Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics

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Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, 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. This safety assessment was prepared by Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR.

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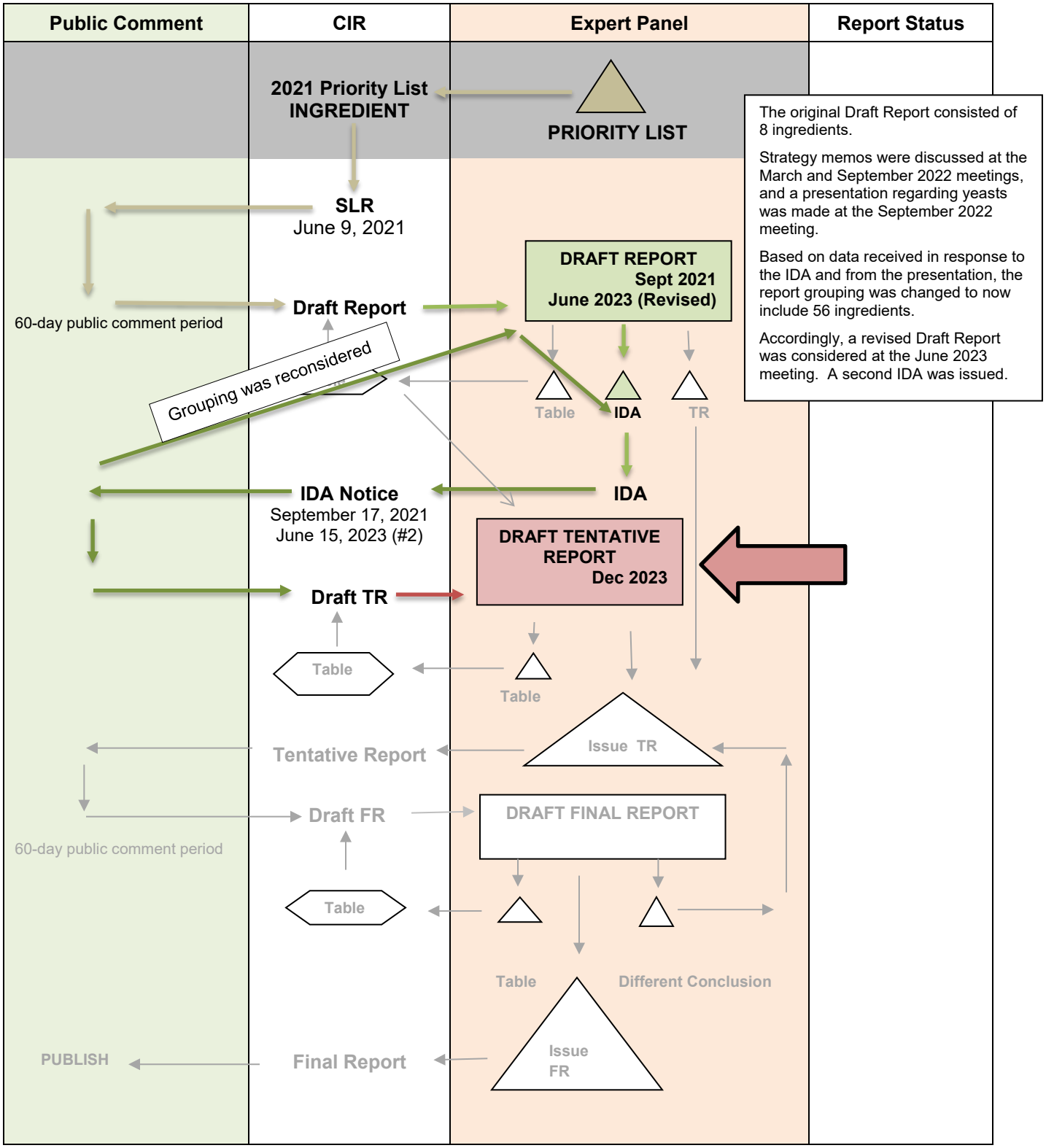
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Yeast-derived ingredients

MEETING December 2023



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR
Date: November 9, 2023
Subject: Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics

Enclosed is the Draft Tentative Report on the Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics (*report_Yeast_122023*). At the June 2023 meeting, the Panel reviewed the Revised Draft Report on these 56 yeast-derived ingredients and issued a second Insufficient Data Announcement (IDA) for this ingredient group. (The first IDA was issued at the September 2021 meeting.) In this IDA, in order to determine the safety of these ingredients, the Panel requested confirmatory dermal sensitization data and data on food use/generally recognized as safe (GRAS) status on the yeast species used to derive these ingredients for all ingredients in which this is absent. In lieu of food use/GRAS status data, 28-day dermal toxicity data may be considered. In addition, at the June meeting, the Panel requested information regarding Qualified Presumption of Safety (QPS) status (as designated by the European Union), in order to determine if this parameter may be used to clear the systemic toxicity/food use data needs for ingredients derived from yeast species that have a QPS status. Information on QPS status and a list of yeast species that have QPS status designation can be found in the packet as *data1_Yeast_122023*.

Since the issuing of the IDA, considerable additional information have been received. A bulleted list of the data endpoints received, per data submission, is provided below.

- *data2_Yeast_122023*
  - data on *Galactomyces* ferment filtrate:
    - acute oral toxicity
    - genotoxicity
    - animal ocular irritation
    - human dermal irritation
    - animal photosensitization and phototoxicity
    - animal dermal sensitization
    - in vitro ocular irritation (facial treatment formulation containing 92.675% Galactomyces Ferment Filtrate)
- *data3_Yeast_122023*
  - HRIPT (104 subjects) of a skincare formulation containing 1.485% Galactomyces Ferment Filtrate
- *data4_Yeast_122023*
  - data on a facial treatment formulation containing 92.675% Galactomyces Ferment Filtrate
    - HRIPT (100 subjects)
    - in vitro ocular irritation (same study as submitted in *data2_Yeast_122023*)
- *data5_Yeast_122023*
  - data on an extract containing 0.4% Hydrolyzed Yeast
    - safety assessment summary
    - in vitro sensitization
    - HRIPT (tested at 0.01%; effective test concentration 0.00004% Hydrolyzed Yeast; 51 subjects)
- *data6_Yeast_122023*
  - data on Lipomyces Lipid Bodies and Lipomyces Oil
    - response to request for dermal data on yeast-derived ingredients (Lipomyces Oil and Lipomyces Lipid Bodies)

- *data7_Yeast_12023*
    - data on trade name mixture containing 49% Phaffia Rhodozyma Extract
        - genotoxicity
        - in vitro dermal and in vitro ocular irritation
        - in chemico skin sensitization
        - in vitro skin sensitization
        - in vitro phototoxicity
        - cellular viability assay analysis
    - data on trade name mixture containing 3% Saccharomyces Cerevisiae Extract
        - in vitro dermal and in vitro ocular irritation
    - data on trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate
        - genotoxicity
        - in vitro dermal and in vitro ocular irritation
        - in chemico skin sensitization
        - in vitro skin sensitization
        - in vitro phototoxicity
    - data on trade name mixture containing 98% Saccharomyces Lysate Extract
        - in vitro dermal and in vitro ocular irritation
    - data on trade name mixture containing 25% Saccharomyces Lysate Extract
        - cellular viability assay analysis
        - HRIPT (50 subjects)
    - data on trade name mixture containing 10% Saccharomyces Lysate Extract
        - in vitro dermal and in vitro ocular irritation
- *data8_Yeast_122023*
    - HRIPT (100 subjects) on Saccharomyces Ferment Lysate Filtrate (2% non-volatile solids in water)
- *data9_Yeast_122023*
    - spreadsheet of summary toxicological and food use information on many yeast species (provided by manufacturer)
- *data10_Yeast_122023*
    - spreadsheet of references for food use on many yeast species (provided by manufacturer)
- *data11_Yeast_122023*
    - concentration of use survey results on Hydrolyzed Saccharomyces Cell Wall, Saccharomyces Ferment Extract, and Saccharomyces Ferment Extract Lysate Filtrate
- *data12_Yeast_122023*
    - HRIPTs on a cream containing 0.0135% Saccharomyces Ferment Lysate Filtrate, 0.028% Saccharomyces Lysate Extract, and a lotion containing 0.0045% Yeast Extract

The data profile (*dataprofile_Yeast_122023*) included herein is composed of three tables. Table 1 of the data profile includes all ingredients derived from a known yeast genus and species. The first column contains the names of the known genus/species used to derive the ingredients, and in the second column, the related ingredients are identified (e.g., column 1: *Phaffia rhodozyma*; column 2: Phaffia Rhodozyma Extract, Phaffia Rhodozyma Ferment Extract). If data were found on the cosmetic ingredient itself (e.g., Phaffia Rhodozyma Extract), or an ingredient derived from that genus and species with unknown cosmetic use (e.g., a *Phaffia rhodozyma* extract), a notation of available data will be present in the ingredient-specific (i.e., Phaffia Rhodozyma Extract) row.

If data were identified as Yeast Extract derived from a known yeast species, but the extract was not identical to the cosmetic ingredient (e.g., data were present for *Metschnikowia reukaufii* extract (not a wINCI ingredient), but not for Hydrolyzed Metschnikowia Reukaufii Extract (the cosmetic ingredient)), a notation of available data will be present in the species only row (i.e., *Metschnikowia reukaufii*) row.

Also in the first table, the “Food Use”, “QPS Status”, and “Dermal Sensitization” columns are highlighted in blue. If a strategy similar to the algae reports is used, ingredients with these types of use and information can be easily identified.

Table 2 of the data profile document lists the generic yeast-derived ingredients. This includes ingredients that, according to the *Dictionary*, do not have a reported genus and species (e.g., Yeast Extract), or, ingredients that have reported genus but no reported species (e.g., Hydrolyzed Saccharomyces Cell Wall). As many species of yeast may be used in the preparation

of these generic ingredients, proper searches could not be performed. However, if data were available on a generic ingredient derived from a specific yeast species (e.g., Yeast Extract derived from *Pichia anomala*), in addition to this being noted in Table 1, a notation was also made in this table indicating available data for that ingredient (e.g., Yeast Extract). Although this information is captured for the generic ingredient, it is unknown whether these data are completely representative for that ingredient since it is demonstrated that various species are used in the manufacture of these generic ingredients. Of note, a column to identify food use is not included in this table due to the generic nature of these ingredients.

Table 3 of the data profile document lists the 12 yeast species known to be used in the preparation of Yeast Extract. This table identifies the use of these yeast species in foods/QPS status and sensitization data.

According to all three data tables, the following 18 ingredients have both food use/QPS status and sensitization data:

Galactomyces Ferment Filtrate
Hydrolyzed Candida Saitoana Extract
Hydrolyzed Metschnikowia Agaves Extract
Hydrolyzed Metschnikowia Reukaufii Extract
Metschnikowia Agaves Extract
Metschnikowia Reukaufii Lysate Extract
Phaffia Rhodozyma Extract
Phaffia Rhodozyma Ferment Extract
Pichia Anomala Extract
Pichia Minuta Extract
Saccharomyces Cerevisiae Extract
Yeast Extract derived from *Candida magnoliae*
Yeast Extract derived from *Candida saitoana*
Yeast Extract derived from *Metschnikowia agaves*
Yeast Extract derived from *Metschnikowia reukaufii*
Yeast Extract derived from *Pichia anomala*
Yeast Extract derived from *Pichia minuta*
Yeast Extract derived from *Saccharomyces cerevisiae*

At the June 2023 meeting, the Panel questioned the removal of the following three yeast-derived ingredients: Hydrolyzed Yeast Protein, Yeast Beta-Glucan, and Yeast Polysaccharides. These ingredients were not included in the updated yeast-derived ingredient grouping, as they are discrete molecules. Historically, when the Panel has assessed the safety of natural complex substances, ingredients comprised of discrete molecules are typically excluded (e.g., rosmarinic acid was excluded from the review of rosemary-derived ingredients).

Also included in this packet are transcripts from the previous reviews of this report, including those meetings at which the strategy memos were discussed (*transcripts_Yeast_122023*), a search strategy (*search_Yeast_122023*), flow chart (*flow_Yeast_062023*), report history (*history_Yeast_122023*), and the presentation given to the Panel at the September 2022 meeting (*presentation_Yeast_122023*).

A draft Abstract and Discussion have been included in this report version. The Panel should carefully consider and discuss the data (or lack thereof), and issue a Tentative Report with a safe, safe with qualifications, insufficient data, unsafe, or split conclusion, and identify any additional items for inclusion in the Discussion.

Yeast-Derived Ingredients History

January 2021

- Concentration of use data received on Hydrolyzed Yeast Extract, Hydrolyzed Yeast, Hydrolyzed Yeast Protein, Yeast, Yeast Beta-Glucan, Yeast Extract, Yeast Polysaccharides, and Saccharomyces Cerevisiae Extract

June 2021

- SLR posted
- Summary manufacturing, physical/chemical properties data received from Council on a Saccharomyces Cerevisiae Extract
- Manufacturing, physical properties, and heavy metal specifications data received from Council on Yeast Extract Beta Glucan

July 2021

- Manufacturing, composition, and impurities data received from Council on several Saccharomyces Cerevisiae Extracts
- Comments received from Council on SLR
- FCC monograph received on Yeast, Dried

September 2021

- Expert Panel reviews Draft Report and issues an IDA
- Comments received on Draft Report from Council
- IDA requests:
    - Clarification on which species of yeast used in the manufacturing of cosmetic ingredients
    - Once clarification made, method of manufacturing data, composition, impurities, sensitization, and irritation data requested
        - If GRAS status/food use not noted for species, systemic toxicity data requested (28-d dermal toxicity, genotoxicity, DART)

October 2021

- In vitro dermal and ocular irritation data received on a trade name mixture containing 1.25% Yeast Extract (derived from *Saccharomyces cerevisiae*)
- In vitro dermal and ocular irritation data receive on a trade name mixture containing 4.5% Yeast Extract (derived from *Saccharomyces cerevisiae*)

December 2021

- Manufacturing data received on a Yeast Extract (derived from *Saccharomyces cerevisiae*)
- Physical and Chemical properties data received on a Yeast Extract (derived from *Saccharomyces cerevisiae*)

January 2022

- 2022 VCRP data received and report updated
    - All ingredients have increased number of uses excluding Yeast Beta-Glucan and Saccharomyces Cerevisae Extract

February 2022

- Data received on Yeast Extracts derived from several species – method of manufacture, comp/impurities, derm abs, irr/sens

March 2022

- Strategy memo issued – asked Panel for guidance on if report should focus only on *Saccharomyces cerevisiae*-derived ingredients, or if all yeasts belonging to the class Saccharomycetes should be included

September 2022

- Strategy memo 2 issued – memo contained list of all yeast ingredients in the Dictionary – Panel decided to create Draft Revised Report on all ingredients, regardless of GRAS/food status or VCRP data
- Presentation from SILAB

February 2023

- Concentration of use data received on newly added ingredients

April 2023

- Polysaccharide, protein, beta-glucan, and octenylsuccinate ingredients removed from listing reviewed

June 2023

- Panel reviews Revised Draft Report and issues Insufficient Data Announcement #2
    - needs: human dermal sensitization data and data on food use/GRAS; in lieu of food use/GRAS data, 28-d dermal toxicity considered
    - HRIPT and in vitro ocular irritation data received on Galactomyces Ferment Filtrate
    - Data on Lipomyces Lipid Bodies (impurities, use assay using body cream containing Lipomyces Lipid Bodies)

July 2023

- Data on *Galactomyces* ferment filtrate (several toxicity endpoints) received
- Dermal, ocular, and phototoxicity data received on several ingredients (Phaffia Rhodozyma Extract, Saccharomyces Cerevisiae Extract, Saccharomyces Ferment Lysate Filtrate, and Saccharomyces Lysate Extract)

August 2023

- QPS information and data table received from SILAB
- HRIPT on Galactomyces Ferment Filtrate received
- Summary safety information and in vitro/human sensitization data received on Hydrolyzed Yeast

September 2023

- HRIPT on Saccharomyces Ferment Lysate Filtrate received

October 2023

- Food use references received from SILAB supporting food use statements made in table received from SILAB in August 2023

December 2023

- Panel reviews Draft Tentative Report

Table 1. Data profile on ingredients with reported species (32 total ingredients) - December 2023 – Writer, Priya Cherian

Gemus/Species[a]	Related Ingredients						Toxicokinetics		Acute Tox			Repeated Dose Tox			DART	Genotox		Carci	Dermal Irritation			Phototox	Derm Sens			Ocular Irr	Clinical Studies
		Reported Use	Method of Mfg	Comp/Impurities	Food Use or Presence	QPS Status	log K_{ow}/Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation		In Vitro	In Vivo		In Vitro	Animal	Human		In Vitro	Animal	Human		Case Reports
Candida bombicola																											
	Hydrolyzed Candida Bombicola Extract				X																						
Candida saitoana					X			X													X				X		
	Hydrolyzed Candida Saitoana Extract	X																									
*Galactomyces candidus*** *Galactomyce fermentans*** *Galctomyces reesii***					X																						
	Galactomyces Ferment Filtrate	X								X						X					X			X	X	X	
*Kluyveromyces fragilis*** *Kluyveromyces lactis***			X	X	X	X																					
	Hydrolyzed Kluyveromyces Extract																										
	Kluyveromyces Extract	X											X														
Lipomyces starkeyi				X		X																					
	Lipomyces Oil		X																								
	Lipomyces Oil Extract																										
Metschnikowia agaves					X																						
	Hydrolyzed Metschnikowia Agaves Extract																										
	Metschnikowia Agaves Extract							X													X				X		
Metschnikowia henanensis																											
	Metschnikowia Henanesis Extract																										
Metschnikowia reukaufii					X			X											X		X				X		
	Hydrolyzed Metschnikowia Reukaufii Extract																										
	Metschnikowia Reukaufii Lysate Extract																										
Metschnikowia shanxiensis																											

Table 1. Data profile on ingredients with reported species (32 total ingredients) - December 2023 – Writer, Priya Cherian

Gemus/Species[a]	Related Ingredients						Toxicokinetics		Acute Tox			Repeated Dose Tox			DART	Genotox		Carci	Dermal Irritation			Phototox	Derm Sens			Ocular Irr	Clinical Studies
		Reported Use	Method of Mfg	Comp/Impurities	Food Use or Presence	QPS Status	log K_{ow}/Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation		In Vitro	In Vivo		In Vitro	Animal	Human		In Vitro	Animal	Human		Case Reports
	Hydrolyzed Mestchnikowia Shanxiensis																										
Mestchnikowia viticola					X																						
	Metschnikowia Viticola Extract																										
Phaffia rhodozyma				X		X																					
	Phaffia Rhodozyma Extract												X			X	X		X			X	X			X	
	Phaffia Rhodozyma Ferment Extract																										
Pichia anomala					X	X																					
	Pichia Anomala Extract	X						X													X				X		
Pichia caribicca					X																						
	Pichia Caribbica Ferment																										
Pichia heedii																											
	Pichia Heedii Extract							X													X				X		
Pichia minuta					X																						
	Pichia Minuta Extract							X								X					X				X		
Pichia pastoris						X		X																			
	Pichia Ferment Extract Filtrate																										
	Pichia Pastoris Ferment Filtrate																										
*Pichia populi** Pichia stipitis***																											
	Pichia Ferment Lysate Filtrate	X																									
Torulaspora delbrueckii					X																						
	Hydrolyzed Torulaspora Delbrueckii Extract																										
	Torulaspora Delbrueckii Extract																										
	Torulaspora Delbrueckii Ferment																										
Saccharomyces cerevisiae			X	X	X	X				X	X		X			X	X			X			X				X
	Saccharomyces Cerevisiae Extract	X	X	X					X										X		X		X			X		
Schizosaccharomyces pombe					X	X																					

Table 1. Data profile on ingredients with reported species (32 total ingredients) - December 2023 – Writer, Priya Cherian

Gemus/Species[a]	Related Ingredients	Reported Use	Method of Mfg	Comp/Impurities	Food Use or Presence	QPS Status	Toxicokinetics: log K_{ow}/Dermal Penetration	Toxicokinetics: ADME	Acute Tox: Dermal	Acute Tox: Oral	Acute Tox: Inhalation	Repeated Dose Tox: Dermal	Repeated Dose Tox: Oral	Repeated Dose Tox: Inhalation	DART	Genotox: In Vitro	Genotox: In Vivo	Carci	Dermal Irritation: In Vitro	Dermal Irritation: Animal	Dermal Irritation: Human	Phototox	Derm Sens: In Vitro	Derm Sens: Animal	Derm Sens: Human	Ocular Irr	Clinical Studies: Case Reports
	Schizosaccharoymces Pombe Extract			X																							
Yarrowia lipolytica			X		X	X																					
	Yarrowia Lipolytica Extract																										
	Yarrowia Lipolytica Ferment Lysate																										
	Yarrowia Lipolytica Oil																										

[a]when data is marked as present in a row that states the species only (e.g., *Candida saitoana*), data was found for the general species (or synonymous species) used in the production of the ingredients, or an ingredient similar to an ingredient in this report, using the relevant species (e.g., data was not found on Hydrolyzed Candida Saitoana Extract, but data was found on a Candida Saitoana Extract; since these are not the same ingredient, but are similar ingredients, the notation of present data would be placed in the species (*Candida saitoana*) row

*in some cases, multiple species are listed in a singular cell – this is because the related ingredient may be derived from either of these species (e.g., Pichia Ferment Lysate Filtrate may be derived from either *Pichia populi* or *Pichia stipitis*)

Table 2. Data profile on generic yeast ingredients*

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Comp./Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	Im Chemico/In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Hydrolyzed Saccharomyces Cell Wall							X	X	X						X	X				X			X						
Hyrdrolyzed Saccharomyces Extract																													
Hydrolyzed Saccharomyces Lysate Extract																													
Hydrolyzed Yeast	X							X			X											X		X					
Hydrolyzed Yeast Extract																													
Lactic Yeasts																													
Lipomyces Lipid Bodies			X																		X								
Pichia Extract																													
Saccharomyces																													
Saccharomyces Extract																													
Saccharomyces Ferment	X						X				X				X														
Saccharomyces Ferment Extract																													
Saccharomyces Ferment Extract Lysate Filtrate	X																												
Saccharomyces Ferment Filtrate	X																												
Saccharomyces Ferment Lysate Extract																													
Saccharomyces Ferment Lysate Filtrate	X														X				X			X		X	X	X			
Saccharomyces Lysate	X																												
Saccharomyces Lysate Extract	X																		X					X		X			
Saccharomyces Lysate Extract Filtrate																													
Saccharomyces Lysate Filtrate																													
Schizosaccharomyces Ferment Extract Filtrate																													
Schizosaccharomyces Ferment Filtrate	X																												
Yeast	X		X																										
Yeast Extract	X	X	X			X		X							X				X	X	X	X		X		X	X		
Yeast Ferment Extract	X																												

As these are generic ingredients, several species of yeast may be used in the preparation of these ingredients; a notation (X) was placed in the table above if toxicity data were present on these ingredients, when derived from a particular yeast species (e.g., Yeast Extract derived from Pichia anomala); it is unknown whether this data is representative of the generic ingredient as a whole, as it is unkown which/how many species are used in the production of these ingredients

It should be noted that searches for most generic yeast ingredients (both ingredients with no reported genus or species, and ingredients with only genus reported (according to the wINCI Dictionary), as presented in Table 2, could not be adequately performed as it is unknown which species are being referred to in the production of these ingredients.

Table 3. Food use and sensitization data for known generic Yeast Extract strains*

	Food		Sensitization		
	Food use/presence/GRAS	QPS status	In Vitro	Animal	Human
Candida magnoliae	X		X		
Candida oleophila	X				
Candida saitoana	X				X
Debaryomyces nepalensis	X				
Metschnikowa agaves	X				X
Metschnikowia reukaufii	X		X		X
Metschnikowia pulcherrima	X				
Pichia anomala	X	X			X
Pichia heedii					X
Pichia minuta	X	X			X
Pichia naganishii			X		
Saccharomyces cerevisiae	X			X	

*The yeast species listed in this table are the only known species of yeast used in the production of Yeast Extract

Yeast-Derived Ingredients – December 2023– Writer, Priya Cherian

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Galactomyces Ferment Filtrate		✓							✓								
Hydrolyzed Candida Bombicola Extract		✓							✓								
Hydrolyzed Candida Saitoana Extract		✓							✓								
Hydrolyzed Kluyveromyces Extract		✓							✓								
Hydrolyzed Mestchnikowia Reufaukii Agaves Extract	1309127-75-0	✓							✓								
Hydrolyzed Metschnikowia Reufaukii Extract									✓								
Hydrolyzed Mestchnikowia Shanxiensis		✓							✓								
Hydrolyzed Torulaspora Delbruekii Extract									✓								
Hydrolyzed Yeast Extract									✓								
Hydrolyzed Yeast		✓							✓								✓
Kluyveromyces Extract		✓	✓						✓								
Lactic Yeasts	68876-77-7								✓								
Lipomyces Lipid Bodies									✓								
Lipomyces Oil									✓								
Lipomyces Oil Extract									✓								
Metschnikowia Agaves Extract									✓								
Metschnikowia Henanensis Extract									✓								
Metschnikowia Reukaufii Lysate Extract									✓								

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Metschnikowia Viticola Extract									✓								
Pichia Caribbica Ferment									✓								
Pichia Ferment									✓								
Pichia Ferment Extract Filtrate		✓							✓								
Pichia Ferment Lysate Filtrate		✓							✓								
Pichia Pastoris Ferment Filtrate		✓							✓								
Phaffia Rhodozyma Filtrate									✓								
Phaffia Rhodozyma Ferment Extract									✓								
Pichia Anomala Extract	1033319-29-7	✓							✓								
Pichia Heedii Extract	1801269-82-8								✓								
Pichia Minuta Extract									✓								
Saccharomyces									✓								
Saccharomyces Cerevisiae Extract	84604-16-0	✓	✓						✓	✓							✓
Saccharomyces Extract		✓							✓								
Saccharomyces Ferment									✓								
Saccharomyces Ferment Filtrate									✓								
Saccharomyces Ferment Lysate Filtrate									✓								
Saccharomyces Lysate	8013-01-2								✓								
Saccharomyces Lysate Extract	8013-01-2								✓								
Saccharomyces Lysate Extract Filtrate	8013-01-2								✓								
Schizosaccharomyces Ferment Extract Filtrate									✓								

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Schizosaccharomyces Ferment Filtrate									✓								
Schizosaccharomyces Ferment Filtrate									✓								
Schizosaccharomyces Pombe Extract		✓							✓								
Torulaspora Delbrueckii Extract	1291071-26-5	✓							✓								
Torulaspora Delbrueckii Ferment	1291071-26-5	✓							✓								
Yarrowia Lipolytica Extract		✓	✓						✓								
Yarrowia Lipolytica Ferment Lysate		✓	✓						✓								
Yarrowia Lipolytica Oil		✓	✓						✓								
Yeast	68876-77-7	✓	✓						✓								✓
Yeast Extract	68876-77-7; 8013-01-2								✓								
Yeast Ferment Extract									✓								

Search Strategy

- All search terms were used in PubMed
- Search terms were searched in the “Pertinent Websites” listed below

Typical Search Terms

- INCI names
- Species names (e.g., Pichia anomala)
- CAS numbers

LINKS

Search Engines

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

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

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

SEPTEMBER 2021 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – September 13, 2021

DR. BELSITO: Okey-doke. Okay, so we now will soon be rising after we do yeast. This is the first time that we're reviewing eight ingredients. It went out in June of 2021, unpublished data from the Council put into the report summarizing manufacturing visible chem property data on Saccharomyces Cerevisiae: manufacturing physical properties, heavy metal specifications on yeast extract made of glucan, and manufacturing, composition, impurities on several other Saccharomyces Cerevisiae Extracts in concentration of use data.

The issue was the term "yeast" which pertains to a wide variety of species, and it's not known what is being used in the cosmetic ingredient. So, you will see how this has been posed to us. We should choose to cite this lack of clarification as a data insufficiency or choose to limit our report conclusion to the uses of the yeast where the ingredient exclusively comprises Saccharomyces Cerevisiae, which would be the only yeast species that would be covered by this report. And I sort of felt like, let's just go with Saccharomyces Cerevisiae but I want to open that up for discussion.

DR. LIEBLER: Well, I think the available information strongly implies that it's Saccharomyces Cerevisiae but it doesn't explicitly state it, so that's our challenge. So that second option is to treat this as if it's a Saccharomyces Cerevisiae report and maybe even change the title.

DR. BELSITO: Yeah.

DR. LIEBLER: And then indicate in the introduction that we are proceeding on the understanding that yeast used in cosmetic ingredients will be Saccharomyces which is widely used in food and is widely regarded as safe in food additives, as food substances, and so forth. So I'm okay with taking that approach.

DR. BELSITO: Paul? You must be muted.

DR. SNYDER: No, I was just -- so what is the basis for that reasoning? The yeast not otherwise specified is somehow being different than Saccharomyces Cerevisiae?

DR. BELSITO: We don't know.

DR. SNYDER: I'm not a yeast person, so I can't imagine there's that much difference across yeast.

DR. LIEBLER: Well, in their genetics and functions but there's some yeast pathogens obviously but the ones that are (Inaudible) yeah.

DR. SNYDER: I'm fine with that then.

DR. LIEBLER: Yep.

DR. BELSITO: Okay, so we're going to change the title of this to Safety Assessment of Saccharomyces Cerevisiae Derived Ingredients. Is that correct?

DR. SNYDER: well, the only tox data we have then is a dermal acute study because all the rest of it is all the other ingredients.

DR. BELSITO: But it's GRAS.

DR. SNYDER: Oh, true, yeah. Okay.

DR. LIEBLER: It's GRAS and it's food.

DR. SNYDER: Yep, yep, yep.

DR. BELSITO: So then some of these, I mean basically all of the -- well, I guess we can deal with beta-glucan right?

DR. LIEBLER: Yeah.

DR. BELSITO: And polysaccharides?

DR. LIEBLER: Yep.

DR. BELSITO: But the hydrolyzed yeast, yeast extract, yeast protein, yeast, yeast extract will get removed, and we'll be left with yeast beta-glucan, yeast polysaccharides, and Saccharomyces Cerevisiae Extract. Then, a note into the introduction why we're deleting, why we're not including these yeast ingredients that are in the *Dictionary*. Is that what I'm hearing us agreeing to?

DR. HELDRETH: Could I propose one different strategy?

DR. BELSITO: Sure.

DR. HELDRETH: So, in a past report we had a single ingredient that was an oligopeptide. However, we found in the process of reviewing it that there were three different sequences that were all folded in under this single oligopeptide ingredient name, but we only had data on the one sequence. And so we went forward with the report concluding safety on that ingredient but only when it was the sequence that we knew something about.

So what we were proposing here in our question to the Panel of choosing a conclusion to use yeast that exclusively comprises of Saccharomyces Cerevisiae was to suggest that you could conclude on all these ingredients if you chose to and have that conclusion only reflect when yeast means Saccharomyces Cerevisiae. Part of the reason we're suggesting that is the highest frequency of yeast ingredient in this report is yeast extract, and so if we delete it we'll have to pick it right back up again in another report.

DR. BELSITO: Right, so how would you word- -- you'd wordsmith that in the introduction, Bart?

DR. HELDRETH: I think you would have to put it in the conclusion like we did with the oligopeptide. You would say something like, let's say we come with a safe conclusion, these ingredients are safe as used when yeast is defined as Saccharomyces Cerevisiae, something to that effect.

DR. BELSITO: That's fine with me. I mean, that solves the issue that Priya had brought up with the problem of the definitions of yeast.

DR. LIEBLER: I'm okay with that.

DR. BELSITO: Paul?

DR. SNYDER: I'm fine. That works.

DR. BELSITO: Okay, good. Good compromise there, Bart.

DR. HELDRETH: Thanks.

MS. FIUME: I think Priya did address some of it in the introduction, the third paragraph after the listed ingredients, also addresses what species we're looking at. So that was a start.

DR. BELSITO: Okay, so she says the Panel could choose to site this lack of clarification as a data insufficiency. I think we should strike that and say the Panel has proceeded with this review on the assumption that these yeast products are derived from Saccharomyces Cerevisiae.

MS. CHERIAN: Yes, I was referring to the introduction on page 10. The third paragraph on page 10 after the list of ingredients. Is that wording okay there as well.

DR. BELSITO: Okay. According to -- majority agreement.

MS. CHERIAN: Because the term yeast pertains to a wide variety of species.

DR. LIEBLER: The third paragraph.

DR. BELSITO: Yes, okay. So, yeah, I actually put a comment on that. Do we limit yeast ingredients to this? If not, how handle? So we're going to limit the yeast ingredients to this.

DR. SNYDER: Now could you just change the wording to just say that yeast, not otherwise specified can refer to a wide variety of species including Saccharomyces Cerevisiae based on the definition in the cosmetic ingredients dictionary, this report is evaluating only Saccharomyces Cerevisiae. Something like that.

DR. BELSITO: Yeah. So, I mean, I think maybe just an intermediary sentence between the first sentence and the second again saying that the Panel is operating on the assumption that all of the yeast-derived products in this report are from Saccharomyces Cerevisiae and then we'll have that in the conclusion as well.

DR. SNYDER: Okay, whatever language we use in our conclusion should just be replicated up here in the intro.

DR. BELSITO: Okay. Okey-doke. So method of manufacture, we only have for the Saccharomyces Cerevisiae extract. Do we need for the other ingredients, Dan, Paul?

DR. LIEBLER: We have it for the beta-glucan.

DR. BELSITO: That's true, okay. But what about the others?

DR. LIEBLER: I think this is sufficient, really.

DR. BELSITO: Okay. Composition and impurities, do we need for the hydrolyzed yeast extract?

DR. LIEBLER: We've got it for hydrolyzed yeast protein.

DR. BELSITO: So you're okay?

DR. LIEBLER: Yeah, again, I don't think their additional content is needed for these, but if the other team pushes for it, I won't put up a fight. Okay?

DR. BELSITO: Okay, but we're going to say that we don't need it based upon the hydrolyzed yeast protein data.

DR. LIEBLER: Right.

DR. SNYDER: So, Don, if we go back to that introduction on page 10.

DR. BELSITO: Yeah.

DR. SNYDER: That first sentence, "This assessment reviews the safety of the following eight ingredients," as derived from Saccharomyces Cerevisiae, you just state it right up there, right up front.

DR. BELSITO: We could do that. What do you think, Dan?

DR. LIEBLER: Say that again, Paul? I'm sorry.

DR. SNYDER: Under the introduction, the first sentence just put it right up front. This assessment reviews the safety of the following eight ingredients as derived from Saccharomyces Cerevisiae.

DR. BELSITO: And as used.

DR. SNYDER: And as used in cosmetic formulation.

DR. LIEBLER: Yeah, that's fine. I don't think we need that paper -- I mean, that other paragraph can actually go away.

DR. BELSITO: Well, I mean, I think it's important that we do point out that we're knowledgeable that yeast could refer to a huge number of species and just to reiterate it again, but I'm fine with deleting the paragraph too. Dan, what do you think?

DR. LIEBLER: The very first paragraph of the introduction after the list?

DR. BELSITO: No, third paragraph, where we go into yeast of various species. We're limiting it to Saccharomyces. So would you --

DR. LIEBLER: Yeah, I think Paul's sentence is a little more succinct than this paragraph. It's sufficient.

DR. BELSITO: Okay, so we'll just get rid of that whole paragraph. Okay. Good job, Paul. That makes it easy. So we'll need the respiratory boilerplate I believe. So the repro DART, we don't need because of GRAS status. Same with Genotox.

So under other relevant studies, the immunomodulatory effects, I just have a comment. It's not the correct grading for IgE prick test studies, but I presume this is just how it's reported so it's probably just me being a little too anal. Okay, so I'll get rid of that. Okay, so --

DR. SNYDER: Don, can we go back to that one? On page 16 at the top there. Oh, okay, never mind. It does. When I first read the list, I didn't see the Saccharomyces in there, but it is in there. Never mind.

DR. BELSITO: Okay, so the irritation and sensitization, we have just for the extract, which is the one that's most used. I didn't really think we needed it on the other components. Are you okay with that?

DR. SNYDER: I am.

DR. LIEBLER: I am too.

DR. BELSITO: (Audio gap) what David says tomorrow. Okay, so PDF page 18, the sentence just above the summary. It says that, "Saccharomyces Cerevisiae is responsible for up to 3.6 percent of all episodes of fungemia" in immunosuppressed patients. Do we need to discuss this in relation to the inhalation issue?

DR. LIEBLER: I can't address that.

DR. BELSITO: Paul, you're muted. Any comments?

DR. LIEBLER: You're muted.

DR. SNYDER: Oh, damnit. What I was going to say, was the data we're missing here is how many non-diarrhea patients also were cultivated for Saccharomyces Cerevisiae in the hospital? They were taking a probiotic.

DR. BELSITO: Okay.

DR. SNYDER: I mean, I don't understand what they're attribu- -- I mean, are they interpreting this to mean that it was the cause of their diarrhea?

DR. BELSITO: Well, if they had fungemia, presumably they cultured it from the product.

DR. SNYDER: That's true, yeah.

DR. BELSITO: But, again, this was nasogastric feeding of a probiotic capsule.

DR. SNYDER: Yeah. I wouldn't put too much weight into that. I wouldn't, I mean --

DR. BELSITO: Do we even discuss it or is that putting too much weight on it?

DR. SNYDER: I think it puts too much weight on it. To me, you just bring too much attention to it.

DR. BELSITO: Dan, are you okay with just ignoring it in the discussion?

DR. LIEBLER: It sounds like that's okay.

DR. BELSITO: Okay. Okay, so discussion. We have the respiratory boilerplate. We're not going to deal with a fungemia. We have this issue of melanogenesis. For some reason, I skipped over that. Where was that?

DR. LIEBLER: PDF 17, top.

DR. BELSITO: Oh yeah, I missed that. So how do we deal with that? Basically, say that cosmetic formulated, you should take caution to avoid this. It would not be a cosmetic. It shouldn't have that activity. I mean, we have some type of boilerplate.

DR. SNYDER: Yeah, we have a language where it's not in the purview. We should be aware of the pigmentary issues or something. We had another report. Didn't we have it in another report we looked at today? That language?

MS. FIUME: We did. We do have some standard language for that.

DR. BELSITO: Okay, so we just need to bring that language into the discussion.

DR. LIEBLER: Once again, when we see these, it's almost always something like this. It's some cells treated with a relatively high concentration of the ingredient we're studying, it affects melanin synthesis in vitro. Without some more convincing evidence that this could be even an in vivo effect in an animal model, I don't think we really have -- at most we can handle it in the discussion by saying that the concentrations used to produce this effect in in vitro models far in excess of expected exposure in cosmetic products. Is that similar to what our boilerplate says?

DR. SNYDER: That's very consistent to the language you used in one other report that we did this time.

DR. LIEBLER: Yeah.

DR. BELSITO: Right. Okay. Anything else that needs to go into the discussion? Okay, so then based upon our limitations with Saccharomyces Cerevisiae, we basically have a safe as used conclusion. Is that what I'm hearing?

DR. LIEBLER: Yes.

DR. SNYDER: Yes.

DR. BELSITO: All right. Anything else that needs to be discussed on this? Okay, hearing no one piping up, although, Paul, you're muted if you're trying to say something. We're not hearing you. We'll see you all tomorrow morning at 8:30.

DR. SNYDER: All right, good job.

DR. LIEBLER: Yes, sir. Thanks. Bye-bye.

DR. BELSITO: Have a good afternoon.

DR. LIEBLER: Bye-bye.

MS. FIUME: Everybody, have a good night.

Cohen Team – September 13, 2021

DR. COHEN: This is a --

DR. BERGFELD: Microorganism.

DR. COHEN: Yes. It's a -- yes, this is a draft report. It's the first time we're reviewing this. The safety assessment has eight derived ingredients, although there's considerable ambiguity in making an assessment or a read across. We are presented specific data on Saccharomyces Cerevisiae. It's used as a skin conditioning agent, hair conditioning agent, film former, protectant, and viscosity increasing agent. We have max use for yeast polysaccharides in leave-on products up to 0.36 percent in face powders, and we have frequency of use reported.

We have to make some decision on what we want to do with this list of eight derived ingredients, and we do have information that the Saccharomyces is GRAS used as a flavor. We have method of manufacturing for Saccharomyces extract and yeast beta-glucan, and we have composition and impurities for Saccharomyces Extract. I think there's a hypopigmentation signal.

I think we need sensitization data on max use concentration. I can open it up. There's a lot to discuss on yeast. Lisa, what do you think about the read across table?

DR. PETERSON: Well, I guess for me the big question was, does the Saccharomyces Cerevisiae represent what's in cosmetics? That is what counsel supplied, but I guess I was just curious if they could make a comment on, is that the predominant strain of yeast that's used or something else?

Then, I thought, what was missing was the method of manufacturing on the hydrolyzed yeast products. I guess I didn't really understand what hydrolyzed yeast would be. How is the hydrolysis done? So that would be for the hydrolyzed yeast, yeast extract, and protein, again, all hydrolyzed. Then, I thought, there's missing yeast -- generally, yeast polysaccharides, but it turns out the beta-glucan is a polysaccharide, so that can probably stand in for the -- I thought the yeast beta-glucan method could probably stand in for the yeast polysaccharides because beta-glucan is a polysaccharide.

My biggest question had to do with the method of manufacturing for the hydrolyzed ingredients. Again, the composition for the hydrolyzed in the report was basically using the non-hydrolyzed yeast protein, which is, I guess, okay, but again, I was curious what hydrolyzed meant. I mean, what are they hydrolyzing with? Are they treating it with a base? Are they giving it an enzyme treatment? What is the hydrolysis supposed to be accomplishing? That was my big question.

DR. COHEN: Lisa, in the uses, the Saccharomyces are used in 74 formulations, but the rest of the 267 are others, right? I'm very confused as to what the term "yeast" means --

DR. SLAGA: Right.

DR. COHEN: -- in this whole thing.

DR. PETERSON: Yeah, I agree.

DR. COHEN: I know Saccharomyces' a yeast, but I'm not an expert in this, but there's a lot of yeasts out there, right?

DR. PETERSON: Right, and you would think that they could provide some additional information. Like, when they say yeast generically, are they really talking about this one that's known?

DR. COHEN: Ron, what do you think?

DR. SHANK: My take was to limit the scope of this report to Saccharomyces Cerevisiae and drop all of the others.

