photoirritation) is defined as an acute toxic response that is elicited after exposure of the skin to certain chemicals and subsequent exposure to light. Under the conditions of this assay, the test article substance was considered to be non-phototoxic at concentrations of 0.5%, 1.5%, 5.0%, and 10.0%. The negative and positive controls performed as anticipated.

There is a slight decrease in viability at the 10% concentration but viability does not decrease more than the acceptable 20%. We can safely say that **ABS Corn Flower Extract** is not a photoirritant when used at the suggested use levels of 1.0% -10.0%.

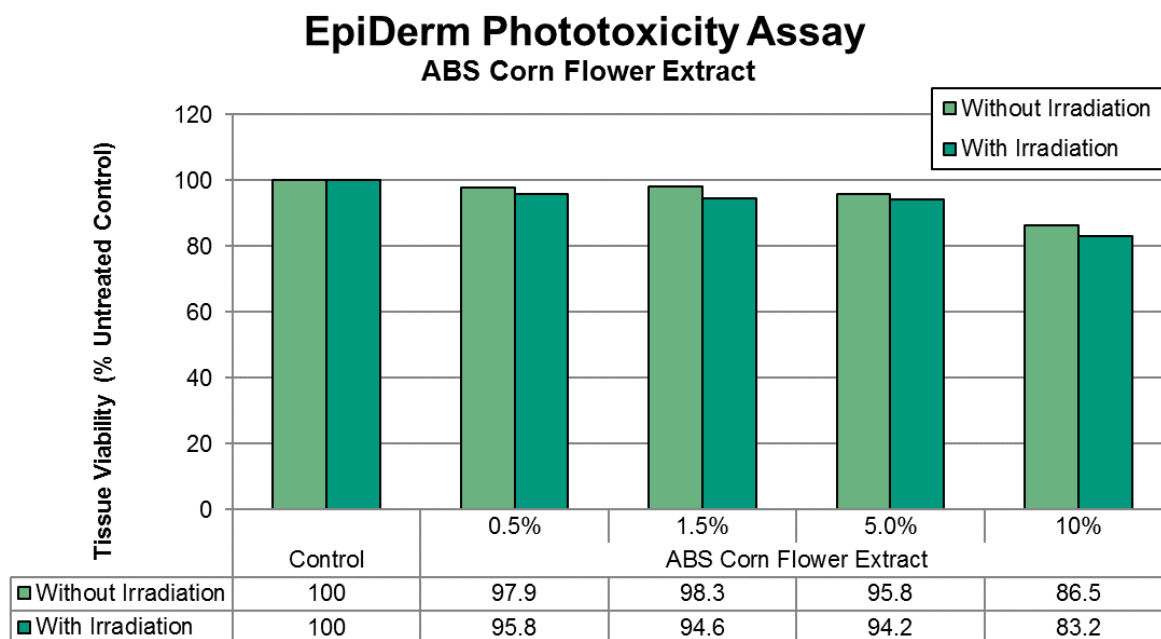


Figure 1: EpiDerm Phototoxicity Graph

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10109REV ABS Corn Flower Extract UV Spectrum