DR. BERGFELD: Right. I agree.

DR. SHANK: Then you have a very neat report.

DR. SLAGA: Right.

DR. SHANK: You can actually conclude it's safe as used.

DR. SLAGA: I agree because there were statements in here stating about some of the other products that could be a mixture. They didn't know what it really was. I would go with Ron, that we pick out something that we know, and call it safe, and take the rest away.

DR. COHEN: So --

DR. BERGFELD: I totally agree with that, and I think that you would clarify that in your title.

DR. SLAGA: Right.

DR. HELDRETH: So, by removing all others, do you mean actually remove ingredients like yeast extract or limit the scope of conclusion of yeast extract to when Saccharomyces Cerevisiae is the species used?

DR. BERGFELD: Right.

DR. SLAGA: I don't understand what you mean.

DR. BERGFELD: You assume that everything's -- that if you limit it to the Saccharomyces, then everything you talk about is that.

DR. HELDRETH: Right, so I was just trying to get clarification. When you said keep Saccharomyces Cerevisiae Extract and get rid of the rest, did you mean that just have that one solitary ingredient, Saccharomyces Cerevisiae Extract, and delete all the others? Or did you mean to look at all of the yeast ingredients that are in here and limit the conclusion so that, per se, like when we're looking at yeast extract safety, it only pertains to those incidences where they used Saccharomyces Cerevisiae as the yeast species?

We did something similar to my second alternative there. Previously, we were looking at a specific oligopeptide. And, under that one name of the oligopeptide, it turns out that the definition allowed for you to have three different sequences, all with the

same ingredient name. But we only had data for one of those sequences, and so the Panel's conclusion was on the safety of that ingredient, but only when the sequence that we knew about was used.

I mean, I'm just suggesting that's one possibility here that you could include it on other ingredients, like yeast, yeast extract, yeast polysaccharides only when Saccharomyces Cerevisiae is used. Or you could delete the other ingredients and have it just be on the one ingredient, Saccharomyces Cerevisiae Extract. The only problem with that is that ingredient's not the one with the highest frequency of use.

DR. BERGFELD: Right.

DR. HELDRETH: The whole reason that this came up on our priority list was because of yeast extract with 267 uses. So then you're still left with the need to review the safety of that ingredient if you cut it out of this report.

DR. BERGFELD: Can I ask a question, a clarification on that? When you say yeast extract, what are you including in that yeast? Everything? Anything?

DR. HELDRETH: No, that's what I'm suggesting. You could either say we're insufficient for yeast extract, or you could say here's our conclusion on yeast extract when Saccharomyces Cerevisiae is used. Those are options.

DR. COHEN: Carol had a comment.

DR. BERGFELD: I assumed that.

DR. EISENMANN: A couple things. Historically, ingredient names came from some food definitions, and yeast, in the Food Chemical Codex, dried yeast has three species in addition to the one that you're talking about. I agree with the general approach that this report you should, in the conclusion, limit it to the one species.

Food is also Saccharomyces fragilis and torula utilis, so I suspect that was the original, but I've also discussed with Joanne what would happen if another species of yeast came in currently. They would give it the new genus-species name. They would not put it under yeast extract. If that makes you -- so any new material, but unfortunately, occasionally you get people that self-name, so I think if you limit it to defining yeast extract for the purposes of your report as only Saccharomyces Cerevisiae that would probably be the best approach.

DR. COHEN: From a technical standpoint, this is a draft report, right? We're issuing an IDA, and, so far, we're asking for methods of manufacturing for the hydrolyzed ingredients. We need -- let's see, we have an irritancy study, but we don't have sensitization data on max use for Saccharomyces. We still need that.

What else are we asking for because we're either going to take out all those other terms, or we're in the draft report stage and we're going to ask for more information to clarify it. We're not late stage here, so do we try to keep it in and ask for greater detail on the definitions of these and what they're including?

DR. SLAGA: That would be helpful.

DR. EISENMANN: Well, you're not going to get more clarification at this point, but I have asked every supplier we have listed, and I've given you the data that has come back. The suppliers did not come back with other species.

DR. COHEN: So we're back to keeping everything in, but our conclusion is just on yeast. Our comments are related to Saccharomyces.

DR. EISENMANN: Correct.

DR. SLAGA: Right.

DR. COHEN: So, in our IDA, right, where we've asked for hydrolyzed ingredients, sensitization data, are we asking for irritancy and sensitization on all of the other components? Right? I mean, we can't -- it's not dead yet, right? This is still a draft early report, so when we issue the IDA, we have to provide some guidance on what we're looking to get back. Is it just going to be those two things, or are we going to ask for everything: method of manufacturing, impurities on the things we don't already have, irritation and sensitization? Are we going to ask for those things for the next iteration?

DR. SHANK: We have irritation and sensitization for Saccharomyces Cerevisiae Extract.

DR. COHEN: Do we have human data on Saccharomyces?

DR. SHANK: No.

DR. BERGFELD: Lymph node assay.

DR. SHANK: The sensitization is a local lymph node assay.

DR. COHEN: So I was going to ask for sensitization in humans at max use. No?

DR. SLAGA: It's early in the game. Go ahead and ask for it.

DR. COHEN: Well, wouldn't we normally ask -- I mean, wouldn't we normally ask for that data?

DR. BERGFELD: Well, sometimes we've used the lymph node assay, but that would be the end. I mean, that would be the final.

DR. COHEN: Okay.

DR. BERGFELD: I'm not sure I understand why all this discussion on the -- which species you're going to use, I guess you'd call it that, because most of the information here is on the Saccharomyces Cerevisiae and why not go with that one since you have most of your information there? Including some of the cell walls and, let me see, what else is in there? The hydrolyzation, the beta-glucan, it's all on the Saccharomyces.

DR. COHEN: Yeah. Yeah, the comment before was we have a pretty good draft report for Saccharomyces.

DR. BERGFELD: Right.

DR. COHEN: So it'll all rest in the conclusion.

DR. BERGFELD: Yeah, so why are you even thinking about adding another one? Or other two species?

DR. COHEN: Not adding the species, just to define the terms, which seem vague.

DR. BERGFELD: Oh. Well, in this case, it's specific because you have a yeast, Saccharomyces Cerevisiae. It's specific.

DR. COHEN: But does that -- is the totality of hydrolyzed yeast extract that seems to include things other than Saccharomyces and in the --

DR. BERGFELD: Then ask for composition and impurities of the hydrolyzes.

DR. COHEN: Yeah. Yeah. Okay.

DR. BERGFELD: There are two mentions there under composition impurities. One does not suggest a species; the other does.

DR. COHEN: Okay. So we're going to have an IDA on this. Ron, is that right?

DR. SHANK: Okay, I'll go along with it, but what are you going to call this report? Yeast?

DR. BERGFELD: No.

DR. SHANK: Or you're going to call it Saccharomyces Cerevisiae?

DR. BERGFELD: Call it that.

DR. COHEN: I thought we were going to call it yeast and then, in the conclusion, hone in on the fact that our conclusions are based on Saccharomyces.

DR. BERGFELD: But, if the new dictionary is coming in with the yeast species, specifically for yeast, then why don't we start there? Start it now.

DR. COHEN: So, Wilma, you're saying we should excise the other seven lines in the read across. My concern is the use, right, where there's heavy use and, of the --

DR. BERGFELD: And it's a food.

DR. COHEN: Yep. There's 267 formulations, of which Saccharomyces only accounts for 74. So, if we excise the rest of them, we're leaving a large portion of products not covered by this report. So I wanted to resist just making this a Saccharomyces report and try to get as much information as we can because we're early in the game.

DR. SLAGA: That's fine. I mean, we may go eventually with the one ingredient, but let's see what we can get.

DR. SHANK: Okay.

DR. COHEN: I think Don's presenting this one tomorrow, so we could see what -- how they adjudicate it. That did come into my mind when I was reviewing this. It's like, how am I going to articulate all this? But our team will remain on standby for this as a seconders.

DR. BERGFELD: So you're sort of leaning towards going to a specific Saccharomyces Cerevisiae, and, if Don offers another option, you go with that? Or you're going to hold out for the, what, 30 or 40 percent that are uncovered?

DR. COHEN: I was, my gut was to hold out to get as much information and to include as much as I could at the next round before we just make this a Saccharomyces report. Bart, any comments?

DR. HELDRETH: I agree. I think it always makes sense to, when we're in the early stages like this, ask for any data that might help the Panel feel more comfortable making a decision. I don't think there's any reason to rush forward and declare safety or lack of safety or some qualifications at this stage.

If there's any missing information or ambiguity to the information we have that the Panel would feel more comfortable with if they had a better explanation or more data, by all means, ask, and we can think about the what the safety conclusion or the scope of that conclusion at a later stage.

DR. BERGFELD: Can Carol give us the list of those that she thinks are included in that group of absent information? She had (audio gap). It's nowhere in the --

DR. EISENMANN: All I was saying, the Food Chemical Codex definition for dried yeast includes two other species.

DR. BERGFELD: Yeah.

DR. EISENMANN: So, in other words, if you saw yeast on a food package, it could mean also Saccharomyces fragilis and torula utilis. I wasn't suggesting that you put a lot of information on it, other than the statement that what the Food Chemical Codex definition includes.

DR. COHEN: That's pretty helpful information, though, don't you think?

DR. BERGFELD: Yeah.

DR. COHEN: I mean, it adds a little color to the GRAS issue, no?

DR. PETERSON: Right, so do we get a -- is there a statement saying that, how yeast is defined as a food in the document under other uses or non-cosmetic? I think a statement like that should be added to the non-cosmetic use, that would be helpful.

DR. COHEN: Yeah, if you look at the screen, it lists those other ingredients: the fragilis and the torula.

DR. BERGFELD: Did you find that, David?

DR. COHEN: No, no, no. Is this -- Priya, did you put this up?

DR. HELDRETH: No, I put it up.

DR. COHEN: Oh.

DR. HELDRETH: It's Bart.

DR. COHEN: I like Lisa's comment. We could put this in the other uses. All right. We'll have Don describe their findings. We can make our comments about trying to keep as much in as possible, ask for further information, and see what we get. So we could put yeast aside, and let it rise later. Couldn't help myself.

DR. BERGFELD: What specific -- you're going to have to have a list of specifics that you want.

DR. COHEN: Yeah. I was going to ask for sensitization data on Saccharomyces at mass use in people, method of manufacturing for the hydrolyzed ingredients, composition impurities for the ones that are not listed already.

DR. PETERSON: Are you going to add composition of the hydrolyzed use protein because there is a list of non-hydrolyzed use protein, but it's not the hydrolyzed? I don't know how, again, if it defines what the hydrolysis method is maybe then you can do the read across, but I wasn't a hundred percent convinced of that.

DR. COHEN: Okay. Are we okay to move on to that one? From that one.

DR. SHANK: Yeah.

DR. SLAGA: Okay.

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DR. BELSITO: Yeah, so, we initially struggled with this, but Priya sort of helped us out as did Bart. So, Yeast is a broad range of ingredients, and there is no idea what if you just say "yeast extract" you're referring to. And so the first thing we wanted to do here is change the title of this assessment to the "Safety Assessment of Saccharomyces Cerevisiae-Derived Ingredients as Used in Cosmetics. And then, once we do that and we restricted it to these yeast products that are derived from Saccharomyces cerevisiae we found that we could go with a safe as used conclusion. And in the discussion include the respiratory boilerplate and the language that we typically use when there are reports of melanogenesis.

DR. BERGFELD: And that's a motion?

DR. BELSITO: That's a motion.

DR. BERGFELD: Dr. Cohen.

DR. COHEN: So, I'm not sure whether we should second that. We grappled with this as well, and, the reason we decided not to limit the report was because of the frequency of use, right. There were 74 formulations for Saccharomyces but the totality had over 250 -- 267. So, we didn't want to close the report, or narrow it too quickly, if we were able to cover those other uses.

We were asking for a high-fidelity definition of the yeast in this assessment other than the Saccharomyces, and it's GRAS, so we may be able to get some more information about the species that fall within the yeast moniker. We wanted method of manufacturing and composition and impurities for the hydrolyzed yeast products. And, we have irritancy data, Don, do we have sensitization data on Saccharomyces? Yeah, we do. So --

DR. BELSITO: I'll past this over to Bart, because I think he was the one who sort of discussed this with us about holding -- that your understanding, if I recall our discussion yesterday, was that most of these yeast-derived products are in fact from Saccharomyces. Is that correct, Bart?

DR. HELDRETH: Yeah, I mean, that is our suspicion, although we don't know. But the proposal that I was making was that your conclusion could say whatever your safety conclusion is, whether it's safe or safe the qualification, but would have a caveat when yeast means this particular species.

So your conclusion would only apply when someone's using yeast extract, they actually meant Saccharomyces cerevisiae Extract. Or when someone's using yeast polysaccharides, what they really meant is Saccharomyces cerevisiae Polysaccharides. So, it's limiting it to Saccharomyces cerevisiae, but it's not limiting it just to the one that has the genus and species in the name. All of the other ones would still be covered in this assessment, but only when the formulator is using that genus and species. That was the proposal, but it's up to the panel to decide if they'd like to use it.

DR. COHEN: In our discussion yesterday about the foods, two other yeasts were discussed. And, we thought we would keep the door open for more information to come in to see if we can expand that. I mean, is your plan not limiting and excluding yeast products that don't have Saccharomyces in them? It seems like it would, and I don't know if all those uses are all Saccharomyces that aren't listed as Saccharomyces. I don't know if that made any sense, but.

DR. BERGFELD: Well, you were actually asking to explore the other two yeasts that are in the dictionary. And that's the leaving the door open to see if there's anything on those two other species. And we also heard yesterday that the dictionary is not going to be using the name "yeast" anymore, but specific to the species.

DR. EISENMANN: No, it is going to be using the name, yeast. If somebody new applied for a name with a different specific species -- I discussed this with Joanne (phonetic) -- they would name it with the genus species name, but the yeast name will stay in the dictionary. Because there's a European name, I think it's Faex (phonetic), which she can't get rid of and it's a general yeast term that they use. So, no, they won't be getting rid of the yeast name.

DR. BERGFELD: Is it true that there are only two other yeast genus and species under the category of yeast in the dictionary?

DR. EISENMANN: No, that's in the food chemical codex, how it's defined. Dried yeast, if you see the name yeast on a food package, there are three species that are used as dried yeast in the definition in the food chemical codex. I was just suggesting that that be put in the other use information. That's all.

DR. BERGFELD: So, any discussion regarding the more restricted presentation?

DR. COHEN: Well, is there a reason to restrict it at this stage in the development of the report? Is there value to that, or, do we see this again?

DR. SNYDER: So, my question is when we did a search for safety data, did we search those other yeast or did we just search Saccharomyces cerevisiae?

DR. HELDRETH: It was all searched; it's a very broad topic to go out and search for all yeast.

DR. SNYDER: No, but, specifically the two that the Cohen team is thinking about including in this assessment, did we search for those two genus and species of yeast, because basically 99 percent of the data is on the Saccharomyces cerevisiae?

MS. CHERIAN: No, we purposely didn't include any information on any other genus or species because it was just such a broad title. And, I mean, in the dictionary there are other yeasts outside of that food chemical codex that I did see that are yeast ingredients. But it was just so broad, so we decided to use this method instead.

And, I think, yesterday, Carol, did you say that even if we did ask for clarification -- we already did -- would we actually receive clarification on what genus and species are being used right now?

DR. EISENMANN: I did ask all the suppliers we have listed under the yeast ingredients, and of course I never get response from everybody. The ones that did respond are using Saccharomyces cerevisiae. They didn't indicate other species to me. For the ones that (audio skip) names registered.

DR. SNYDER: My concern here is that we have an unintended bias for Saccharomyces cerevisiae-derived yeast extracts or whatever, because we only looked for that. And, if we bring those others forward and say they're insufficient, well, then we didn't really look for those. Is that not correct?

DR. HELDRETH: I mean, the panel can go whichever direction you want, but my suggestion was not to say insufficient for the other species, but to simply conclude on Saccharomyces cerevisiae as the only species in this report. And then if someone comes forward in the future and says, hey, hey, I'm using one of these other species that is listed in the food chemical codex, like the Saccharomyces fragilis, or the Torula utilis, then those can be brought back into the report assuming that data comes with it. I mean, we're only at the draft report stage.

DR. COHEN: Well, for a couple of questions. If we knew this was the dataset, why weren't we presented just Saccharomyces? And, in that table why did all those other ones show up there for us to look at? And then, to your other point, Bart, we have two other yeasts that are in the food codex that I don't know how they relate to the other uses that are not listed as Saccharomyces, why limit it now in the draft report? Why not talk about this later?

DR. HELDRETH: Yeah, I mean, it's certainly the panel's choice to limit it or not limit it. The reason that we brought in yeast extract specifically is because that is the one that has the highest frequency of use. So, that was actually the driving ingredient that brought this ingredient group to the priority list.

So, ultimately, if we wanted to start cutting this report apart and taking ingredients out, if we take the generic yeast name out of the report, then we're going to have to have a separate report on it somewhere else. So, it's really the cerevisiae that was added into this report as we thought it belonged with it. And, ultimately the data that we found relating to yeast ingredients was almost exclusively on the Saccharomyces cerevisiae. So, that is why we suggested possibly limit the scope of this report to that genus and species, but, again, it's your choice.

DR. BERGFELD: So, it's easy to limit it but it's harder to expand it. So, David, you're up for a second to this motion to limiting it to this species, or do we open it up. We have to have a consensus here.

DR. COHEN: I'll look to our team. I don't know if it's that convincing to limit the report at this stage. Lisa, Ron, Tom?

DR. SHANK: This is the first time we've seen the report, and the search was done just for Saccharomyces cerevisiae. So, I think we should keep it open and see if we can get any information submitted to the panel on the other strains of yeast. If we don't, then we limit it to just Saccharomyces cerevisiae. But I think it's premature to do it now.

DR. SLAGA: I agree.

DR. PETERSON: I agree with Ron.

DR. BERGFELD: Okay, so, the Cohen team agrees. What's with the Belsito team?

DR. BELSITO: I'm fine. This is the first time we're looking at it.

DR. LIEBLER: Yeah.

DR. BELSITO: If we wanted to -- I just got the impression from Priya and Bart yesterday that if we ask them to proceed looking at anything other than Saccharomyces cerevisiae that we'd be spinning a lot of wheels and wasting a lot of time.

DR. COHEN: Let's just limit it to the other two food yeast for now.

DR. BERGFELD: Is that agreeable?

DR. BELSITO: So what are we specifically asking for, that Carol go out and ask manufacturers whether they produce yeast extract from those two species as well? How do we get -- what is our IDA?

DR. SNYDER: Well, Priya said there were other genus and species in the dictionary. So, why would we restrict it to food ones if there're other ones in the dictionary, unless they're also the food ones? So, that's what I'd like to know, if we going to expand it.

DR. COHEN: Well, I guess you'd have -- as GRAS it'd just be an easier way to go through the report for tox.

DR. BERGFELD: Dan?

DR. LIEBLER: So I agree with my distinguish colleagues on the Cohen team to keep it wider open at this point. And, I think we just trust Priya and Bart to make best judgements as to -- or make our best efforts to data gathering for us. And then when we discuss this next time we can decide if we need to close this down a little bit.

I mean, we're going to have to -- aside from the selection of the ingredients, the supporting data are always going to have this level of ambiguity because much of the data is with yeast. It's not really labeled as the species. So we're simply going to have to, I think in the final report we're probably going to have to outline our assumptions that led to our evaluation of the totality of the data for the report so.

DR. BERGFELD: Well, I'm going to ask Bart. Bart, if it's a consensus that we're opening it and we need some information, this will be done by Carol. Do we need an IDA yet, or do we go for the IDA with the insufficient?

DR. HELDRETH: Yeah, I think if you have insufficiencies, especially at a draft report stage like this, I would issue an IDA with whatever specific needs you have. And then, the CIR staff will do what we can to gather information that we can. And, of course, industry will also do their part to try to find what's out there, if there's anything out there, in addition to what we found.

DR. BERGFELD: So, let's see, Dr. Belsito, you did your motion that wasn't seconded. So, are you rescinding your motion at this point and time?

DR. BELSITO: Yes, so the data will be insufficient for determination of what other yeast species could be used in the formulation of these yeast-derived ingredients.

DR. BERGFELD: Is there a second to that?

DR. COHEN: Second.

DR. BERGFELD: Okay. And, the needs that would be then listed in our discussion under IDA?

DR. BELSITO: Well, the first need would be what are the ingredients that we're adding, are they GRAS, if not, then we may need to look at other toxicity data. We may want sensitization and irritation. So, I mean, I think that it's hard to give a list when we don't know what we're dealing with. So I would say that the IDA is for what other genus and species of yeast might be used in these yeast-derived products, if they're not GRAS, a 28-day dermal or other toxicity endpoints to be satisfied, sensitization and irritation, composition, manufacturing, impurities. I mean, the list goes on and on.

DR. BERGFELD: The whole list, okay. David, you want to add something to that?

DR. COHEN: No, Don actually summarized it. But I think I recall Lisa wanted specifically method of manufacturing, composition and impurities of the hydrolyzed yeast products.

DR. BERGFELD: Okay.

DR. COHEN: Yeah, how were they hydrolyzed, what are the impurities and composition?

DR. BERGFELD: And, I'm sorry, I don't have the scientific writer for this one at my fingertips.

DR. LIEBLER: It's Priya.

DR. BERGFELD: Priya, have you got what you need?

MS. CHERIAN: I've got what I need, thank you.

DR. BERGFELD: Okay. So, the motion has been made and seconded. Discussion regarding the needs for the IDA have been stated and understood. So, I'm going to call for the question unless there's another comment to be made. Seeing none, all those that oppose? Abstain? A unanimous agreement to proceed with an IDA. Okay, so our next biggie, Barley, Dr. Cohen.

MARCH 2022 MEETING – STRATEGY MEMO 1

Belsito Team – March 14, 2022

Dr. Donald Belsito
OK, we're back. Well, maybe we can at least start this discussion cause we got some tough ones coming up. So, the major discussion is how to handle these yeasts? Should we just consider Saccharomyces cerevisiae? And that's what we feel represents yeast. Or should we add other yeast ingredients like PGonArmada extract in the assessment, which I guess has what, 4 uses or something? Or 4 reported uses? I can't even keep it straight. I mean, I just felt we should go with Saccharomyces cerevisiae. I just I don't know how we can wrap our heads around all of the yeast, but maybe chemist like Dan can help me out.

Dr. Dan Liebler
Oh, I don't. I don't think this is really a chemistry issue. I think that I came down on the side of including the other yeasts. Because of the very broad, INCI definition and the fact that there are at least some uses, and I thought that we could essentially apply the same logic we use for allergy. Which is if we've got food uses to cover, you know, the broad safety endpoints and we had sensitization data then we're going to be able to clear these. There will be lots of data for SarahBCA. So I'm trying to read Priya's face here. I don't know if that was smirk or and itch, but anyway that that's what I thought we could do. I think we could

take an allergy type approach to this. I don't know if you guys think that that this fits the same framework is Algae in terms of the available information Pryia to the extent you've looked. Do you think that makes any sense?

Priya Cherian (CIR)
The problem is that there are other species of yeast being used right now and the dictionary and then in that supplement that we got there were different unison species that weren't in the dictionary, so it would just depend on what exactly are we going by which genus and species of any sort or being used right now and what are we including?

Dr. Dan Liebler
I'm my suggestion ass ed that we only would include what's in the dictionary.

Priya Cherian (CIR)
OK.

Dr. Dan Liebler
Yeah. So, if it's not included in the dictionary, it's off limits for us and you know, but I mean still what's in the dictionary is still broad enough that it's more than Sarahvca.

Dr. Donald Belsito
What? Exactly is in the dictionary. Can someone read that?

Priya Cherian (CIR)
I made a documents a while back about the yeast that I found in the dictionary. And I can probably find that and send that out.

Dr. Dan Liebler
I mean if the if the panel all kind of came in on let's just do Saccharomycesservice, then I'm going to argue for the others. But I think that we could handle the ones that are in the dictionary based on that sort of the algae framework which is if there are food uses and if we have sensitization data, we can clear them or we can at least that that's the approach we could take to clearing them.

Dr. Paul Snyder
I had the same approaches, Dan, I said. If they're in the dictionary and their use, let's just add them and get them off the table.

Dr. Curtis Klaassen
Further question is do you want to divide the yeast up into three or four different groupings?

Dr. Dan Liebler
Different reports.

Dr. Curtis Klaassen
Yeah.

Dr. Dan Liebler
I personally don't think that's necessary, but you know because we are again the Algae approach was to avoid having to do that, that's.

Monice Fiume (CIR)
We just lost, Don.

Dr. Dan Liebler
OK.

Monice Fiume (CIR)
Create do you have a number? While we're waiting, maybe for Don to come back, on. How many ingredients there are in the dictionary under that yeast? Family.

Priya Cherian (CIR)
I'm trying to. Am I allowed to share my screen here?

Monice Fiume (CIR)
Yes, you should be able to share.

Priya Cherian (CIR)
OK.

Dr. Donald Belsito
I got. I got kicked out. Can you hear me now?

Priya Cherian (CIR)
So when I.

Dr. Curtis Klaassen
Yeah.

Priya Cherian (CIR)
Yes.

Dr. Curtis Klaassen
Yes.

Dr. Donald Belsito
OK, sorry. Go ahead Priya.

Priya Cherian (CIR)
So when I was looking through the dictionary, all of these ingredients, all these yeast ingredients or they ingredients that I've found this was last year. I can look again and see if there are any is if there's anything new and this is also according to 2021 VCRP the ones that are also recorded to be used are these ingredients. And that's according to 2021. I'll have to double check with 2022.

Dr. Donald Belsito
And what's the red mean?

Priya Cherian (CIR)
These are the ones that are included right now in our report.

Dr. Donald Belsito
OK. So Dan, you're saying include all of them?

Dr. Dan Liebler
The ones that are maybe I can get it. Could you leave your screen up, Priya? Sorry.

Priya Cherian (CIR)
Oh yeah, sorry.

Dr. Dan Liebler
So if you scroll up so we can see that first group. OK, so you've got potential ingredients. The everything listed here is in the INCI Dictionary.

Priya Cherian (CIR)
Yes, as of 2021.

Dr. Dan Liebler
OK. So, and then the ones that are red are currently part of the ingredient group. I see. OK, so we've got maybe less than a dozen. In the ingredient group, the red ones, and then all of these others, setting aside what's in use, just staying out in the upper grouping, we've got all of these others. This is similar to the scope of the of the red algae. I think in terms of numbers of substances to be considered.

Dr. Dan Liebler
Most of these are like hydrolyzed. You know other stuff like the Candida, Banda cola, etc. Anyway, it it's approachable by these sort of the LG type framework. I notice that you've got some Saccharomyces cerevisiae that are not included, like the cirlarsa extract lysate extract filtrate etc., it could be brought in because they'd be under sarahvca. Yeah, and I would expect once we learn a little bit more. Or about the sarahvca and some of the extracts and manufacturing and such. We probably be able to include many of these uses again using the same framework we did with algea, where we knew that these were sub components of a larger group that have food uses or you know or acceptable uses that allow us to clear, you know, most of the safety endpoints and then we can have our discussions about, you know, sensitization. That's kind of what it would boil down to keeping, you know, to clearing these.

Dr. Donald Belsito
And then it sounds like a plan. Or we can try it. Paul, Curt.

Dr. Paul Snyder
Yeah, that was my that was my initial take is just to include them all. If they're, if they're in the dictionary and there used.

Dr. Curtis Klaassen
Yeah, give it a try. See how it works.

Priya Cherian (CIR)
So are we including all of these in the dictionary because these ones are just in the dictionary, the ones at the bottom are in the dictionary and reported having use.

Dr. Donald Belsito
No, I think what I heard is all that are in the dictionary.

Priya Cherian (CIR)
OK.

Dr. Dan Liebler
Correct. I think if we don't have uses, we can deal with that. You know later on. But to start with, I think this upper group is starting list.

Priya Cherian (CIR)
OK. And so? In that documents that we send out and it was sent to us from the Council with the yeast extract and all of those genus and species. What do I do with those genus and species? Because some of those don't correspond to an ingredient that's in the dictionary right now.

Dr. Dan Liebler
I think we only do it in the dictionary. Right. Its not the dictionary. It's not our problem.

Priya Cherian (CIR)
Well, the problem is that we haven't ingredient that's called yeast extract in the dictionary.

Dr. Dan Liebler
Oh, I see. Well, if Council is, you know, sending things our way that that they think there are producers and users of uses of and they're not on your list, but they're on that other list, which I don't remember looking at but, then we should include them because of the broader dictionary definition. But if they're just sending us every name that they can come up with. You know it I mean, if it's arguably within the dictionary, then it belongs on the list that you had and then we still apply their framework, food use and sensitization. We can, you know, we can get them through. And if there's no food use and no sensitization, then will simply be insufficient.

Dr. Donald Belsito
OK, so approach it like we approach the algea.

Dr. Dan Liebler
Yeah.

Dr. Donald Belsito
OK. Is that clear Priya.

Priya Cherian (CIR)
Yep.

Dr. Donald Belsito
OK, good. So, let's move on to the priorities for 2023. So, the list needs to be publicly made June 1. Comments on the list.

Cohen Team – March 14, 2022

Dr. David Cohen
And Yeast. And this is a bit complicated, so this was for additional information and clarification. In that, you know Priya went through a lot of this data and the definition of yeast is extremely broad and it's not very informative. And a lot of this data is on Saccharomyces. And with two additional species you mentioned toriola and candidate Utilis? I know Toriola is candidate you

tillison Saccharomyces fragilis. But you indicated that there's no evidence that they're being used in cosmetics. So the question is, are we lumpers or splitters on this? Is it just Saccharomyces? Or is it going to be yeast extract?

Dr. Ron Shank
I would limit it too only. To only this species used in cosmetics.

Dr. Thomas Slaga
I totally agree. I think we ought to go with the 1st. One and only go with.

Dr. Wilma Bergfeld
Sacrifices.

Dr. Thomas Slaga
Yeah, it it's used in cosmetics and leave the other two out.

Dr. David Cohen
So. That, that that wasn't my initial impression, but I could be persuaded, which is when you look at the constituents of these things, is there anything in there from my perspective that was going to be an irritant or contact sensitizer I couldn't come up with anything just on the top of my head. And if we do it in such a narrow way, or we going to have to have reports in the future if another.. Yeah, Bart.

Dr. Bart Heldreth
So maybe I misread it, but I I've read the situation very differently. So at the last meeting it was brought to our attention that we should look to the Food Chemical Codex to see what species are considered for a yeast that type that we consume and food. And so we looked at the Food Chemicals Codex that that's where we found that truly utilities and the Saccharomyces fragilis. And so that's why we brought those in. Not really a problem, at least from our, you know, amateur staff side. What did strike us as something we didn't know what to do with is, we made the assumption at the last meeting that we would just assume whenever we looked at least extract or yeast anything that didn't have a genus and species that we were going to only you look at Saccharomyces Servasa. If somebody was using something else other than Saccharomyces Servasa, we weren't concluding on it. However, we got this document back from industry and if you look the page 4 of that strategy memo you see listed under the generic yeast extract cosmetic name we have Candida Sitona anDeborah, *(inaudible) and the list goes on and it goes back to the notion we had before the yeast meant a whole slew of not only species, but geniuses. And so our question is you want to continue and just head down or only going to review Saccharomyces surveysay? When we're talking about yeast and yeast extract or do we want to include these other genus and species in our review? That's the impression I got.

Dr. David Cohen
Now I think we had similar impressions. I we I saw those lists and I'm saying all right, there's a lot here. Are we going to have individual reports for each one of these when most of it is protein, sugars and this it.

Dr. Wilma Bergfeld
General ash.

Dr. David Cohen
Yeah. So. Why not take the opportunity to lump them together if we, if we can, I suppose?

Dr. Bart Heldreth
OK.

Dr. Wilma Bergfeld
Well, we always have the opportunity later to split.

Carol Eisenmann (PCPC)
Where do you?

Dr. David Cohen
Yeah. Hi, Carol.

Carol Eisenmann (PCPC)
I just wonder where you stop because that list probably is not I didn't try to look and see, but I suspect there's a lot more. I mean this this is partly a naming issue.

Dr. Bart Heldreth
Mostly.

Carol Eisenmann (PCPC)
Currently. If you if you wanted a new name for a for your material made of yeast, you'd have to tell him the genus species, and they would name it using the genus species name. So there's going to be a lot more. There's always going to be more. This is like allergy. There's always going to be a new yeast coming in. So that that's me is a difficulty how do you stop but the main ones still, of the ones that? That they reported, they hardly have any uses reported to the VCRP. I still think that Saccharomyces surveysay is the one with the most uses. And you could limit it just because you're going to focus on the one with the most uses. And you already have a report pretty much prepared, which you could finish up and be done with.

Dr. Thomas Slaga
Right.

Dr. David Cohen
OK. I mean it makes sense and it's expedient certainly.

Dr. Wilma Bergfeld
Yep. It's still doable.

Dr. Bart Heldreth
Yeah. And that and.

Dr. Wilma Bergfeld
Alge wasn't doable.

Dr. Bart Heldreth
Thats great. That's easy for us. But then that leads to another question. What do we do with this data on CandidaSitona and DeborahMyhineas that if we just say thanks and put it in a folder? Or is it relatable to Saccharomyces surveyssay?

Dr. Ron Shank
Are they used in cosmetics?

Dr. Bart Heldreth
Yes, and under the name, yeast extract.

Carol Eisenmann (PCPC)
But I can also say that supplier has names, has trade name, materials under the genus, species names also. So. But I think you could say yes, thank you. But we're going to wait and review them.

Dr. Thomas Slaga
Later.

Carol Eisenmann (PCPC)
Right. When the when I when the genus species name comes.

Dr. Bart Heldreth
So then we would have a conclusion whether it's safe or safe with qualifications or unsafe or whatever for yeast extract when the species is Saccharomyces surveysay. Is that?

Carol Eisenmann (PCPC)
And you might change the name to Saccharomyces surveysay, and then do it the opposite way.

Dr. Thomas Slaga
Right.

Dr. David Cohen
You mean the title of the report?

Carol Eisenmann (PCPC)
Right to try to learn.

Dr. David Cohen
With these Saccharomyces.

Carol Eisenmann (PCPC)
Right. Whether or not it's called if it's called, yeast extract or the Saccharomyces surveysay extract.

Dr. David Cohen
I think if we kept it as yeast extract, it's going to be pretty confusing if the whole report is on Saccharomyces.

Dr. Thomas Slaga
Yeah.

Dr. David Cohen
I think we have to call it Saccharomyces, if that's what we're if we're deciding to split. That's what we need to do. But then that leaves all these other yeasts. Dangling.

Dr. Wilma Bergfeld
We've done that before. We can also open up in 15 years and add a few. I mean, they're all kinds of ways of handling the additions.

Dr. Ron Shank
Can the other yeast species be handled in the discussion? Or an appendix. To the report. Or do they have a lot of uses? The other species.

Dr. Bart Heldreth
At least from my perspective. It's absolutely impossible to know, so a little history on this, I think it was nine years ago I started pushing to put yeast extract on the priority list. At that time yeast extracts was one of the very few yeast ingredients in the dictionary, and it had about 1000 uses. And I kept getting pushed back. Oh, we're going to change the name. We're going to make be more specific. And nothing really happened there. And so I kept bringing it forward because it had very high frequency of use. So. My best understanding is right now, there's flooding of products on the market that's a yeast extract on the label. At some of them, say Saccharomyces surveysays the species they use, some of them are DeborahCS,. I don't think there's, at least I haven't seen any data to show us how many products have this one and how many products have that species. It's unknown.

Dr. Wilma Bergfeld
Pandora's box.

Dr. Bart Heldreth
That's right.

Priya Cherian (CIR)
So. Just to be thorough, before this report started, I did go through the Winky Dictionary and I looked and try to find every single yeast species that's currently reported to be in the Winky Dictionary. And then I have a document with those I can share my screen. I can show you. Great. So yeast at the top, are the ones that I found to be in the Winky dictionary in 2021. I haven't done another search this year. And then these at the bottom are ones that are in the Winky dictionary and have at least one reported using the VCRP according to 2021. But not all of the genus and species that were reported to be under the yeast extract, and that data supplement that you got, correspond to a Winky ingredient as of now.

Dr. David Cohen
Right. We don't know what yeast extract really means in that VCRP list.

Dr. Bart Heldreth
Right.

Priya Cherian (CIR)
Right.

Dr. Bart Heldreth
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Dr. Wilma Bergfeld
So the intent is to change the name of this yeast Saccharomyces?

Dr. David Cohen
Change the name of the report, right?

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Yeah.

Dr. David Cohen
K.

Dr. Bart Heldreth
Right.

Dr. David Cohen
OK, I think that. Brings us to the conclusion any. Comments. Advice. Suggestions. For tomorrow.

Dr. Thomas Slaga
You did a great job. Just continue to Marvel.

Dr. Wilma Bergfeld
Yeah.

Dr. David Cohen
Thank you.

Dr. David Cohen
Well, thank you. It's only cuts of the team. OK. I think tomorrow we're all going to need to sort of rally. There'll be a couple of, issues that are going to take some discussion, not the least of which will be glucosamine.

Dr. Thomas Slaga
Yeah.

Dr. Bart Heldreth
Yep.

Dr. David Cohen
Alright.

Dr. Wilma Bergfeld
All righty.

Dr. Thomas Slaga
Tomorrow.

Dr. Wilma Bergfeld
See you tomorrow at 8:30.

Dr. David Cohen
See you tomorrow, 838 thirty.

Dr. Ron Shank
See you tomorrow.

Dr. Thomas Slaga
Overall.

Dr. Wilma Bergfeld
Be ready.

Dr. Bart Heldreth
See you then.

Dr. David Cohen
Take care. Bye.

Full Panel – March 15, 2022

Dr. David Cohen
And Yeast. And this is a bit complicated, so this was for additional information and clarification. In that, you know Priya went through a lot of this data and the definition of yeast is extremely broad and it's not very informative. And a lot of this data is on Saccharomyces. And with two additional species you mentioned toriola and candidate Utilis? I know Toriola is candidate you tillison Saccharomyces fragilis. But you indicated that there's no evidence that they're being used in cosmetics. So the question is, are we lumpers or splitters on this? Is it just Saccharomyces? Or is it going to be yeast extract?

Dr. Ron Shank
I would limit it too only. To only this species used in cosmetics.

Dr. Thomas Slaga
I totally agree. I think we ought to go with the 1st. One and only go with.

Dr. Wilma Bergfeld
Sacrifices.

Dr. Thomas Slaga
Yeah, it it's used in cosmetics and leave the other two out.

Dr. David Cohen
So. That, that that wasn't my initial impression, but I could be persuaded, which is when you look at the constituents of these things, is there anything in there from my perspective that was going to be an irritant or contact sensitizer I couldn't come up with anything just on the top of my head. And if we do it in such a narrow way, or we going to have to have reports in the future if another.. Yeah, Bart.

Dr. Bart Heldreth
So maybe I misread it, but I I've read the situation very differently. So at the last meeting it was brought to our attention that we should look to the Food Chemical Codex to see what species are considered for a yeast that type that we consume and food. And so we looked at the Food Chemicals Codex that that's where we found that truly utilities and the Saccharomyces fragilis. And so that's why we brought those in. Not really a problem, at least from our, you know, amateur staff side. What did strike us as something we didn't know what to do with is, we made the assumption at the last meeting that we would just assume whenever we looked at least extract or yeast anything that didn't have a genus and species that we were going to only you look at Saccharomyces Servasa. If somebody was using something else other than Saccharomyces Servasa, we weren't concluding on it. However, we got this document back from industry and if you look the page 4 of that strategy memo you see listed under the generic yeast extract cosmetic name we have Candida Sitona anDeborah, *(inaudible) and the list goes on and it goes back to the notion we had before the yeast meant a whole slew of not only species, but geniuses. And so our question is you want to continue and just head down or only going to review Saccharomyces surveysay? When we're talking about yeast and yeast extract or do we want to include these other genus and species in our review? That's the impression I got.

Dr. David Cohen
Now I think we had similar impressions. I we I saw those lists and I'm saying all right, there's a lot here. Are we going to have individual reports for each one of these when most of it is protein, sugars and this it.

Dr. Wilma Bergfeld
General ash.

Dr. David Cohen
Yeah. So. Why not take the opportunity to lump them together if we, if we can, I suppose?

Dr. Bart Heldreth
OK.

Dr. Wilma Bergfeld
Well, we always have the opportunity later to split.

Carol Eisenmann (PCPC)
Where do you?

Dr. David Cohen
Yeah. Hi, Carol.

Carol Eisenmann (PCPC)
I just wonder where you stop because that list probably is not I didn't try to look and see, but I suspect there's a lot more. I mean this this is partly a naming issue.

Dr. Bart Heldreth
Mostly.

Carol Eisenmann (PCPC)
Currently. If you if you wanted a new name for a for your material made of yeast, you'd have to tell him the genus species, and they would name it using the genus species name. So there's going to be a lot more. There's always going to be more. This is like allergy. There's always going to be a new yeast coming in. So that that's me is a difficulty how do you stop but the main ones still, of the ones that? That they reported, they hardly have any uses reported to the VCRP. I still think that Saccharomyces surveysay is the one with the most uses. And you could limit it just because you're going to focus on the one with the most uses. And you already have a report pretty much prepared, which you could finish up and be done with.

Dr. Thomas Slaga
Right.

Dr. David Cohen
OK. I mean it makes sense and it's expedient certainly.

Dr. Wilma Bergfeld
Yep. It's still doable.

Dr. Bart Heldreth
Yeah. And that and.

Dr. Wilma Bergfeld
Alge wasn't doable.

Dr. Bart Heldreth
Thats great. That's easy for us. But then that leads to another question. What do we do with this data on CandidaSitona and DeborahMyhineas that if we just say thanks and put it in a folder? Or is it relatable to Saccharomyces surveyssay?

Dr. Ron Shank
Are they used in cosmetics?

Dr. Bart Heldreth
Yes, and under the name, yeast extract.

Carol Eisenmann (PCPC)
But I can also say that supplier has names, has trade name, materials under the genus, species names also. So. But I think you could say yes, thank you. But we're going to wait and review them.

Dr. Thomas Slaga
Later.

Carol Eisenmann (PCPC)
Right. When the when I when the genus species name comes.