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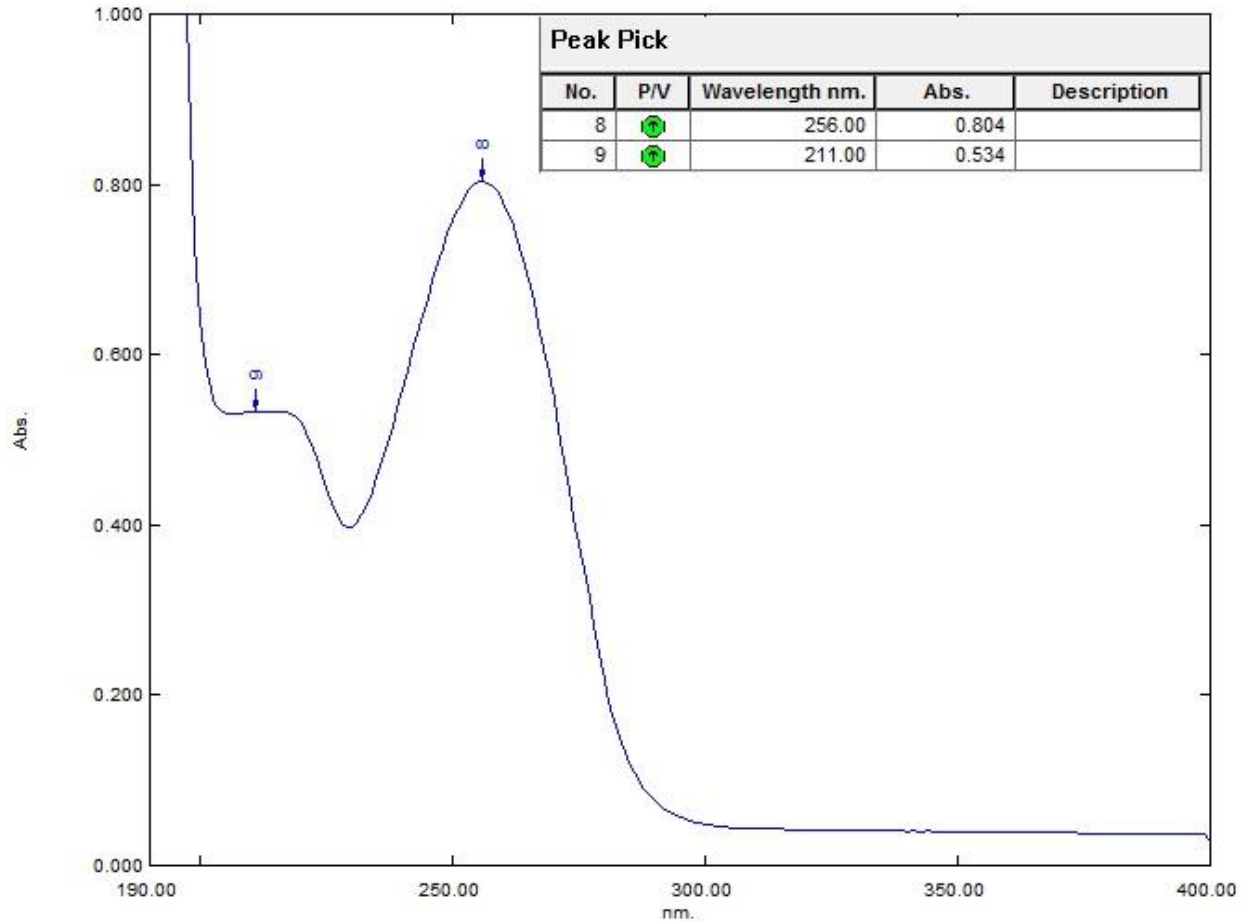


Figure 1—UV spectrum of 10109REV ABS Corn Flower Extract lot 59950, 0.05% in water.



Bacterial Reverse Mutation Test

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Test Article: ABS Corn Flower Extract
Code Number: 10109REV
CAS #: 107-88-0 & 84012-18-0

Sponsor:
Active Concepts, LLC
107 Technology Drive
Lincolnton, NC 28092

Study Director: Maureen Danaher
Principle Investigator: Monica Beltran

Test Performed:
Genotoxicity: Bacterial Reverse Mutation Test

Reference:
OECD471/ISO10993.Part 3

Test Request Number: 4934

SUMMARY

A *Salmonella typhimurium*/*Escherichia coli* reverse mutation standard plate incorporation study described by Ames *et al.* (1975) was conducted to evaluate whether a test article solution **ABS Corn Flower Extract** would cause mutagenic changes in the average number of revertants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2uvrA in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The stock test article was tested at eight doses levels along with appropriate vehicle control and positive controls with overnight cultures of tester strains. The test article solution was found to be noninhibitory to growth of tester strain TA98, TA100, TA1537, TA1535 and WP2uvrA after Sport Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45°C supplemented with histidine-biotin solution for the *Salmonella typhimurium* strains and supplemented with tryptophan for *Escherichia coli* strain were inoculated with 100 µl of tester strains, 100 µl of vehicle or test article dilution were added and 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. After vortexing, the mixture was poured across the Minimal Glucose Agar (GMA) plates. Parallel testing was also conducted with positive control correspond to each strain, replacing the test article aliquot with 50µl aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for 48 hours at 37°C. The mean numbers of revertants of the test plates were compared to the mean number of revertants of the negative control plates for each of the strains tested. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* tester strain WP2uvrA. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

All *Salmonella* tester strain cultures demonstrated the presence of the deep rough mutation (*rfa*) and the deletion in the *uvrB* gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvrA* cultures demonstrated the deletion in the *uvrA* gene. All cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2uvrA, 10-60.

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Bacterial Reverse Mutation Test

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I. Introduction

A. Purpose

A *Salmonella typhimurium*/*Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a test article solution would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvrA* in the presence and absences of the S9 metabolic activation. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards.

II. Materials

- A. **Storage Conditions:** Room temperature (23-25C).
- B. **Vehicle:** Sterile DI Water.
- C. **Preparation:** Eight different doses level were prepared immediately before use with sterile DI water.
- D. **Solubility/Stability:** 100% Soluble and Stable.
- E. **Toxicity:** No significant inhibition was observed.