Dr. Bart Heldreth
So then we would have a conclusion whether it's safe or safe with qualifications or unsafe or whatever for yeast extract when the species is Saccharomyces surveysay. Is that?

Carol Eisenmann (PCPC)
And you might change the name to Saccharomyces surveysay, and then do it the opposite way.

Dr. Thomas Slaga
Right.

Dr. David Cohen
You mean the title of the report?

Carol Eisenmann (PCPC)
Right to try to learn.

Dr. David Cohen
With these Saccharomyces.

Carol Eisenmann (PCPC)
Right. Whether or not it's called if it's called, yeast extract or the Saccharomyces surveysay extract.

Dr. David Cohen
I think if we kept it as yeast extract, it's going to be pretty confusing if the whole report is on Saccharomyces.

Dr. Thomas Slaga
Yeah.

Dr. David Cohen
I think we have to call it Saccharomyces, if that's what we're if we're deciding to split. That's what we need to do. But then that leaves all these other yeasts. Dangling.

Dr. Wilma Bergfeld
We've done that before. We can also open up in 15 years and add a few. I mean, they're all kinds of ways of handling the additions.

Dr. Ron Shank
Can the other yeast species be handled in the discussion? Or an appendix. To the report. Or do they have a lot of uses? The other species.

Dr. Bart Heldreth
At least from my perspective. It's absolutely impossible to know, so a little history on this, I think it was nine years ago I started pushing to put yeast extract on the priority list. At that time yeast extracts was one of the very few yeast ingredients in the dictionary, and it had about 1000 uses. And I kept getting pushed back. Oh, we're going to change the name. We're going to make be more specific. And nothing really happened there. And so I kept bringing it forward because it had very high frequency of use. So. My best understanding is right now, there's flooding of products on the market that's a yeast extract on the label. At some of them, say Saccharomyces surveysays the species they use, some of them are DeborahCS,. I don't think there's, at least I haven't seen any data to show us how many products have this one and how many products have that species. It's unknown.

Dr. Wilma Bergfeld
Pandora's box.

Dr. Bart Heldreth
That's right.

Priya Cherian (CIR)
So. Just to be thorough, before this report started, I did go through the Winky Dictionary and I looked and try to find every single yeast species that's currently reported to be in the Winky Dictionary. And then I have a document with those I can share my screen. I can show you. Great. So yeast at the top, are the ones that I found to be in the Winky dictionary in 2021. I haven't done another search this year. And then these at the bottom are ones that are in the Winky dictionary and have at least one reported using the VCRP according to 2021. But not all of the genus and species that were reported to be under the yeast extract, and that data supplement that you got, correspond to a Winky ingredient as of now.

Dr. David Cohen
Right. We don't know what yeast extract really means in that VCRP list.

Dr. Bart Heldreth
Right.

Priya Cherian (CIR)
Right.

Dr. Bart Heldreth
So I you know if you're looking for a direction to go, I think you're right to narrow it down to two a species you can handle instead of looking at all of them at once. When we don't even know you know what the uses are for all these other ones. So it does make sense, I think, to stay with just the Saccharomyces surveysay and will save this data for a future date and the maybe things will be cleared out further at that point.

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Dr. Thomas Slaga
You did a great job. Just continue to Marvel.

SEPTEMBER 2022 MEETING – STRATEGY MEMO 2

Belsito Team – September 26, 2022

Minutes not available.

Cohen Team – September 26, 2022

Dr. David Cohen - OK. I think, we got through our summaries. OK. Yeast.

Dr. Tom Slaga - Yeah.

Dr. David Cohen - Well, that gosh.

Dr. Tom Slaga - I wish them stated that can we put it at the end?

Dr. David Cohen - I thought I knew where we were going to go. Look, so I think the CIR staff was great in just focusing us a bit, right. I guess the question is ultimately, are we going to include all of those yeasts in a future in a future review or are we going to keep it narrow to the species Saccharomyces cerevisiae?

Any top blind comments from the group after the lecture today?

Dr. Tom Slaga - Well, if we were sure, did we could. That only a species would use, but my understanding, several different species could be used at any time and you know how? How can we separate that out unless we do all of them? It's just a comment.

Dr. David Cohen - It seemed to me.

Dr. Tom Slaga - It's a very difficult when you don't, you know.

Dr. David Cohen - Look, I.

Dr. Tom Slaga - If we were only dealing one species, it would be fine, but we're really not. Right Monice?

Monice Fiume (CIR) - It sound to me that they've grouped every species under the name yeast.

Dr. Tom Slaga - Yeah.

Dr. Wilma Bergfeld - The class of Saccharomyces.

Dr. David Cohen - Well.

Dr. Tom Slaga - Yeah.

Dr. David Cohen - So class is really high up right? It has all the genus and all the species.

Dr. Tom Slaga - Yeah.

Dr. Wilma Bergfeld - Alright.

Dr. David Cohen - It did. I read it wrong, or did it seem to me? Well, I don't think it should have been any surprise, one species versus another is going to have some similarities. I mean, if you ground me and Don up and did an analysis, we would not be the same. Right? Would be a little bit different. And we're in the same species ostensively, right?

Dr. Tom Slaga - Right. A good bit of difference.

Dr. David Cohen - Thomas, you had your hand up. Maybe you can help.

Thomas Gremillion (CFA) - I don't. I don't know. I don't feel this is going to be but it. I just wanted to ask the question, are the pathogenic yeast in the same class as they're not OK?

Dr. Wilma Bergfeld - No.

Alex Kowcz (PCPC) - No, they're not.

Dr. David Cohen - It seemed to me that we could put them all in a single report, right? Understanding that the systemic talks would probably have a lot of data on. And then the question would be how much dermal tox would we really need to clear the whole group, right? Because it seemed it sounded like there when they're declaring something safe, they're doing some sensitization data and they're looking to make sure that everything falls into this class the way it's supposed to be and everything is, is inactive. There's no live material. And the class that broadly is used in food so, I guess would we go with, Yes, let's put them all together. And then when we get the report, we'll have to see what sensitization and irritation data and we would want. I remember we what did we have to do this with some was it wasn't Carl was it.

Monice Fiume (CIR) - Algae.

Dr. David Cohen - With some algae. Yeah. Thank you.

Dr. Wilma Bergfeld – (*inaudible).

Dr. David Cohen - We had to do it with algae, so when we handle this the same way.

Susan Tilton - David, can you or can I get a clarification just on the question that we're trying to answer. So one option is to only review data for the species cerevisiae. And the other option is to include other species in the evaluation. Would it be evaluated under yeast as a together, not differentiating amongst what data is included? Or would we be discriminating? Like would be. Would they be listed like they were different ingredients in terms of how they're evaluated?

Dr. Wilma Bergfeld - I think it's going to be due to the chemistry of the protein.

Dr. Susan Tilton - Or how we would evaluate?

Dr. David Cohen - But the report's going to be yeast, right? Not I. I don't think we're shoot. I'm moving away from saying we're just going to have a report on Saccharomyces cerevisiae when moving to a report that says yeast. Right?

Dr. David Ross - Well.

Dr. Wilma Bergfeld - Yeah.

Dr. David Ross - Because you're in products that you used is a yeast. It's yeast and it contains everything. My understanding in the presentation was that. Yeah, these different things, these different yeasts are going to be different. They've got, you know, ask the question on cast members. They're going to have different chemical and protein properties and they go to induce different effects. But the product you're using is them all mixed up altogether, right. So that's what we're going to be considering with respect to dermal and ocular irritation.

Dr. Tom Slaga – Right.

Dr. David Ross - And doing sensitization.

Dr. Tom Slaga – Well, we could try it with all and see what happens.

Dr. David Cohen - Monice, so we answering the question that you guys want us to answer, I hope we're. Monice, so we answering the question that you guys want us to answer, I hope we're getting close.

Monice Fiume (CIR) - It's good. So I think yes and no. I think the panel is in a very tough situation when we did algae, those ingredients were separate ingredients, so each Algae ingredient had its own INCI name, so you could go through and see, does this genus species have systemic tox have sensitization data or topical what the other the dermal aspect and make a decision? For this, they're telling us that the name yeast is the INCI name, but it could be any of these genus species under this class, so it makes it a little more difficult, I think, in determining safety. I know in the past when we've had a situation where. What's in the ingredient may not have been clear. The discussion address the fact that this is what we found safe the information if it, if it's this genus and species, and we had information on it, we can rule on the safety because that's the information we have in the report. If it is different than the specifications listed in the report, then either the data or insufficient or whatever conclusion you would draw. So we would the panel would craft the discussion to say. Say it. It's not I'm Saccharomyces cerevisiae. Say it's something else and you had information on it and it was enough for you to say yes, that's genus and species would be fine. It would be covered, but if it is not included in the report, you can't comment on it. So a lot of times we would have a table in the report that would show say exactly which genus and species were referred to in the document that you had information on. That would be OK and that if industry was using something different, they would either have to independently have safety data for that ingredient because the CIR report does not cover that genus and species, even though it's under the umbrella of yeast or yeast extract. Does that make sense? What I'm trying to say?

Dr. Wilma Bergfeld - That's the only way you can go.

Dr. David Cohen - It actually. It does make sense. The during the lecture though in the conclusion slide they said we can group the class of Saccharomyces together right, which would include enumerable, genus and species, right?

Monice Fiume (CIR) - But they also did say for systemic, but for the dermal like irritation and sensitization. Those data would be needed.

Dr. Tom Slaga - Right.

Monice Fiume (CIR) - So I think that might be where it would come into play as you've done in the past where you know which you do have a full complement of safety data that you would need for a report in which you want it. And so it wouldn't be that you would have to say. These are not, if it you're yeast extract includes this genus, and this species is insufficient, I think you could probably flip it and say if you're yeast extract includes this genus and species, then it is sufficient we have sufficient safety data and we know a yeast ingredient that is manufactured using this genus and species. From a CIR standpoint, has a conclusion.

Dr. David Cohen - Yeah. And so that's a discussion item we could, we might consider going out with after adjudication safe as used, right? But in the discussion say, hey, we based it on these, the data on this genus and species, if you have another genus and species, you're going to have to do some additional safety work on it. That that's what you're saying, right?

Monice Fiume (CIR) - Yes, that's what we've done in the past and that's why the conclusion goes to say, as described in this report, to point people to yes, you really need to look and see what we're saying here.

Dr. David Cohen - I think we would have to really Illite the unique nature of this because that kind of sort of loose language could come up. You know, when we have, you know, 18 derived chemicals and you know, we may not have data on some of them or there's a 19th one that's kind of close. So yeah, alright it is it is tricky.

Monice Fiume (CIR) - And I will say the panel has become very creative as you've encountered these issues because brown algae, the first meeting or so was very vague and very confusing. And then the panel did develop a strategy, so that was the

strategy that was done for that. Maybe, maybe not for this. You know, I don't know if anyone has, you know, you may come up with a better strategy to Illite it, but that's one thing that we've definitely done in the past.

Dr. David Ross - And it's just one question, but I don't really understand the extracts is as a whole here, but you know, are we likely the things we're going to get are going to be mixtures of yeasts, is that correct? Or they're going to be, they're going to be Peaky or they're going to be Saccharomyces. They're going to be mixed?

Dr. Wilma Bergfeld - You don't know that actually.

Dr. David Cohen - I know I that I don't know. We heard that either way.

Dr. Wilma Bergfeld - Don't know that.

Carol Eisenmann (PCPC) - That's my understanding. They use a specific Organism for each for a specific ingredient they don't for at least for the ones that you're reviewing now, they're one that part of the problem is INCI names have evolved, so they used to name everything just by yeast. So a number of specific species got named under yeast. Now they are naming them using at least the genus name.

I'm sometimes the genus species name. So I think you're just looking at I a single species at a time I don't think you they're. I mean, yes, there are other ingredients that are specifically named where they, They're doing these ferments with multiple yeast and bacteria and they may have different fruits and vegetables. We're not looking at those. I think we're just looking at yeast, a single yeast in standard media and then, they're extracting or they're looking at the filtrates of the ferments, something general for this report. There are more complex permutations going on. But that's not going to be what's in this report.

Monice Fiume (CIR) - And David, the only other thing I was going to say is, if the panel is not comfortable on ruling on safety, there is the insufficient data conclusion is always a valid conclusion. If you really don't understand the compass, because I know Dan Liebler is.

Dr. David Cohen - Of course.

Monice Fiume (CIR) - You know, made this point if we don't understand the composition, how do we rule on safety? So that is also another valid conclusion.

Dr. David Ross - Or another approach that I thought of when I read the information was that we would restrict it to the to use the were A used in cosmetics and B how to define CAS number. And that's why I asked the question on CAS number, and I don't even know if that's a valid approach or not. Everything is used as a mixture of and it's not, but if they're separate, then you know it potential is.

Dr. David Cohen - Yeah, we split decisions we've, we've put out insufficient data. We do that all the time. Right? I mean we could in the come out and just say this genus and species is what we feel comfortable with and we don't feel comfortable with the rest of them based on what we look at.

Dr. Wilma Bergfeld - Unless they can show us the composition.

Monice Fiume (CIR) - I wish Priya was here because she's more familiar. So I'm to remember if one of the options on PDF page four of the Yeast Strategy memo lists all the INCI ingredients in the dictionary that are yeast, and right now the highest frequency of use does fall to those that are named a yeast ingredient or Saccharomyces cerevisiae so you can see there are other genus and species that are named as individual ingredients.

Dr. David Cohen - Yes, I saw that. I think they fall under the family of Saccharomyces, right?

Dr. Wilma Bergfeld - Right the class.

Dr. David Cohen - Under the family.

Monice Fiume (CIR) - I think so, yes.

Dr. Wilma Bergfeld - The family, rather than the class.

Dr. David Cohen - Yeah, I think the, I don't know if they fault.

Dr. Wilma Bergfeld - The class is the is the broadest. I mean, I just looked that up.

Dr. David Cohen - Yeah. No, no, you're right. But I think I think when we review them, we should have that level of detail like what, where is it in the order? Well, what I shouldn't use that term, where is it in the table of organization? In there so we could figure out how close they may be.

Monice Fiume (CIR) - Yes. So Saccharomyces is the family, but the other?

Dr. David Cohen - They used class I think in their conclusion.

Dr. Wilma Bergfeld - I ask. And there outline use a class.

Monice Fiume (CIR) - Yes, so it is, it is the class.

Dr. David Cohen – The class. Yeah. The conclusion was in their class we could group them together. So I remember it was a we didn't have a long time to look at that slide. These other associated genus and species were under that class.

Monice Fiume (CIR) - Yes.

Dr. Wilma Bergfeld - Yes, all of it. I have it here. So I'm looking at it.

Dr. David Ross - Yeah.

Dr. David Cohen - So. We're going to go out as a team right now as groupers, as opposed to splitters for now, right? Is that? Is that fair?

Dr. Susan Tilton - I agree.

Dr. David Cohen - OK. Tom, David, any other further comments about yeast?

Monice Fiume (CIR) - And so David, they will still be yeast and not include any of the other name genus, species ingredients, even though they fall under that class?

Dr. David Cohen - No, no, I thought we were going to. We were going to include them.

Dr. Wilma Bergfeld - Yeah.

Monice Fiume (CIR) - Oh. Oh, OK. That's why I just wanted to be clear. Thank you.

Dr. David Cohen - Yeah.

Dr. Wilma Bergfeld - We're going to include the cosmetic grade.

Monice Fiume (CIR) - So that would be all of the ingredients listed on PDF pages four and five?

Dr. Wilma Bergfeld - Like it?

Dr. David Cohen - That that was my thought. It was. Did anyone have a different thought on that? There was certainly in the class.

Dr. Susan Tilton - No, I agree. And So what that? What that would mean is that, data that's available for any yeast within the class would be included as available within a report for evaluation. Is that right? It we wouldn't be limiting ourselves to just data cerevisiae for instance. And then we can make a decision based on what's under evaluation as to whether we feel that it's in the scope of this data set for the class?

Dr. David Cohen - Yeah, I think Monice's point, the hydrolyzed yeast protein and yeast extract, they're they're a major part of the in use products. And if we if we just go too tight, we we're not going to cover really important uses.

Dr. Susan Tilton - Yeah.

Dr. David Cohen - Or yeast extract.

Monice Fiume (CIR) - Yes, because those.

Dr. Susan Tilton - Alright, that that is the largest category.

Monice Fiume (CIR) - And those were the ingredients that were originally in the report, I think. And I have to look back for sure. The ones that are in the yellow were part of the original grouping of the yeast report. All of the others would be added into the document now. For the next iteration.

Dr. David Cohen - Yes, we'll need a lot of time with that one.

Monice Fiume (CIR) - OK, great. I'll make a note of that.

Dr. Wilma Bergfeld – Oh dear.

Dr. David Cohen - That was a hint Monice that was just like a yeah, that was like a that's just a subtle remark.

Monice Fiume (CIR) - I have it in big letters in my notes, David. It is noted.

Dr. David Cohen - OK. So let's move on to glycol lactones. In March we reviewed this and we concluded that Gluconolactone was safe as used and we had insufficient data for the remaining other derived ingredients and we asked for impurities. A method of man and method of manufacturing specifically for, glucarolactone, glucarolactone and we received no additional information. I think if when we look back on our judication of the glycol lactones, I think we were a little bit less restrictive on it. We've we thought we might be able to read across but when we got to group together Don and his team had maintained their IDA for the insufficiencies. And we agreed with them. Now that we have no additional information, our heels as dug in. Because now this is this is a draft final, right?

Monice Fiume (CIR) - Yes.

Dr. David Ross - Yeah.

Dr. David Cohen - Yeah.

Dr. Dr. Tom Slaga - I agree final.

Dr. David Cohen - Yeah. So, Tom, what are what are your thoughts? Are we splitting this decision or are we going to utilize what we have on, gluconolactone?

Dr. Tom Slaga - Use what we have.

Dr. Wilma Bergfeld - Well, that means splitting is. Is that what you mean Tom?

Dr. Tom Slaga - No.

Dr. Wilma Bergfeld - There's 1 (*inaudible) for so you're going back to the original. So that makes a difference because this is gone out already for review.

Dr. Tom Slaga - Yeah.

Dr. Wilma Bergfeld - You're changing the conclusion.

Dr. Tom Slaga - We can't change conclusion.

Dr. David Cohen - Well.

Dr. Wilma Bergfeld - You can change it, but just understand it would have to go out for review again.

Dr. Tom Slaga - Yeah. No, no, I understand that, but.

Dr. David Ross - I thought, you know, David said we didn't get any new data, right? And so, you know, in my notes I just said in conclusion safe as used for gluconolactone insufficient for the others? I don't. I'm not. Not sure why you do read across now when you didn't do read across before because you have no new data.

Dr. David Cohen - Well, I listen. I'm still, I think this is a continuing learning process. But we do ask for things I'm hoping will get additional information. Sometimes it's a bit aspirational on what we ask for and then when we get to a certain point, we settle in with what we have and make conclusions on that. Am I overstating it, Wilma?

Dr. Wilma Bergfeld - No, we you can do anything just to know that you're going to delay it another 60 days. That's all. That's all I'm stating. You can do anything you want. You can say I'm not comfortable with this conclusion.

Dr. David Cohen - Susan, any thoughts on your read? Because this is more of a first read for you.

Dr. Susan Tilton C - It is a first read I was comfortable with the split conclusion moving forward based on the data available for. Gluconolactone but insufficient data for the others. With lack of a read across to apply that one data set to the others.

Dr. David Ross - So first read for me too, and so just to recap, read lack of read across because was because of the lack of impurities. Was that correct?

Dr. David Cohen - Yeah, the from, from my recollection of the transcripts and the meetings, right, we didn't have impurities and some method of manufacturing. And I think Priya am I right that that kind of hold up the Belsito team from clearing the group.

Dr. Tom Slaga - Yeah, that was it.

Priya Cherian (CIR)- I'm so sorry. I just jumped into this meeting. They just talked to me about yeast.

Dr. David Cohen - No, no, no, that's OK.

Monice Fiume (CIR) - I'll answer for.

Dr. David Cohen - No, no, no. We're past yeast. We definitely don't want to hit replay on yeast, but we're on glucono lactones.

Priya Cherian (CIR)- OK.

Monice Fiume (CIR) - So yes, David, on PDF page 32, the discussion, the second paragraph saves that requires impurities, data and cosmetic specific method of manufacture.

Dr. David Cohen - And. Yeah. Yeah, that's, that's what the held it up. So it sounds like from the team, we're going to carry the last motion to final.

Dr. Tom Slaga - That that's what I say.

Dr. Wilma Bergfeld - They can. They can always come back. That industry can always come back and say ohh here it is. Then we'd have to amend.

Dr. David Cohen - Yeah. I just.

Dr. David Ross - What's that?

Dr. David Cohen - I look, that was my gut. But I want to make sure that we don't do I just a pro forma.

But you know, carry the motion when we're going into final. Because sometimes there are things that we'd like to have, but we may be able to imply from others, so we will carry the motion. From last time because we don't have anything new.

Dr. David Ross - Are you (*inaudible) ending that one David?

Dr. David Cohen - No.

Monice Fiume (CIR) - And David, if it's OK if I jump , since there are new members just to let everyone know, when we have an insufficient conclusion. That puts a two year clock on those ingredients. And then after two years, if ingredients have 0 uses and were insufficient data and we've received nothing new, they go to a category called 0 use and the four that are listed here. Unless something changes will eventually change to that category. If any of the ingredients that were insufficient as a final conclusion. If we don't receive data and they do have use, it switches category to called use not supported, which implies that these are ingredients are in use and there are no data to support use in cosmetics, so it's not called insufficient data at that point, but use not supported.

Dr. David Cohen - And that happens automatically. That's not a we don't adjudicate that at all, right?

Monice Fiume (CIR) - Bart will provide the updates at some point during each year as to which ingredients are changing category. But it does give industry two years to submit data before the conclusion switches.

Full Panel – September 27, 2022

Dr. Don Belsito - Yeah. So in addition to the almost one hour presentation on yeast or panel spent probably more than one hour discussing these and going around in circles and you know noting that the vast majority of them were largely undefined as yeast or Saccharomyces. And how would we deal with these and that manufacturing seem to be the same, but composition might be different. So in the end we decided to look at only those knowing despite everything I've said before at this point where either industry or VCRP has told us that they are actually being used and that we would look at, we were trying in a sense to have Priya do the same type of thing she did with red algae and to look at where there are food uses. That might give us confidence and lack of systemic toxicity data and whether where there's a dermal sensitization and irritation, but we are not going to look at all the yeasts that are listed in the chemical dictionary, only those where there are reported uses either VCRP or industry. Take a dive into that and maybe based upon what we see, want to split them off like we did with algae. I think we started with algae and then we went to red algae blue algae and different colored brown algae. So that's where we ended up with the Yeast.

Dr. David Cohen - That's Don. We use the algae.

Dr. Don Belsito - We didn't quite rise to the occasion.

Dr. David Cohen - We use the exact same analogy of the algae in our in our group. It's interesting the that's a good idea. With the VCRP data. And we thought based on the presentation, we could review up to the class of saccharomyces because that last slide or that summary slide when it's high as class, right and some of the yeasts that were mentioned, some of the genus and species were not saccharomyces, they had other names, but they belong to the class of saccharomyces. So we could include that in in one review. If I don't have an issue with you using the VCRP as a guide.

Dr. Wilma Bergfeld - But they will also ask industry.

Dr. Don Belsito - What's your question, Wilma?

Dr. Wilma Bergfeld - I just adding to the VCRP that you were asking industry as well for the use of yeast and information on these. So there were two prongs.

Dr. Don Belsito - Yeah, we would. What we suggested is, is any materials reported to be used by industry or VCRP. The problem we had, David would going up to saccharomyces's was that in the end our understanding was that cell wall lysates from these different saccharomyces's could be chemically very different and you can't we could not read across from them. So.

Dr. David Cohen - When we had that problem before, so the point is, I don't know if we would read across, remember what we did with the algae. We said if they're eaten and we have dermal tox or sensitization, we cleared them. And if they didn't, we didn't clear them. We I think Dan mentioned it before we could keep them in the same report, it just didn't mean we had to drag all the data across for all of them.

Dr. Don Belsito - I mean the this is a beginning. You know, so poor Priya, she did the algaes too. She's doing this. I mean, we can start that way and take a look and then decide to split it up. I mean I don't have a problem. We're just trying to make it easier for Priya. This was Bart's suggestion that we finally agreed with. So Bart, maybe you want to chime in here.

Dr. Bart Heldreth Yeah. I mean hearing, I only got to hear of course the Belsito teams discussion on this yesterday. But one thing that I thought was interesting was you know, within that saccharomyces class, we do have some pathogenic yeast like the Candida albicans. And so one suggestion was that we have a table that says, hey, here's these pathogenic saccharomyces, (*inaudible). But then from the tox we had yesterday, I think a question that I had was maybe we should consider in addition to looking for grass status for these, these ingredients, since they are all Organism based, should we consider a in our safety assessment whether each Organism is BSL, one level, another word a very safe Organism? Could that considered?

Dr. Wilma Bergfeld - I think that's important, yeah.

Dr. Bart Heldreth - In instances where we don't know about GRAS status.

Dr. Don Belsito - Or weren't we told by the manufacturers that that's their, that's their first step with the cosmetic ingredients. So by definition, anything in cosmetics would be BSL1?

Dr. Wilma Bergfeld - Yes. That's correct.

Dr. Don Belsito – Paul, you were in our group, had the most to say about this. You want to chime in here?

Dr. Paul Snyder - Sure. I think you've already captured it. I mean the only issue to me was that there's classification we know about pathogenic yeast and it's based upon their exoenzymes or phospholipases proteinases and things like that as an issue. And so I really want to see profiles of the constituents in there, the mathematic fracturing and composition of those only as it pertains because there are pathogenic yeast and those are typically pathogenic as opportunistic infections. And were normal barriers are breached. I mean, we're in the normal immune response is compromised or something. And so if people are, if there's ingredients containing these constituents that are the sort of the pathogenic factors, I mean, even if they're not in the pathogen, we just don't know. I don't know them that well. I'm not a yeast person. So and then of course the cross linking of IGE and bypassing again like on inhalation and stuff like that. So that was that was the only issues that, that I talked about, I thought we should start like, like Bart said with the use that are in the VCRP in 2022 and kind of see how it goes and instead of trying to make too much of a cumbersome process.

Dr. Wilma Bergfeld - Paul, does that negate asking industry for information on the yeast?

Dr. Paul Snyder – No, I think we need to have a clarification. I wasn't clear in the discussion, (*inaudible). I did have some trouble understanding her. I even spent some time last night trying to see if the pathangenic yeast rose to a BSL2 level. And I actually couldn't find that information. But I was trying to do it hurriedly so, those are some of the questions we need to ask. If they are in fact BSL1's, then I think we're fine other than the composition and knowing where they contain peptides sufficient enough to cross link IGE molecules on the surface of mast cells.

Dr. Don Belsito - But then we have the, you know, hydrolysis. And we also have already resolved that issue with hydrolyzed wheat. So all of that information from hydrolyzed wheat in terms of, you know, the likely their weight and the peptide size that it takes to link the FCFsalon receptors on mast cells, we know about from that data. So that would be brought in for these.

Dr. Paul Snyder - Yeah, we kind of laid the road map of how to do it and what to look for.

Dr. Don Belsito - Right.

Dr. Wilma Bergfeld - David, do you have anything to offer here or add?

Dr. David Cohen - No, I think. We've already suggested that we start up high and we'll use those filtering criteria. I would have expected. Pathogenic yeast to be more than BSL one. But we'll be able to review that as we see them come in and if we could keep them in one report it you know, the algae were very difficult to get through, but I think it would be even more

difficult if we if we initially started breaking them up. Don, you've made a number of suggestions over the years to break out groups like the clays and they worked out very well. But I think starting with them all together is better.

Dr. Wilma Bergfeld - Anyone else have any comments to make Bart? Do you do hear the marching orders for this?

Dr. Bart Heldreth - Heard.

Dr. Wilma Bergfeld - I think that won't.

Dr. Don Belsito – Priya I can see you crying now.

Dr. Wilma Bergfeld - The poor thing she may need help.

Dr. Bart Heldreth - We will help her.

JUNE 2023 MEETING – REVISED DRAFT REPORT

Belsito Team – June 12, 2023

DR. BELSITO: Okay. Yeast. So, we got a Wave 2 on this which was just PCPC comments. Just look at those first, whether we agreed with them. Are there any questions to be asked? So basically, their comments that we got information on ingredients sold under yeast on candida oleophila, candida magnoliae, debaryomyces, nepalensis, metschnikowia, metschnikowia pulcherrima and pichia naganishii that weren't included in here. So, was there a reason why they weren't included?

MS. CHERIAN: They were included in the report, it just wasn't included in the data profile. Because all of those ingredients don't correspond to a similar yeast ingredient in the report that have a related genus and species.

DR. BELSITO: Okay.

MS. CHERIAN: Those species only fall under yeast. The generic name yeast extract.

DR. BELSITO: Okay. So how do we suggest we handle that? So, they could be components of a generic yeast extract?

MS. CHERIAN: Correct.

DR. BELSITO: So, they need to be in the report someplace, no?

DR. SNYDER: I found this to be extremely confusing, the nomenclature and how --

DR. BELSITO: And then what the product name is and --

DR. SNYDER: Yeah, yeah. I mean, I defer to that table on page 107, the taxonomy table, because I thought that was kind of helpful. But I wish that table had the GRAS status and more information in it because I was trying to decipher what I was actually looking at.

DR. BELSITO: My eyesight is gone.

DR. SNYDER: Yeah.

DR. SNYDER: I mean --

DR. BELSITO: Yeah, if you look at the GRAS status, you know, you have the exits there but then there is -- in our presentation that we got, there's something called -- where's my note? Sorry, I'm on the wrong document here. Where they had some of the yeast as QPS which stands for Qualified Presumption of Safety and how does QPS relate to GRAS?.

DR. RETTIE: Is that a term you use?

DR. SNYDER: I've never used it before.

DR. BELSITO: Well, if you go back and you look at the presentation document that we have at the end of this, that we saw like a year ago that I hardly remember, they talk about these -- some of the yeast as being QPS. And I honestly didn't remember that because I would've asked what that meant, and I don't know what it means.

MS. CHERIAN: From my understanding it's a European term used by the EFSA. So, if it had a QPS status I didn't include it as a GRAS or food use. It only had a GRAS or food use label if it was from a journal or actual GRAS from FDA.

DR. BELSITO: Right. I realize that because when I was looking to see the QPSs that they had noted they weren't, you know.

MS. CHERIAN: Because I wasn't sure how relevant it was to us or how reli- -- you know.

DR. BELSITO: I'm not either. Maybe we should query back those presenters and then ask them exactly what's meant by QPS.

DR. EISENMANN: Audrey is here.

DR. BELSITO: Okay.

DR. EISENMANN: I think it's really more of a European thing versus GRAS is a U.S.

DR. BELSITO: Who is Audrey? Yes please. That's the problem with the original, it doesn't sink in as well.

MS. POKRZYWA: Is it a (inaudible) of GRAS status in Europe. And this is a way to not ensure the safety of the yeast, but to advance some information about the safety of this yeast. As there is several yeasts in QPS status, I may send you more information about QPS status together with (inaudible). It would be some more useful as my explanation of everything. I will share other information about this.

DR. KLAASSEN: Okay. So, is it fair to say that QPS in Europe is similar to GRAS in the United States?

MS. POKRZYWA: It's similar but it's not exactly the same. It's a good way to identify the yeast, which can be considered as safe, but it's not actually safe. It's a good progress to consider them as safe. But it's not exactly the same as GRAS status, which is more precise.

DR. SNYDER: Okay.

DR. BELSITO: So, if in Europe something is qualified as QPS, could it be used in a food?

MS. POKRZYWA: Yes. Yes. Again, sometimes yeasts used in food may have QPS, but it's not always. Some QPS that use strain may have not been used in cosmetics or food. It's more of a general statutes of QPS that's not only for yeast used in food.

DR. BELSITO: Okay.

DR. RETTIE: Do you have any information about the term qualified? Why is it qualified?

MS. POKRZYWA: I need to do more research on this.

DR. RETTIE: Is there a PS designation, presumed safe, as opposed to QPS?

MS. POKRZYWA: Yes, QPS.

DR. RETTIE: No, no. Is there a separate PS designation, Presumed Safe? Not qualified, but presumed? I was just curious if there were multiple -- just wondered what qualified meant.

DR. KLAASSEN: It just seems like two words that kind of mean the same thing.

DR. RETTIE: Yes. Need antoher vowel in there.

MS. ZANG: I have a question for QPS. Does the QPS status associate with a specific use like GRAS or is just a general statement?

MS. POKRZYWA: It's a general statement.

DR. RETTIE: I had a clarification question on use concentrations. I was reading galactomyces ferment filtrate at 91 percent. Is that right?

DR. SNYDER: Yes, that's what it says.

DR. EISENMANN: Yes, that is correct. And yes, you will get data on that product and that ingredient, but it's being translated and we didn't want to overwhelm you with a lot more data. So, yes, that is correct and there's data on its way.

DR. SNYDER: Eye lotion at 37 percent.

DR. BELSITO: Yeah. What surprised me is I think we all thought that saccharomyces cerevisiae was going to be the most frequently used, and it is not, it's that species.

DR. RETTIE: Yeah -- no, 77 uses? Somewhere up at nearly 400.

DR. SNYDER: 343 leave ons, 55 rinse offs.

DR. BELSITO: Okay. So, let's just go back to the Wave 2 comments and then we can move back into the main document. Is that fair? So, the first comment we're going to somehow have to include those specific yeasts as being used to produce, just general product yeast extract.

MS. CHERIAN: That should be included in the document already. The only place it wasn't included was the data profile.

DR. BELSITO: Okay.

MS. CHERIAN: But that data, saying that those species are used in yeast extract -- the generic yeast extract -- that's included.

DR. BELSITO: Okay.

MS. CHERIAN: The composition and the taxonomy table.

DR. BELSITO: Okie doke. And then what other comments were there before we go?

DR. RETTIE: I have a question on Table 9?

DR. BELSITO: Can we just try and go through the PCP Wave 2 comments? I think it's easier. Then we can go into the main document just to see. So, hydrolyzed yeast protein, beta-glucan and polysaccharides were removed from the report. I don't remember why that was.

MS. CHERIAN: That was Bart's decision. But I think because he found them not chemically similar to the remainder of the ingredients and they were generic ingredients. And I think Carol might be able to answer better, but I think they'll eventually be removed -- those generic ingredients might be removed from the dictionary eventually and replaced with species-related ingredients.

DR. EISENMANN: Certainly not the beta-glucan, but the other ones, possibly, I don't know. Right now, I don't think Joann's (phonetic) planning on necessarily removing them because people don't like name changes, but I don't know.

DR. BELSITO: Because I didn't have any notes on that. Monice, do you know why they were removed?

MS. FIUME: No. Unfortunately, that is my other notebook that is at home from when we had our staff meeting. But I believe what happened -- so, do you remember, I think it was something like eight ingredients the first time the report was brought to you and then we went through numerous --

DR. BELSITO: Fifty-six --

MS. FIUME: Yeah. And so, the last time it was include everything that seems to fit. So, we had been playing with the groupings to try and decide what all fits. I'm trying to remember exactly why we pulled them out. It had something to do -- that they didn't appear to be the same as the others when we were going through it.

I do wish I had my other notes to give you. I don't want to speak off the top of my head and tell you something incorrectly. I know that is something Bart can answer with clarity. We did pull it out. Actually, let me see if I even have email about it.

DR. SNYDER: These would all be components from the extracts so why wouldn't they be included. I don't understand it.

DR. BELSITO: Yeah.

MS. FIUME: Let me see.

DR. BELSITO: I'm just going to move this over to the main document, so.

MS. FIUME: Sorry, I do not have those in my email. So rather than misspeak, I'd rather let Bart address this one.

DR. BELSITO: Yeah, I'm putting a note right at the beginning of the main panel meeting here to find out why that was. Okay. And then Wave 2. So, method of manufacture, unpublished data we've submitted describing methods for some. So, I wasn't sure what that was a referral to in Wave 2, but apparently PCPC felt that they had submitted documents on manufacturing unpublished data that weren't included in the original. Is that correct?

DR. EISENMANN: It's just an incomplete sentence. That's all, it's not anything.

MS. FIUME: It's editorial.

DR. EISENMANN: It's editorial.

DR. BELSITO: Oh, okay. Okay. Okay. So, everything else here is just editorial. Is that correct if I'm reading it right? Okay. So then that's the only thing that we need to discuss in Wave 2. Okay. So now let's get into the yeast documents, the original one. Allan, you had a comment on page 9?

DR. RETTIE: It was a clarification. On Table 9, I was just curious --

DR. BELSITO: PDF page please?

DR. RETTIE: PDF 123. It was the in vitro dermal absorption studies. I was just curious how we'd go about measuring absorption of a yeast extract when applied to the surface. But I see there's a test guideline on OECD, so I can just look that up.

DR. SNYDER: It's pretty standard.

DR. KLAASSEN: The question is, what do they actually quantify? I mean, it's not, you know, this is -- they're putting soup on the skin, so what part of the soup do you quantify in the blood? But I don't understand that either.

DR. BELSITO: So, I had a question. There were two of these that are not saccharomyces. There's the schizosaccharomyces and the tremellomycetes that are not saccharomyces. Do we want to include those? I thought we were just going to include the saccharomyces.

DR. SNYDER: I go to -- that's the taxonomy. I can't make heads or tails of it. It's very confusing.

DR. BELSITO: Are there significant differences in those yeasts from saccharomyces that you're aware of?

MS. POKRZYWA: Yeast extract are also yeast belonging to the sacchromycetes class. And both the strains you speak about are not from the saccharomycetes class. So, I was surprised to see them, these ones, in this review because with the class of saccharomyces, I think, existed enough in one class of the list. And the whole list can be studied in the same class. But these two yeast, I'm not expert on these ones, but I think they're a little different from the (inaudible).

DR. BELSITO: Priya, any idea why they were included here?

MS. CHERIAN: It's just because they were yeasts under the wINCI ingredients. So, all of the yeasts that were in the dictionary were included in this report.

DR. BELSITO: Okay. Are there uses for these? I mean, I didn't look at that. I'm not a microbiologist. I mean, I'm sort of operating under the assumption that there must be similarities in cell wall and other compositions that put these into the same species. And that if they have a different name there may be differences in their proteins and their carbohydrates and their whatever.

But in the end, it seems when you look at all of these things, other than potential impurities, what you're ending up with are amino acids, fatty acids --

DR. RETTIE: The bids.

DR. BELSITO: -- yeah. I mean, stuff that we've already looked at that are fairly innocuous.

DR. RETTIE: Yeah. There's a pie chart that kind of makes that point, or tries to make that point that a yeast is a yeast is a yeast. My son should be here, he's a microbiologist, he would know this.

DR. BELSITO: And we know that's based upon the manufacturing that we're given, that there's not going to be any live organisms.

DR. RETTIE: That's a very important part and it's hammered home.

DR. BELSITO: Only thing that bothered me, is when you start seeing things like -- if you look at PDF Page 92, it says this is for kluyveromyces. And it says you're looking at the extract including hexadecane, pentanoic acid, phenol, as contaminants. Like what were the levels of those? The PDF Page 92, the last two lines.

DR. RETTIE: So that one, which is difficult to pronounce for sure, I was wondering why it was in there. But it's used for the production of renin in cheese processing, so I'm assuming that's pretty safe.

DR. BELSITO: I understand, but when you look at it, it says that the extract includes. And then you look at the list of things that it can include and they're volatile, but then it goes on to say other volatile compounds found in to a lesser amount. But we don't know the amounts of those.

I mean, I would be concerned about -- although these yeasts are used in very high concentrations. So, you know, phenol can be present in a ten percent concentration of a yeast extract. Again, I doubt it, but we don't have that information.

DR. RETTIE: We don't know.

DR. BELSITO: We're just told that in this particular one, here are some compounds that are found in the extract. And then it goes on to say in lesser amounts. Are we talking about going from 200 parts per million to less than one part per million? Or are we talking about going from four percent to one percent? I mean, that's also a lesser amount.

DR. RETTIE: So, you're looking for clarification of these impurities?

DR. BELSITO: Yeah, I think so.

DR. SNYDER: It's a ton of data. There's a ton of data in this report but --

DR. BELSITO: And, you know, again we're seeing that -- on PDF Page 93, the third line down, we're seeing benzaldehyde and other benzyl alcohol --

DR. SNYDER: But to your point, no concentration.

DR. BELSITO: No concentration. It says these are impurities that can be present, and we don't know the amounts.

DR. RETTIE: But isn't the term volatile helpful to us?

DR BELSITO: Yeah. I mean, they should volatilize out, right? I mean, it would be nice to have -- you know, sometimes we'll get that information. You know, it's present in its impurity, but it volatilizes out in the final marketed product, you know, dah, dah, dah. But we don't have that. The statement says they're present.

DR. KLAASSEN: Most likely these are pretty low -- I would guess these are very low concentrations.

DR. BELSITO: I would too.

DR. KLAASSEN: Otherwise, the organism wouldn't be alive.

DR. BELSITO: Well, no, the organism is no longer alive, it's been killed.

DR. KLAASSEN: Well, yeah. But we didn't add it to it after it died, so it was in them when they were alive I would guess.

DR. BELSITO: No, I think they were part of the --

DR. SNYDER: Extraction process.

DR. BELSITO: -- process of extracting.

DR. KLAASSEN: You aren't going to use 20 different chemicals to extract something.

DR. BELSITO: Well benzaldehyde, I don't think is going to be in a yeast, do you?

DR. KLAASSEN: Oh, I don't know. Again, it depends how much. You know, I probably have benzaldehyde in myself. I don't know. I guess, what we need to do is see if we can find any quantitative data for this, but I don't think we're probably going to get that data easily.

DR. SNYDER: I mean, normally it would be very little concern but at 91 percent, that's not an insignificant concentration of use.

DR. KLAASSEN: Yeah. Well, all we can do is ask for it.

DR. RETTIE: I looked up the OECD test that's what you would expect the paragraph to say, used radio labeled material, put in two chambers, measure what's left. But I just can't see how we can have any idea how that test was done on an extract from yeast.

DR. KLAASSEN: Yeah.

DR. BELSITO: Let's try and recap where we are because it's 10:36 and we probably need a break because my mind is blowing up after all of we've been discussing. So, have we agreed as to whether we're going to get rid of the two non-saccharomyces species from this report, that I won't even try to repronounce?

DR. RIETTE: Yes.

MS. CHERIAN: Just a comment on that, too, before you make a decision. Another reason why they were included in the report, is because even though yeast extract and yeast report saccharomyces are the class being used, hydrolyzed yeast has a lower case use of the word yeast, which means it's not directly correlating to saccharomyces that we know. So we just kind of included all the yeast that are in the dictionary to be safe, because it might be referring to the phaffia rhodozyma or a class that's not saccharomyces. But I'm not sure. It might be saccharomyces.

DR. BELSITO: Actually, saccharomyces is not the one -- well, I mean the saccharomyces species. But saccharomyces cerevisiae is not -- we thought that was going to be the one that was used extensively. Okay, so Curt is saying we should get rid of those two that aren't saccharomyces.

DR. RETTIE: I like it because it's cleaner.

DR. BELSITO: Paul?

DR. SNYDER: Well, I want to hear what the other team thinks.

DR. BELSITO: Okay.

DR. SNYDER: I mean, if they're not dissimilar then why exclude? Then we have two hanging out there, so.

DR. BELSITO: Right. Based upon everything we have read in this report, do we think that we can read across? Is there enough similarity, in terms of amino acids and fatty acids, that we're seeing from these chemicals that we can read across to these large number of other ones that we have no data on?

DR. RETTIE: I think so in the general sense.

DR. SNYDER: Yeah. I mean, the extracts are just lipids, proteins and carbohydrates. I mean, there's nothing in there that I had any concern about. So, we have enough. Like I said, there's a lot of data in here. Yeah, we don't have exact specifics on

percentages, but I can't imagine that those volatile organics are in there any significant level. I do have pause for concern because it is at 91 percent, but I'm not seeing any flags.

DR. BELSITO: Okay. Priya, on PDF Page 95, the information that you have on absorption, distribution, metabolism, that sounds to me like it was an infectious disease study where they inoculated, and I think it should just be dropped. I mean, this was looking at when you infect someone with this particular yeast, where does it go, and it goes to the brain. So, it has --

DR. RETTIE: That's what my notes say, delete the ADME section.

DR. BELSITO: Yeah. I think the biggest problem for us is going to be anaphylaxis and pneumonitis. Because it is known -- there is bakers' asthma, there is bakers' pneumonitis that's caused from baking yeast. And it's used in a face powder if I recall. So that's something that clearly could be inhaled.

Now, granted, bakers are getting this stuff every day, but then we also have reports of consumers having experienced these reactions. So, how do we go forward based upon that kind of toxicity where you get a pneumonitis, hypersensitivity pneumonitis or asthma?

And I throw that out for discussion because --

DR. RETTIE: So, it happens at what frequency?

DR. BELSITO: Low, but it happens.

DR. RETTIE: So, like, to the profession?

DR. BELSITO: No, it happens in non-bakers. I mean, we have a couple of reports in the literature of consumers getting it, having hypersensitivity. I mean, in one they did have pneumonitis. It wasn't just asthma, right?

DR. RETTIE: Is that something you deal with in the discussion, to note the rarity of it? Caution against whatever you can caution against?

DR. BELSITO: But the development of this allergy comes with exposure, right. I mean, we're sort of all born with the allergies that we could develop, but if we're not exposed, we will never get them. But if we're genetically predisposed, and we're repeatedly exposed, then the allergy will come out.

So, like if you are genetically predisposed to be allergic to poison ivy, but have never contacted the plant you wouldn't have that allergy. But if you started to contact the plant, you would.

I mean, I don't think we know anything about the -- I mean, the mechanism is IGE-mediated. And in the case of hypersensitivity pneumonitis, probably there's a component of a cell-mediated immunity, otherwise you wouldn't be getting a pneumonitis type of picture, you'd simply be getting an asthma type of picture.

But we don't know why this happens, but it happens in a small number of people. And it's not an insignificant reaction, so how do we deal with that?

DR. RETTIE: The history of use of these preparations, so it would give you some measure of comfort. I mean, serious when it occurs. I understand what you're saying. Just wondering if you can bring in history here somehow.

DR. BELSITO: Or say that it shouldn't be used in products that could potentially be inhaled.

DR. RETTIE: Is that practical?

DR. BELSITO: We've done it before.

DR. RETTIE: Okay.

DR. BELSITO: I mean, I'm just throwing this out here. I mean, I --

DR. RETTIE: So, David's presenting tomorrow. He might have a lot to say about that.

DR. BELSITO: I'm one vote here. But, I mean, I just think that, is it really needed in a face powder or another product that could potentially be inhaled? And we're looking at -- yet it's allowed on the market and bakers work with it all the time, right? And people use it in their house all the time. I mean, so I don't know the answer to this.

You know, clearly the U.S. government has allowed it to continue to be used and you can -- I mean, many households have it sitting in their kitchen cabinet, right? I think it bears at least discussion.

DR. KLAASSEN: Right.

DR. BELSITO: That's all I had to say. So, I don't know where we are with this, sufficient, insufficient, safe as used, get rid of the two that aren't saccharomyces.

DR. SNYDER: I had safe as used.

DR. BELSITO: Okay.

DR. SNYDER: I thought there was just a lot of data, and it was enough similar across, you know, the composition and all those issues. I think the hypersensitivity thing would be something we probably don't need to go to because anybody who has a sensitivity to saccharomyces would probably know about it. I doubt you're going to become sensitized. The exposures -- I looked up the inhalation exposures, they're pretty low concentrations.

DR. BELSITO: Right.

DR. SNYDER: The high ones are in the lotions and things. So that would not result in sensitizing somebody or likely elicit a sensitization reaction in somebody who is already allergic to it. So, I think we have a thorough discussion about it, but I don't think that it warrants any greater level than that because as you know anybody can be allergic to anything.

DR. BELSITO: Right. Like aquagenic urticaria, right, from water.

DR. SNYDER: Exactly. Yep.

DR. BELSITO: And we can't band that.

DR. SNYDER: Yep.

DR. BELSITO: Okay, so safe as used. Discussion the --

DR. SNYDER: Clear the two, whether we're going to clear them or not.

DR. BELSITO: Discussion, the organic solvents that we would expect to volatilize off.

DR. SNYDER: We can just put that in the discussion, those appear to be --

DR. BELSITO: Yeah. And discussion the as --

MS. CHERIAN: So, since these -- so in bakers' yeast, the yeast is alive.

DR. BELSITO: Pardon?

MS. CHERIAN: In bakers' yeast, the yeast it's alive.

DR. BELSITO: Yeah, that's true.

MS. CHERIAN: So, do you want to make a statement about that, too?

DR. BELSITO: That's a good point, Priya.

DR. SNYDER: That is a good point. And because many of these extracts don't have the cell wall component, right?

DR. BELSITO: Yeah, that's right.

DR. SNYDER: Yeah, so I'll bet it's the cell wall that's the problem.

DR. BELSITO: It is.

DR. SNYDER: Yep. So, we can bring that into the discussion.

DR. BELSITO: Yeah.

DR. SNYDER: Yeah.

DR. BELSITO: Thank you. I didn't think about that. I should've. And there are live yeasts. Okay, and we are or are not including the two non-saccharomyces?

DR. SNYDER: See what the other group thinks. I think --

DR. BELSITO: Include the non-two and discuss.

DR. SNYDER: Yeah.

DR. BELSITO: So David is presenting this tomorrow?

DR. SNYDER: Yes.

DR. BELSITO: Okay. It's 10:47, like a ten-minute bio-break?

DR. SNYDER: Sure.

DR. BELSITO: Clean our brains.

DR. COHEN: Okay. So we've reviewed this before in September of 2021. And we since then have gotten a review about yeast from an expert. And now the revised draft report has 56 yeast-derived ingredients, which we're reviewing. And I think just to summarize, we've taken sort of this algae algorithm to suggest that if we have its use in food, whereas GRAS, and we have sensitization data, that's what we would use to clear.

And we had a bolus of information since the last report. And mercifully we have a table that you made that was very helpful, that was color coded. And it took us a while to get through the algae, through this mechanism, but it did work, we did get to land that plane too. And so, I guess we could just open it up. I see it looked like we had the data needs for Pichia anomala. That seemed to work. I'm not sure we had it for anything else.

MS. CHERIAN: We had them for three species. The Metschnikowia agave, Pichia anomala and Saccharomyces Cerevisiae. And that corresponds to four ingredients.

DR. COHEN: Wait, so --

DR. ROSS: Those were my notes, too, but I have a specific question on that.

DR. COHEN: So, okay, I see how you came across that. So, the agaves, M agaves, right.

DR. TILTON: Hydrolyzed.

DR. COHEN: What's that again?

DR. TILTON: The hydrolyzed form for two of them in agave.

DR. COHEN: Yeah, there's just two ingredients for that one, right, because We're going to have that section cover itself.

DR. ROSS: And we have it for Ru coffee. Is that how you pronounce it, Ru coffee? That's the bottom of the first page in Tables.

DR. COHEN: Which one is it?

DR. ROSS: Ru coffee, but it's not used in foods. So that wouldn't be covered.

DR. COHEN: All right, so we have M-agaves, we have Pichia anomala, those are three.

DR. TILTON: And Saccharomycetes cerevisiae.

DR. COHEN: Yeah. Wait, for Saccharomycetes, where's the human sensitization data? I might have gotten lost here, so help me navigate there.

DR. ROSS: Yeah, I have it at max here.

MS. CHERIAN: Page 127.

DR. COHEN: 127.

MS. CHERIAN: Animal LLNA.

DR. COHEN: Right. So were we clearing algae on animal or in vitro data? We were -- I thought we were using human data on that. That's why I didn't clear it.

DR. TILTON: I don't recall making that distinction before.

DR. COHEN: I think maybe we need to go back to the algae report and see. Do you recall, Bart?

DR. BERGFELD: I don't remember. I think that we did, but I don't remember specifically. See, I didn't have it cleared for that reason.

MS. CHERIAN: That might be because we didn't have any animal data. So when we were asking for data, we were asking for HRIPTs. I don't remember clearly.

DR. COHEN: So these are just -- these are --

DR. ROSS: So, David, what was your specific question? You were after sensitizing data for Saccharomyces cerevisiae extract?

DR. COHEN: Yes.

DR. ROSS: Yes. There in animals, you're right, and then in humans.

DR. COHEN: Like we have, in Table 13, human data, which is what I was relying on. And I just didn't think the LLNA was going to be sufficient for us to clear it. Anyways.

DR. ROSS: There is a lot of animal data. You are right.

DR. COHEN: You don't happen to have the algae report?

MS. CHERIAN: I can try and find it.

DR. COHEN: Because I'd like to be consistent now. If we didn't have -- like, it's strange that there would be absolutely no animal data on any of the algae. If we could find it, that's great. If not, I could look at it tonight, because I'm presenting this tomorrow.

Would that be okay with the group? But the others, we have not passed muster. And I think this information will start to just trickle in, particularly if we wait enough time before we look at it again.

DR. SLAGA: It's fine with me.

DR. BERGFELD: So which ones already has it?

DR. COHEN: Two M agaves and one P anomala. And it's interesting because I think the most commonly used one is the one we're talking about.

MS. CHERIAN: For the red algae report, there was only human HRIPTs in the report for sensitization data. Let me look back at brown.

DR. COHEN: I feel we've gone very animal forward at this meeting, more so than I've noticed at any of the other meetings.

DR. TILTON: You mean in terms of --

DR. COHEN: With the reliance on the data. I mean, we've reviewed ani- -- I mean, I'm not doing this that long, right. But for the last two and a half years, we've looked at the animal data and said, okay, great, but let's look at the human data. And we've asked for human data. We've never gone back with an Insufficient Data Announcement that says we need more guinea pig data. Never. We've never said that.

DR. BERGFELD: Never. No, we've always gone to human if we needed data, but if we had animal, we have passed things on animal.

DR. ANSELL: Yeah.

DR. BERGFELD: And you know there are a few animals, rabbits, guinea pigs.

DR. COHEN: I don't know of recently how many I recall where we've had no human data and we've said okay.

DR. BERGFELD: Past, I said.

DR. COHEN: We've had some in vitro data, right, that we've used.

DR. ROSS: Yeah.

DR. COHEN: DARPA, that kind of thing. But we don't have that here. Right?

DR. ROSS: I didn't see one.

DR. COHEN: Okay. So algae was human.

DR. ROSS: Can I ask you a question on the yeast extract, the generic yeast extract, which is in the list?

MS. CHERIAN: Yeah.

DR. ROSS: 398 uses. And then what we're doing is we wouldn't be clearing that, right? I mean, I realize that the yeast extract can be made up of lots of different things.

MS. CHERIAN: Right.

DR. ROSS: So there may be some Pichia, maybe some saccharomyces. Maybe not in a mixture, but they could be different extracts.

MS. CHERIAN: Right.

DR. ROSS: But we're not clearing yeast extract, specifically. Correct, David?

DR. COHEN: I think that's right. That's why it broken down like this.

DR. ROSS: Okay.

MS. CHERIAN: Yeah. So even if you did clear the ingredients, the M agaves or Pichia anomala extract, the generic yeast ingredient isn't part of these ingredient list that would be cleared.

DR. TILTON: Are they not considered GRAS? The generic?

MS. CHERIAN: The generic? It depends because the generic does include Saccharomyces cerevisiae. That's considered GRAS. We don't know which species they're using in that generic ingredient. And even though we list a bunch of species, I don't even know if that's all encompassing of the ingredient.

DR. ROSS: Because that's the majority of uses, right, 398 uses for "yeast extract."

DR. COHEN: Yeah. It's very generic.

DR. ROSS: And it's defined actually in our method of manufacturer as -- to give an example with Candida saitoana, I seem to recall, without looking at my notes, but I think that's correct. So, we wouldn't be clearing that?

DR. HELDRETH: Yeah. I mean, there's kind of two strategies there. You can either have it pass or not pass for any species. Or another strategy that the Panel has used in the past is remark on safety in the conclusion for a subset of the possibility.

So let's say we have four species that the Panel feels confident about the safety of, they could say safe as used in the present practices of use and concentration when derived from one of these four species. Then you're not saying the others ones are unsafe, but you're just not providing --

DR. BERGFELD: Then the next paragraph is insufficient data for blah, blah, blah.

DR. HELDRETH: You could. You could do it either way. I mean, the Panel has done it both ways. They'd either just not remarked on the other species, or say it's insufficient data.

DR. ROSS: And just a follow up question, on those yeast extracts, the generic term yeast extract are still a little bit fuzzy in my mind, which is not unusual. But with respect, is that always a pure extract of one yeast or is there a mix of many different yeasts or do we know that?

MS. CHERIAN: We're not sure.

DR. COHEN: But it does say in Table 13 that it says similar to Hydrolyzed Candida Saitoana, similar to M Reukaufii. I mean, how did they just come up with that?

MS. CHERIAN: That was specific to the species given. So, if it was yeast extract derived from?

DR. COHEN: Yeah. Okay. And the only Saccharomyces human data we have is irritation. And it was the only one that had one slight irritation. So, I don't think it's unreasonable to ask for human data on this.

MS. CHERIAN: So, I finally found brown algae report. And in the discussion it says, "or sensitization data." So, I think it was just regular sensitization data, because we do have sensitization data in vitro, animal, and human in this report. But when we asked for it, we asked for HRIPTs.

DR. COHEN: But more importantly, did we clear any --

MS. CHERIAN: Yes.

DR. COHEN: -- with no HRIPT?

MS. CHERIAN: Let me double check.

DR. COHEN: That's the question. We might have had in vitro data and HRIPT.

DR. BERGFELD: Well that would be a gradual involvement of clearing it with that kind of testing. Because we've put it in, but the Panel has not been totally comfortable with it, without human. But we're moving towards that to be the testing system, the in vitro.

DR. COHEN: I agree. But we don't have in vitro sensitization data at all. We have in vitro irritation data.

DR. BERGFELD: Irritation. Yeah. You have animal sensitization.

DR. COHEN: If animal sensitization data did the job, we would never be doing HRIPTs for the next 50 years.

MR. BJERKE: Can I make a comment?

DR. COHEN: Yes, please.

MR. BJERKE: Yeah. So, for the animal data, I think it is probably wise to look at the OECD 406 Guidance. Because those animal data is correlated with what you see in humans. I think the advantage, perhaps, of using some of the animal data is you can take the dose really high, whereas in humans it's unethical to basically try to find the limit. So you're really doing it as a confirmatory test and only going so high.

Whereas -- like, for example, the local lymph node assay, when we looked at CAPB, we ended up running a local lymph node assay for one of those impurities. I can't remember if it was amidoamine or DMAPA. And the benefit there was it gives you a potency so you have a threshold. So, I think there's some advantages to the animal data, sometimes over the HRIPT.

Sometimes they're complimentary to each other. You run the animal data first and then do a confirmatory HRIPT at lower concentrations.

DR. COHEN: Yeah, no, I buy that. But industry has still relied on the HRIPT as the finale of their tox data. I mean, almost everything we look at has it, right?

DR. BERGFELD: That's past data, though, it's not the ongoing data.

DR. COHEN: I know.

DR. BJERKE: Yeah. I think if you look at the correlations based on the method, would that help you? Kind of the accuracy relative to human data.

DR. COHEN: Yeah. I guess the question is how fungible is that -- how generalizable is that? Is that chemical-group specific, or is that span everything we're looking at? We're looking at yeast and then before we're looking at MIBK. Right? Like, can you take that all the way through?

MR. BJERKE: Yeah. So, when we actually do a quantitative risk assessment for skin sensitization, we look at all the data. And you're right, the human data typically trumps the animal data. But we don't always have human data.

And, you know, preservatives are a great example. But we'll look at the wealth of the data, human data has greater relevance, obviously. But if the animal data has a lower threshold, we'll default for that.

DR. COHEN: We have a lot of admin data on Saccharomyces.

DR. TILTON: With different species.

DR. ROSS: Yeah.

MR. BJERKE: And I think historically used in baker's yeast, brewer's yeast, occupational setting.

DR. COHEN: You know I have it highlighted in my report on PDF 128; on the Saccharomyces, the first one, the comment is the test substance was considered to be sensitized.

DR. ROSS: It's Table 13, right?

DR. COHEN: Table 13. First Saccharomyces animal sensitization study. Go to the far right, look at the last sentence of the results.

DR. BERGFELD: Yeah, I have it highlight, too.

DR. COHEN: Yeah. I highlighted it in yellow on my report.

DR. TILTON: So it says that was the case in one assay. But then in four additional assays, it was considered to be non-sensitizing.

DR. ROSS: Correct.

DR. COHEN: Right. Okay. Were we waiting on anything? I've lost track.

DR. ROSS: No, I think we've got what we need. We're not clearing the rest, we're clearing it based on food use.

DR. BERGFELD: Four out of 56.

DR. ROSS: Yeah. So, we're not clearing anything.

DR. COHEN: I still have three out of 56.

DR. BERGFELD: Three? I thought you said four.

DR. COHEN: I have three, right.

DR. ROSS: Yeah, three.

DR. BERGFELD: Okay.

DR. COHEN: Tomorrow will be fun.

MR. BJERKE: More data is coming.

MR. CHERIAN: Three species, but four ingredients.

MR. BJERKE: Didn't want to overwhelm you in Wave 3.

DR. COHEN: Wait, wait, wait. Which three species?

MR. CHERIAN: Three species are the M agaves, Pichia anomala and Saccharomyces Cerevisiae.

DR. COHEN: No, we didn't clear Saccharomyces.

MS. CHERIAN: Okay, so we're not doing Saccharomyces?

DR. COHEN: I don't think we cleared it. That's what we're cogitating.

MS. CHERIAN: Okay.

DR. BERGFELD: So, we're discussing the merits of the animals, versus the human, versus in vitro.

DR. COHEN: Which is ironic that we're having the conversation here about yeast, because it's like the age old conversation, right?

DR. ROSS: And particularly about baker's yeast.

DR. COHEN: Yeah, baker's yeast. We do have a lot of data. We'll have a conversation tomorrow or come to a conclusion.

DR. BERGFELD: No, the rest you're calling insufficient for what reasons, so we have that clear?

DR. COHEN: They're insufficient either because we don't have sensitization data on them, or we don't have evidence of them being food, GRAS.

DR. BERGFELD: But no tox data? No insufficiency in the tox?

DR. ROSS: Not if it's food use.

DR. BERGFELD: Not if it's food.

MS. CHERIAN: For brown algae we either did systemic tox, like a 28-day or oral.

DR. COHEN: Yeah, I remember that.

MS. CHERIAN: Yeah.

DR. COHEN: Do we have sufficient tox data on any of them that trumps food data? I tried searching for that. I didn't think I found that. But this is a morass of information.

DR. ROSS: Go back to the notes.

DR. COHEN: We have oral tox data on Saccharomyces.

DR. ROSS: Saccharomyces cerevisiae. Yeah. Table 10. Oral tox -- some inhalation I noted tox. And there was some (inaudible) with Pichia.

DR. COHEN: But you know what, that gets us back to the same exact issue because we already know it's GRAS, right? So the tox was superfluous. It was the sensitization data that we got held up on then.

DR. ROSS: Yeah.

DR. COHEN: The question is are there any species that we have sensitization data on, but not GRAS where we have tox?

DR. ROSS: (Inaudible) extract.

DR. TILTON: I group them together, but if we did, we don't have irritation or sensitization data.

DR. COHEN: Right. It's either or.

DR. TILTON: It's either or. Yeah.

DR. ROSS: Yeah.

DR. COHEN: Okay.

DR. TILTON: So we did discuss the generic yeast extract. So, is the conclusion -- did I understand correctly that we can say, as long as it's derived from one of the approved cleared species, then the yeast extract is also cleared?

DR. ROSS: That's what I understood.

DR. COHEN: Wait. So yeast extract generically gets cleared by just one species?

DR. TILTON: Not one.

MS. CHERIAN: So are you saying to have with the safe ingredients add those generic yeast and say that if they're derived from M agaves or Pichia anomala?

DR. TILTON: Right.

MS. CHERIAN: Okay.

DR. ROSS: Solely derived.

MS. CHERIAN: Yes.

DR. TILTON: Right.

DR. COHEN: Why do we even need to say that?

MS. CHERIAN: Because then you would need to add on the insufficiencies that the generic yeast ingredients aren't safe. But for what -- you would have to add the insufficiencies for those. And I don't think you can ever complete that because we don't know which species are used.

DR. ROSS: Could you cover that in the discussion?

DR. COHEN: Yeah.

DR. HELDRETH: And ultimately, since this is going to be an IDA, we're punting.

DR. COHEN: They're all extras. They're all extras, right?

DR. HELDRETH: There's the generically named one, just yeast extract that could be any or all of the species. So, we're suggesting if we feel comfortable with those two species, then are we comfortable with, say, the generically-named yeast extract when they mean they're using those two species?

DR. ROSS: Sounds logical to me.

DR. HELDRETH: We've done that splitting out before.

DR. COHEN: When we clear Pichia anomala, we're clearing Pichia anomala extract.

MS. CHERIAN: Right.

DR. COHEN: Pichia is a yeast. It's an extract made from this yeast. Why do we need to use a generic term like yeast?

MS. CHERIAN: We don't. It's actually an old name, I think. And so, I think, eventually they'll all be cleared out and named instead of yeast extract, they'll be named by the species.

DR. ROSS: The only reason is that it's in there with (inaudible).

MS. CHERIAN: Yeah.

DR. ROSS: You know, the maximum number of uses we have is with the generic "yeast extract." And that's the only reason I would support putting it in.

DR. COHEN: That's simply guidance for us to take this on. It doesn't have to inform our conclusion.

DR. HELDRETH: There's still many, many products out on the market that say yeast extract on the label. Are we saying all of those are insufficient data to conclude on safety?

DR. ROSS: You know, if it's made from purely agaves or the anomala, then I think you're fine. But I would imagine that's a very, very small percentage.

DR. HELDRETH: It may be zero. Maybe everybody that's using those two species already switched over to the specific names. But we don't know.

DR. COHEN: Would a manufacturer supplier, a finisher, have an issue if they used a yeast extract from a cleared species? I can't imagine that being a problem.

DR. ROSS: But if someone is used to picking up a bottle with yeast extract on it, and now you suddenly say it's Pichia anomala extract, they may not do it. So, it may continue.

DR. HELDRETH: I think you're spot on, right, from the manufacturer side. But what about from the consumer side when they pick up their bottle and it says yeast extract on it. What does CIR say about it? CIR says there's not enough data to conclude on safety.

DR. COHEN: Okay, I dig that.

DR. TILTON: Can I put this away now?

DR. COHEN: It's going to come back. It's coming back. Any other comments? There being none. It's complicated because there's so many of them. That's all.

DR. HELDRETH: We do have one of the yeast expert presenters here if you have any questions for her. Audrey is here.

DR. COHEN: Any comment?

DR. SLAGA: I'm having a very tough time hearing you all. It's a very poor connection. I hear some of it, but I piece it together. I don't have any other comments other than what you all have been discussing. The ones that are used as food that have sensitivity data are fine. And the rest we need a lot of sensitivity data.

DR. COHEN: Yep. We agree.

DR. SLAGA: And you know, some of them are GRAS. What are we -- the means to recognized as being safe. How do we --

DR. COHEN: Well, if they're not GRAS and we don't have overwhelming tox data, they're not passing, right?

DR. SLAGA: Yeah. No. Other than -- we have genotox for several and some irritation for several, but not many.

DR. COHEN: Yeah.

DR. SLAGA: We need a lot of data.

DR. COHEN: Any commentary?

MS. POKRZYWA: Yes. If I may participate. Yeast extract can be defined by the definition of PCPC, which is the Saccharomycetes class. And if we studying all the yeast in the Saccharomycetes class, including (inaudible) it can be exhaustive (inaudible) on this class.

Because consumer know this this extracts are so -- the strain more or less known by the consumer. If indeed maybe some additional data will be supplied by the manufactures. But there is another one list, which is GRAS. It's the (inaudible) in this class. And some of the yeast are the QPS status, which is a Qualified Presumption of Safety recorded by the EAFI, which is the European Agency of Food Ingredients. So maybe this kind of data can be used also for this.

DR. COHEN: Which additional data would it be? What additional data would it be?

MS. POKRZYWA: The QPS status. QPS.

MR. BJERKE: QPS for food use EAFA. So it's Qualified something safety?

DR. COHEN: So that's for ingestion? That's for ingestion?

DR. ANSELL: Yeah.

DR. COHEN: I don't think we have a problem with that, though.

DR. ANSELL: No. I mean, you use the word GRAS but you use it inconsistently and wrongly. I mean, what we're talking about is approved food use. And FDA is not the only group through the GRAS regulatory approach to approve materials used in food. Actually not even all FDA approved food use are GRAS. So, the European approach would similarly be, we would argue to have the systemic tox issues addressed through their food use.

MR. BJERKE: Qualified Presumption of Safety.

DR. ANSELL: Right.

DR. COHEN: So, would that increase our ability to deal with this and put European GRAS in here?

DR. ANSELL: Well, I'm just curious. Are there materials which are European food use that we haven't included?

MS. POKRZYWA: Yes. I think because (inaudible) is the same, is a similar (inaudible) this data were provided by our presentation (inaudible). But maybe I can send it again.

DR. COHEN: That would be very helpful. If we knew there were European food uses --

DR. ROSS: Yes.

DR. COHEN: -- we would put that in here and check that box. And then if we had the sensitization data it would go through.

DR. ANSELL: Right.

DR. COHEN: We're good with that.

DR. ROSS: So we just need clarity on the food use.

MS. POKRZYWA: Excuse me?

DR. ROSS: We need some clarity on the food use, and are we missing any strains with respect to their food use?

MS. POKRZYWA: Yes. All the strains are not in this QPS that you list, but several of them. And maybe we can provide you some additional data about skin sensitization. Because I think supplier of yeast ingredients (inaudible) this kind of information generally when we market the product, so we have all this data. So I think the industry (inaudible).

DR. COHEN: That would be most helpful.

DR. BERGFELD: Thank you.

DR. COHEN: Most welcomed. We would take that, right, food use, not GRAS.

DR. ANSELL: Right.

DR. COHEN: Yeah. They fall like dominos after that. When we have it and the things line up. It just happens. So it's just data gathering. And if we can get that information, we'd update your very wonderful chart, Table one.

DR. BERGFELD: I would like to see the table a little bit differently. I'd like to see all those that are food use in a line. And where they had human sensation also. Just that group.

DR. COHEN: How about this? I like the blue line on the column. But just for simplicity, if we can have like a yellow bar going across where they match. Guess that's what I was trying to do.

MS. CHERIAN: You're talking about the data profile?

DR. COHEN: The data profile.

MS. CHERIAN: Okay.

DR. BERGFELD: Well, that was difficult though, because somewhere down below the category, and I didn't know if that meant that it was different.

DR. COHEN: That's why I got a little tied up as well. Then I looked at it again here and saw what everyone was talking about. So, it would just be a broader bar, right? Not just the name, but the hydrolyzed one or the extract. And so, if you had that bar going across that would -- that would be the clear bar.

DR. BERGFELD: That would be fine too. Yeah.

DR. COHEN: Okay.

DR. COHEN: Well, that was great. Let's move on from yeast. I think we need more on the animal -- the in vitro. We're very predisposed to hearing more on the in vitro. We had a lecture last year, which I thought was really good and moved me off the needle. And so, if we had some more of that, we can rely on more of that information.

MR. BJERKE: Would it help to recirculate the 2010 presentation that we gave on CAPB? Because in there, there's a breakdown for amidoamine and DMAPA where the threshold data is coming from. In one case it was, I think we had eight local lymph node assays, so we derived a nestle from that.

And in the other case there was one HRIPT and one animal local lymph node assay. And we defaulted to the more conservative human data that was shown to be protective. But I think it gives an overall approach that we use for CAPB that might be reapplied.

DR. COHEN: Yeah. So I mean, we know that the amidoamine and the other amine, dimethylaminopropylamine are human sensitizers. We see them positive. And the coco betaine, you don't really get much from.

DR. BERGFELD: I have a few.

DR. COHEN: Yeah. The question is, is the patch test material free of those? I don't know.

MR. BJERKE: It's not, they're not. Actually CAPB was considered allergen of the year by the North America Contact Dermatitis, which triggered a lot of this review.

DR. COHEN: Yes. It tends to do that. So sometimes you'll see patients with all three positives. But more often than not you'll just see an amidoamine pop up or a DMAPA pop up, but the CPB is negative.

But that's a situation where we know we have a human sensitizer and we have an animal model that matches up. The question is, what about the times when the animal model is negative and the human model is positive? Or the degree of -- there's an order of magnitude difference so we miss it. Right? I think if we look back, you're going to show me that it works, right. But we know the endpoints already. Okay.

Listen, we all have to move in this direction anyway. We're not going to have the animal data, we're not going to have a lot of human data anymore. So, we have to get used to it and fast.

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DR. COHEN: Yes, so Yeast. Our journey with yeast-derived ingredients started at the September 2021 meeting, when the Panel reviewed the draft report on eight yeast-derived ingredients mostly labeled with the generic term "yeast-derived ingredient," and the inclusion of a single genus specie, Saccharomyces Cerevisiae.

Subsequently, after panel discussion and the generation of two strategic memos, we came to a conclusion to include all yeast ingredients currently listed in the dictionary, along with notations of whether or not these ingredients or their corresponding species are used in foods, and their frequency of use in cosmetics.

At the September 2022 meeting, an expert presented on the manufacturing general characteristics and classification of yeast-derived cosmetic ingredients. We now have a revised draft report on 56 yeast-derived ingredients. Given the volume of the material, and the precedence of clearing organisms-derived ingredients, the Panel has elected to streamline the process by adopting a strategy to evaluate the toxicology by way of their use in food or through adequate classical toxicologic data, coupled with irritation and sensitization data respectively for each genus and species included in their derived ingredients.

We understand that there may be additional data on the use of yeast in food in Europe, and perhaps other data on irritation and sensitization that may be forthcoming. As a result of this analysis we propose the motion of safe as used in the current concentration of practice for two M. agaves-derived ingredients and one Pichia Anomala ingredient.

After that motion, we wish to enter into a discussion with the Belsito Team on three items under consideration, and reserve our right to amend the motion after the review of the Saccharomyces data. How you like that one, Don?

DR. BELSITO: We thought they were all safe as used. When you look at what eventually came out of the processing, it was just fatty acids and carbohydrates and amino acids. There were slight variations in compositions. And, there were some organic solvents that we felt would volatilize off. We would put in our discussion that the asthma of lung hypersensitivity is with live organism; these are completely dead. So, we thought they were all safe as used.

DR. COHEN: So, Don, I got the impression that we're going to get more information about food use. This is very much an algae-like process, right. We're using the same mechanism that we use for algae. And we didn't roll them all up based on composition and impurities last time. We waited for both of those data points to align. And, I think, not just for precedence, but to give us just more information on the safety of these, we can wait to see what other additional information we get on these, if there are any red flags.

DR. BELSITO: But the algae were being added as ground products, they weren't being totally lysed and dissolved like these yeast organisms are. It's quite a different --

DR. COHEN: We still had composition that was pretty inert, right, with the algae?

DR. BELSITO: Yeah.

DR. COHEN: So, might you indulge us to wait, perhaps, for another cycle to get more food data?

DR. BELSITO: Yeah.

DR. SNYDER: I don't think we need it.

DR. BELSITO: Right.

DR. SNYDER: I mean we're just going to get more of the same, so.

DR. BELSITO: Curt? Allan? I mean --

DR. KLAASSEN: I'm fine with it.

DR. BELSITO: Fine with what?

DR. BERGFELD: Well, you're fine with what, going safe?

DR. SNYDER: Safe as used for all of them.

DR. KLAASSEN: Safe as used.

DR. BERGFELD: Okay.

DR. RETTIE: Could I ask David what specifically he might be looking for in the added data that might come?

DR. COHEN: I think knowing that more of these -- there are a lot of these here, right. We've only got food data and sensitization data on two species. All the rest we either have one or the other, or it's absolutely nothing. And, when we had a conversation with an expert on yeast, we were under the impression that we could get more information about the use of these yeasts in food in Europe, not just GRAS classical, but just any use of yeast in food.

DR. ROSS: We were sticking to the food use and the sensitization and irritation in humans. And that's how we derive those three. We also pointed out that the yeast extract, just the generic "yeast extract," which is 100's of uses, and its most frequently used was quite non-defined with respect to what was in it. And, you know, it could be multiple strains or mixtures, and so we wanted a bit more information on some of the major components of that generic yeast extract before we approved it. So that's where we came down.

DR. BERGFELD: Okay.

MS. GRIFFIN: I'm interested to listen to the Panel's consideration regarding the sensitization potential in food powder, potential inhalation from the inhalation route.

DR. BELSITO: That's baker's yeast, which is live yeast. These are killed.

DR. COHEN: I just don't know enough, but you might still have proteins that can cause immediate-type hyposensitivity, right, even in the killed organs?

DR. BELSITO: They were amino acids.

DR. COHEN: Pollens are dead, right. But I don't see the harm in waiting for additional -- listen, I guess, based on your analysis, you didn't need any sensitization data or food data, you could've just gone right to this. And we agreed on a process that would parallel the algae process. So we were going along that process that we all sort of agreed on last time.

I'm not suggesting that your scientific argument is without merit, it's highly meritorious and I understand it. But, it's a big leap from where we were to where you guys are going. Because we only cleared two.

DR. TILTON: We only really even discussed safe as used for those that had been designated in food, and then, secondarily, consider the sensitization data.

DR. BERGFELD: Is there any other discussion? I may have to call the question to resolve how we'll deal with this. And Dr. Cohen has a motion; it has not been seconded, though. His motion is to go sufficient for three, insufficient for the rest. I understand it will be 53.

DR. ROSS: Well, I'm not hearing anything from the Belsito team, so I'll second it.

DR. BERGFELD: Second it? Okay. So we'll call the question, all those in favor of the Cohen conclusion please indicate by raising your hand.

DR. COHEN: Got Tom's hand up.

DR. BERGFELD: Four-four. Against, oppose? For, so it's up to me to do this. Well, I'm going to go with the Cohen Team on this, only because it just delays it for a little bit to definitely resolve this question.

DR. SNYDER: Can we have a gentlemen's agreement, then when we get ten more of them that we'll clear all of them? We won't just keep going?

DR. COHEN: Listen, I just think --

DR. SNYDER: I understand.

DR. COHEN: I completely get it. And if we can get a preponderance of the evidence, I think we're going to go with that exactly. But, two species, I'd like a little more.

DR. ROSS: We didn't even clear Saccharomyces Cerevisiae, because we felt there were some issues then that needed to be resolved.

DR. COHEN: Yes, so, we'll -- thank you for your consideration.

DR. BERGFELD: What we've done with this vote is to delay a bit to satisfy the Cohen Team, and then we'll move forward in December, you think, for this ingredient, or later?

MS. FIUME: Being that it's an IDA, it would likely be December.

DR. BERGFELD: December, so we have a timeline on it.

MS. FIUME: Priya, are you good on the list of the IDA, or does it need to be repeated?

MS. CHERIAN: It's just like algae, so I'm good on the list. I think the European data we're talking about is that QPS status. So in that PowerPoint there were eight species that had QPS status. And, I have a question for Audrey. Do you know if any of the other species listed have QPS status to them, or no?

MS. **POKRZYWA:** Some of the --

DR. HELDRETH: Audrey, can you come forward and speak on the microphone so that we can get it on the record, thank you.

MS. POKRZYWA: (Inaudible) numbers --

DR. BERGFELD: We can't hear you.

MS. POKRZYWA: -- which has a QPS. But I will send you the full list of all of the QPSs.

MS. CHERIAN: Great. Thank you so much. So, in the next iteration I'll have listed the QPS status ingredients as well.

DR. BERGFELD: Okay, I think that we've resolved this.

DR. SLAGA: I think we need a little better explanation of what QPS really means.

MS. CHERIAN: Okay.

DR. SLAGA: And how similar is that to GRAS, or how dissimilar I guess, which we don't know.

DR. HELDRETH: We'll provide that in the next iteration.

DR. BERGFELD: Any other comments regarding clarification or needs? Seeing none, I think we'll move on then to Dr. Belsito, Amphocarboxylates.

Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics

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ABBREVIATIONS

2-AA	2-aminoanthracene
2-NF	2-nitrofluorene
9-AA	9-aminoadridine
ADME	absorption, distribution, metabolism, and excretion
AF-2	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide
ALT	alanine aminotransferase
ARE	antioxidant response element
BAL	bronchoalveolar lavage
BSL	biosafety level
B16F10	melanocytes
Caco-2	adenocarcinoma of the colon
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CFU	colony-forming units
CIR	Cosmetic Ingredient Review
CL	chemiluminescence
Council	Personal Care Products Council
DART	Developmental and Reproductive Toxicity
DLD1	adenocarcinoma of the colon
DNA	deoxyribonucleic acid
dpm	disintegrations per minute
DPRA	direct peptide reactivity assay
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
ENNG	1-ethyl-2-nitro-3-nitrosguanidine
EP-2	natural yeast extract isolated by ethanol precipitation
FDA	Food and Drug Administration
GRAS	generally recognized as safe
GST	glutathione S-transferase
HaCaT	human keratinocytes
HCC70	non-metastatic breast cancer cell line
HCT116	adenocarcinoma of the colon
HeLa	human cervical cancer cells
HRIPT	human repeated-insult patch test
HSCAS	hydrated sodium calcium aluminosilicate
ICU	intensive care unit
IFN	interferon
IgA	immunoglobulin A
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
kDa	kilodaltons
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LLNA	local lymph node assay
MCF-7	human metastatic breast cancer cell line
α-MSH	α-melanocyte-stimulating hormone
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCBI	National Center for Biotechnology Information
NOAEL	no-observed-adverse-effect-level
NR	not reported
Nrf2	nuclear factor erythroid 2-related factor 2
OECD	Organisation for Economic Cooperation and Development
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PEFR	peak expiratory flow rate
PMN	polymorphonuclear leukocytes
QPS	qualified presumption of safety
RAST	radioallergosorbent test
SI	stimulation index
S180	murine sarcoma cancer cell line
SCC-4	squamous cell carcinoma of the tongue
SPF	specific pathogen-free
TG	test guidelines
TGF	transforming growth factor
T_{max}	time to maximum blood perfusion
t_{50}	duration of exposure resulting in a 50% decrease in MTT conversion
THP-1	human monocytic cell line
US	United States
UVA	ultraviolet A
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*
ZR-75-1	human metastatic breast cancer cell line

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 56 yeast-derived ingredients. These ingredients are mostly reported to function in cosmetics as skin protectants or skin-conditioning agents. Industry should continue to use good manufacturing practices to minimize impurities that could be present in yeast-derived ingredients. The Panel reviewed the available data to determine the safety of these ingredients and concluded that… [to be determined].

INTRODUCTION

This assessment reviews the safety of the following 56 yeast-derived ingredients as used in cosmetic formulations:

Galactomyces Ferment Filtrate
Hydrolyzed Candida Bombicola Extract
Hydrolyzed Candida Saitoana Extract
Hydrolyzed Kluyveromyces Extract
Hydrolyzed Metschnikowia Agaves Extract
Hydrolyzed Metschnikowia Reukaufii Extract
Hydrolyzed Metschnikowia Shanxiensis Extract
Hydrolyzed Saccharomyces Cell Wall
Hydrolyzed Saccharomyces Extract
Hydrolyzed Saccharomyces Lysate Extract
Hydrolyzed Torulaspora Delbruekii Extract
Hydrolyzed Yeast
Hydrolyzed Yeast Extract
Kluyveromyces Extract
Lactic Yeasts
Lipomyces Lipid Bodies
Lipomyces Oil
Lipomyces Oil Extract
Metschnikowia Agaves Extract
Metschnikowia Henanensis Extract
Metschnikowia Reukaufii Lysate Extract
Metschnikowia viticola Extract
Pichia Anomala Extract
Pichia Caribbica Ferment
Pichia Extract
Pichia Ferment Extract Filtrate
Pichia Ferment Lysate Filtrate
Pichia Heedii Extract
Pichia Minuta Extract
Pichia Pastoris Ferment Filtrate
Phaffia Rhodozyma Extract
Phaffia Rhodozyma Ferment Extract
Saccharomyces
Saccharomyces Cerevisiae Extract
Saccharomyces Extract
Saccharomyces Ferment
Saccharomyces Ferment Extract
Saccharomyces Ferment Extract Lysate Filtrate
Saccharomyces Ferment Filtrate
Saccharomyces Ferment Lysate Extract
Saccharomyces Ferment Lysate Filtrate
Saccharomyces Lysate
Saccharomyces Lysate Extract
Saccharomyces Lysate Extract Filtrate
Saccharomyces Lysate Filtrate
Schizosaccharomyces Ferment Extract Filtrate
Schizosaccharomyces Ferment Filtrate
Schizosaccharomyces Pombe Extract
Torulaspora Delbrueckii Extract
Torulaspora Delbrueckii Ferment
Yarrowia Lipolytica Extract
Yarrowia Lipolytica Ferment Lysate
Yarrowia Lipolytica Oil
Yeast
Yeast Extract
Yeast Ferment Extract

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the majority of these ingredients are reported to function in cosmetics as skin protectants or skin-conditioning agents (Table 1).[1] Other reported functions for this ingredient group include hair-conditioning agent, surfactant, humectant, antioxidant, colorant, anti-acne agent, anti-microbial agent, film former, and viscosity-increasing agent.