III. Test System

A. Test System

Each *Salmonella typhimurium* and *Escherichia coli* tester strain contains a specific deep rough mutation (*rfa*), the deletion of *uvrB* gene and the deletion in the *uvrA* gene that increase their ability to detect mutagens, respectively. These genetically altered *Salmonella typhimurium* strains (TA98, TA100, TA1537 and TA1535) and *Escherichia coli* strain (WP2*uvrA*) cannot grow in the absence of histidine and tryptophan, respectively. When placed in a histidine-tryptophan free medium, only those cells which mutate spontaneously back to their wild type states are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

<u>Tester strain</u>	<u>Mutations/Genotypic Relevance</u>
TA98	hisD3052, Dgal chID bio <i>uvrB rfa</i> pKM101
TA100	hisG46, Dgal chID BIO <i>uvrB rfa</i> pKM101
TA1537	hisC3076, <i>rfa</i> , Dgal chID bio <i>uvrB</i>
TA 1535	hisG46, Dgal chID bio <i>uvrB rfa</i>
WP2 <i>uvrA</i>	trpE, <i>uvrA</i>

<i>rfa</i>	=	causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
<i>uvrB</i>	=	deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
<i>uvrA</i>	=	All possible transitions and transversions, small deletions.

B. Metabolic Activation

Aroclor induced rat liver (S9) homogenate was used as metabolic activation. The S9 homogenate is prepared from male Sprague Dawley rats. Material is supplied by MOLTOX, Molecular Toxicology, Inc.

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C. Preparation of Tester strains

Cultures of *Salmonella typhimurium* TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2uvrA were inoculated to individual flasks containing Oxoid broth No.2. The inoculated broth cultures were incubated at 37°C in an incubator shaker operating at 140-150 rpm for 12-16 hours.

D. Negative Control

Sterile DI water (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of revertants colonies that developed in each test plate were compared to determine whether the test material had significant mutagenic properties.

E. Positive Control

A known mutagen for each strain was used as a positive control to demonstrate that tester strains were sensitive to mutation to the wild type state. The positive controls are tested with and without the presence of S9 homogenate.

F. Titer of the Strain Cultures:

Fresh cultures of bacteria were grown up to the late exponential or early stationary phase of growth; to confirm this, serial dilutions from each strain were conducted, indicating that the initial population was in the range of 1 to 2×10^9 /ml.

IV. Method

A. Standard Plate Incorporation Assay:

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for *Escherichia coli* were inoculated with 100 μ l of culture for each strain and 100 μ l of testing solution or vehicle without test material. A 500 μ l aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across Minimal Glucose Agar plates labeled with strain number and S9 activation (+/-). When plating the positive controls, the test article aliquot was replaced by 50 μ l aliquot of appropriate positive control. The test was conducted per duplicate. The plates were incubated for 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of revertants was determined. The mean numbers of revertants of the test plates were compared to the mean number of revertants of the negative control of each strain used.

V. Criteria for a Valid Test

For the test solution to be evaluated as a test failure or "potential mutagen" there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for any or all strains. Each positive control mean must have exhibited at least a 3-fold increase over the respective negative control mean of the *Salmonella* and *Escherichia coli* tester strains used.

All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvrB* gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvrA* cultures must demonstrate the deletion in the *uvrA* gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2uvrA, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to 0.3×10^9 cells/ml.

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The mean of each positive control must exhibit at least 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels is required to evaluate assay data. A dose level is considered toxic if one of both of the following criteria are met: (1). A >50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2). At least a moderate reduction in the background lawn.

VI. Results and Discussion

A. Solubility:

Water was used as a solvent. Solutions from the test article were made from 0.015 to 50mg/ml.

B. Dose levels tested:

The maximum dose tested was 5000 µg per plate. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate.

C. Titer (Organisms/ml):

5×10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2×10^9 UFC/ml.

D. Standard Plate Incorporation Assay

In no case was there a 2-fold or greater increase in the mean number of revertant testing strains TA98, TA100, TA1537, TA1535 and WP2*uvrA* in the presence of the test solution compared with the mean of vehicle control value. The positive controls mean exhibited at least a 3-fold increase over the respective mean of the *Salmonella typhimurium* and *Escherichia coli* tester strains used. The results are summarized in Appendix 2.

All *Salmonella* tester strain cultures demonstrated the presence of the deep rough mutation (*rfa*) and the deletion in the *uvrB* gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvrA* cultures demonstrated the deletion in the *uvrA* gene. All cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvrA*, 10-60.