Some of the species of yeast reviewed in this report are naturally present or are used in foods (e.g., *Saccharomyces cerevisiae* is generally recognized as safe (GRAS) as a flavoring agent and adjuvant at a level not to exceed 5% in food [21CFR184.1983]). For the ingredients that are affirmed GRAS or are used/present in foods, systemic toxicity via the oral route will not be the focus of this safety assessment. Although oral exposure data are included in this report, the primary focus for the safety of such ingredients is topical exposure and local effects.

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 October 2023. 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.

Some of the data included in this safety assessment were found on the European Chemicals Agency (ECHA) website.[2] Please note that the ECHA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited.

The cosmetic ingredient names, according to the *Dictionary*, are written as listed above, without italics and by capitalizing the first letter of each word in the name. In many of the published studies, it is not known how the substance being tested compares to the ingredient as used in cosmetics. Therefore, if it is not known whether the ingredient being discussed is a cosmetic ingredient, for the generic yeast ingredients, the name of the test substance will be written using all lower-case letters (e.g., yeast extract); however, if it is known that the substance is a cosmetic ingredient, the first letter of each word in the name will be capitalized (e.g., Yeast Extract). For the genus/species ingredients, if it is not known whether the ingredient being discussed is a cosmetic ingredient, the standard scientific practice of using italics will be followed (e.g., *Saccharomyces cerevisiae* extract); if it is known that the substance is a cosmetic ingredient, the *Dictionary* terminology (e.g., Saccharomyces Cerevisiae Extract) will be used.

In many instances, data were found on the species of yeast (e.g., *Yarrowia lipolytica*), and not on specific ingredients that are reviewed in this report (e.g., Yarrowia Lipolytica Ferment Lysate). Because of this, information is primarily organized by species names, rather than ingredient names, throughout the report. However, when it is known that the test substance used is a cosmetic ingredient, the INCI name will be used. It should be noted that some ingredients reviewed in this report (e.g., Galactomyces Ferment Filtrate) may be derived from more than one species of yeast (i.e., Galactomyces Ferment Filtrate may be derived from *Galactomyces candidus*, *Galactomyces fermentans*, or *Galactomyces reessii*).

In addition, many of the species of yeast reviewed in this report have synonymous names, according to the National Center for Biotechnology Information (NCBI) taxonomy database. When studies state the use of a yeast species (e.g., *Starmerella bombicola*) that is synonymous to a species reviewed in this report (e.g., *Candida bombicola*), the species name stated in the study is used as the header (e.g., *Starmerella bombicola*), with a notation stating the synonymous species that is relevant to this report (e.g., *Starmerella bombicola* (synonymous to *Candida bombicola*)).

It should also be noted that the generic yeast ingredients (e.g., Yeast Extract) named in this report may refer to several different species of yeast under the class Saccharomycetes. (Species known to be used in the formulation of Yeast Extract are listed in the Composition section of this report.) When the species of a generic ingredient is known (e.g., *Candida saitoana*), and the ingredient is a known cosmetic ingredient, it will be stated in text (e.g., Yeast Extract derived from *Candida saitoana*), and data will be associated with the specific ingredients derived from the species. Data on any species that is reported to be used in generic yeast ingredients, and is not known to be a cosmetic ingredient, will be named in the report as the species name (e.g., *Candida oleophila*). In addition, because the *Dictionary* does not define the species of yeast used in the production of these generic ingredients, when data are provided on these ingredients, the generic ingredient name will be used as the header, instead of a species name.

CHEMISTRY

Definition

According to the *Dictionary*, Yeast (CAS No. 68876-77-7) is a class of microorganisms (Saccharomycetes) characterized by a lack of photosynthetic ability, existence as unicellular or simple irregular filaments, and reproduction by budding or direct division.[1] *Saccharomyces cerevisiae,* a yeast strain widely used in the preparation of foods and cosmetics, is a highly adaptable, unicellular fungus, capable of growth both aerobic and anaerobically.[3-5] All ingredients reviewed in this report are derived from various yeast species. The definitions of the ingredients included in this report are provided in Table 1.

Yeasts are ubiquitous microorganisms that may be present in a diverse range of habitats, including the air, animals, water, and plants.[6,7] Yeasts are typically nomadic, resilient, and are able to survive in a wide range of conditions. In addition, phenotypic characteristics of yeasts may vary dependent upon environment.[8] Although yeasts can be found in natural habitats, they are typically laboratory-grown for industrial purposes.

Chemical Properties

Dried yeast (derived from *Saccharomyces cerevisiae*) occurs in the form of powder, granules, or flakes, and is typically light brown to buff in color.[9] According to a supplier, a Saccharomyces Cerevisiae Extract was reported to be a clear, yellow-colored liquid, with a pH value of 4.0 - 5.0, and a density of 1.035 - 1.055 (at 20° C).[10] The water solubility of a *Saccharomyces cerevisiae* extract is reported to be > 200 g/l, with the majority of particle sizes ranging from 50 to 220 µm (only 3% of particles < 10 µm in size).[2] Other properties of yeast-derived ingredients can be found in Table 2.

Taxonomy

The majority of the ingredients in this report, including the generic yeast ingredients (e.g., Yeast Extract), correspond to yeasts that are part of the Saccharomycetes class.[1] However, ingredients derived from the species *Phaffia rhodozyma* and the genus *Schizosaccharomyces* belong to the class Tremellomycetes and Schizosaccharomycetes, respectively.[11] The taxonomic profile, as well as relevant synonymous genus/species names of these ingredients, are provided in Table 3.

Yeast Strain Identification and Biosafety

In order to ensure the proper strain of yeast is used in manufacturing, taxonomic identification is performed, typically via r-28S deoxyribonucleic acid (DNA) sequencing and Internal Transcribed Space.[12] According to the US Centers for Disease Control and Prevention, biosafety level (BSL) classifications are given to biological agents, including yeasts, based

on the level of protection provided to workers, the environment, and the public. These levels range from 1 (no or low individual and community risk; e.g., baker's yeast) to 4 (high individual and community risk; e.g., Ebola virus). According to a manufacturer, only BSL-1 yeast species should be used in the manufacture of cosmetic ingredients. In Europe and the US, pathogenic yeasts under the Saccharomycetes class with a BSL-2 categorization include *Candida auris*, *Candida albicans*, *Candida dubliensis*, *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*, none of which are used in the manufacturing of cosmetic ingredients.

Method of Manufacture

Unpublished data were submitted describing methods of manufacture for some of these ingredients. Additionally, general methods of manufacture were found in the published literature; it is unknown if the general methodologies described herein apply to the manufacture of cosmetic ingredients.

According to a manufacturer, yeast ingredients are manufactured via atomization, high temperature enzymatic inactivation (80°C), addition of preservatives, freezing, mechanical grinding, ultrafiltration (0.45 µm or sterilizing filtration (0.22 µm), autolysis/lysis, and acid pH adjustment.[12] Because yeasts are only viable at temperatures < 50°C, no live yeasts would be present in the finished cosmetic product.

Kluyveromyces marxianus (synonymous to *Kluyveromyces fragilis*) and *Saccharomyces cerevisiae*

Extract powders (derived from *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*) are created by first producing yeast biomass via molasses (medium of cultivation).[13] Molasses solutions (molasses and distilled water) are subjected to heavy metal removal, boiled, autoclaved, cooled, filtered, and fermented. Yeast cultures are inoculated into the bioreactor and subjected to a fermentation process under aerobic conditions. After fermentation, the fermentation medium is centrifuged, and the supernatant is decanted and the pellet is washed with saline and centrifuged again. Yeast cells are autolyzed, cooled, and centrifuged to remove cell wall components. The supernatant is then dried in a freeze-dryer, yielding the extract powder.

Lipomyces starkeyi

Lipomyces starkeyi oil is prepared by first culturing the yeast, followed by cell crushing, filtration, organic solvent extraction, and oil purification.[14] The cell crushing process is performed using a high-pressure homogenizer, and performed until particle sizes are less than 3 µm. Examples of organic solvents used for extraction include hexane, ethanol, and 2-propanol.

Saccharomyces cerevisiae

In order to obtain a baker's yeast extract (derived from *Saccharomyces cerevisiae*), dry baker's yeast (50 g) is ground using a mortar, and stirred overnight with water (100 ml).[15] The mixture is then centrifuged for 30 min, filtered, dialyzed, and freeze-dried, ultimately obtaining approximately 1 g baker's yeast extract.

Saccharomyces Cerevisiae Extract

According to data submitted by industry, Saccharomyces Cerevisiae Extract is prepared via an extraction using 1,2-propylene glycol.[10] The extract is sterile filtered and combined with 0.35% potassium sorbate and 0.35% sodium benzoate for preservation. According to a different industry submission, Saccharomyces Cerevisiae Extract is prepared by first concentrating or spray-drying a solution obtained via yeast autodigestion.[16] The resulting solution is extracted with purified water, filtered, and evaporated. The remaining substance is then combined with either ethanol or 1,3-butylene glycol, followed by sedimentation, filtration, and combination with 50% ethanol or a 50% butylene glycol solution.

Yarrowia lipolytica

A biomass of *Yarrowia lipolytica* is prepared by first grafting the yeast from an agar slant.[17] Proliferation of the yeast is continued in tanks of increasing capacity with consistent culture conditions. Yeast is harvested (centrifuged, rinsed with water, and again centrifuged) after the appropriate concentration of yeast dry matter is reached, followed by drying until a moisture content of < 5% is reached (yeast are killed during this step).

Yeast Extract

According to a manufacturer, Yeast Extract is prepared via extraction with a specified eluent (e.g., water, butylene glycol, glycerin, propylene glycol, carthamus tinctorius (safflower) seed oil), to yield a concentrate.[18] The concentrate is then blended with a diluent and preservation system to produce the final result. According to a different manufacturer, Yeast Extract is prepared via solubilization of yeast (e.g., *Candida saitoana*) in water, separation of soluble and insoluble phases, filtration, followed by sterile filtration.[19]

Composition and Impurities

Candida kefyr (synonymous to *Kluyveromyces fragilis*)

The total saturated, monounsaturated, and polyunsaturated fatty acid composition of *Candida kefyr* was determined to be 23.79, 52.79, and 23.42% (of total fatty acids), respectively (measured via gas chromatography mass spectrometry).[20] The specific fatty acids observed can be found in Table 4.

Kluyveromyces fragilis

The composition of a biomass of *Kluyveromyces fragilis* grown on deproteinized whey supplemented with 0.8% diammonium hydrogen phosphate and 10 ppm indole-3 acetic acid was evaluated.[21] The biomass was reported to consist of 37 g/100 g crude protein, 16 g/100 g ash, 4.9 g/100 g crude fiber, 7.8 g/100 g fat, and 34.3 g/100 g carbohydrates. Also reported was a total nitrogen content of 5.92% and total nucleic acid content of 4.82% in *Kluyveromyces fragilis* cells. The essential amino acid profile of the biomass is as follows: arginine (4.30 g/100 g protein), histidine (1.98 g/100 g protein), isoleucine (3.82 g/100 g protein), leucine (5.47 g/100 g protein), lysine (6.91 g/100 g protein), methionine (0.38 g/100 g protein), phenylalanine (3.98 g/100 g protein), threonine (4.45 g/100 g protein), tryptophan (1.07 g/100 g protein), and valine (5.02 g/100 g protein).

Kluyveromyces lactis

A quantitative analysis of sterols in *Kluyveromyces lactis* cells was performed using high-performance liquid chromatography.[22] Ergosterol represented more than 80% of the total amount of yeast sterols.

Kluyveromyces marxianus

Prominent volatile compounds found in a *Kluyveromyces marxianus* extract include hexadecane, pentanoic acid, phenol, γ-decalactone, 3-octanone, and 2-methylpentanal.[13] Other volatile compounds found in this extract in lesser amounts include acetic acid, 2-phenylethyl ester, benzaldehyde, 2,3-butanediol, 2-ethyl,3,5-dimethylpyrazine, nonanal, benzyl alcohol, 2-phenylethanol, (-)-citronellol, geranyl acetate, 2,3,5-trimethylpyrazine, pentadecane, 2-phenyl-2-butenal, tetradecane, 2-nonanone, ethyl phenylacetate, β-myrcene, 2-ethyl-2,5-dimethylpyrazine, and 2-ethyl-6-methylpyrazine. This extract was reported to contain amino acids in an amount of 42.31 g/100 g protein). Alpha-mannans are reported to be present in *Kluyveromyces marxianus* cell walls.[23]

Lipomyces Lipid Bodies

Full genomic sequencing and polymerase chain reaction tests were performed on a cream containing 100% Lipomyces Lipid Bodies.[24] This cream contained no foreign genes or antibiotic resistance traits.

Lipomyces starkeyi

The main component of *Lipomyces starkeyi* is triacylglycerides.[14] Yeast oil derived from this species is rich in palmitic and oleic acid.

Phaffia rhodozyma

The sterol, ubiquinone, and carotenoid content of a *Phaffia rhodozyma* yeast biomass sample consisted of the following: ergosterol 1.121 ± 0.013 mg/g, ubiquinone 1.548 ± 0.009 mg/g, torularhodin 0.856 ± 0.009 mg/g, torulen 0.058 ± 0.002 mg/g, and beta-carotene 0.024 ± 0.001 mg/g.[25] This biomass sample contained 20% saturated fatty acids, 42% monounsaturated fatty acids, and 38% saturated fatty acids.

Saccharomyces cerevisiae

In order for baker's yeast extract (mechanically ruptured cells of *Saccharomyces cerevisiae*) to meet GRAS specifications for food use, the ingredient must contain, on a dry weight basis, < 0.4 ppm arsenic, < 0.13 ppm cadmium, < 0.2 ppm lead, < 0.05 ppm mercury, < 0.09 ppm selenium, and < 10 ppm zinc [21CFR184.1983]. In addition, dried yeast (*Saccharomyces cerevisiae*) may be safely used in food provided the total folic acid content of the yeast does not exceed 0.04 mg/g yeast [21CFR172.896]. The composition of a cleaned natural yeast (*Saccharomyces cerevisiae*; g/100 g dry yeast) was reported to be 42.83 ± 0.11 protein, 1.45 ± 0.40 total lipids, 1.74 ± 0.17 ashes, and 53.91 carbohydrates.[26] This sample of yeast contained moisture in an amount of approximately 0.07 g/100 g dry yeast.

The essential amino acid profile, amount of mineral elements, and fatty acid composition of whole yeast cells (*Saccharomyces cerevisiae*) was evaluated.[27] The mineral elements observed in the largest quantities were phosphorous (1516.0 mg/100 g) and potassium (2035 mg/100 g). All other mineral elements were present in amounts of 147.7 mg/100 g or less. The essential amino acids observed were threonine (4.7 g/100 g protein), methionine + half-cystine (2.4 g/100 g protein), valine (4.8 g/100 g protein), isoleucine (4.2 g/100 g protein), leucine (6.0 g/100 g protein), tyrosine + phenylalanine (6.5 g/100 g protein), lysine (8.0 g/100 g protein), histidine (4.2 g/100 g protein), and tryptophan (1.2 g/100 g protein). The total saturated and monounsaturated fatty acid composition in *Saccharomyces cerevisiae* was determined to be 29.32 and 70.69% (of total fatty acids), respectively (measured via gas chromatography mass spectrometry). The specific fatty acids observed can be found in Table 4. In addition, the nutrient, amino acid, and mineral composition of a *Saccharomyces cerevisiae* sample can be found in Table 5.

The main classes of lipids observed in *Saccharomyces cerevisiae* extracts were determined to be glycerophospholipids, sphingolipids, sterols, and glycerolipids.[28] Forty percent of the identified lipids were polar lipids, while the remaining 60% were neutral lipids. In addition, the cell wall of *Saccharomyces cerevisiae* contains layers predominantly consisting of beta-glucans.[29] The inner layer of the cell wall contains (1→3) β- and (1→6) β-linked glucose residues, and chitin. The outer layer of the cell wall is mainly composed of α-mannan and glycoproteins.

Prominent volatile compounds found in a *Saccharomyces cerevisiae* extract include acetic acid, 2-phenylethyl ester, benzaldehyde, 2,3-butanediol, 2-ethyl-3,5-dimethylpyrazine, nonanal, benzyl alcohol, 2-phenylethanol, (-)-citronellol, hexadecane, and pentanoic acid.[13] Other volatile compounds found in lesser amounts include phenol, γ-decalactone, 3-octanone, 2-methylpentanal, geranyl acetate, 2,3,5-trimethylpyrazine, pentadecane, 2-phenyl-2-butenal, tetradecane, 2-nonanone, ethyl phenylacetate, β-myrcene, 3-ethyl-2,5-dimethylpyrazine, and 2-ethyl-6-methylpyrazine. This extract was reported to be rich in amino acids (47.41 g/100 g protein).

The chemical composition of yeast hydrolysate obtained from *Saccharomyces cerevisiae* was reported to be 4.7% moisture, 68.3% crude protein, 0.3% crude lipid, 3.1% crude ash, and 23.6% carbohydrate.[30]

According to the Food Chemicals Codex, dried yeast (*Saccharomyces cerevisiae*) may not contain more than 1 mg/kg lead.[9] In addition, dried yeast may not contain more than 8% ash.

Saccharomyces Cerevisiae Extract

According to a supplier, Saccharomyces Cerevisiae Extract may not contain more than 20 ppm heavy metals or 2 ppm arsenic.[16]

Schizosaccharomyces pombe

The fatty acid profile of a *Schizosaccharomyces pombe* extract was evaluated via gas chromatography.[31] These fatty acids include palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and oleic acid (C18:1). The *Schizosaccharomyces pombe* cell wall contains two electron-dense layers formed by galactomannan and a central electron-transparent layer consisting of β- and α-glucans (e.g., β-(1,3)-, β-(1,6)-, and α-(1,3)-glucan).[32]

Yarrowia lipolytica

Yeast biomass derived from *Yarrowia lipolytica* (a novel food according to the European Food Safety Authority (EFSA)) is reported to consist primarily of proteins (45 - 55 g/100 g), dietary fiber (25 g/100 g), and fat (7 - 10 g/100 g (the majority being mono-and polyunsaturated fatty acids).[17] When pesticide evaluations were performed on yeast biomass samples, the analyzed pesticides (e.g., organochlorinated and organophosphate pesticides, pyrethroids) were below limits of quantification. Specifications for yeast biomass derived from *Yarrowia lipolytica* as a novel food include the following: ≤ 3.0 mg/kg lead, ≤ 1.0 mg/kg cadmium, ≤ 0.1 mg/kg, ≤ 5000 colony-forming units (CFU)/g total aerobic microbial count, ≤ 100 CFU/g total yeast and mold count, < 10 CFU/g viable *Yarrowia lipolytica* cells, and ≤ 10 CFU/g coliforms.

The total saturated, monounsaturated, and polyunsaturated fatty acid composition of *Candida lipolytica* (synonymous to *Yarrowia lipolytica*) was determined to be 13.63, 63.36, and 23.01% (of total fatty acids), respectively (measured via gas chromatography mass spectrometry).[20] The specific fatty acids observed can be found in Table 4. In addition, the nutrient, amino acid, and mineral composition of a *Yarrowia lipolytica* sample can be found in Table 5.

Yarrowia lipolytica can accumulate lipids to levels > 50% of cell dry weight.[33] These lipids consist mostly of triglycerides and steryl esters. This accumulation, however, depends on multiple factors including environmental conditions, temperature, pH, production of secondary metabolites, nutrient limitation, and microorganism physiology.

Yeast Extract

According to a supplier, a Yeast Extract derived from several different yeast species (*Candida magnoliae, Candida oleophilia, Candida saitoana, Debaryomyces nepalensis, Metschnikowia agaves, Metschnikowia reukaufii, Metschnikowia pulcherrima, Pichia anomala, Pichia heedii, Picha minuta, and Pichia naganishii*) contained 10-53% sugars, 38-39% mineral ashes, and 7-60% proteins.[19] The sum of heavy metals in these extracts were reported to be < 20 ppm.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, 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.

According to 2023 VCRP survey data, Yeast Extract is reported to be used in 398 formulations (343 leave-on formulations and 55 rinse-off formulations; Table 6).[34] All other in-use ingredients are reported to be used 81 formulations or less. The results of the concentration of use survey conducted by the Council indicate Galactomyces Ferment Filtrate has

the highest concentration of use in a leave-on formulation; it is used at up to 90.7% in moisturizing products (not spray).[35] Based on VCRP data and concentration of use survey results, 18 yeast-derived ingredients are reported to be used; the 38 ingredients not in use according to the VCRP and industry survey are listed in Table 7.

Incidental ingestion of several of these ingredients may occur as they are reported to be used in lipstick formulations (e.g., Saccharomyces Ferment is used in lipstick formulations at 0.00013%). These ingredients are also reported to be used in products that may result in mucus membrane (e.g., Saccharomyces Ferment Filtrate is used at up to 0.038% in feminine deodorants) and eye exposure (e.g., Galactomyces Ferment Filtrate is used in eye lotions at up to 37.5%). Saccharomyces Lysate Extract is used at up to 0.067% in baby lotions/oils/powders/creams.

Some of these ingredients are used in cosmetic sprays and powders, and could possibly be inhaled; for example, Saccharomyces Ferment Filtrate and Yeast Extract are used in colognes and toilet waters at 0.065% and Galactomyces Ferment Filtrate is reported to be used at 1.1% in face powders. 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.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The yeast-derived ingredients reviewed in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[36]

Non-Cosmetic

Yeasts are commonly used worldwide in the food and beverage industry, mainly in baking and alcohol production as a fermentative agent.[37] The use/presence of several of the species reviewed in this report in foods, their GRAS status, their Qualified Presumption of Safety (QPS) status (as designated by the EFSA), and information regarding other non-cosmetic uses of these species are provided in Table 8. Specifications required for the GRAS ingredients derived from *Saccharomyces cerevisiae* are described in the Composition and Impurities section of this report.

TOXICOKINETIC STUDIES

Dermal Absorption

Details of the in vitro dermal absorption studies summarized below can be found in Table 9.

Several in vitro dermal absorption assays were performed according to Organisation for Economic Cooperation and Development test guideline (OECD TG) 428 on 30% emulsions of Metschnikowia Agaves Extract, Pichia Anomala Extract, Pichia Heedii Extract, Pichia Minuta Extract, a Yeast Extract derived from *Candida saitoana*, and a Yeast Extract derived from *Metschnikowia reukaufii*.[19] Dermal absorption in these studies ranged from 0.2 to 4.6% of the applied dose 24 h after application.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Details on the acute toxicity studies summarized below can be found in Table 10.

Median lethal doses (LD_{50}s) of > 2000 mg/kg were predicted in 3T3 neutral red uptake assays performed using Pichia Minuta Extract and Yeast Extract (derived from *Pichia naganishii*).[38] An LD_{50} of > 2000 mg/kg was established in rats in acute dermal toxicity assays at a test concentration of 49.5% *Saccharomyces cerevisiae* cell wall in hydrated sodium calcium aluminosilicate (HSCAS) and a *Saccharomyces cerevisiae* extract (in water).[2,4] Similarly, no toxicity was observed in acute oral toxicity assays performed in mice using a *Galactomyces* ferment filtrate (up to 60,000 mg/kg) or in rats with a yeast hydrolysate obtained from *Saccharomyces cerevisiae* (5000 mg/kg bw), 49.5% *Saccharomyces cerevisiae* cell wall (2000 mg/kg bw), a fermentate powder derived from *Saccharomyces cerevisiae* (2000 mg/kg), or *Candida oleophila* strain O (2.3 - 3.8 x 10^8 CFU).[4,30,39-41] Acute inhalation toxicity was evaluated in rats using 49.5% *Saccharomyces cerevisiae* cell wall (2.09 mg/l).[4] The median lethal concentration (LC_{50}) was determined to be > 2.09 mg/l. *Candida oleophila* strain O was not toxic at 1.2 - 5.2 x 10^8 CFU in an inhalation study or 1.1 - 2.0 x 10^7 CFU in a parenteral study performed in rats.[41] No adverse effects were observed in an acute toxicity assay performed in mice inoculated with live *Pichia pastoris* cells (in saline; 1 × 10^6 CFU).[42]

Repeated-Dose Toxicity Studies

Details on the repeated-dose oral toxicity studies summarized below can be found in Table 11.

No significant adverse effects were noted in a 14-d assay in which rats (5/sex/group) were orally administered 1000 mg/kg bw/d yeast hydrolysate derived from *Saccharomyces cerevisiae* (method of oral administration and vehicle not stated).[30] In a different 14-d study, *Kluyveromyces marxianus* extracts (strains A4 and A5; 1.0 x 10^6 CFU/ml or 1.0 x 10^8 CFU/ml; in sterilized saline) were orally administered to female mice (6/group; method of oral administration not stated).[43] Statistically significant lower spleen to body ratios and liver to body ratios were noted in mice treated with the high concentration of the A5 strain, and the low concentration of the A4 strain, respectively. No other adverse effects were observed. *Phaffia rhodozyma* extract (up to 1000 mg/kg) in corn oil was given to rats (6/sex/group), via gavage, for 28 d.[44] The no-observed-adverse-effect-level (NOAEL) was determined to be > 1000 mg/kg. Fermentate powder derived from *Saccharomyces cerevisiae* (in methylcellulose and water) was given to rats (20/sex/group) in a 90-d study (rats given up to 1500 mg/kg bw/d; via gavage), and a 1-yr study (rats given up to 800 mg/kg bw/d; via gavage).[40] All administrations were performed via gavage. The NOAELs for the 90-d and 1-yr study were determined to be 1500 mg/kg bw/d and 800 mg/kg bw/d (the highest dose administered in each study), respectively.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No relevant developmental and reproductive toxicity studies on the yeast-derived ingredients evaluated in this report were found in the published literature, and unpublished data were not submitted.

GENOTOXICITY STUDIES

Details on the genotoxicity studies summarized below can be found in Table 12.

Negative results were obtained for Ames assays performed on *Galactomyces* ferment filtrate (in water; up to 10,000 µg/plate), 90% yeast (*Saccharomyces cerevisiae*) cell wall (in HSCAS; up to 3500 µg/plate), *Phaffia rhodozyma* extract (in acetone; up to 5000 µg/plate), a trade name mixture containing 49% Phaffia Rhodozyma Extract (in water; up to 5000 µg/plate), Pichia Minuta Extract (concentration not stated), fermentate powder derived from *Saccharomyces cerevisiae* (in methylcellulose and water; up to 5000 µg/plate), a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate (in water; up to 5000 µg/plate), *Candida oleophila* strain O (concentration not stated), and a Yeast Extract derived from *Pichia naganishii* (concentration not stated).[4,38,40,44-47] Negative results were also obtained in mammalian cell gene mutation assays performed using a fermentate powder derived from *Saccharomyces cerevisiae* (in methylcellulose and water; up to 5000 µg/plate) and *Candida oleophila* strain O (concentration not stated). No mutagenicity was observed in micronucleus assays performed using Pichia Minuta Extract (concentration not stated) and Yeast Extract derived from *Pichia naganishii* (concentration not stated). Mammalian bone marrow chromosomal assays were performed using a *Phaffia rhodozyma* extract (in corn oil; up to 2000 mg/kg bw/d; performed in 3 male mice/group; oral administration) and 90% yeast (*Saccharomyces cerevisiae*) cell wall (in HSCAS; up to 2000 mg/kg bw/d; performed 28 mice/sex/group; via gavage). Both test substances were considered to be non-clastogenic.

CARCINOGENICITY STUDIES

No relevant carcinogenicity studies on the yeast-derived ingredients evaluated in this report were found in the published literature, and unpublished data were not submitted.

ANTI-CARCINOGENICITY STUDIES

In Vitro

Saccharomyces cerevisiae

Treatment with *Saccharomyces cerevisiae* resulted in the growth inhibition or apoptosis of several cancer cell types in multiple anti-carcinogenicity assays.[48-51] Cell lines that were inhibited by *Saccharomyces cerevisiae* include human metastatic breast cancer cells (MCF-7 and ZR-75-1), non-metastatic breast cancer cells (HCC70), squamous cell carcinoma of the tongue (SCC-4), adenocarcinomas of the colon (Caco-2, DLD1, and HCT116; concentrations not reported), and cervical cancer cells (HeLa; up to 1000 µg/ml yeast cells).

OTHER RELEVANT STUDIES

Anti-Inflammatory Effects

The following study is included as it may help in providing information regarding dermal irritation/allergy alleviation following exposure to Saccharomyces Ferment, when derived from *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae

The anti-inflammatory properties from a dried fermentate derived from *Saccharomyces cerevisiae* was evaluated using a single-blind, placebo-controlled assay (n = 12 subjects). To induce inflammation, 0.01 ml of a dilute solution of histamine was applied to the forearm of each subject, and a scratch was performed using a sterilized lancet. One min after the scratch, the histamine solution was removed, and 0.01 ml dried fermentate (0.1 g/ml) was applied to the site. After 1 min, the dried fermentate was removed, and laser Doppler probes evaluated skin sites (evaluation for 10 min). Doppler probe measured parameters included the time to maximum blood perfusion (T_{max}), and the slope of the curve generated during the resolution phase over time, as a measure of the speed of resolution. This same procedure was performed on the other forearm using

saline (negative control) instead of dried fermentate. After probes were removed, each subject was asked to score the level of itching on each skin site using a 100 mm Visual Analogue Scale. Among the 12 test subjects, the observed average time to T_{max} on sites treated with dried fermentate were significantly shorter than sites treated with saline ($p < 0.05$). In addition, the slope of the curve after T_{max} was significantly lower compared to saline treated site s ($p < 0.05$), indicating that treatment with dried fermentate resulted in a faster process of inflammation resolution.

Immunomodulatory Effects

The following studies are included as they may be helpful in providing information regarding potential allergenicity/ hypersensitivity of the yeast-derived ingredients evaluated in this report.

Candida pseudotropicalis (synonymous to *Kluyveromyces fragilis*), *Geotrichum candidum* (synonymous to *Galactomyces candidus*), and *Saccharomyces cerevisiae*

Immunological cross-reactivity of several yeast species (*Candida albicans*, *Candida pseudotropicalis*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Candida guilliermondi*, *Candida humicola*, *Canidida norwegica*, *Candida utilis*, *Cryptococcus albidus*, *Geotrichum candidum, Pityrosporon pachydermatis, Pityrosporon ovale, Rhodotorula minuta, Rhodotorula rubra, Saccharomyces cerevisiae, Torulopsis glabrata*, and *Trichosporon cutaneum*) was evaluated.[52] Cross-reactive components of yeast extracts were measured via an enzyme immunoassay using rabbit anti-*Candida albicans* antiserum. Results were expressed relative to the absorbance observed with *Candida albicans* extract. Significant cross-reactivity was only observed between Candida species. Skin prick tests were performed in 67 atopic patients using whole cell and disrupted cell extracts several yeast species including *Saccharomyces cerevisiae*. Whole cell and disrupted cell extracts of *Saccharomyces cerevisiae* resulted in positive results in 41 and 31% of patients, respectively.

Pichia pastoris

A delayed-type hypersensitivity test was performed in female BALB/c mice to evaluate cell-mediated immunity to live *Pichia pastoris* cells.[42] Four groups of 5 adult mice were anesthetized and abdominal skin was shaved. Approximately 50% of the stratum corneum was removed, and *Pichia pastoris* cells (2×10^8 CFU in 50 µl sterile saline) were applied epicutaneously. Vehicle group mice received applications of 50 µl sterile saline on stratum corneum-removed skin. Another group of control mice consisted of shaved animals without disruption of the stratum corneum, and were used to evaluate baseline measures. Seven days after administration, ear thickness was measured with a micrometer. To achieve the efferent phase of the delayed-type hypersensitivity response, mice were challenged with inoculation into the ears with heat-killed *Pichia pastoris* cells (1×10^7 CFU). Swelling was calculated by subtracting the ear thickness 24 h after the challenge from the baseline thickness. Results between control, vehicle-control, and *Pichia pastoris*-treated groups were similar, indicating that *Pichia pastoris* did not induce a cell-mediated immune response.

Saccharomyces cerevisiae

Forty-seven patients with inhalant allergy to fungi were tested for allergic sensitivity to baker's yeast (*Saccharomyces cerevisiae*).[15] Baker's yeast extract and purified enolase obtained from baker's yeast were each formulated at concentrations of 1 and 10 mg/ml in a diluent of 50% glycerin in sterile saline. Skin prick testing was performed using both the baker's yeast extract and purified enolase on each of the 47 patients. Non-fungi allergic control subjects (10 non-allergic subjects and 10 grass-pollen and/or mite-allergic patients) were subjected to skin prick tests with baker's yeast extract. Wheal sizes were recorded 15 min following skin prick. Clear wheal and flare skin reactions to baker's yeast extract were observed at both test concentrations (wheal sizes of at least 3 mm) in fungi-allergic patients. No skin reactions were seen at either test concentration in control subjects that were not reported to have fungi allergy. Twenty-three of the fungi-allergic patients showed an allergic response to baker's yeast enolase. Sera from all 47 fungi-allergic patients were subjected to radioallergo-sorbent testing (RAST) using both baker's yeast extract and enolase. Sera from 10 of these patients were RAST-negative to baker's yeast extract and enolase, and 5 other sera were considered doubtful positives. Thirty-two patients were RAST-positive, 22 of which showed RAST uptakes with enolase that were equal to, or higher than, the uptakes recorded with baker's yeast extract. Skin prick tests for these 32 RAST-positive patients revealed that in 25 subjects, wheal sizes to enolase were equal to, or greater than, wheal sizes recorded for baker's yeast extract.

In a different study, the potential sensitizing effects of a *Saccharomyces cerevisiae* extract was evaluated in 449 patients (226 with atopic dermatitis, 50 with allergic rhinitis and/or asthma, and 173 non-atopic controls) via a skin prick test.[53] Skin prick tests were performed in duplicate, and the results were evaluated after 15 min. Serum samples were taken for total serum immunoglobulin E (IgE) determinations. Twenty percent of patients (92) had positive skin prick tests to the extract. Of these subjects, 85 were atopic dermatitis patients, 4 had allergic rhinitis and/or asthma, and 3 were nonatopic controls. There was a significant correlation between the severity of eczema and frequency of positive skin test results to *Saccharomyces cerevisiae*. Patients with moderate to severe dermatitis displayed positive skin prick test reactions significantly more frequently than allergic rhinitis/asthma patients or nonatopic controls ($p < 0.001$). In addition, a parallel skin reactivity assay was performed with other yeasts and common allergens. Parallel skin reactivity was observed with yeasts (*Pitryosporum ovale* and *Candida albicans*), molds, and animal dander, but not with pollen or dust mites. In addition, a significant correlation between total serum IgE and positive skin prick test results with *Saccharomyces cerevisiae* was seen ($r = 0.53$, $p < 0.001$).

Allergens of *Saccharomyces cerevisiae* were evaluated via an IgE-immunoblotting assay performed on 83 subjects.[54] Sixty-three of these patients were previously diagnosed with atopic dermatitis with positive skin prick tests or RAST for *Saccharomyces cerevisiae*, and 7 subjects were diagnosed with atopic dermatitis, but did not have positive skin prick tests or RAST for *Saccharomyces cerevisiae*. The remaining 13 subjects were non-atopic controls. A disrupted whole-body extract of *Saccharomyces cerevisiae* was used for evaluation. Forty-one atopic subjects were positive in the IgE immunoblotting assay, revealing 22 IgE stained bands (10 bands represented immediate allergens, and 12 bands represented minor allergens). In 39% of positive subjects, staining of the 48 kD band was observed. Non-atopic (control-subject serum) and sera from atopic patients with negative skin prick tests to *Saccharomyces cerevisiae* were IgE negative in this experiment.

IgE, IgA, and IgG responses to common yeasts, including *Candida albicans*, *Candida utilis*, *Cryptococcus albidus*, *Rhodotorula rubra*, and *Saccharomyces cerevisiae*, were evaluated via an immunoblotting assay.[55] In addition, the cross-reactivity of their IgE-binding components were also evaluated. Twenty atopic subjects with asthma, allergic rhinitis, or atopic dermatitis, were included in the study (16 patients skin prick test-positive to yeast, 4 were not and served as controls). IgE immunoblotting revealed IgE-binding bands in all species (*Candida albicans* (11 bands), *Candida utilis* (8 bands), *Saccharomyces cerevisiae* (5 bands), *Rhodotorula rubra* (5 bands), and *Cryptococcus albidus* (4 bands)). The 46-kDa band was shared by all 5 yeasts, and the 13-kDa band was shared by 4 yeasts. Prominent IgE binding was seen to a 46-kDa band of *Candida albicans* (7 subjects), *Candida utilis* (5 patients), and *Saccharomyces cerevisiae* (1 patient). Strong IgG responses were observed against *Saccharomyces cerevisiae* (19 patients had a response; 14 patients had a response to *Saccharomyces cerevisiae* mannans) and *Candida albicans* (18 patients had a response; 17 patients had a response to *Candida albicans* mannans). The corresponding patient numbers in IgA immunoblotting were 17 (*Candida albicans*), 17 (*Candida albicans* mannans), 15 (*Saccharomyces cerevisiae*), 7 (*Saccharomyces cerevisiae* mannans), 5 (*Rhodotorula rubra*), 11 (*Cryptococcus albidus*), and 2 (*Cryptococcus albidus* mannans). An IgA response to the 20-kDa band of *Saccharomyces cerevisiae* was observed in 12 patients.

Pulmonary Toxicity

The following studies are included in this report as they may be helpful in evaluating the inhalation toxicity potential of yeast-derived ingredients.

Geotrichum candidum (synonymous to *Galactomyces candidus*)

The cause of allergic alveolitis was evaluated in 12 Australian patients.[56] The houses of all patients were evaluated and inspected. Extensive wood decay was found in 10/12 houses, while 4/12 also had obvious fungal growth on damp walls. Twelve fungal species were observed in homes, including *Geotrichum candidum* (synonymous to *Galactomyces candidus*). Precipitin tests were performed on the 12 patients, along with 14 controls, using freeze-dried fungal extracts (30 mg/ml) of the 12 observed fungal species, in addition to several other species and allergens. If results were negative, tests were repeated using serum that had been concentrated to 20% of the original volume by desiccation. Six of the 12 patients exhibited positive precipitins to one or more of the fungi when unconcentrated serum was used. Nine of 12 patients displayed positive precipitins with concentrated serum (2 positive reactions to *Geotrichum candidum* extract). No precipitins were found to any of the fungal groups in control subjects. Skin prick tests were performed in all patients (number of control subjects not specified) using freeze-dried fungal extracts (10 mg/ml) and other allergens. One patient displayed a positive reaction to *Geotrichum candidum* extract. Inhalation tests were performed with 3 control subjects and 6 patients with alveolitis using solutions of nebulized yeast (*Serpula lacrymans*, *Geotrichum candidum*, and *Aspergillus fumigatus*; 1 mg/ml). Measurements (spirometry and single breath diffusion capacity) were taken every 15 min for the first hour, and every 30 min for at least 8 h. No immediate positive responses were observed; however, positive late responses were obtained to *Serpula lacrymans* (3 positive reactions), *Geotrichum candidum* (2 positive reactions), and *Aspergillus fumigatus* (2 positive responses). Relocation of patients resulted in improvement of symptoms in all cases.

Effect on Pigmentation

The following study is included in this report as it may be helpful in evaluating the potential anti-pigmentation effects of yeast-derived ingredients.

Galactomyces Ferment Filtrate

The effect of Galactomyces Ferment Filtrate on melanization was evaluated in vitro.[57] Cultured normal human melanocytes were exposed to Galactomyces Ferment Filtrate in concentrations of 15, 20, and 30%. Galactomyces Ferment Filtrate at a concentration of 15% did not affect melanocyte viability; however, concentrations of 20 and 30% reduced melanocyte viability by 20 and 50%, respectively. Human melanoma cells and normal human melanocytes (derived from both light and dark skin) were treated with either 5 or 10% Galactomyces Ferment Filtrate, every other day, and evaluated for melanin content. In melanoma cells, a 60% reduction in melanin was noted after treatment with both 5 and 10% Galactomyces Ferment Filtrate, within 12 d. In normal human melanocytes, melanin was reduced by 30 and 55%, after treatment with 5 and 10% Galactomyces Ferment Filtrate, respectively, within 25 d. Galactomyces Ferment Filtrate appeared slightly more effective on normal human melanocytes from dark skin as opposed to light skin. According to this study, Galactomyces Ferment Filtrate did not influence the expression of tyrosinase related protein 1 or premalanosome protein 17, and had a minimal effect on reducing the expression of tyrosinase. In order to determine the mechanism of action of Galactomyces Ferment Filtrate, the effect of Galactomyces Ferment Filtrate on the expression of nuclear factor erythroid 2-

related factor 2 (Nrf2) and glutathione S-transferase (GST) was evaluated in human melanoma cells. Galactomyces Ferment Filtrate (10%) increased the expression of Nrf2, over 70%, within 16 d. In addition, an 8-d treatment of 10% Galactomyes Ferment Filtrate on human melanoma cells increased the expression of GST.

The effect of three Galactomyces Ferment Filtrate-containing skin care products (concentration of Galactomyces Ferment Filtrate in product not stated) on hyperpigmented spots (as induced by skin aging) was evaluated in 86 volunteers over a 1-yr treatment period.[58] An original evaluation was performed in 1999. In 2010 (11 yr later), subjects were instructed to apply all three products (2 essence preparations and 1 cream preparation) twice daily for 1 yr. Skin was evaluated at 2, 8, and 12 mo during this period. Hyperpigmented spots were significantly aggravated when evaluated in 2010 prior to the 12 mo treatment with Galactomyces Ferment Filtrate-containing products ($p < 0.01$). Hyperpigmentation gradually decreased during the 12-mo treatment period, and eventually recovered to a level close to that in 1999.

Saccharomyces cerevisiae

The effect of a natural yeast extract isolated by ethanol precipitation from *Saccharomyces cerevisiae* on melanogenesis was evaluated in an in vitro assay.[59] To evaluate the melanin synthesis inhibition, B16F10 cells (melanocytes) were exposed to the extract (50, 100, and 200 µg/ml) for 72 h. The test substance inhibited melanin synthesis from α-melanocyte-stimulating-hormone (α-MSH)-stimulated B16F10 cells in a dose-dependent manner. Melanin synthesis was also evaluated in melanocytes co-cultured with human keratinocytes (HaCaT), and treatment with the same test substance at concentrations of 50, 100, and 500 µg/ml. Melanin synthesis in these co-cultured melanocytes was also decreased in a dose-dependent manner. The inhibitory effect of the same *Saccharomyces cerevisiae* extract on tyrosinase was examined by a cell-free tyrosinase assay with mushroom tyrosinase, and by an intracellular tyrosinase assay in B16F10 cells. Cells were treated with the test substance (50, 100, and 500 µg/ml), or the positive control, arbutin. The test substance decreased the activity of intracellular tyrosinase in a dose-dependent manner, but had no direct inhibitory effect on tyrosinase itself. The positive control showed significant inhibitory effect on tyrosinase activity in the cell-free assay, in a dose-dependent manner.

Cytotoxicity

Cellular viability assays were performed using a trade name mixture containing 49% Phaffia Rhodozyma Extract and a trade name mixture containing 25% Saccharomyces Lysate Extract (both test substances tested at concentrations of 0.1 and 0.01%).[60,61] Assays were performed using normal human dermal fibroblasts (24 h incubation). Neither test substance was considered to be cytotoxic.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details of the irritation, sensitization, and phototoxicity/photosensitization studies summarized below are provided in Table 13.

In vitro dermal irritation assays yielded negative results.[2,62-68] Tests were performed using a trade name mixture containing 49% Phaffia Rhodozyma Extract, a powdered *Saccharomyces cerevisiae* extract, trade name mixtures containing 1.25, 3, and 4.5% Saccharomyces Cerevisiae Extract, a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate, and trade name mixtures containing 10% and 98% Saccharomyces Lysate Extract, and all materials were tested as supplied. Slight irritation was observed in an irritation assay performed in rabbits using a mixture containing 90% yeast (*Saccharomyces cerevisiae*) cell wall in 10% HSCAS (tested at 55% in water under semi-occlusive conditions).[4] No irritation as observed in a primary dermal irritation assay in which a non-cosmetic product containing 57% *Candida oleophila* strain O was applied to the skin of rabbits.[38] In dermal patch tests in humans, the following were tested and found to be non-irritating: a *Galactomyces* ferment filtrate (test concentration not stated); Metschnikowia Agaves Extract, Pichia Anomala Extract, Pichia Heedii Extract, Pichia Minuta Extract, and Yeast Extract derived from *Candida mangoliae, Candida saitoana*, *Metschnikowia pulcherrima*, and *Metschnikowia reukaufii* (all tested at 15% aq.); a cosmetic formulation containing 1% *Saccharomyces cerevisiae* extract (tested neat); and a cream consisting of 100% Lipomyces Lipid Bodies (tested neat).[19,24,69,70]

No sensitization potential was observed in several in chemico/in vitro sensitization assays performed using a trade name mixture containing 0.4% Hydrolyzed Yeast, a trade name mixture containing 49% Phaffia Rhodozyma Extract, Pichia Minuta Extract (concentration not stated), a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate, and Yeast Extract derived from *Candida magnoliae*, *Metschnikowia reukaufii,* and *Pichia naganishii*.[19,38,71-75] The majority of test substances were tested at up to 2000 µM in antioxidant response element (ARE)-nuclear factor erythroid 2-related factor 2 (Nrf2) luciferase assays and up to 100 mM, in acetonitrile, in direct peptide reactivity assays (DPRA). A trade name mixture containing 0.4% Hydrolyzed Yeast was also tested at up to 5000 µg/ml in a human cell line activation test. In guinea pig studies, no sensitization was observed in a guinea pig maximization assay of *Galactomyces* ferment filtrate (tested neat),[76] and no sensitization was observed in an assay performed using a mixture containing 90% yeast (*Saccharomyces cerevisiae*) cell wall in 10% HSCAS (tested at 49.5% in water and carboxymethylcellulose).[4] Local lymph node assays (LLNAs) were performed in mice using *Saccharomyces cerevisiae* extract, at concentrations of up to 50%.[2] In one assay, the test substance was considered to be sensitizing at concentrations > 10%; however, in four other assays performed according to the same procedures, the test substance was considered to be non-sensitizing. Human repeated-insult patch tests (HRIPTs) of a trade name mixture containing 0.4% Hydrolyzed Yeast (tested at 0.01%; final test concentration of Hydrolyzed Yeast: 0.00004%),

a skincare product containing 1.485% Galactomyces Ferment Filtrate (tested neat), a facial treatment essence containing 92.675% Galactomyces Ferment Filtrate (tested neat), a cream containing 0.0135% Saccharomyces Ferment Lysate Filtrate, a trade name mixture containing 2% Saccharomyces Ferment Lysate Filtrate (tested neat), a cream containing 0.028% Saccharomyces Lysate Extract (tested neat), and a trade name mixture containing 25% Saccharomyces Lysate Extract (tested at 10% in water; final test concentration of Saccharomyces Lysate Extract: 2.5%); a lotion containing 0.0045% Yeast Extract, 15% aq. Metschnikowia Agaves Extract, Pichia Anomala Extract, Pichia Heedii Extract, Pichia Minuta Extract, a Yeast Extract derived from *Candida saitoana*, and a Yeast Extract derived from *Metschnikowia reukaufii* were negative for sensitization.[19,77-84]

No phototoxicity was observed in EpiDerm™ assays performed using a trade name mixture containing 49% Phaffia Rhodozyma Extract or a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate (both test substances tested at up to 10%).[85,86] Similarly, no phototoxicity or photosensitization was observed in assays performed on animals using *Galactomyces* ferment filtrate (concentration not stated).[87,88]

OCULAR IRRITATION STUDIES

Details on the ocular irritation studies summarized below can be found in Table 14.

Several in vitro assays were performed. The following test substances were predicted to be either minimally or non-irritating in in vitro ocular assays: a facial treatment essence containing 92.675% *Galactomyces* ferment filtrate, a trade name mixture containing 49% Phaffia Rhodozyma Extract, Pichia Minuta Extract (concentration not stated), several trade name mixtures containing Saccharomyces Cerevisiae Extract (up to 20%), a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate, two trade name mixtures containing Saccharomyces Lysate Extract (up to 98%), and Yeast Extract derived from *Pichia naganishii*.[2,38,62-68,89]

No irritation was observed in an ocular irritation assay performed in rabbits using *Galactomyces* ferment filtrate (concentration not stated).[90] Minimal irritation was observed in an ocular irritation assay performed in rabbits using a mixture containing 90% *Saccharomyces cerevisiae* cell wall in HSCAS and in an assay performed in rabbits using a non-cosmetic product containing 57% *Candida oleophila* strain O.[4,38] Resolvable irritation was observed in rabbits treated with an undiluted powdered *Saccharomyces cerevisiae* extract.[2]

CLINICAL STUDIES

Case Reports

Case reports were found in the literature describing infection relating to several of the yeast species reviewed in this report.[91-114] These reports, however, were found in immunocompromised or post-surgical patients; therefore, their relevancy to cosmetic safety is unlikely.

Candida oleophila

During a pilot-plant production trial of a product containing *Candida oleophila* strain O (as an active ingredient at 57% by weight), 3 out of 6 workers not wearing personal protective equipment reported clinical symptoms of a respiratory reaction.[41] No adverse dermal effects were observed.

Saccharomyces cerevisiae

A 29-yr-old woman presented to the hospital with multiple severe anaphylactic reactions induced by food.[115] The patient reported a pollen and animal dander allergy, and previous anaphylactic reactions after exposure to contrast media, beer, wine, spaghetti Bolognese sauce, pasta, and bread. Skin prick tests revealed positive results for soya, various nuts and seeds, anthocyanin, and beer malt containing barley. The next anaphylactic reaction took place following ingestion of a meal consisting of industrial-made olive sauce, pasta, and feta cheese. The patient experienced severe allergic symptoms including angioedema of the throat, difficulty breathing, and near loss of consciousness, and was treated in the emergency department. Three wk after the reaction, the patient was examined using skin prick tests and serum allergen-specific IgE/inhibition tests. Various yeasts and molds were tested as well as 2 pasta sauces, individual sauce ingredients, commercial yeast extract preparations, and wines. Skin prick and serum IgE test results were positive to several molds (*Cladosporium herbarum*, *Alternaria alternata*, *Aspergillus fumigatus*, and *Penicillium notatum*), baker's yeast (*Saccharomyces cerevisiae*), *Malassezia furfur*, champignon and the 2 pasta sauces, the yeast ingredient, and a food-quality yeast extract.

A 33-yr-old with a history of allergic rhinoconjunctivitis with exercise-induced asthma reported experiencing episodes of anaphylaxis with no associated exercise over a period of 3 yr.[116] These reactions were successfully treated with epinephrine. The patient related the episodes to ingestion to beer, chips, olives, and wine. Skin prick tests with common aeroallergens, beer extracts, wine, yeast (including several *Saccharomyces cerevisiae* extracts), cereal extracts, and fruits were performed. Results were positive with beer extract, *Saccharomyces cerevisiae* extracts, *Pencillium nalgiovense*, and mushrooms. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting assay was performed with several beer extracts, *Saccharomyces cerevisiae* extract, and the patient's serum. The main IgE-reactive bands detected in the beer extracts were 97 kDa, 80 kDa, 55 kDa, 40 kDa, 32 kDa, and 17 kDa. In the *Saccharomyces cerevisiae* extract, a high intensity IgE-binding zone was observed between 100 kDa and 29 kDa, and a band around 17 kDa. In order to determine

whether *Saccharomyces cerevisiae* was the allergenic source of IgE-reactive proteins detected in beer extracts, an immunoblotting-inhibition assay was performed using a trappist style beer extract in the solid phase and beer extracts and *Saccharomyces cerevisiae* extracts as inhibitors. Both beer extracts and Saccharomyces cerevisiae extracts produced total inhibition of IgE-binding in the trappist style beer extract.

A 25-yr-old woman was admitted to the hospital with a dry cough, low-grade fever, and focal patchy shadow of pulmonary infiltrates.[117] The patient had no previous history of atopic diseases. Because *Saccharomyces cerevisiae* was detected in patient sputum, eosinic bronchitis caused by *Saccharomyces cerevisiae* was suspected. Fungal antigenic solutions were prepared by culturing fungus on medium containing 0.5% yeast extract. Skin tests with the fungal antigens were performed via intradermal injection of the antigen solution (1 mg/ml). Reactions to the injections were observed 15 min and 48 h post-administration. The patient displayed an immediate positive skin reaction to *Saccharomyces cerevisiae*, but both the immediate and delayed skin reactions were negative for *Penicillin janthinellum* as a control. After 7 d of beclomethasone dipropionate inhalation therapy, the patient's symptoms improved, and *Saccharomyces cerevisiae* was no longer present in sputum. Three mo later, the patient was readmitted for bronchoprovocation testing using *Saccharomyces cerevisiae* and *Penicillin janthinellum* antigens. Antigen solutions were administered via a nebulizer. Test results were negative following *Penicillin janthinellum* antigen exposure, but positive following *Saccharomyces cerevisiae* exposure. The patient exhibited a coughing attack, high fever, and ticklish throat within 15 min of exposure. Serum C-reactive protein and sputum eosinophils were increased on the day after provocation testing with *Saccharomyces cerevisiae* antigen. Symptoms disappeared 3 d after testing.

A 48-yr-old bakery worker presented with repeated episodes of hydrorrhea, sneezing, nasal obstruction, wheezing, spasmodic cough, and dyspnea, with symptoms occurring 1-2 h after the start of a workday.[118] Treatment with budesonide and salbutamol was started; however, symptoms were not fully controlled. Skin prick tests were performed using extracts of dehydrated yeast in dry powder form (*Saccharomyces cerevisiae*), conventional wet yeast (*Saccharomyces cerevisiae*), a commercial mixture of baking additives, a battery of inhalant allergies and pollens, flours (wheat, soybean, and barley), and alpha-amylase. Yeast extracts were evaluated at dilutions of 10^{-4} – 10^{-2}. Negative reactions were observed for all non-yeast test substances and the 10^{-4} and 10^{-3} dilutions of the yeast extracts (both wet and dry); however, positive responses to the wet and dry yeast extracts were observed at the 10^{-2} dilution. In addition, baseline peak expiratory flow rates (PEFR) were evaluated when the patient was at the workplace versus away from the workplace. On the patient's workdays the PEFR measurements showed significant decreases from baseline values (>25%). During time away from the workplace, PEFR values did not fall more than 20%. During a nonspecific bronchial provocation test using a dry *Saccharomyces cerevisiae* extract (dilution of 10^{-3}), a drop in forced expiratory volume and shortness of breath/wheezing was observed. These symptoms were not observed when the extract was tested at a 10^{-4} dilution. The patient was diagnosed with occupational asthma caused by *Saccharomyces cerevisiae* sensitization, and began to use conventional wet yeast without symptoms.

SUMMARY

The safety of 56 yeast-derived ingredients as used in cosmetics is reviewed in this safety assessment. According to the *Dictionary*, the majority of these ingredients are reported to function in cosmetics as skin protectants or skin conditioning agents. Several of the species reviewed in this report are used in foods (e.g., *Saccharomyces cerevisiae* is GRAS as a flavoring agent and adjuvant at a level not to exceed 5% in food [21CFR184.1983]).

According to 2023 VCRP survey data, Yeast Extract is reported to be used in 398 formulations (343 leave-on formulations and 55 rinse-off formulations). All other in-use ingredients are reported to be used in 81 formulations or less. The results of a concentration of use survey conducted by the Council indicate Galactomyces Ferment Filtrate has the highest concentration of use in a leave on formulation; it is used at up to 90.7% in moisturizing products. Based on VCRP data and concentration of use survey results, 18 of the yeast-derived ingredients are reported to be in use, and 38 are not.

Several in vitro dermal absorption assays were performed using 30% emulsions of Metschnikowia Agaves Extract, Pichia Anomala Extract, Pichia Heedii Extract, Pichia Minuta Extract, a Yeast Extract derived from *Candida saitoana*, and a Yeast Extract derived from *Metschnikowia reukaufii*. Dermal absorption in these studies ranged from 0.2 to 4.6% of the applied dose 24 h after application.

Median lethal doses (LD_{50}s) of > 2000 mg/kg were predicted in 3T3 neutral red uptake assays performed using Pichia Minuta Extract and Yeast Extract (derived from *Pichia naganishii*). An LD_{50} of > 2000 mg/kg was established in rats in acute dermal toxicity assays using 49.5% *Saccharomyces cerevisiae* cell wall (in HSCAS) and a *Saccharomyces cerevisiae* extract (in water). Similarly, no toxicity was observed in acute oral toxicity assays performed in mice s using a *Galactomyces* ferment filtrate (up to 60000 mg/kg) or in rats with a yeast hydrolysate obtained from *Saccharomyces cerevisiae* (5000 mg/kg bw), 49.5% *Saccharomyces cerevisiae* cell wall (2000 mg/kg bw), a fermentate powder derived from *Saccharomyces cerevisiae* (2000 mg/kg), or *Candida oleophila* strain O (2.3-3.8 x 10^8 CFU). Acute inhalation toxicity was evaluated in rats using 49.5% *Saccharomyces cerevisiae* cell wall (2.09 mg/l). The median lethal concentration (LC_{50}) was determined to be > 2.09 mg/l. *Candida oleophila* strain O (1.2-5.2 x 10^8 CFU (in inhalation study); 1.1-2.0 x 10^7 CFU (in parenteral study)) was considered to be non-toxic in acute inhalation and acute parenteral assays performed in rats. No

adverse effects were observed in an acute toxicity assay performed in mice inoculated with live *Pichia pastoris* cells (in saline; 1×10^6 CFU).

No significant adverse effects were noted in a 14-d assay in which rats were orally administered 1000 mg/kg bw/d yeast hydrolysate derived from *Saccharomyces cerevisiae*. In a different 14-d study, *Kluyveromyces marxianus* extracts (strains A4 and A5; 1.0 x 10^6 CFU/ml or 1.0 x 10^8 CFU/ml; in sterilized saline) were orally administered to female mice. Statistically significant lower spleen to body ratios and liver to body ratios were noted in mice treated with the high concentration of the A5 strain, and the low concentration of the A4 strain, respectively. *Phaffia rhodozyma* extract (up to 1000 mg/kg) in corn oil was given to rats, via gavage, for 28 d. The NOAEL was determined to be > 1000 mg/kg. Fermentate powder derived from *Saccharomyces cerevisiae* (in methylcellulose and water) was given to rats (20/sex/group) in a 90-d oral toxicity study (rats given up to 1500 mg/kg bw/d), and a 1-yr oral toxicity study (rats given up to 800 mg/kg bw/d). The NOAELs for the 90-d and 1-yr study were determined to be 1500 mg/kg bw/d and 800 mg/kg bw/d, respectively.

No mutagenicity was observed in in vitro genotoxicity studies performed on several yeast-derived ingredients (*Galactomyces* ferment filtrate, 90% yeast (*Saccharomyces cerevisiae*) cell wall, *Phaffia rhodozyma* extract, a trade name mixture containing 49% Phaffia Rhodozyma Extract, Pichia Minuta Extract, fermentate powder derived from *Saccharomyces cerevisiae*, trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate, *Candida oleophila* strain O, Yeast Extract derived from *Pichia naganishii*. Similarly, negative results were also obtained in in vivo assays using a *Phaffia rhodozyma* extract, and 90% yeast (*Saccharomyces cerevisiae*) cell wall.

Treatment with *Saccharomyces cerevisiae* resulted in the growth inhibition or apoptosis of several cancer cell types in multiple anti-carcinogenicity assays. Cell lines that were inhibited by *Saccharomyces cerevisiae* include human metastatic breast cancer cells (MCF-7 and ZR-75-1), non-metastatic breast cancer cells (HCC70), squamous cell carcinoma of the tongue (SCC-4), adenocarcinomas of the colon (Caco-2, DLD1, and HCT116), and cervical cancer cells (HeLa).

The anti-inflammatory properties of a dried *Saccharomyces cerevisiae* fermentate was evaluated in 23 subjects. Inflammation was induced via histamine scratches in all subjects (saline used as control). Treatment with the fermentate resulted in faster and more effective inflammation reduction compared to the control.

The immunological cross-reactivity of several yeast species (including *Candida psuedotropicalis* (synonymous to *Kluyveromyces fragilis*), *Geotrichum candidum* (synonymous to *Galactomyces candidus*), and *Saccharomyces cerevisiae*) was evaluated in vitro. Significant cross-reactivity was only observed between *Candida* species. When skin prick tests were performed in 67 atopic patients using whole cell and disrupted cell extracts several yeast species including *Saccharomyces cerevisiae*, whole cell and disrupted cell extracts of *Saccharomyces cerevisiae* resulted in positive results in 41 and 31% of patients, respectively.

A delayed-type hypersensitivity test was performed in female mice using *Pichia pastoris* cells (in saline) on stratum corneum-removed skin. One control group was exposed to the same test substance on regular, intact, shaved skin, and another control group received saline only, on stratum corneum-removed skin. Seven days after administration, ear thickness was measured. Delayed type hypersensitivity was evaluated by inoculating ears with heat-killed *Pichia pastoris* cells. Results between control, vehicle-control, and *Pichia pastoris*-treated groups were similar.

Skin prick tests were performed in 47 individuals with an inhalant allergy to fungi; 10 non-allergic subjects were used as controls. Tests were performed using baker's yeast (*Saccharomyces cerevisiae*) extract and purified enolase obtained from baker's yeast. Clear reactions to the baker's yeast extract were noted in all fungi-allergic patients. Twenty-three patients showed a reaction to the baker's yeast enolase. No reactions were noted for either test substance in control subjects. Skin prick tests using a *Saccharomyces cerevisiae* extract were also performed in a different study, using 449 patients (226 with atopic dermatitis, 50 with allergic rhinitis and/or asthma, and 173 nonatopic controls). Ninety-two patients had positive skin prick tests to the extract. Patients with moderate to severe dermatitis displayed positive skin prick test reactions significantly more frequently than allergic rhinitis/asthma patients or nonatopic controls ($p < 0.001$). A significant correlation between total serum IgE and positive skin prick test results with *Saccharomyces cerevisiae* was seen ($r = 0.53$, $p < 0.001$).

Allergens of *Saccharomyces cerevisiae* were evaluated via an IgE-immunoblotting assay performed on 83 patients (70 atopic patients, 13 non-atopic controls). Forty-one atopic patients were positive in the IgE immunoblotting assay, revealing 22 IgE stained bands. Non-atopic serum and sera from atopic patients with negative skin prick tests to *Saccharomyces cerevisiae* were IgE negative in this experiment. In a similar assay, 20 patients (16 atopic, 4 non-atopic controls) were evaluated for IgE, IgA, and IgG responses to several common yeasts including *Saccharomyces cerevisiae*. Immunoblotting assays revealed IgE binding in all species (5 IgE binding bands in *Saccharomyces cerevisiae*). Prominent IgE binding was seen to a 46-kDa band of several species, including *Saccharomyces cerevisiae*. In addition, IgA and IgG responses were observed against *Saccharomyces cerevisiae*.

The cause of allergic alveolitis was evaluated in 12 Australian patients after a home evaluation for fungal growth. Twelve fungal species, including *Geotrichum candidum (*synonymous to *Galactomyces candidus*) was found in homes. When a precipitin test was performed on the subjects using freeze-dried fungal extracts and other allergens, 2 displayed positive reactions to *Geotrichum candidum* extract. Skin prick tests performed in the same patients resulted in one positive

reaction to *Geotrichum candidum* extract. In an inhalation test performed in 6 of these patients, positive late responses were observed in 2 patients.

Normal human melanocytes treated with Galactomyces Ferment Filtrate (at concentrations of 20% or greater) exhibited a reduction in cell viability. Galactomyces Ferment Filtrate (5 and 10%) resulted in a reduction in melanin in human melanoma cells and normal human melanocytes. When the mechanism of action of Galactomyces Ferment Filtrate was evaluated, it was observed that 10% Galactomyces Ferment Filtrate increases the expression of Nrf2 and GST in human melanoma cells. The hyperpigmentation-reversal potential of Galactomyces Ferment Filtrate-containing skin care products was evaluated in 86 volunteers after a 1 yr treatment period. Treatment with Galactomyces Ferment Filtrate-containing products resulted in significant age-induced hyperpigmentation reversal.

The inhibitory effects of a *Saccharomyces cerevisiae* extract on melanogenesis were evaluated in B16F10 cells (melanocytes), alone, at doses of up to 200 µg/ml, and in melanocytes co-cultured with human keratinocytes, at doses of up to 500 µg/ml. Melanin synthesis decreased in a dose-dependent manner in melanocytes cultured with and without human keratinocytes. The inhibitory effect of *Saccharomyces cerevisiae* extract (up to 500 µg/ml) on tyrosinase was examined by a cell-free tyrosinase assay with mushroom tyrosinase, and by an intracellular tyrosinase assay in B16F10 cells. The test substance decreased the activity of intracellular tyrosinase in a dose-dependent manner, but had no direct inhibitory effect on tyrosinase itself.

Cellular viability analyses were performed using a trade name mixture containing 49% Phaffia Rhodozyma Extract and a trade name mixture containing 25% Saccharomyces Lysate Extract. Neither test substance was considered to be cytotoxic.

All in vitro dermal irritation assays yielded negative results (performed using a trade name mixture containing 49% Phaffia Rhodozyma Extract (tested neat), powdered *Saccharomyces cerevisiae* extract (tested neat), three trade name mixtures containing up to 4.5% Saccharomyces Cerevisiae Extract (concentration tested unknown), a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate (tested neat), and two trade name mixtures containing 10% and 98% Saccharomyces Lysate Extract (both tested neat)). Slight irritation was observed in an irritation assay performed in rabbits using a mixture containing 90% yeast (*Saccharomyces cerevisiae*) cell wall in 10% HSCAS (tested at 55% in water under semi-occlusive conditions). No dermal irritation was observed in an assay performed in rabbits using a non-cosmetic product containing 57% *Candida oleophila* strain O. All test substances were considered to be non-irritating in dermal irritation assays performed in humans using a *Galactomyces* ferment filtrate (tested concentration not stated), a cream consisting of 100% Lipomyces Lipid Bodies (tested neat), Metschnikowia Agaves Extract (15% in water), Pichia Anomala Extract (15% in water), Pichia Heedii Extract (15% in water), Pichia Minuta Extract (15% in water), a cosmetic formulation containing 1% *Saccharomyces cerevisiae* extract (tested neat), a Yeast Extract derived from *Candida mangoliae* (15% in water), a Yeast Extract derived from *Candida saitoana* (15% in water), a Yeast Extract derived from *Metschnikowia pulcherrima* (15% in water), and a Yeast Extract derived from *Metschnikowia reukaufii* (15% in water).

No sensitization potential was observed in several in chemico/in vitro sensitization assays performed using a trade name mixture containing 0.4% Hydrolyzed Yeast, a trade name mixture containing 49% Phaffia Rhodozyma Extract, Pichia Minuta Extract, a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate, and Yeast Extracts derived from *Candida magnoliae*, *Metschnikowia reukaufii*, and *Pichia naganishii*. Several LLNAs were performed in mice using *Saccharomyces cerevisiae* extract, at concentrations of up to 50%. In one assay, the test substance was considered to be sensitizing at concentrations > 10%; however, in four other assays performed according to the same procedures, the test substance was considered to be non-sensitizing. No sensitization was observed in an assay performed in guinea pigs using a mixture containing 90% yeast (*Saccharomyces cerevisiae*) cell wall in 10% HSCAS (tested at 49.5% in water and carboxymethylcellulose). HRIPTs were negative in assays performed using a trade name mixture containing 0.4% Hydrolyzed Yeast (tested at 0.01%; final test concentration of Hydrolyzed Yeast: 0.00004%), a skincare product containing 1.485% Galactomyces Ferment Filtrate (tested neat), a facial treatment essence containing 92.675% Galactomyces Ferment Filtrate (tested neat), Metschnikowia Agaves Extract (15% in water), Pichia Anomala Extract (15% in water), Pichia Heedii Extract (15% in water), Pichia Minuta Extract (15% in water), a cream containing 0.0135% Saccharomyces Ferment Lysate Filtrate (tested neat), a trade name mixture containing 2% Saccharomyces Ferment Lysate Filtrate (tested neat), a cream containing 0.028% Saccharomyces Lysate Extract (tested neat), a trade name mixture containing 25% Saccharomyces Lysate Extract (tested at 10% in water; final test concentration of Saccharomyces Lysate Extract: 2.5%), a lotion containing 0.0045% Yeast Extract, a Yeast Extract derived from *Candida saitoana* (15% in water), and a Yeast Extract derived from *Metschnikowia reukaufii* (15% in water).

No phototoxicity was observed in EpiDerm™ assays performed using a trade name mixture containing 49% Phaffia Rhodozyma Extract and a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate (both test substances tested at up to 10%. Similarly, no phototoxicity or photosensitization was observed in assays performed on animals using *Galactomyces* ferment filtrate (concentration not stated).

All test substances were considered to be either minimally or non-irritating in in vitro ocular assays performed using a facial treatment essence containing 92.675% *Galactomyces* ferment filtrate, a trade name mixture containing 49% Phaffia Rhodozyma Extract, Pichia Minuta Extract, several trade name mixtures containing Saccharomyces Cerevisiae Extract (up to 20%), a trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate, two trade name mixtures containing

Saccharomyces Lysate Extract (up to 98%), and Yeast Extract derived from *Pichia naganishii*. No irritation was observed in an ocular irritation assay performed in rabbits using *Galactomyces* ferment filtrate (concentration not stated). Minimal irritation was observed in an ocular irritation assay performed in rabbits using a mixture containing 90% *Saccharomyces cerevisiae* cell wall in HSCAS and in an assay performed in rabbits using a non-cosmetic product containing 57% *Candida oleophila* strain O. Resolvable irritation was observed in rabbits treated with an undiluted powdered *Saccharomyces cerevisiae* extract.

Three out of 6 pilot-plant production workers not wearing personal protective equipment displayed respiratory reactions when working in a facility manufacturing a product containing *Candida oleophila* strain O (as an active ingredient at 57% by weight). A 29-yr-old woman suffered from multiple severe anaphylactic reactions following a meal of olive sauce, pasta, and feta cheese. Skin prick and serum immunologic E (IgE) tests revealed were positive to several molds including baker's yeast (*Saccharomyces cerevisiae*). A 33-yr-old woman with a history of allergies and asthma reported anaphylaxis episodes that were related to ingestion of beer, chips, olive, and wine. An immunoblotting assay revealed a high-intensity IgE-binding zone, when evaluating *Saccharomyces cerevisiae* extract, between 100 kDa and 29 kDa, and a band around 17 kDa. In a different case report, a 25-yr-old woman was admitted to the hospital with a dry cough, low-grade fever, and focal patchy shadow of pulmonary infiltrates. Skin prick tests were positive to *Saccharomyces cerevisiae*. Bronchoprovocation testing performed 3 mo later using *Saccharomyces cerevisiae* antigens yielded positive results, and the patient exhibited a coughing attack, high fever, and ticklish throat within 15 min of exposure. Serum C-reactive protein and sputum eosinophils were increased on the day after provocation testing with *Saccharomyces cerevisiae* antigen. A 48-yr-old baker reported respiratory symptoms 1-2 h after the start of a workday. Skin prick test were performed using extracts of wet and dry yeast (at dilutions of 10^{-4} – 10^{-2}), as well as other potential allergens. Positive responses to the wet and dry yeast extracts were observed at the 10^{-2} dilution. The patient was diagnosed with occupational asthma caused by *Saccharomyces cerevisiae* sensitization, and began to use conventional wet yeast without symptoms

DRAFT DISCUSSION

[**Note**: This Discussion is in draft form, and changes may be made following the Panel meeting.]

The ingredients in this report are derived from various species of yeast, the majority of which, are from the Saccharomycetes class. The Panel noted that elevated levels of heavy metals and pesticide residues may be present in these yeast-derived ingredients. The cosmetics industry should continue to use current good manufacturing processes to limit these impurities. In addition, the Panel noted that volatile compounds (e.g., benzaldehyde) may be present in yeast-derived ingredients. However, these compounds are expected to become volatilized prior to the preparation of the final cosmetic product containing these ingredients, and thus would be present in none to minimal amounts.

In addition, it was noted that several species of yeast evaluated in this report are GRAS, have a QPS status, and/or used in/naturally present in foods. Because exposure via ingestion would be far greater than exposure via cosmetics, the Panel deferred the need for systemic toxicity data.

The Panel also noted incidences of IgE-mediated hypersensitivity following exposure to certain yeast species (e.g., Saccharomyces cerevisiae), along with the potential inhalation of these ingredients in cosmetics. However, these reactions were observed in subjects exposed to live yeasts at high concentrations. Yeasts in cosmetic ingredients are lysed and inactivated, and are reported to be used in inhalable cosmetic products at very low concentrations (≤ 1.1%). In addition, safety of these ingredients were supported by the minimal amount of hypersensitivity case reports present in the literature in comparison to the widespread historical use and consumption of various species of yeast.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (e.g., Galactomyces Ferment Filtrate is reported to be used at 1.1% in face powders). Inhalation toxicity data were limited; however, the Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which the ingredients are used in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

CONCLUSION

To be determined.

TABLES

Table 1. INCI names, definitions, and reported functions of the yeast-derived ingredients in this safety assessment[1]

Ingredient (CAS No.)	Definition	Function
Galactomyces Ferment Filtrate	Galactomyces Ferment Filtrate is a filtrate of the product obtained by the fermentation of a growth media by the microorganism, *Galactomyces candidus*, *Galactomyces fermentans*, or *Galactomyces reessii*.	Skin-Conditioning agents - Humectant
Hydrolyzed Candida Bombicola Extract	Hydrolyzed Candida Bombicola Extract is the hydrolysate of an extract of *Candida bombicola* obtained by acid, enzyme or other method of hydrolysis.	Surfactants – Cleansing Agents
Hydrolyzed Candida Saitoana Extract	Hydrolyzed Candida Saitoana Extract is the hydrolysate of an extract of *Candida saitoana* derived by acid, enzyme or other method of hydrolysis.	Skin Protectants
Hydrolyzed Kluyveromyces Extract	Hydrolyzed Kluyveromyces Extract is the hydrolysate of Kluyveromyces Extract derived by acid, enzyme or other method of hydrolysis.	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Metschnikowia Agaves Extract [1309127-75-0]	Hydrolyzed Metschnikowia Agaves Extract is the hydrolysate of an extract of the yeast, *Metschnikowia agaves* derived by acid, enzyme or other method of hydrolysis.	Skin Protectants
Hydrolyzed Metschnikowia Reukaufii Extract	Hydrolyzed Metschnikowia Reukaufii Extract is the extract of the hydrolysate of Metschnikowia Reukaufii Lysate Extract derived by acid, enzyme or other method of hydrolysis.	Skin Protectants
Hydrolyzed Metschnikowia Shanxiensis Extract	Hydrolyzed Metschnikowia Shanxiensis Extract is the hydrolysate of an extract of the microorganism, *Metschnikowia shanxiensis.*	Skin Protectants
Hydrolyzed Saccharomyces Cell Wall	Hydrolyzed Saccharomyces Cell Wall is the hydrolysate of the cell walls of *Saccharomyces* derived by acid, enzyme or other method of hydrolysis.	Film Formers Hair Conditioning Agents Skin-Conditioning Agents - Humectant Slip Modifiers
Hydrolyzed Saccharomyces Extract	Hydrolyzed Saccharomyces Extract is the hydrolysate of an extract of Saccharomyces derived by acid, enzyme or other method of hydrolysis.	Skin-Conditioning Agents - Emollient
Hydrolyzed Saccharomyces Lysate Extract	Hydrolyzed Saccharomyces Lysate Extract is the extract of the product obtained by the hydrolysis of Saccharomyces Lysate Extract.	Skin-Conditioning Agents - Humectant
Hydrolyzed Torulaspora Delbrueckii Extract	Hydrolyzed Torulaspora Delbrueckii Extract is the hydrolysate of an extract of *Torulaspora delbrueckii* derived by acid, enzyme or other method of hydrolysis.	Skin Protectants
Hydrolyzed Yeast	Hydrolyzed Yeast is the hydrolysate of yeast derived by acid, enzyme or other method of hydrolysis.	Hair-Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Yeast Extract	Hydrolyzed Yeast Extract is the hydrolysate of Yeast Extract derived by acid, enzyme or other method of hydrolysis.	Skin-Conditioning Agents - Miscellaneous
Kluyveromyces Extract	Kluyveromyces Extract is the extract of *Kluyveromyces lactis* or *Kluyveromyces fragilis*.	Skin-Conditioning Agents - Humectant
Lactic Yeasts [68876-77-7]	Lactic Yeasts is a Yeast obtained from milk.	Not Reported
Lipomyces Lipid Bodies	Lipomyces Lipid Bodies are the lipid-rich organelles produced through fermentation by *Lipomyces*.	Skin-Conditioning Agents - Emollient
Lipomyces Oil	Lipomyces Oil is the oil produced through fermentation by the fungus, *Lipomyces starkeyi*.	Hair-Conditioning Agents; Skin-Conditioning Agents – Humectant; Surfactants-Cleansing Agents; Surfactants-Emulsifying Agents
Lipomyces Oil Extract	Lipomyces Oil Extract is the extract of Lipomyces Oil	Skin-Conditioning Agents - Emollient
Metschnikowia Agaves Extract	Metschnikowia Agaves Extract is the extract of the yeast, *Metschnikowia agaves*.	Skin Protectants
Metschnikowia Henanesis Extract	Metschnikowia Henanensis Extract is the extract of the fungus, *Metschnikowia henanensis.*	Skin-Conditioning Agents - Humectants
Metschnikowia Reukaufii Lysate Extract	Metschnikowia Reukaufii Lysate Extract is the extract of a lysate of the cultured cells of *Metschnikowia reukaufii.*	Skin Protectants
Metschnikowia Viticola Extract	Metschnikowia Viticola Extract is the extract of the yeast, *Metschnikowia viticola.*	Skin-Conditioning Agents - Humectant
Pichia Caribbica Ferment	Pichia Caribbica Ferment is the product obtained by the fermentation of *Pichia caribbica*.	Skin-Conditioning Agents - Humectant
Pichia Extract	Pichia Extract is the extract of various species of the microorganism, *Pichia.*	Skin Protectants
Pichia Ferment Extract Filtrate	Pichia Ferment Extract Filtrate is a filtrate of an extract of the product obtained through fermentation by the microorganism, *Pichia pastoris.*	Skin Protectants; Skin-Conditioning Agents – Emollient; Skin-Conditioning Agents - Humectant
Pichia Ferment Lysate Filtrate	Pichia Ferment Lysate Filtrate is a filtrate of a lysate of the product obtained by the fermentation of *Pichia pastoris*, *Pichia populi* or *Pichia stipitis.*	Humectants; Skin Protectants; Skin-Conditioning Agents – Miscellaneous

Table 1. INCI names, definitions, and reported functions of the yeast-derived ingredients in this safety assessment[1]

Ingredient (CAS No.)	Definition	Function
Pichia Pastoris Ferment Filtrate	Pichia Pastoris Ferment Filtrate is a filtrate of the product obtained by the fermentation of a growth media by the microorganism, *Pichia pastoris*.	Skin-Conditioning Agents – Miscellaneous
Phaffia Rhodozyma Extract	Phaffia Rhodozyma Extract is the extract of the microorganism, *Phaffia rhodozyma*.	Hair-Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Phaffia Rhodozyma Ferment Extract	Phaffia Rhodozyma Ferment Extract is the extract of the fermentation product of *Phaffia rhodozyma*.	Antioxidants; Colorants; Skin-Conditioning Agents - Emollient
Pichia Anomala Extract [1033319-29-7]	Pichia Anomala Extract is the extract of the yeast, *Pichia anomala*.	Skin Protectants
Pichia Heedii Extract [1801269-82-8]	Pichia Heedii Extract is the extract of the yeast, *Pichia heedii*.	Skin Protectants
Pichia Minuta Extract [2009239-94-3]	Pichia Minuta Extract is the extract of the microorganism, *Pichia minuta.*	Skin Protectants
Saccharomyces	Saccharomyces is one or more species of the microorganism, *Saccharomyces*	Anti-Acne Agents; Anti-Microbial Agents; Binders; Skin Protectants
Saccharomyces Cerevisiae Extract [84604-16-0]	Saccharomyces Cerevisiae Extract is the extract of the yeast cells of *Saccharomyces cerevisiae*.	
Saccharomyces Extract	Saccharomyces Extract is the extract of Saccharomyces	Antioxidants; Hair-Conditioning Agents; Skin Protectants; Skin-Conditioning Agents - Miscellaneous
Saccharomyces Ferment	Saccharomyces Ferment is the product obtained through fermentation by the microorganism, *Saccharomyces*.	Not Reported
Saccharomyces Ferment Extract	Saccharomyces Ferment Extract is the extract of the product obtained by the fermentation of media by *Saccharomyces*.	Flavoring Agents Fragrance Ingredients
Saccharomyces Ferment Extract Lysate Filtrate	Saccharomyces Ferment Extract Lysate Filtrate is the filtrate of the product obtained after the lysis of the cultured cells of the microorganism, *Saccharomyces*.	Skin Protectants
Saccharomyces Ferment Filtrate	Saccharomyces Ferment Filtrate is a filtrate of the product obtained by the fermentation of a growth media by the microorganism, *Saccharomyces*.	Skin-Conditioning Agents - Humectant
Saccharomyces Ferment Lysate Extract	Saccharomyces Ferment Lysate Extract is the extract of the lysed cells of *Saccharomyces* grown in culture.	Skin Protectants
Saccharomyces Ferment Lysate Filtrate	Saccharomyces Ferment Lysate Filtrate is the filtrate of a lysate of the product obtained by the fermentation of *Saccharomyces*.	Skin Protectants
Saccharomyces Lysate [8013-01-2]	Saccharomyces Lysate is a lysate of the product obtained by the fermentation of *Saccharomyces*.	Not Reported
Saccharomyces Lysate Extract [8013-01-2]	Saccharomyces Lysate Extract is the extract of Saccharomyces Lysate	Skin-Conditioning Agents – Humectant; Skin-Conditioning Agents - Miscellaneous
Saccharomyces Lysate Extract Filtrate	Saccharomyces Lysate Extract Filtrate is a filtrate of the extract of the product obtained by the lysis of *Saccharomyces* cells.	Skin-Conditioning Agents - Miscellaneous
Saccharomyces Lysate Filtrate	Saccharomyces Lysate Filtrate is a filtrate of lysed *Saccharomyces* grown in culture.	Hair-Conditioning Agents; Skin Protectants
Schizosaccharomyces Ferment Extract Filtrate	Schizosaccharomyces Ferment Extract Filtrate is a filtrate of an extract obtained by the fermentation of *Schizosaccharomyces*.	Humectants; Skin-Conditioning Agents - Miscellaneous
Schizosaccharomyces Ferment Filtrate	Schizosaccharomyces Ferment Filtrate is a filtrate of the product obtained by the fermentation of a growth media by the microorganism, *Schizosaccharomyces*.	Hair-Conditioning Agents; Humectants; Skin-Conditioning Agents – Miscellaneous
Schizosaccharomyces Pombe Extract	Schizosaccharomyces Pombe Extract is the extract of the yeast, *Schizosaccharomyces pombe*.	Skin-Conditioning Agents – Miscellaneous
Torulaspora Delbrueckii Extract [1291071-26-5]	Torulaspora Delbrueckii Extract is the extract of the yeast, *Torulaspora delbrueckii*.	Skin Protectants
Torulaspora Delbrueckii Ferment [1291071-26-5]	Torulaspora Delbrueckii Ferment is the product obtained by the fermentation of *Torulaspora delbrueckii*.	Skin-Conditioning Agents - Miscellaneous
Yarrowia Lipolytica Extract	Yarrowia Lipolytica Extract is the extract of the microorganism, *Yarrowia lipolytica* obtained through fermentation.	Skin-Conditioning Agents - Humectant
Yarrowia Lipolytica Ferment Lysate	Yarrowia Lipolytica Ferment Lysate is the product obtained after the lysis of the cultured cells of the microorganism, *Yarrowia lipolytica.*	Skin-Conditioning Agent – Humectant
Yarrowia Lipolytica Oil	Yarrowia Lipolytica Oil is the oil derived from the fermentation of the fungus, *Yarrowia lipolytica* grown in culture.	Skin-Conditioning Agent - Emollient
Yeast [68876-77-7]	Yeast is a class of microorganisms (Saccharomycetes) characterized by their lack of photosynthetic ability, existence as unicellular or simple irregular filaments, and reproduction by budding or direct division.	Not Reported
Yeast Extract [68876-77-7; 8013-01-2]	Yeast Extract is the extract of Yeast.	Skin Protectants; Skin-Conditioning Agents - Miscellaneous
Yeast Ferment Extract	Yeast Ferment Extract is the extract of the product obtained by the fermentation of *Saccharomyces cerevisiae*.	Skin-Conditioning Agents – Miscellaneous