VII. Conclusion

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvrA*. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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Appendix 2:

Bacterial Mutation Assay Plate Incorporation Assay Results

	Concentration µg per Plate	TA98		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	12	14	13
	1500	14	17	16
	500	10	12	11
	150	25	25	25
	50	13	16	15
	15	12	24	18
	5.0	31	30	31
	1.5	42	37	40
Test Solution w/o S9	5000	13	16	15
	1500	25	23	24
	500	18	24	21
	150	22	29	26
	50	25	29	27
	15	13	14	14
	5.0	39	37	38
	1.5	21	21	21
DI Water w/S9		20	19	20
DI Water w/o S9		68	62	65
2-aminoanthracen w/ S9		142	139	141
2-nitrofluorene w/o S9		125	131	128
Historical Count Positive w/S9		43-1893		
Historical Count Positive w/o S9		39-1871		
Historical Count Negative w/S9		4-69		
Historical Count Negative w/o S9		3-59		

*CFU = Colony Forming Units

*Mean = Average of duplicate plates

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	Concentration µg per Plate	TA100		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	115	123	119
	1500	143	149	146
	500	165	142	154
	150	143	159	151
	50	162	151	157
	15	163	149	156
	5.0	132	133	133
	1.5	116	112	114
Test Solution w/o S9	5000	133	136	135
	1500	137	129	133
	500	145	188	167
	150	149	141	145
	50	111	127	119
	15	116	145	131
	5.0	122	116	119
	1.5	110	125	118
DI Water w/S9		98	116	107
DI Water w/o S9		77	84	81
2-aminoanthracen w/ S9		616	626	621
Sodium azide w/o S9		551	607	579
Historical Count Positive w/S9		224-3206		
Historical Count Positive w/o S9		226-1837		
Historical Count Negative w/S9		55-268		
Historical Count Negative w/o S9		47-250		

*CFU = Colony Forming Units

*Mean = Average of duplicate plates

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	Concentration µg per Plate	TA1537		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	15	14	15
	1500	8	8	8
	500	14	18	16
	150	26	11	19
	50	15	14	15
	15	13	10	12
	5.0	8	7	8
	1.5	13	17	15
Test Solution w/o S9	5000	22	23	23
	1500	10	16	13
	500	15	19	17
	150	14	16	15
	50	17	15	16
	15	22	23	23
	5.0	29	26	28
	1.5	15	9	12
DI Water w/S9		18	14	16
DI Water w/o S9		28	22	25
2-aminoanthracen w/ S9		355	370	363
2-aminoacridine w/o S9		128	127	128
Historical Count Positive w/S9		13-1934		
Historical Count Positive w/o S9		17-4814		
Historical Count Negative w/S9		0-41		
Historical Count Negative w/o S9		0-29		

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*Mean = Average of duplicate plates

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	Concentration µg per Plate	TA1535		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	9	17	13
	1500	14	10	12
	500	14	18	16
	150	9	13	11
	50	12	12	12
	15	19	11	15
	5.0	20	22	21
	1.5	7	5	6
Test Solution w/o S9	5000	20	15	17
	1500	13	16	15
	500	16	12	14
	150	17	10	14
	50	22	12	17
	15	16	19	18
	5.0	16	8	12
	1.5	11	16	14
DI Water w/S9		38	39	39
DI Water w/o S9		11	14	13
2-aminoanthracen w/ S9		145	166	156
Sodium azide w/o S9		744	732	738
Historical Count Positive w/S9		22-1216		
Historical Count Positive w/o S9		47-1409		
Historical Count Negative w/S9		1-50		
Historical Count Negative w/o S9		1-45		

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*Mean = Average of duplicate plates

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	Concentration µg per Plate	WP2uvrA		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	8	14	11
	1500	16	13	15
	500	26	17	22
	150	16	15	16
	50	16	13	15
	15	8	9	9
	5.0	22	19	21
	1.5	6	12	9
Test Solution w/o S9	5000	30	27	29
	1500	20	25	23
	500	26	24	25
	150	12	15	14
	50	28	29	29
	15	14	22	18
	5.0	26	21	24
	1.5	16	9	13
DI Water w/S9		25	30	28
DI Water w/o S9		25	27	26
2-aminoanthracen w/ S9		158	161	160
Methylmethanesulfonate w/o S9		240	264	252
Historical Count Positive w/S9		44-1118		
Historical Count Positive w/o S9		42-1796		
Historical Count Negative w/S9		8-80		
Historical Count Negative w/o S9		8-84		

*CFU = Colony Forming Units

*Mean = Average of duplicate plates

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