Table 2. Chemical properties of yeast-derived cosmetic ingredients

Property	Value	Reference
Saccharomyces Cerevisiae Extract		
Physical Form	liquid	10
Color	clear-yellow	10
Odor	faint	10
Specific Gravity (@ 20ºC)	1.035 – 1.055	10
Vapor pressure (mmHg @ 105ºC)	3.83	2
Refraction Index (RIU (@ 20°C))	1.035 – 1.055	10
Yeast		
Physical Form	powder, granules, or flakes	9
Color	light brown - buff	9
Yeast Extract*		
Physical Form	liquid	18
Color	clear-pale yellow	18
Odor	characteristic	18
Water Solubility	soluble	18
Specific Gravity (@ 25ºC)	1.05 – 1.15	18
Refraction Index (RIU (@ 25°C))	1.3920 – 1.5000	18

*derived from *Saccharomyces cerevisiae*

Table 3. Taxonomy of yeast-derived ingredients[1,119]

INCI Ingredient	Class	Order	Family	Genus	Associated Genus and Species/Synonyms	Synonyms**
Galactomyces Ferment Filtrate*	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Geotrichum*	*Galactomyces candidus*	*Dipodascus geotrichum* *Endomyces geotrichum* *Galactomyces geotrichum* *Geotrichum candidum*
	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Dipoascus*	*Galactomyces fermentans*	-
	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Galactomyces*	*Galactomyces reessii*	*Endomyces reessii* *Dipodascus reessii*
Hydrolyzed Candida Bombicola Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetales*	*Starmerella*	*Candida bombicola*	*Starmerella bombicola*
Hydrolyzed Candida Saitoana Extract	Saccharomycetes	Saccharomycetales	*Debaryomycetaceae*	*Candida*	*Candida saitoana*	-
Hydrolyzed Kluyveromyces Extract*	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Kluyveromyces*	*Kluyveromyces fragilis*	*Candida kefyr* *Candida pseudotropicalis* *Dekkeromyces marxianus* *Guilliermondella marxiana* *Kluyveromyces cicerisporus* *Kluyveromyces marxianus* *Saccharomyces marxianus* *Zygofabospora marxiana* *Zygorenospora marxiana* *Zygosaccharomyces marxianus*
	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Kluyveromyces*	*Kluyveromyces lactis*	*Torulaspora lactis* *Saccharomyces lactis* *Kluyveromyces drosophilarum* *Candida sphaerica*
Hydrolyzed Metschnikowia Agaves Extract	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia agaves*	-
Hydrolyzed Metschnikowia Reukaufii Extract	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia reukaufii*	*Candida reukaufii*
Hydrolyzed Metschnikowia Shanxiensis	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia shanxiensis*	-
Hydrolyzed Saccharomyces Cell Wall	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Hydrolyzed Saccharomyces Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Hydrolyzed Saccharomyces Lysate Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Hydrolyzed Torulaspora Delbrueckii Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Torulaspora*	*Torulaspora delbrueckii*	*Saccharomyces delbrueckii* *Saccharomyces fermentati* *Saccharomyces rosei* *Candida colliculosa*
Hydrolyzed Yeast	Saccharomycetes	-	-	-	-	-
Hydrolyzed Yeast Extract	Saccharomycetes	-	-	-	-	-

Table 3. Taxonomy of yeast-derived ingredients[1,119]

INCI Ingredient	Class	Order	Family	Genus	Associated Genus and Species/Synonyms	Synonyms**
Kluyveromyces Extract*	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Kluyveromyces*	*Kluyveromyces fragilis*	*Candida kefyr* *Candida pseudotropicalis* *Dekkeromyces marxianus* *Guilliermondella marxiana* *Kluyveromyces cicerisporus* *Kluyveromyces marxianus* *Saccharomyces marxianus* *Zygofabospora marxiana* *Zygorenospora marxiana* *Zygosaccharomyces marxianus*
	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Kluyveromyces*	*Kluyveromyces lactis*	*Torulaspora lactis* *Saccharomyces lactis* *Kluyveromyces drosophilarum* *Candida sphaerica*
Lactic Yeasts	Saccharomycetes	-	-	-	-	-
Lipomyces Lipid Bodies	Saccharomycetes	Saccharomycetales	*Lipomycetaceae*	*Lipomyces*	*Lipomyces sp.*	-
Lipomyces Oil	Saccharomycetes	Saccharomycetales	*Lipomycetaceae*	*Lipomyces*	*Lipomyces starkeyi*	-
Lipomyces Oil Extract	Saccharomycetes	Saccharomycetales	*Lipomycetaceae*	*Lipomyces*	*Lipomyces starkeyi*	-
Metschnikowia Agaves Extract	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia agaves*	-
Metschnikowia Henanensis Extract	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia henanensis*	-
Metschnikowia Reukaufii Lysate Extract	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia reukaufii*	*Candida reukaufii*
Metschnikowia Viticola Extract	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia viticola*	-
Pichia Anomala Extract	Saccharomycetes	Saccharomycetales	*Phaffomycetaceae*	*Wickerhamomyces*	*Pichia anomala*	*Whickerhamomyces anomalus* *Saccharomyces anomalus* *Endomyces anomalus* *Hansenula anomala* *Pichia anomalus* *Willia anomala*
Pichia Caribbica Ferment	Saccharomycetes	Saccharomycetales	*Debaryomycetaceae*	*Meyerozyma*	*Pichia caribbica*	*Meyerozyma caribbica* *Candida fermentati* *Torula fermentati*
Pichia Extract	Saccharomycetes	Saccharomycetales	*Pichiaceae*	-	-	-
Pichia Ferment Extract Filtrate	Saccharomycetes	Saccharomycetales	*Phaffomycetaceae*	*Komagatella*	*Pichia pastoris*	*Komagataella pastoris* *Zygosaccharomyces pastoris*
Pichia Ferment Lysate Filtrate*	Saccharomycetes	Saccharomycetales	*Phaffomycetaceae*	*Barnettozyma*	*Pichia populi*	*Barnettozyma populi* *Hansenula populi*
Pichia Ferment Lysate Filtrate*	Saccharomycetes	Saccharomycetales	*Debaryomycetaceae*	*Scheffersomyces*	*Pichia stipitis*	*Scheffersomyces stipitis* *Yamadazyma stipitis*
Pichia Heedii Extract	Saccharomycetes	Saccharomycetales	*Pichiaceae*	*Pichia*	*Pichia heedii*	-
Pichia Minuta Extract	Saccharomycetes	Saccharomycetales	*Pichiaceae*	*Ogataea*	*Pichia minuta*	*Ogataea minuta* *Hansenula minuta* *Candida methanolovescens* *Torulopsis methanolovescens*
Pichia Pastoris Ferment Filtrate	Saccharomycetes	Saccharomycetales	*Phaffomycetaceae*	*Komagatella*	*Pichia pastoris*	*Komagataella pastoris* *Zygosaccharomyces pastoris*

Table 3. Taxonomy of yeast-derived ingredients[1,119]

INCI Ingredient	Class	Order	Family	Genus	Associated Genus and Species/Synonyms	Synonyms**
Phaffia Rhodozyma Extract	Tremellomycetes	Cystofilobasidales	*Mrakiaceae*	*Phaffia*	*Phaffia rhodozyma*	*Cryptococcus rhodozymus* *Rhodomyces dendrorhous* *Xanthophyllomyces dendrorhous*
Phaffia Rhodozyma Ferment Extract	Tremellomycetes	Cystofilobasidales	*Mrakiaceae*	*Phaffia*	*Phaffia rhodozyma*	*Cryptococcus rhodozymus* *Rhodomyces dendrorhous* *Xanthophyllomyces dendrorhous*
Saccharomyces	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Cerevisiae Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	*Saccharomyces cerevisiae*	*Mycoderma cerevisiae* *Candida robusta* *Saccharomyces capensis* *Saccharomyces italicus* *Saccharomyces oviformis* *Saccharomyces uvarum var. melibiosus*
Saccharomyces Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Ferment	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Ferment Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Ferment Extract Lysate Filtrate	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Ferment Filtrate	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Ferment Lysate Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Ferment Lysate Filtrate	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Lysate	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Lysate Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Lysate Extract Filtrate	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Saccharomyces Lysate Filtrate	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Schizosaccharomyces Ferment Extract Filtrate	Schizosaccharomycetes	Schizosaccharomycetales	*Schizosaccharomycetaceae*	*Schizosaccharomyces*	-	-
Schizosaccharomyces Ferment Filtrate	Schizosaccharomycetes	Schizosaccharomycetales	*Schizosaccharomycetaceae*	*Schizosaccharomyces*	-	-
Schizosaccharomyces Pombe Extract	Schizosaccharomycetes	Schizosaccharomycetales	*Schizosaccharomycetaceae*	*Schizosaccharomyces*	*Schizosaccharomyces pombe*	*Schizosaccharomyces malidevorans*
Torulaspora Delbrueckii Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Torulaspora*	*Torulapora delbrueckii*	*Saccharomyces delbrueckii* *Saccharomyces fermentati* *Saccharomyces rosei* *Candida colliculosa*
Torulaspora Delbrueckii Ferment	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Torulaspora*	*Torulapora delbrueckii*	*Saccharomyces delbrueckii* *Saccharomyces fermentati* *Saccharomyces rosei* *Candida colliculosa*

Table 3. Taxonomy of yeast-derived ingredients[1,119]

INCI Ingredient	Class	Order	Family	Genus	Associated Genus and Species/Synonyms	Synonyms**
Yarrowia Lipolytica Extract	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Yarrowia*	*Yarrowia lipolytica*	*Endomycopsis lipolytica* *Mycotorula lipolytica* *Candida lipolytica*
Yarrowia Lipolytica Ferment Lysate	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Yarrowia*	*Yarrowia lipolytica*	*Endomycopsis lipolytica* *Mycotorula lipolytica* *Candida lipolytica*
Yarrowia Lipolytica Oil	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Yarrowia*	*Yarrowia lipolytica*	*Endomycopsis lipolytica* *Mycotorula lipolytica* *Candida lipolytica*
Yeast	Saccharomycetes	-	-	-	-	-
Yeast Extract***	Saccharomycetes	-	-	-	-	-
	Saccharomycetes	Saccharomycetales	*NR*	*Starmerella*	*Candida magnoliae*	*Starmerella magnoliae* *Torulopsis magnoliae*
	Saccharomycetes	Saccharomycetales	*Debaryomycetaceae*	*Kurtzmaniella*	*Candida oleophila*	-
	Saccharomycetes	Saccharomycetales	*Debaryomycetaceae*	*Candida*	*Candida saitoana*	-
	Saccharomycetes	Saccharomycetales	*Debaryomycetaceae*	*Debaryomyces*	*Debaryomyces nepalensis*	-
	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia agaves*	-
	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia reukaufii*	*Candida reukaufii*
	Saccharomycetes	Saccharomycetales	*Metschnikowiaceae*	*Metschnikowia*	*Metschnikowia pulcherrima*	*Candida pulcherrima*
	Saccharomycetes	Saccharomycetales	*Phaffomycetaceae*	*Wickerhamomyces*	*Pichia anomala*	*Whickerhamomyces anomalus* *Saccharomyces anomalus* *Endomyces anomalus* *Hansenula anomala* *Pichia anomalus* *Willia anomala*
	Saccharomycetes	Saccharomycetales	*Pichiaceae*	*Pichia*	*Pichia heedii*	-
	Saccharomycetes	Saccharomycetales	*Pichiaceae*	*Ogataea*	*Pichia minuta*	*Ogataea minuta* *Hansenula minuta* *Candida methanolovescens* *Torulopsis methanolovescens*
	Saccharomycetes	Saccharomycetales	*Pichiaceae*	*Ogataea*	*Pichia naganishii*	*Ogataea naganishii*
	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	*Saccharomyces cerevisiae*	*Mycoderma cerevisiae* *Candida robusta* *Saccharomyces capensis* *Saccharomyces italicus* *Saccharomyces oviformis* *Saccharomyces uvarum var. melibiosus*
Yeast Ferment Extract	Saccharomycetes	-	-	-	-	-

*ingredient has more than one associated genus and species according to the *Dictionary*, and therefore has multiple entries in this table

**synonyms include heterotypic synonyms, homotypic synonyms, and basionyms

***although this is a generic yeast ingredient, several species have been identified in unpublished literature[18,19] that correspond to "Yeast Extract"; it is unknown whether or not these species are the only species used in the formulation of Yeast Extract

NR = not reported

Table 4. Fatty acid composition of several yeast species (measured as % of total fatty acids)[20]

Fatty acid	*Candida kefyr* (synonymous to *Kluyveromyces fragilis*)	*Candida lipolytica* (synonymous to *Yarrowia lipolytica*)	*Saccharomyces cerevisiae*
decanoic (C10:0)	0.06 ± 0.01	-	6.15 ± 1.18
lauric (C12:0)	0.22 ± 0.02	-	7.59 ± 1.35
myristic (C14:0)	2.05 ± 0.13	-	1.90 ± 0.05
myristoleic (C14:1)	0.24 ± 0.05	-	0.98 ± 0.04
pentadecanoic (C15:0)	0.25 ± 0.06	0.87 ± 0.11	-
palmitic (C16:0)	20.06 ± 1.55	11.99 ± 2.23	12.72 ± 1.45
palmitoleic (C16:1)	27.46 ± 2.48	17.22 ± 1.12	51.21 ± 2.25
heptadecanoic (C17:1)	0.08 ± 0.01	2.71 ± 0.43	-
stearic (C18:0)	1.15 ± 0.04	0.77 ± 0.02	0.95 ± 0.02
cis-9-octadecanoic (C18:1(9))	24.61 ± 2.38	42.85 ± 3.65	18.50 ± 1.33
cis-11-octadecanoic (C18:1(11))	0.40 ± 0.02	0.58 ± 0.04	-
linoleic (C18:2)	19.41 ± 2.13	23.01 ± 2.15	-
linolenic (C18:3)	4.01 ± 0.66	-	-

Table 5. Nutrient, amino acid, and mineral composition of *Saccharomyces cerevisiae* and *Yarrowia lipolytica*[120]

Nutrient (%)	*Yarrowia lipolytica*	*Saccharomyces cerevisiae*
crude protein	45.5	40.34
crude fat	1.47	0.51
dry matter	97.30	97.44
ash	7.71	8.03
Amino acids (g/kg dry matter)		
lysine	30.5	7.71
methionine	6.94	6.01
threonine	15.85	13.21
tryptophan	4.01	3.98
cysteine	4.23	4.66
leucine	28.0	24.55
isoleucine	18.9	14.77
histidine	9.78	8.98
arginine	17.51	20.98
phenylalanine	18.53	19.31
Minerals (g/kg)		
calcium	4.11	2.98
phosphorous	4.87	9.44
magnesium	1.77	1.69
iron	0.111	0.099
zinc	0.071	0.066
copper	0.01	0.012

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Galactomyces Ferment Filtrate		Hydrolyzed Candida Saitoana Extract		Hydrolyzed Yeast		Hydrolyzed Yeast Extract	
Totals*	**77**	**0.072 – 90.7**	**10**	**0.02 – 3.8**	**2**	**0.00038 – 0.004**	**26**	**0.000018 – 0.035**
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	*70*	*0.072 – 90.7*	*9*	*0.02 – 3.8*	*2*	*0.00038 – 0.004*	*25*	*0.00003 – 0.035*
Rinse-Off	*7*	*5*	*1*	*NR*	*NR*	*NR*	*1*	*0.000018 – 0.0011*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type*								
Eye Area	5	0.072 – 37.5	2	0.02	NR	0.0005	1	NR
Incidental Ingestion	NR	NR	NR	NR	1	NR	NR	NR
Incidental Inhalation-Spray	31[a]; 24[b]	NR	2[a]; 4[b]	NR	1[a]	NR	10[a]; 13[b]	0.00043 – 0.0035[a]
Incidental Inhalation-Powder	24[b]	1.1	4[b]	3.8[c]	NR	0.0005[c]	13[b]	0.02[c]
Dermal Contact	76	1.1 – 90.7	10	0.02 – 3.8	1	0.00038 – 0.004	26	0.00003 – 0.02
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	1	NR	NR	NR	NR	NR	NR	0.000035 – 0.035
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	0.000018 – 0.000035
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	3	NR	NR	NR	1	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams								
Eye Makeup Preparations								
Eyeliner								
Eye Shadow								
Eye Lotion	4	37.5	1	0.02	NR	0.0005		
Eye Makeup Remover								
Mascara	NR	0.072						
Other Eye Makeup Preparations	1	NR	1	NR			1	NR
Fragrance Preparations								
Cologne and Toilet Water								
Hair Preparations (non-coloring)								
Hair Conditioner							NR	0.0011
Hair Spray (aerosol fixatives)								
Permanent Waves								
Shampoos (non-coloring)	1	NR					NR	0.000035
Tonics, Dressings, and Other Hair Grooming Aids							NR	0.00043 – 0.0035
Wave Sets								
Other Hair Preparations							NR	0.035
Hair Coloring Preparations								
Hair Dyes/Colors (all types requiring caution statements and patch tests)							NR	0.000018
Hair Rinses (coloring)							NR	0.000035
Makeup Preparations								
Blushers (all types)								
Face Powders	NR	1.1						
Foundations	NR	17.6			NR	0.00038		
Lipstick					1	NR		
Makeup Bases								

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Rouges								
Makeup Fixatives								
Other Makeup Preparations	1	NR						
Manicuring Preparations (Nail)								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices								
Personal Cleanliness Products								
Bath Soaps and Detergents	1	NR						
Deodorants (underarm)								
Feminine Deodorants	1	NR						
Other Personal Cleanliness Products	1	NR						
Shaving Preparations								
Aftershave Lotion								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing	4	5	1	NR			1	NR
Depilatories								
Face and Neck (exc shave)	23	NR	4	3.8 (not spray)	NR	0.0005 (not spray)	10	0.02 (not spray)
Body and Hand (exc shave)							3	NR
Moisturizing	24	90.7 (not spray)	1	0.02 (not spray)	1	NR	7	NR
Night		83.1 (not spray)	1	NR			1	NR
Paste Masks (mud packs)								
Skin Fresheners	7	NR					2	NR
Other Skin Care Preparations	9	NR	1	0.02	NR	0.004	1	0.00003
Suntan Preparations								
Suntan Gels, Creams, and Liquids								
	Kluyveromyces Extract		**Pichia Anomala Extract**		**Pichia Ferment Lysate Filtrate**		**Saccharomyces Cerevisiae Extract**	
Totals*	**5**	**NR**	**2**	**0.05 – 0.1**	**3**	**NR**	**56**	**0.0001 – 0.3**
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	*5*	*NR*	*2*	*0.05 – 0.1*	*3*	*NR*	*50*	*0.001 – 0.18*
Rinse-Off	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*6*	*0.0001 – 0.3*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	1	NR	NR	NR	NR	NR	16	0.00083 – 0.15
Incidental Ingestion	NR	NR	NR	NR	NR	NR	1	NR
Incidental Inhalation-Spray	1[a]; 1[b]	NR	2[a]	NR	1[a]; 2[b]	NR	11[a]; 18[b]	0.045; 0.1[a]
Incidental Inhalation-Powder	1[a]	NR	NR	NR	2[b]	NR	2; 18[b]	0.001 – 0.18[c]
Dermal Contact	5	NR	2	0.05 – 0.1	3	NR	50	0.00083 – 0.3
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	4	0.0001 – 0.001
Hair-Coloring	NR	NR	NR	NR	NR	NR	1	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	1	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams								
Eye Makeup Preparations								
Eyeliner								
Eye Shadow								
Eye Lotion	9	0.0005 – 0.0036					6	0.001 – 0.15
Eye Makeup Remover							NR	0.00083
Mascara								
Other Eye Makeup Preparations	6	NR	1	NR			10	NR
Fragrance Preparations								
Cologne and Toilet Water								
Hair Preparations (non-coloring)								
Hair Conditioner	4	0.005					NR	0.001
Hair Spray (aerosol fixatives)								
Permanent Waves								
Shampoos (non-coloring)	2	0.00025					4	0.0001
Tonics, Dressings, and Other Hair Grooming Aids	2	NR						
Wave Sets								
Other Hair Preparations	1	0.005						
Hair Coloring Preparations								
Hair Dyes/Colors (all types requiring caution statements and patch tests)							1	NR
Hair Rinses (coloring)								
Makeup Preparations								
Blushers (all types)								
Face Powders							2	NR
Foundations	NR	0.000038						
Lipstick							1	NR
Makeup Bases								
Rouges								
Makeup Fixatives								
Other Makeup Preparations							1	NR
Manicuring Preparations (Nail)								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices								
Personal Cleanliness Products								
Bath Soaps and Detergents								
Deodorants (underarm)								
Feminine Deodorants								
Other Personal Cleanliness Products								
Shaving Preparations								
Aftershave Lotion	1	NR					NR	0.025
Other Shaving Preparations	1	NR						
Skin Care Preparations								
Cleansing	4	NR					1	0.3
Depilatories								
Face and Neck (exc shave)	40	0.0005 – 0.12 (not spray)	1	NR	2	NR	18	0.001 – 0.18 (not spray)

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Body and Hand (exc shave)	3	0.19 (not spray)					NR	0.01 (not spray)
Moisturizing	19	NR	2	0.1 (not spray)	1	NR	9	0.045 (spray)
Night	4	0.002 (not spray)	NR	0.05 (not spray)			2	0.045 (not spray)
Paste Masks (mud packs)								
Skin Fresheners	2	NR					NR	0.1
Other Skin Care Preparations	11	NR					1	0.09
Suntan Preparations								
Suntan Gels, Creams, and Liquids								
	Saccharomyces Ferment		**Saccharomyces Ferment Extract Lysate Filtrate**		**Saccharomyces Ferment Filtrate**		**Saccharomyces Ferment Lysate Filtrate**	
Totals*	**42**	**0.00013 – 1.2**	**NR**	**0.25**	**48**	**0.01 – 8**	**38**	**0.0035**
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	*38*	*0.00013 – 1.2*	*NR*	*0.25*	*39*	*0.03 – 0.065*	*37*	*0.0035*
Rinse-Off	*4*	*0.002*	*NR*	*NR*	*9*	*0.01 – 8*	*1*	*0.0035*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	3	NR	NR	NR	NR	NR	6	NR
Incidental Ingestion	NR	0.00013	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	20[a]; 1[b]	NR	NR	NR	16[a]; 12[b]	0.065; 0.03[a]; 0.038[b]	2; 12[a]; 14[b]	NR
Incidental Inhalation-Powder	1[b]	NR	NR	NR	1; 12[b]	0.038[b]	14[b]	NR
Dermal Contact	41	0.72 – 1.2	NR	0.25	48	0.01 – 2.1	36	0.0035
Deodorant (underarm)	8[a]	NR	NR	NR	4[a]	NR	NR	NR
Hair - Non-Coloring	1	0.002	NR	NR	NR	0.03 – 8	2	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	1	0.00013	NR	NR	2	0.01 – 0.038	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams								
Eye Makeup Preparations								
Eyeliner								
Eye Shadow							1	NR
Eye Lotion	2	NR					3	NR
Eye Makeup Remover								
Mascara								
Other Eye Makeup Preparations	1	NR					2	NR
Fragrance Preparations								
Cologne and Toilet Water					NR	0.065		
Hair Preparations (non-coloring)								
Hair Conditioner	NR	0.002			NR	8		
Hair Spray (aerosol fixatives)							2	NR
Permanent Waves								
Shampoos (non-coloring)	1	NR						
Tonics, Dressings, and Other Hair Grooming Aids					NR	0.03		
Wave Sets								
Other Hair Preparations								

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Hair Coloring Preparations								
Hair Dyes/Colors (all types requiring caution statements and patch tests)								
Hair Rinses (coloring)								
Makeup Preparations								
Blushers (all types)	NR	1.2						
Face Powders					1	NR		
Foundations					NR	0.045		
Lipstick	NR	0.00013						
Makeup Bases								
Rouges							1	NR
Makeup Fixatives								
Other Makeup Preparations							1	NR
Manicuring Preparations (Nail)								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices								
Personal Cleanliness Products								
Bath Soaps and Detergents	8	NR						
Deodorants (underarm)					4	NR		
Feminine Deodorants					NR	0.038		
Other Personal Cleanliness Products	1	NR			2	0.01		
Shaving Preparations								
Aftershave Lotion								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing	2	NR			5	2.1	1	0.0035
Depilatories								
Face and Neck (exc shave)					11	NR	13	NR
Body and Hand (exc shave)	1	NR			1	NR	1	NR
Moisturizing	19	NR	NR	0.25 (not spray)			12	NR
Night					15	NR		
Paste Masks (mud packs)								
Skin Fresheners					2	NR		
Other Skin Care Preparations	6	0.72			6	NR	1	NR
Suntan Preparations								
Suntan Gels, Creams, and Liquids	1	NR			1	NR		

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Saccharomyces Lysate		Saccharomyces Lysate Extract		Schizosaccharomyces Ferment Filtrate		Yeast	
Totals*	**14**	**NR**	**81**	**0.0007 – 0.71**	**5**	**NR**	**11**	**NR**
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	*8*	*NR*	*76*	*0.01 – 0.71*	*5*	*NR*	*10*	*NR*
Rinse-Off	*6*	*NR*	*5*	*0.0007 – 0.0025*	*NR*	*NR*	*1*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	1	NR	10	0.013 – 0.67	NR	NR	NR	NR
Incidental Ingestion	6	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	3[a]; 3[b]	NR	20[a]; 26[b]	NR	2[a]; 1[b]	NR	1[b]	NR
Incidental Inhalation-Powder	3[b]	NR	26[b]	0.01 – 0.71[c]	1[b]	NR	1[b]	NR
Dermal Contact	8	NR	78	0.0023 – 0.71	5	NR	11	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	3	0.0007 – 0.002	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	6	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	0.067	NR	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams			NR	0.067				
Eye Makeup Preparations								
Eyeliner								
Eye Shadow								
Eye Lotion			1	0.013 – 0.67				
Eye Makeup Remover								
Mascara								
Other Eye Makeup Preparations	1	NR	9	NR				
Fragrance Preparations								
Cologne and Toilet Water								
Hair Preparations (non-coloring)								
Hair Conditioner			1	0.0007 – 0.002				
Hair Spray (aerosol fixatives)								
Permanent Waves								
Shampoos (non-coloring)			1	0.0007 – 0.002				
Tonics, Dressings, and Other Hair Grooming Aids			1	NR				
Wave Sets								
Other Hair Preparations								
Hair Coloring Preparations								
Hair Dyes/Colors (all types requiring caution statements and patch tests)								
Hair Rinses (coloring)								
Makeup Preparations								
Blushers (all types)								
Face Powders								
Foundations			1	NR				
Lipstick								
Makeup Bases			1	NR				

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Rouges								
Makeup Fixatives			1	NR				
Other Makeup Preparations			1	0.23				
Manicuring Preparations (Nail)								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices	6	NR						
Personal Cleanliness Products								
Bath Soaps and Detergents								
Deodorants (underarm)								
Feminine Deodorants								
Other Personal Cleanliness Products								
Shaving Preparations								
Aftershave Lotion			1	NR				
Other Shaving Preparations			2	NR				
Skin Care Preparations								
Cleansing			NR	0.0023 – 0.0025				
Depilatories								
Face and Neck (exc shave)	3	NR	25	0.18 – 0.71 (not spray)	1	NR	1	NR
Body and Hand (exc shave)			1	0.01 (not spray)				
Moisturizing	3	NR	15	0.025 (not spray)	2	NR		
Night			3	NR				
Paste Masks (mud packs)			1	NR			1	NR
Skin Fresheners			1	NR				
Other Skin Care Preparations	1	NR	15	NR	2	NR	9	NR
Suntan Preparations								
Suntan Gels, Creams, and Liquids								
	Yeast Extract		**Yeast Ferment Extract**					
Totals*	**398**	**0.0000036 – 0.16**	**15**	**NR**				
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	*343*	*0.0000036 – 0.16*	*12*	*NR*				
Rinse-Off	*55*	*0.0001 – 0.01*	*3*	*NR*				
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*				
Exposure Type								
Eye Area	25	0.001 – 0.15	NR	NR				
Incidental Ingestion	1	0.00072 – 0.002	NR	NR				
Incidental Inhalation-Spray	2; 125[a]; 133[b]	0.065; 0.00001 – 0.03[a]; 0.038[b]	6[a]; 4[b]	NR				
Incidental Inhalation-Powder	133[b]	0.0000036 – 0.021; 0.038[b]; 0.0036 – 0.16[c]	4[b]	NR				
Dermal Contact	334	0.0000036 – 0.16	14	NR				
Deodorant (underarm)	NR	NR	NR	NR				
Hair - Non-Coloring	62	0.0001 – 0.03	1	NR				
Hair-Coloring	NR	NR	NR	NR				
Nail	1	NR	NR	NR				
Mucous Membrane	1	0.0007 – 0.038	1	NR				
Baby Products	NR	NR	NR	NR				

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Baby Products								
Baby Lotions/Oils/Powders/Creams								
Eye Makeup Preparations								
Eyeliner	NR	0.002						
Eye Shadow	NR	0.001 – 0.002						
Eye Lotion	12	0.038 – 0.15						
Eye Makeup Remover	NR	0.0048 – 0.0048						
Mascara	NR	0.024						
Other Eye Makeup Preparations	13	NR						
Fragrance Preparations								
Cologne and Toilet Water	NR	0.065						
Hair Preparations (non-coloring)								
Hair Conditioner	22	0.0001						
Hair Spray (aerosol fixatives)	2	NR						
Permanent Waves	NR	0.01						
Rinses (non-coloring)								
Tonics, Dressings, and Other Hair Grooming Aids	13	0.009 – 0.03						
Wave Sets								
Other Hair Preparations	11	0.01	1	NR				
Hair Coloring Preparations								
Hair Dyes/Colors (all types requiring caution statements and patch tests)								
Hair Rinses (coloring)								
Makeup Preparations								
Blushers (all types)								
Face Powders	NR	0.0000036 – 0.021						
Foundations	5	0.0014 – 0.038						
Lipstick	NR	0.00072 – 0.002						
Makeup Bases	6	NR						
Rouges								
Makeup Fixatives	1	NR						
Other Makeup Preparations	4	NR						
Manicuring Preparations (Nail)								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices								
Personal Cleanliness Products								
Bath Soaps and Detergents	NR	0.0007	1	NR				
Deodorants (underarm)								
Feminine Deodorants	NR	0.038						
Other Personal Cleanliness Products	NR	0.01						
Shaving Preparations								
Aftershave Lotion	NR	0.025						
Other Shaving Preparations	1	NR						
Skin Care Preparations								
Cleansing	12	0.0007 – 0.0036	2	NR				
Depilatories								
Face and Neck (exc shave)	117	0.0036 – 0.16 (not spray)	4	NR				

Table 6. Frequency (2023)[34] and concentration (2021/2023)[35,121,122] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Body and Hand (exc shave)	16	0.0074 – 0.042 (not spray)						
Moisturizing	83	NR	6	NR				
Night	22	NR						
Paste Masks (mud packs)	5	NR						
Skin Fresheners	6	0.00001 – 0.0036						
Other Skin Care Preparations	31	0.0036 – 0.14	1	NR				
Suntan Preparations								
Suntan Gels, Creams, and Liquids								

NR – not reported

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

**likely duration and exposure is derived 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 7. Yeast-derived not reported to be use according to 2023 frequency of use and 2021/2023 concentration of use data

Hydrolyzed Candida Bombicola Extract	Pichia Heedii Extract
Hydrolyzed Kluyveromyces Extract	Pichia Minuta Extract
Hydrolyzed Metschnikowia Agaves Extract	Pichia Pastoris Ferment Filtrate
Hydrolyzed Metschnikowia Reukaufii Extract	Phaffia Rhodozyma Extract
Hydrolyzed Metschnikowia Shanxiensis Extract	Phaffia Rhodozyma Ferment Extract
Hydrolyzed Saccharomyces Cell Wall	Saccharomyces
Hydrolyzed Saccharomyces Extract	Saccharomyces Extract
Hydrolyzed Saccharomyces Lysate Extract	Saccharomyces Ferment Extract
Hydrolyzed Torulaspora Delbruekii Extract	Saccharomyces Ferment Lysate Extract
Lactic Yeasts	Saccharomyces Lysate Extract Filtrate
Lipomyces Lipid Bodies	Saccharomyces Lysate Filtrate
Lipomyces Oil	Schizosaccharomyces Ferment Extract Filtrate
Lipomyces Oil Extract	Schizosaccharomyces Pombe Extract
Metschnikowia Agaves Extract	Torulaspora Delbrueckii Extract
Metschnikowia Henanensis Extract	Torulaspora Delbrueckii Ferment
Metschnikowia Reukaufii Lysate Extract	Yarrowia Lipolytica Extract
Metschnikowia Viticola Extract	Yarrowia Lipolytica Ferment Lysate
Pichia Caribbica Ferment	Yarrowia Lipolytica Oil
Pichia Extract	
Pichia Ferment Extract Filtrate	

`Table 8. Food use/presence and non-cosmetic uses of yeast species

Associated Ingredients	Food Use/Presence	Other Non-Cosmetic Uses	Reference
Galactomyces Ferment Filtrate	*Geotrichum candidum* is used as an adjunct culture in the maturation of cheese *Galactomyces geotrichum* is found in alcohols and dairy products	*Galactomyces geotrichum* is used in biodegradation and bioremediation processes	123,124
Hydrolyzed Candida Bombicola Extract	*Starmerella bombicola* is naturally present in concentrated grape juice and in high-sugar fermented vegetables and honey	*Candida bombicola* produces sophorolipids which may be used as a biosurfactant in food, pharmaceutical, and cleaning industries	125
Hydrolyzed Candida Saitoana Extract	*Candida saitoana* may be found in plant-based fermented foods	*Candida saitoana* is used as a biocontrol treatment of post-harvest disease in apples and citrus fruit	126,127
Hydrolyzed Kluyveromyces Extract Kluyveromyces Extract	*Kluyveromyces marxianus* is present in Korean kefir and other dairy products Lactase enzyme preparation from *Kluyveromyces lactis* is GRAS for use in hydrolyzing lactose in milk [21CFR184] Rennet and chymosin preparation from *Kluyveromyces marxianus* to coagulate milk in cheeses and other dairy products is considered GRAS [21CFR184] *Kluyveromyces lactis* - QPS status *Kluyveromyces marxianus* – QPS status	*Kluyveromyces marxianus* is used in biotechnological (e.g., native enzyme production, inulinase production) and environmental applications (e.g., heavy metal recovery from agricultural industry wastewater) *Kluyveromyces marxianus* may be used as a probiotic	43,128-131
Hydrolyzed Metschnikowia Agaves Extract Metschnikowia Agaves Extract	*Metschnikowia agaves* can be found in blue agave used to make tequila	-	132
Hydrolyzed Metschnikowia Reukaufii Extract Metschnikowia Reukaufii Lysate Extract Yeast Extract derived from *Metschnikowia reukaufii*	*Metschnikowia reukaufii* is used in beer fermentation	-	133
Hydrolyzed Torulaspora Delbrueckii Extract Torulaspora Delbrueckii Extract Torulaspora Delbrueckii Ferment	*Torulaspora delbrueckii* is used in the production of breads/bakery products, chocolate, coffee, and fermented beverages *Torulaspora delbrueckii* may be present in cheese	-	134-136
Lipomyces Oil Lipomyces Oil Extract	*Lipomyces starkeyi* is GRAS in probiotics	-	131,137
Metschnikowia Viticola Extract	*Metschnikowia viticola may be present in wine* *Metschnikowia viticola has been isolated from grapes grown in Hungary*	-	138,139
Pichia Anomala Extract	*Wickerhamomyces anomalus* is used in Chinese liquor production and soy sauce *Pichia anomala* is commonly found in fermented food and beverages and may be used as a food-flavoring agent *Pichia anomala* – QPS status	*Pichia anomala* may be used as a biopreservative	131,140-143
Pichia Caribbica Ferment	Kombucha tea culture is a symbiosis of several substances, including *Pichia caribbica* *Pichia caribbica* may be used in the production of alcoholic beverages *Pichia caribbica* has been isolated from Brazilian fermented table olives	*Picha caribbica* may be used to produce malic acid	144-146
Pichia Ferment Extract Filtrate Pichia Pastoris Ferment Filtrate	The following substances are considered GRAS: -Pepsin A enzyme preparation produced by *Pichia pastoris* to overexpress the gene encoding pepsin A -Myoglobin preparation from a strain of *Pichia pastoris* expressing the myoglobin gene from *Bos taurus*	-	147

`Table 8. Food use/presence and non-cosmetic uses of yeast species

Associated Ingredients	Food Use/Presence	Other Non-Cosmetic Uses	Reference
	-Soy leghemoglobin preparation from a strain of *Pichia pastoris* -Soybean leghemoglobin from *Pichia pastoris* -Phospholipase C enzyme preparation from *Pichia pastoris* expressing a heterologous phospholipase C gene *Pichia pastoris* – QPS status		
Pichia Ferment Lysate Filtrate	-	-*Pichia stipitis* is capable of fermenting glucose, xylose, galactose, cellulobiose, and fermentose -*Pichia stipitis* may be used in the production of bioethanol -*Pichia populi* has been used in the production of arabitol-free xylitol	148-152
Pichia Heedii Extract	-	*Pichia heedii* may be used to assimilate D-xylose	153
Pichia Minuta Extract	*Pichia minuta* may be found in wine	*Pichia minuta* has been isolated from olive tree cultures	154,155
Phaffia Rhodozyma Extract Phaffia Rhodozyma Ferment Extract	*Phaffia rhodozyma* – QPS status	Astaxanthin-rich *Phaffia rhodozyma* may be used in feed for salmon and trout	131,156
Saccharomyces Cerevisiae Extract	*Saccharomyces cerevisiae* is used in baking and alcohol production as a fermentative agent Baker's yeast extract (mechanically ruptured cells of *Saccharomyces cerevisiae*) is GRAS as a flavoring agent and adjuvant at a level not to exceed 5% in food [21CFR184.1983] Dried yeast (*Saccharomyces cerevisiae*) is considered to be GRAS as a multipurpose food additive [21CFR172.896] Baker's yeast glycan (derived from dried cell walls of *Saccharomyces cerevisiae*) is approved as a direct food additive for human consumption when used as described in 21CFR172.898 (e.g., not to exceed a concentration of 5% in finished salad dressing) *Saccharomyces cerevisiae* – QPS status	Inactivated yeast (*Saccharomyces cerevisiae*) cells are used in animal feed and over-the-counter nutritional supplements	37,131
Schizosaccharomyces Pombe Extract	*Schizosaccharomyces pombe* is used in cachaça (alcoholic beverage made from fermented sugarcane juice) and kombucha *Schizosaccharomyces pombe* – QPS status	-	131,134
Yarrowia Lipolytica Extract Yarrowia Lipolytica Ferment Lysate Yarrowia Lipolytica Oil	*Yarrowia lipolytica* has been found in a variety of different cheeses; predominantly ewe, goat, and buffalo cheese *Yarrowia lipolytica* is also found in other fermented dairy (e.g., yogurt) and meat (e.g., salami) products Eicosapentaenoic acid -rich triglyceride oil *from Yarrowia lipolytica* is considered GRAS at a maximum intake of 3.0 g per person per day eicosapentaenoic acid and not to be combined or augmented with any other food ingredient containing eicosapentaenoic acid and/or another omega-3 fatty acid, docosahexaenoic acid [21 CFR 184.1472] *Yarrowia lipolytica* is GRAS for commercial production of food grade citric acid [21 CFR 173.165] *Yarrowia lipolytica* – QPS status	*Yarrowia lipolytica* is used in livestock feed, a biotechnological production host for organic acids or hydrophobic substances or carotenoids, a heterologous production host for pharmaceutical and industrial proteins and enzymes, for the mass production of biofuels, and for bioremediation purposes Oil produced by *Yarrowia lipolytica* may be used in the agro-alimentary, pharmaceutical, and bioenergy industry	131,157,158

`Table 8. Food use/presence and non-cosmetic uses of yeast species

Associated Ingredients	Food Use/Presence	Other Non-Cosmetic Uses	Reference
Yeast Extract (when derived from *Candida oleophila*)	*Candida oleophila* is naturally found on plant tissues that are commonly consumed (e.g., apples) – this species is also used in fruits to control fungal pathogens *Candida oleophila* may be present in alcoholic beverages	-	41,127
Yeast Extract (when derived from *Candida magnoliae*)	*Candida magnoliae* has been isolated from lime honey and honeycomb	-	159-161
Yeast Extract (when derived from *Debaryomyces nepalensis*)	*Debaryomyces nepalensis* has been isolated from persimmon fruit, passion fruit, avocado, and cape gooseberry	*Debaryomyces nepalensis* may be used in the production of solutes, haloenzymes, alcoholic beverages, and in biological waste treatment *Debaryomyces nepalensis* may be used as a biocontrol agent in fruit and cheese *Debaryomyces nepalensis* may be used in the production of xylitol	162-167
Yeast Extract (when derived from *Metschnikowia pulcherrima*)	*Metschnikowia pulcherrima* may be present in alcoholic beverages and coffee	*Metschnikowia pulcherrima* may be used to produce D-arabitol	168,169

GRAS = generally recognized as safe; QPS = qualified presumption of safety

Table 9. In vitro dermal absorption studies

Ingredient	Test Article	Concentration/Dose	Protocol	Results	References
Metschnikowia Agaves Extract	emulsion containing Metschnikowia Agaves Extract	30%	OECD TG 428	Absorption of 2.4% of the total quantity applied to the surface of the epidermis after 24 h	[19]
Pichia Anomala Extract	emulsion containing Pichia Anomala Extract	30%	OECD TG 428	Absorption of 0.7% of the total quantity applied to the surface of the epidermis after 24 h	[19]
Pichia Anomala Extract	emulsion containing Pichia Anomala Extract	30%	OECD TG 428	Absorption of 0.41% of the total quantity applied to the surface of the epidermis after 24 h	[19]
Pichia Heedii Extract	emulsion containing Pichia Heedii Extract	30%	OECD TG 428	Absorption of 0.2% of the total quantity applied to the surface of the epidermis after 24 h	[19]
Pichia Minuta Extract	emulsion containing Pichia Minuta Extract	30%	OECD TG 428	Absorption of 0.6% of the total quantity applied to the surface of the epidermis after 24 h	[19]
Yeast Extract (may also be chemically similar to Hydrolyzed Candida Saitoana Extract)	emulsion containing Yeast Extract derived from *Candida saitoana*	30%	OECD TG 428	Absorption of 1.1% of the total quantity applied to the surface of the epidermis after 24 h	[19]
Yeast Extract (may also be chemically similar to Hydrolyzed Metschnikowia Reukaufii Extract)	emulsion containing Yeast Extract derived from *Metschnikowia reukaufii*	30%	OECD TG 428	Absorption of 4.6% of the total quantity applied to the surface of the epidermis after 24 h	[19]

NR = not reported; OECD TG = Organisation for Economic Co-operation and Development test guidelines

Table 10. Acute toxicity studies*

Ingredient	Test Article	Vehicle	Test Population	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
IN VITRO							
Pichia Minuta Extract	Pichia Minuta Extract	NR	murine fibroblast cell line, BALB/c 3T3 cells, clone 31	8 test concentrations (specific concentrations not stated)	3T3 neutral red uptake assay; OECD TG 129	LD_{50} > 2000 mg/kg	[38]
Yeast Extract (when derived from *Pichia naganishii*)	Yeast Extract (derived from *Pichia naganishii*)	NR	murine fibroblast cell line, BALB/c 3T3 cells, clone 31	8 test concentrations (specific concentrations not stated)	3T3 neutral red uptake assay; OECD TG 129	LD_{50} > 2000 mg/kg	[38]
ANIMAL							
Dermal							
Hydrolyzed Saccharomyces Cell Wall	90% *Saccharomyces cerevisiae* cell wall (containing 24% glucan and 7% mannan)**	10% HSCAS	Sprague-Dawley rats (5/sex/group)	2000 mg/kg bw; 55% dilution (final test concentration of 49.5% yeast cell wall)	Test article applied to gauze pad and placed on clipped, dorsal/trunk area of animal; pads wrapped; 24 h administration period; 14 d evaluation period	No mortalities or signs or gross toxicity, dermal irritation, adverse pharmacological effects, or abnormal behaviors were noted. The acute dermal LD_{50} of a 55% dilution of the test article was determined to be > 2000 mg/kg bw.	[4]
Saccharomyces Cerevisiae Extract	*Saccharomyces cerevisiae* extract**	Water	Crl:WI (Han) rats (5/sex)	2000 mg/kg	OECD TG 402; occlusive conditions; 24 h administration period; observation for 14 d	Two males and two females showed chromodacryorrhoea on day 1 (24 h after treatment). In addition, one male showed hunched posture on day 1. Two females had scales or focal erythema in the treated skin area during the observation period. No other abnormalities were noted; LD_{50} was determined to be > 2000 mg/kg bw.	[2]

Table 10. Acute toxicity studies*

Ingredient	Test Article	Vehicle	Test Population	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
Oral							
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate**	NR	ddY-N mice (10/sex/group)	34,730, 41,670, 50,000, 60,000 mg/kg	Administration via gavage	No mortality or adverse effects observed; LD_{50} determined to be > 60,000 mg/kg	39
Hydrolyzed Yeast	Yeast hydrolysate obtained from *Saccharomyces cerevisiae***	NR	Sprague-Dawley rats (5/sex/group)	5000 mg/kg bw	OECD TG 420; gavage administration; 14-d observation period	No mortality or adverse effects observed.	30
Hydrolyzed Saccharomyces Cell Wall	90% *Saccharomyces cerevisiae* cell wall (containing 24% glucan and 7% mannan)**	10 HSCAS and distilled water	Sprague-Dawley rats (5/sex/group)	2000 mg/kg bw; 55% dilution (final test concentration of 49.5% yeast cell wall)	Administration via gavage; 14-d observation period	No mortalities were observed throughout the study. One female exhibited reduced fecal volume, however, this animal recovered by day 2. No other signs of toxicity were noted.	4
Saccharomyces Ferment	Fermentate powder derived from *Saccharomyces cerevisiae***	methylcellulose and water	Sprague-Dawley rats (10/sex/group)	2000 mg/kg bw	OECD TG 423; gavage administration; 14-d observation period	No signs of toxicity observed.	40
Yeast Extract (when derived from *Candida oleophila*)	*Candida oleophila* strain O**	NR	rats (species, sex, and number of animals not specified)	2.3 - 3.8 x 10^8 CFU	Animals given single oral dose of the test substance (method of oral administration not stated). Animals were observed for 22 d.	Test substance was not considered to be toxic, infective, or pathogenic	41
Inhalation							
Hydrolyzed Saccharomyces Cell Wall	90% *Saccharomyces cerevisiae* cell wall (containing 24% glucan and 7% mannan)**	10% HSCAS and distilled water	Sprague-Dawley rats (5/sex/group)	Gravimetric and nominal chamber concentrations were 2.09 and 5.81 mg/l, respectively	OECD TG 403; mass median aerodynamic diameter estimated to be 3.75 µm; 14-d observation period	Two males and 2 females exhibited irregular respiration and hypoactive behavior following exposure; however, these animals recovered by day 5. No gross abnormalities were observed upon necropsy, and no other adverse effects were noted; LC_{50} was determined to be > 2.09 mg/l in male and female rats.	4
Yeast Extract (when derived from *Candida oleophila*)	*Candida oleophila* strain O**	NR	rats (species, sex, and number of animals not specified)	1.2 -5.2 x 10^8 CFU	Animals exposed to test substance via intratracheal route and observed for 22 d	Test substance was not considered to be toxic, infective, or pathogenic	41
Parenteral							
Pichia Ferment Extract Filtrate and Pichia Pastoris Ferment Filtrate	Live *Pichia pastoris* cells**	sterile saline	female BALB/c mice (20/group)	1 × 10^6 CFU	Intravenous administration of the test substance via the lateral tail vein; control group one received inoculation with saline; control group two was left untreated; body weight and behavior monitored; 5 mice/group were euthanized at 4, 24, and 48 h and 6 d post-administration; samples of sera and tissues (kidney, liver, brain, spleen, heart, and lung) were collected	Results were similar among control and treated groups (no adverse effects relating to body weight, survival, or locomotion changes); no adverse effects related to pathology in tissues were noted	42
Yeast Extract (when derived from *Candida oleophila*)	*Candida oleophila* strain O**	NR	rats (species, sex, and number of animals not specified)	1.1-2.0 x 10^7 CFU	Animals subcutaneously injected with test substance and observed for 22 d	Test substance was not considered to be toxic, infective, or pathogenic	41

CFU = colony-forming units; LC_{50} = median lethal concentration; LD_{50} = median lethal dose; NR = not reported; OECD TG = Organisation for Economic Co-operation and Development test guidelines

*It should be noted that the test articles evaluated in these studies may not be identical to the wINCI ingredients reviewed in this report; however, as they may be similar, both test articles and potentially-related wINCI ingredients have been included in the table

**unknown if test substance is a cosmetic ingredient (e.g., *Candida oleophila* strain O); however, ingredient relates to INCI ingredient reviewed in this report (Yeast Extract (when derived from *Candida oleophila*)

Table 11. Repeated dose oral toxicity studies*

Ingredient	Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Hydrolyzed Yeast	Yeast hydrolysate obtained from *Saccharomyces cerevisiae***	NR	Sprague-Dawley rats (5/sex/group)	14 d	1000 mg/kg bw/d	OECD TG 407; animals administered test substance orally (method of oral administration not stated); animals killed after treatment period; control animals given water; satellite group treated with the test substance, at the same dose, at the same time period, and kept for another 14 d post-treatment for observation	No significant differences in organ weights between control and treated groups were noted. No adverse hematological effects, gross abnormalities, or histopathological changes were observed. Treatment with the test substance induced significant increases in body weight compared to the control group ($p < 0.05$).	30
Kluyveromyces Extract	*Kluyveromyces marxianus* strains A4 and A5**	sterilized saline	female SPF BALB/c mice (6/group)	14 d	1.0 x 10^6 CFU/ml or 1.0 x 10^8 CFU/ml	Animals were orally administered the test substance (method of oral administration not stated); negative control group left untreated; another negative control group treated with saline only	No adverse effects relating to body weight or food and water intake were observed. The spleen to body ratio of the A5 strain (high concentration)-treated group was significantly lower than that of the untreated negative control group ($p < 0.05$). The liver to body weight ration of the A4 strain (low concentration)-treated group was significantly lower than that of the untreated negative control group ($p < 0.05$). All blood parameters and cytokine parameters (interleukin-1β and tumor necrosis factor-α) were comparable between treated and negative control groups.	43
Phaffia Rhodozyma Extract and Phaffia Rhodozyma Ferment Extract	*Phaffia rhodozyma* extract**	corn oil	Sprague-Dawley rats (6/sex/group)	28 d	3 ml/kg; 500 and 1000 mg/kg	OECD TG 407; gavage administration 6 d/wk; control group given corn oil	Decreased body weight was observed in females in the 1000 mg/kg treated group; increased ALT levels and relative liver weights were observed in females in the 1000 mg/kg group ($p < 0.05$); absolute and relative thymus weights tended to increase in males of the 1000 mg/kg group; no other toxicologically-relevant adverse effects were observed; NOAEL > 1000 mg/kg	44
Saccharomyces Ferment	Fermentate powder derived from *Saccharomyces cerevisiae***	methylcellulose and water	Sprague-Dawley rats (20/sex/group)	90 d	30, 200, and 1500 mg/kg bw/d	OECD TG 408; gavage treatment once per day; control group used, however, details regarding treatment not provided	No treatment-related toxicity was observed regarding general state, behavior, external appearance, body weight, ophthalmologic changes, urine analysis, organ weights, or histopathology. A dose-related slight decrease in total cholesterol was observed in male rats of the high-dose (not observed in females); NOAEL = 1500 mg/kg bw/d	40
Saccharomyces Ferment	Fermentate powder derived from *Saccharomyces cerevisiae***	methylcellulose and water	Sprague-Dawley rats (20/sex/group)	1 yr	20, 200, and 800 mg/kg bw/d	OECD TG 408 and 452; gavage administration; control group used, however, details regarding treatment not provided	No macroscopic or microscopic, serum chemistry, hematological, urinary, or histological adverse effects were observed to be of clinical significance. A statistically significant decrease in water consumption over nonconsecutive weeks was observed in the highest dose group; NOAEL = 800 mg/kg bw/d	40

ALT = alanine aminotransferase; CFU = colony-forming units; NOAEL = no-observed-adverse-effect level; OECD = Organisation for Economic Co-operation and Development; TG = test guidelines

*It should be noted that the test articles evaluated in these studies may not be identical to the wINCI ingredients reviewed in this report; however, as they may be similar, both test articles and potentially related wINCI ingredients have been included in the table

**unknown if test substance is a cosmetic ingredient (e.g., *Candida oleophila* strain O); however, ingredient relates to INCI ingredient reviewed in this report (Yeast Extract (when derived from *Candida oleophila*)

Table 12. Genotoxicity studies*

Ingredient	Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VITRO				
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate**	sterile water	10, 50, 100, 500, 1000, 2500, 5000, and 10,000 µg/plate	*S. typhimurium strains* TA98, TA100, TA1538, and TA1535*; E. coli* WP2 urvA	Ames assay; performed with and without metabolic activation; vehicle used as negative control; positive controls: AF-2, ENNG, 9-AA, and 2-NF	Non-genotoxic; controls gave expected results	47
Hydrolyzed Saccharomyces Cell Wall	90% yeast (*Saccharomyces cerevisiae*) cell wall (containing 24% glucan and 7% mannan)**	HSCAS	3.4, 10.3, 30.98, 92.6, 277.8, 833.3, and 2500 µg/plate	*S. typhimurium* strains TA1535, TA1537, TA98, and TA102	Ames assay; OECD TG 471; performed with and without metabolic activation; vehicle used as negative control; positive controls: sodium azide, 9-aminoacridine, 2-nitro fluorene, mitomycin C, 2-anthramine, and benzo[a]pyrene	Non-genotoxic; controls gave expected results	4
Phaffia Rhodozyma Extract and Phaffia Rhodozyma Ferment Extract	*Phaffia rhodozyma* extract**	acetone	25 µl; 1.22 – 5000 µg/ plate	*S. typhimurium* strains TA 98 and TA100	Ames assay; OECD TG 471; performed with and without metabolic activation; vehicle used as negative control; positive controls: AF-2 and 2-AA	Non-genotoxic; controls gave expected results	44
Phaffia Rhodozyma Extract and Phaffia Rhodozyma Ferment Extract	trade name mixture containing 49% Phaffia Rhodozyma Extract	sterile water	1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate	*S. typhimurium* strains TA98, TA100, TA1537, and TA1535*; E. coli* WP2 *urvA*	Ames assay; OECD TG 471; performed with and without metabolic activation; vehicle used as negative control; positive controls: 2-AA and 2-NF, sodium azide, 2-aminoacridine, methylmethanesulfonate	Non-mutagenic; controls gave expected results	45
Pichia Minuta Extract	Pichia Minuta Extract	NR	At least 5 concentrations tested	4 strains of *S. typhimurium*; one strain of *E. coli* (specific strains not stated)	Ames assay; OECD TG 471	Non-mutagenic	38
Pichia Minuta Extract	Pichia Minuta Extract	NR	NR	TK6 lymphoblastoid human cells	micronucleus assay	Non-mutagenic	38
Saccharomyces Ferment	fermentate powder derived from *Saccharomyces cerevisiae***	methylcellulose and water	5, 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate	*S. typhimurium* strains TA97a, TA98, TA100, and TA1535; *E. coli* WP2 *urvA*	Ames assay; OECD TG 471; performed with and without metabolic activation; negative control: sterile water	Non-genotoxic; controls gave expected results	40
Saccharomyces Ferment	fermentate powder derived from *Saccharomyces cerevisiae***	methylcellulose and water	up to 5000 µg/ml (specific concentrations tested not stated)	mouse lymphoma L5178Y cell line	mammalian cell gene mutation assay; OECD TG 476; positive controls: methyl methanesulfonate and cyclophosphamide	Non-genotoxic; controls gave expected results	40
Saccharomyces Ferment Lysate Filtrate	trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate	sterile water	1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate	*S. typhimurium* strains TA98, TA100, TA1537, and TA1535*; E. coli* WP2 *urvA*	Ames assay; OECD TG 471; performed with and without metabolic activation; vehicle used as negative control; positive controls: 2-AA and 2-NF, sodium azide, 2-aminoacridine, methylmethanesulfonate	Non-mutagenic; controls gave expected results	46
Yeast Extract (when derived from *Candida oleophila*)	*Candida oleophila* strain O**	NR	at least 5 concentrations tested	4 strains of *S. typhimurium*; one strain of *E. coli* (specific strains not stated)	Ames assay performed with and without metabolic activation; OPPTS Guideline 870.5100	Non-mutagenic	41
Yeast Extract (when derived from *Candida oleophila*)	*Candida oleophila* strain O**	NR	at least 4 concentrations tested	NR	mammalian cell gene mutation assay performed with and without metabolic activation; OPPTS Guideline 870.5300	Non-mutagenic	41
Yeast Extract (when derived from *Pichia naganishii*)	Yeast Extract (derived from *Pichia naganishii*)	NR	at least 5 concentrations tested	4 strains of *S. typhimurium*; one strain of *E. coli* (specific strains not stated)	Ames assay; OECD TG 471	Non-mutagenic	38
Yeast Extract (when derived from *Pichia naganishii*)	Yeast Extract (derived from *Pichia naganishii*)	NR	NR	L5178Y TK+/- mouse lymphoma cells	micronucleus assay	Non-mutagenic	38

Table 12. Genotoxicity studies*

Ingredient	Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VIVO				
Phaffia Rhodozyma Extract and Phaffia Rhodozyma Ferment Extract	*Phaffia rhodozyma* extract**	corn oil	500, 1000, and 2000 mg/kg bw/d	male ICR mice (3/group)	mammalian bone marrow chromosomal aberration assay; OECD TG 475; negative control group received corn oil orally (method of oral administration not stated); once a day treatment for 2 d; positive control group received injection of mitomycin C	Non-clastogenic; controls gave expected results	44
Hydrolyzed Saccharomyces Cell Wall	90% yeast (*Saccharomyces cerevisiae*) cell wall (containing 24% glucan and 7% mannan)**	HSCAS	500, 1000, and 2000 mg/kg bw/d	Swiss ICO OF1 mice (28/sex/group)	mammalian bone marrow chromosomal aberration assay; OECD TG 475; gavage administration; once a day treatment for 2 d; negative control: 0.5% methylcellulose in purified water; positive control group: cyclophosphamide in 0.9% saline	Non-clastogenic; controls gave expected results	4

2-AA = 2-aminoanthracene; 2-NF = 2-nitrofluorene; 9-AA = 9-aminoadridine; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide; ENNG = 1-ethyl-2-nitro-3-nitrosguanidine; NR = not reported; OECD TG = Organisation for Economic Co-operation and Development test guidelines; OPPTS = Office of Prevention, Pesticides, and Toxic Substances

*It should be noted that the test articles evaluated in these studies may not be identical to the wINCI ingredients reviewed in this report; however, as they may be similar, both test articles and potentially related wINCI ingredients have been included in the table

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			IRRITATION			
			In Vitro			
Phaffia Rhodozyma Extract	trade name mixture containing 49% Phaffia Rhodozyma Extract	tested neat; 30 µl	reconstructed human epidermal model (EpiDerm™)	EpiDerm™ assay; 3 tissue inserts incubated with test substance for 60 min, followed by washing, re-plating, and MTT assay; negative control of PBS; positive control of sodium dodecyl sulfate	non-irritating	63
Saccharomyces Cerevisiae Extract	powdered *Saccharomyces cerevisiae* extract****	tested neat; 10 mg moistened with 5 µl water	human three-dimensional epidermal model (EpiSkin™)	human epidermis model; negative control of PBS; positive control of sodium dodecyl sulfate; 15 min exposure followed by 42-h recovery period; colorimetric measurement of MTT reduction was used as index of cell viability	non-irritating	2
Saccharomyces Cerevisiae Extract	trade name mixture containing 1.25% Saccharomyces Cerevisiae Extract	tested neat; 30 µl	reconstructed human epidermal model (EpiDerm™)	EpiDerm™ assay; 3 tissue inserts incubated with test substance for 60 min, followed by washing, re-plating, and MTT assay; negative control of PBS; positive control of sodium dodecyl sulfate	non-irritating	64
Saccharomyces Cerevisiae Extract	trade name mixture containing 3% Saccharomyces Cerevisiae Extract	tested neat; 30 µl	reconstructed human epidermal model (EpiDerm™)	EpiDerm™ assay; 3 tissue inserts incubated with test substance for 60 min, followed by washing, re-plating, and MTT assay; negative control of PBS; positive control of sodium dodecyl sulfate	non-irritating	97
Saccharomyces Cerevisiae Extract	trade name mixture containing 4.5% Saccharomyces Cerevisiae Extract	25, 50, 75, 100, and 135 µl	Irritection® system**	Test substance applied to membrane for 24 h; irritancy measured via a spectrophotometer	non-irritating	62

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Saccharomyces Ferment Lysate Filtrate	trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate	tested neat; 30 µl	reconstructed human epidermal model (EpiDerm™)	EpiDerm™ assay; 3 tissue inserts incubated with test substance for 60 min, followed by washing, re-plating, and MTT assay; negative control of PBS; positive control of sodium dodecyl sulfate	non-irritating	65
Saccharomyces Lysate Extract	trade name mixture containing 10% Saccharomyces Lysate Extract	tested neat; 30 µl	reconstructed human epidermal model (EpiDerm™)	EpiDerm™ assay; 3 tissue inserts incubated with test substance for 60 min, followed by washing, re-plating, and MTT assay; negative control of PBS; positive control of sodium dodecyl sulfate	non-irritating	68
Saccharomyces Lysate Extract	trade name mixture containing 98% Saccharomyces Lysate Extract	tested neat; 30 µl	reconstructed human epidermal model (EpiDerm™)	EpiDerm™ assay; 3 tissue inserts incubated with test substance for 60 min, followed by washing, re-plating, and MTT assay; negative control of PBS; positive control of sodium dodecyl sulfate	non-irritating	66
Animal						
Hydrolyzed Saccharomyces Cell Wall	mixture containing 90% yeast (*Saccharomyces cerevisiae*) cell wall (24% glucan and 7% mannan) in 10% HSCAS****	55%; moistened with distilled water	3 male New Zealand albino rabbits	Test substance mixture (0.91 g) was placed on gauze pad and applied to one 6 cm^2 dose site on each animal. The pad was wrapped under semi-occlusive conditions. Pads were kept on for 4 h. Erythema and edema were evaluated 30 - 60 min, 24, 48, and 72 h after patch removal. Sites were scored according to the Draize scoring system.	Slight erythema noted within 30 - 60 min after dressing removal; primary dermal irritation of 0.1; classified as slightly irritating	4
Yeast Extract (when derived from *Candida oleophila*)	non-cosmetic product containing *Candida oleophila* strain O (as an active ingredient at 57% by weight)****	100%; 0.5 g	3 rabbits (sex and strain not stated)	primary dermal irritation study; application to 25 mm x 25 mm area for 4 h; level of occlusion not stated; animals observed for 72 h; irritation scored by Draize method	non-irritating; primary irritation index: 0	41
Human						
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate****	NR	45 subjects	continuous skin irritation test; gauze (10 cm^2) containing test substance applied to cheek for 15 min, once per day, for 40 d; level of occlusion not stated	No adverse reactions observed.	70
Lipomyces Lipid Bodies and Lipomyces Oil	cream consisting of 100% Lipomyces Lipid Bodies***	100%	NR	4-wk dermal exposure; subjects used cream on face and hands for an average period of 27.6 d	The test substance was considered to be well-tolerated	24
Metschnikowia Agaves Extract	Metschnikowia Agaves Extract	15% in water	11 subjects	patch test; no other details provided	non-irritating	19
Pichia Anomala Extract	Pichia Anomala Extract	15% in water	10 subjects	patch test; no other details provided	non-irritating	19
Pichia Anomala Extract	Pichia Anomala Extract	15% in water	10 subjects	patch test; no other details provided	non-irritating	19
Pichia Heedii Extract	Pichia Heedii Extract	15% in water	10 subjects	patch test; no other details provided	non-irritating	19
Pichia Minuta Extract	Pichia Minuta Extract	15% in water	11 subjects	patch test; no other details provided	non-irritating	19
Saccharomyces Cerevisiae Extract	cosmetic formulation containing 1% Saccharomyces Cerevisiae Extract	tested neat	28 subjects	20 µl were applied to the skin, under an occlusive patch, for 48 h; skin irritation was evaluated for irritation 15 min and 48 h after patch removal	Slight erythema noted in one volunteer 15 min after patch removal; however, no reaction was noted 48 h after patch removal	69
Yeast Extract	Yeast Extract derived from *Candida magnoliae*	15% in water	10 subjects	patch test; no other details provided	non-irritating	19
Yeast Extract (may also be chemically similar to Hydrolyzed Candida Saitoana Extract)	Yeast Extract derived from *Candida saitoana*	15% in water	10 subjects	patch test; no other details provided	non-irritating	19
Yeast Extract derived from *Metschnikowia pulcherrima*	Yeast Extract derived from *Metschnikowia pulcherrima*	15% in water	10 subjects	patch test; no other details provided	non-irritating	19

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Yeast Extract (may also be chemically similar to Hydrolyzed Metschnikowia Reukaufii Extract)	Yeast Extract derived from *Metschnikowia reukaufii*	15% in water	11 subjects	patch test; no other details provided	non-irritating	19
SENSITIZATION						
In Chemico/In Vitro						
Hydrolyzed Yeast	trade name mixture containing 0.4% Hydrolyzed Yeast, 30% 1,3-butylene glycol, 0.08% polysorbate 20, and 69.52% water)	up to 2000 µM	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	71
Hydrolyzed Yeast	trade name mixture containing 0.4% Hydrolyzed Yeast, 30% 1,3-butylene glycol, 0.08% polysorbate 20, and 69.52% water)	up to 5000 µg/ml	THP-1 cell line	human cell line activation test; OECD TG 442E	no sensitization potential	71
Phaffia Rhodozyma Extract	trade name mixture containing 49% Phaffia Rhodozyma Extract	100 mM in acetonitrile	lysine and cysteine peptides	DPRA; OECD TG 442C	no sensitization potential	72
Phaffia Rhodozyma Extract	trade name mixture containing 49% Phaffia Rhodozyma Extract	up to 2000 µM	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	73
Pichia Minuta Extract	Pichia Minuta Extract	NR	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	38
Pichia Minuta Extract	Pichia Minuta Extract	NR	U937 cell line	U937 cell line activation test; OECD TG 442E	no sensitization potential	38
Saccharomyces Ferment Lysate Filtrate	trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate	100 mM in acetonitrile	Lysine and cysteine peptides	DPRA; OECD TG 442C	no sensitization potential	75
Saccharomyces Ferment Lysate Filtrate	trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate	up to 2000 µM	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	75
Yeast Extract (when derived from *Candida magnoliae)*	Yeast Extract (derived from *Candida magnoliae)*	NR	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	38
Yeast Extract (may also be chemically similar to Hydrolyzed Metschnikowia Reukaufii Extract)	Yeast Extract derived from *Metschnikowia reukaufii*	100%	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	19
Yeast Extract (when derived from Pichia *naganishii*)	Yeast Extract (derived from Pichia *naganishii*)	NR	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	38
Yeast Extract (when derived from Pichia *naganishii*)	Yeast Extract (derived from Pichia *naganishii*)	NR	THP-1 cell line	Human cell line activation test; OECD TG 442E	no sensitization potential	38

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			Animal			
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate****	100%	10 female Hartley guinea pigs/group	Guinea pig maximization assay: intradermal induction: 3 pairs of injections on day 1: 1.) adjuvant + distilled water 2.) test article 3.) test article + adjuvant/distilled water topical induction: 48-h occlusive patch (2 x 4 cm patch) on day 7 challenge: 24-h occlusive patch (20 mm x 20 mm) on day 21	0% sensitization rate	[76]
Hydrolyzed Saccharomyces Cell Wall	mixture containing 90% yeast (*Saccharomyces cerevisiae*) cell wall (24% glucan and 7% mannan) in 10% HSCAS****	55%; vehicle of 2% carboxymethylcellulose in distilled water	male Hartley guinea pigs (20 test group, 10 control group)	OECD TG 406; Once each week for 3 wk, the test substance was applied to the animal's left side under an occlusive patch and left on for 6 h. Readings were made 24 and 48 h after each induction period. Twenty-seven days after the first induction dose, the test substance was applied, under an occlusive patch, on a naïve site on the right side of the animal as a challenge dose. Sites were evaluated for a sensitization response 24 and 48 h after challenge application. A control group was treated with HSCAS, only.	non-irritating; non-sensitizing	[4]
Saccharomyces Cerevisiae Extract	*Saccharomyces cerevisiae* extract****	0, 10, 25, and 50% in propylene glycol	female CBA/J mice (5/group)	LLNA; OECD TG 429; The dorsal surface of both ears were epidermally treated (25 µl/ear) with the test substance, once a day for 3 d. Control animals were treated with the vehicle only. On day 6, animals were injected via the tail vein with 0.25 ml PBS containing 3H-methyl thymidine, and 5 h later ,killed. The auricular lymph node was excised, evaluated, and drained. Radioactivity measurements were performed. The SI was evaluated for each group. The SI is the ratio of the dpm/group compared to dpm/vehicle control group. An SI ≥ 3 indicates potential skin sensitization.	SI values at the 10, 25, and 50% concentration levels were 2.1, 5, and 28.9, respectively. The estimated test substance concentration that would give an SI = 3 was calculated to be 14.7%. The test substance was considered to be sensitizing.	[2]
Saccharomyces Cerevisiae Extract	*Saccharomyces cerevisiae* extract****	0, 10, 25, and 50% in propylene glycol	female CBA/J mice (5/group)	LLNA performed according to the same procedure as above	SI values at the 10, 25, and 50% concentration levels were 1.1, 2, and 1.7, respectively. The test substance was considered to be non-sensitizing.	[2]
Saccharomyces Cerevisiae Extract	*Saccharomyces cerevisiae* extract****	0, 10, 25, and 50% in propylene glycol	female CBA/J mice (5/group)	LLNA performed according to the same procedure as above	SI values at the 10, 25, and 50% concentration levels were 2.5, 2.5, and 1.8, respectively. The test substance was considered to be non-sensitizing.	[2]
Saccharomyces Cerevisiae Extract	*Saccharomyces cerevisiae* extract****	0, 10, 25, and 50% in propylene glycol	female CBA/J mice (5/group)	LLNA performed according to the same procedure as above	SI values at the 10, 25, and 50% concentration levels were 1.4, 1.7, and 2.6, respectively. The test substance was considered to be non-sensitizing.	[2]

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Saccharomyces Cerevisiae Extract	*Saccharomyces cerevisiae* extract**	0, 2.5, 5, 10, 25, and 50% in acetone and olive oil	female CBA mice (4/group)	LLNA performed according to the same procedure as above	SI values at the 2.5, 5, 10, 25, and 50% concentration levels were 0.87, 0.49, 1.36, 0.71, and 0.63, respectively. The test substance was considered to be non-sensitizing.	2
Human						
Hydrolyzed Yeast	trade name mixture containing 0.4% Hydrolyzed Yeast, 30% 1,3-butylene glycol, 0.08% polysorbate 20, and 69.52% water	0.01%	51 subjects	HRIPT; occlusive condition (patch size: 4 cm^2); 9 induction patches; challenge patch applied 2 wk after last induction patch	non-irritating and non-sensitizing	79
Galactomyces Ferment Filtrate	skincare product containing 1.485% Galactomyces Ferment Filtrate	100%	104 subjects	HRIPT; semi-occlusive conditions (patch size 8 mm); 9 induction patches; challenge patch applied 10-14 d after last induction patch	non-irritating and non-sensitizing	78
Galactomyces Ferment Filtrate	facial treatment essence containing 92.675% Galactomyces Ferment Filtrate	100%	100 subjects	HRIPT; occlusive conditions (patch size: 4 cm^2); 9 induction patches; challenge patch applied 12-20 d after last induction patch	non-sensitizing	83
Metschnikowia Agaves Extract	Metschnikowia Agaves Extract	15% in water	112 subjects	HRIPT; no other details provided	non-sensitizing	19
Pichia Anomala Extract	Pichia Anomala Extract	15% in water	104 subjects	HRIPT; no other details provided	non-sensitizing	19
Pichia Anomala Extract	Pichia Anomala Extract	15% in water	100 subjects	HRIPT; no other details provided	non-irritating; non-sensitizing	19
Pichia Heedii Extract	Pichia Heedii Extract	15% in water	106 subjects	HRIPT; no other details provided	non-irritating; non-sensitizing	19
Pichia Minuta Extract	Pichia Minuta Extract	15% in water	107 subjects	HRIPT; no other details provided	non-sensitizing	19
Saccharomyces Ferment Lysate Filtrate	cream containing 0.0135% Saccharomyces Ferment Lysate Filtrate	100%	52 subjects	HRIPT; occlusive conditions (patch size: 2 cm^2); 9 induction patches; challenge patch applied 2 wk after last induction patch	non-irritating and non-sensitizing	80
Saccharomyces Ferment Lysate Filtrate	trade name mixture containing 2% Saccharomyces Ferment Lysate Filtrate non-volatile solids in water	100%	105 subjects	HRIPT; semi-occlusive conditions (patch size 8 mm^2); 9 induction patches; challenge patch applied 10 - 14 d after last induction patch	non-irritating and non-sensitizing	82
Saccharomyces Lysate Extract	cream containing 0.028% Saccharomyces Lysate Extract	100%	50 subjects	HRIPT; occlusive conditions (patch size: 2 cm^2); 9 induction patches; challenge patch applied 2 wk after last induction patch	non-irritating and non-sensitizing	77
Saccharomyces Lysate Extract	trade name mixture containing 25% Saccharomyces Lysate Extract	10% in water	50 subjects	open patch repeat patch test; 0.2 ml applied to back per application and allowed to air dry; 9 induction patches; challenge patch 10 - 14 d after last induction patch	non-irritating and non-sensitizing	84
Yeast Extract	lotion containing 0.0045% Yeast Extract	100%	52 subjects	HRIPT; occlusive conditions (patch size: 2 cm^2); 9 induction patches; challenge patch applied 2 wk after last induction patch	non-irritating and non-sensitizing	81
Yeast Extract (may also be chemically similar to Hydrolyzed Candida Saitoana Extract)	Yeast Extract derived from *Candida saitoana*	15% in water	112 subjects	HRIPT; no other details provided	non-sensitizing	19

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Yeast Extract (may also be chemically similar to Hydrolyzed Metschnikowia Reukaufii Extract)	Yeast Extract derived from *Metschnikowia reukaufii*	15% in water	104 subjects	HRIPT; no other details provided	non-sensitizing	[19]
PHOTOTOXICITY						
In Vitro						
Phaffia Rhodozyma Extract	trade name mixture containing 49% Phaffia Rhodozyma Extract	0.5, 1.5, 5, and 10%	reconstructed human epidermal model (EpiDerm™)	EpiDerm™ phototoxicity assay; incubated tissue inserts irradiated with UVA for 60 min (6 J/cm^2); controls not exposed to UVA; cell viability measured via MTT assay; chloropromazine used for positive control	predicted to be non-phototoxic	[85]
Saccharomyces Ferment Lysate Filtrate	trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate	0.5, 1.5, 5, and 10%	reconstructed human epidermal model (EpiDerm™)	EpiDerm™ phototoxicity assay; incubated tissue inserts irradiated with UVA for 60 min (6 J/cm^2); controls not exposed to UVA; cell viability measured via MTT assay; chloropromazine used for positive control	predicted to be non-phototoxic	[86]
Animal						
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate****	NR	3 male New Zealand white rabbits	Test material (0.8 ml) applied to shaved skin under 4 cm^2 flannel cloth lined with surgical tape for 24 h (level of occlusion not stated); irradiation with long-wavelength ultraviolet rays (1.2 x 10^8 erg/cm^2) for 3 h; observations performed 24 and 48 h after irradiation	non-phototoxic	[88]
PHOTOSENSITIZATION						
Animal						
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate****	NR	female Hartley albino guinea pigs (10/group)	Guinea pig photosensitization assay: 1) animals injected with adjuvant 2) 20% aqueous solution of sodium lauryl sulfate applied, 24 h later, cellophane tape adhered and removed 7 times 3) test material (0.4) applied, animals irradiated with long-wavelength ultraviolet rays (1.2 x 10^8 erg/cm^2) for 3 h Steps 2 and 3 were repeated 5 times every other day. For the challenge test, on the 4th week of the study, 0.8 ml of the test substance was applied to the back, and animals were irradiated for 1 h; potential photosensitization observed 24 and 48 h after treatment	non-photosensitizing	[87]

ARE = antioxidant response element; dpm = disintegrations per minute; DPRA = direct peptide reactivity assay; HSCAS = hydrated sodium calcium aluminosilicate; HRIPT = human repeat insult patch test; LLNA = local lymph node assay; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Nrf2 = nuclear factor erythroid 2-related factor 2; OECD = Organisation for Economic Co-operation and Development; PBS = phosphate-buffered saline; SI = stimulation index; TG = test guideline; THP-1 = human monocytic cell line; UVA = ultraviolet A

*It should be noted that the test articles evaluated in these studies may not be identical to the wINCI ingredients reviewed in this report; however, as they may be similar, both test articles and potentially related wINCI ingredients have been included in the table

**the Irritection® system involved the use of a proprietary solution comprised of both proteins and macromolecules in a well that is covered by a membrane. The test material is applied to the membrane and diffuses into the well. The proteins and macromolecules within the well undergo conformational changes depending on the irritation potential of the test substance that mimic the biomolecular changes that occur when irritants are placed on the skin and eyes. The more turbid the solution becomes, the higher the irritancy level. Irritancy is measured using a spectrophotometer.

***Lipomyces Lipid Bodies naturally contain 87% Lipomyces Oil per lipid body

****unknown if test substance is a cosmetic ingredient (e.g., *Candida oleophila* strain O); however, ingredient relates to INCI ingredient reviewed in this report (Yeast Extract (when derived from *Candida oleophila*)

Table 14. Ocular irritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
				IN VITRO			
Galactomyces Ferment Filtrate	facial treatment essence containing 92.675% *Galactomyces* ferment filtrate	NR	100%	human cell construct model (EpiOcular™)	tissue equivalent assay with EpiOcular™ cultures; MTT assay used to evaluate cellular metabolism after exposure to test article for various exposure times (10, 30, 60, and 180 min); sterile deionized water used as negative control; octoxynol-9 used as positive control	non-irritating; definitive t_{50} determined to be >240; controls gave expected results in definitive assay	89
Phaffia Rhodozyma Extract	trade name mixture containing 49% Phaffia Rhodozyma Extract	NR	100%	corneal epithelial model (EpiOcular™)	EpiOcular™ assay; 30 min incubation; MTT assay performed; sterile deionized water used as negative control; methyl acetate used as positive control	non-irritating; controls gave expected results	63
Pichia Minuta Extract	Pichia Minuta Extract	NR	NR	bovine eyes	bovine corneal opacity and permeability test method; OECD TG 437	Test substance did not require classification of eye irritation or serious eye damage	38
Saccharomyces Cerevisiae Extract	trade name mixture containing 1.25% Saccharomyces Cerevisiae Extract	NR	100%: 50 µl	corneal epithelial model (EpiOcular™)	EpiOcular™ assay; tissues treated and incubated for 90 min; PBS used as negative control; methyl acetate used as positive control	non-irritating; controls gave expected results	64
Saccharomyces Cerevisiae Extract	trade name mixture containing 3% Saccharomyces Cerevisiae Extract	NR	100%	corneal epithelial model (EpiOcular™)	EpiOcular™ assay; 30 min incubation; MTT assay performed; sterile deionized water used as negative control; methyl acetate used as positive control	non-irritating; controls gave expected results	67
Saccharomyces Cerevisiae Extract	trade name mixture containing 4.5% Saccharomyces Cerevisiae Extract	NR	25, 50, 75, 100, and 125 µl	Irritection® systems	Irritection® assay*	Test substance was considered to be minimally irritating at all tested concentrations (all scores under 12.5 are considered to be minimally irritating). Irritation scores resulting from doses of 25, 50, 75, 100, and 125 µl were 5.2, 5.5., 6.1, 6.4, and 7.2, respectively.	62
Saccharomyces Cerevisiae Extract	powdered *Saccharomyces cerevisiae* extract***	physiological saline	20%; 750 µl	bovine corneas	bovine corneal opacity and permeability test; OECD TG 437; negative control: physiological saline; positive control: 20% imidazole	Test substance not considered to be severe irritant or corrosive. Mean irritation score of test substance: 3.3 Mean irritation score of negative control: below upper limits of laboratory historical range Mean irritation score of positive control: 119	2

Table 14. Ocular irritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Saccharomyces Ferment Lysate Filtrate	trade name mixture containing 24.5% Saccharomyces Ferment Lysate Filtrate	NR	100%	corneal epithelial model (EpiOcular™)	EpiOcular™ assay; 30 min incubation; MTT assay performed; sterile deionized water used as negative control; methyl acetate used as positive control	non-irritating; controls gave expected results	65
Saccharomyces Lysate Extract	trade name mixture containing 98% Saccharomyces Lysate Extract	NR	100%	corneal epithelial model (EpiOcular™)	EpiOcular™ assay; 30 min incubation; MTT assay performed; sterile deionized water used as negative control; methyl acetate used as positive control	non-irritating; controls gave expected results	66
Saccharomyces Lysate Extract	trade name mixture containing 10% Saccharomyces Lysate Extract	NR	100%	corneal epithelial model (EpiOcular™)	EpiOcular™ assay; 30 min incubation; MTT assay performed; sterile deionized water used as negative control; methyl acetate used as positive control	non-irritating; controls gave expected results	68
Yeast Extract (when derived from *Pichia naganishii*)	Yeast Extract derived from *Pichia naganishii*)	NR	NR	bovine eyes	bovine corneal opacity and permeability test method; OECD TG 437	test substance did not require classification of eye irritation or serious eye damage	38
ANIMAL							
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate***	NR	0.1 ml (concentration not stated)	3 Japanese white rabbits (sex not stated)	test substance instilled in right eye; control substance instilled in left eye (control substance used not stated); eyes evaluated immediately after, 3, 6, 24, 48, and 72 h after administration	non-irritating	90
Hydrolyzed Saccharomyces Cell Wall (when derived from *Saccharomyces cerevisiae*)	mixture containing 90% yeast (*Saccharomyces cerevisiae*) cell wall*****	HSCAS	100%; 0.09 g	3 male New Zealand albino rabbits	One eye of each animal anesthetized and test substance instilled into conjunctival sac; irritation evaluated using high-intensity white light at 1, 24, 48, and 72 h post-instillation	mildly irritating; no corneal opacity or iritis was observed in any treated eye during the study. One hour following test substance administration, all treated eyes exhibited positive conjunctivitis. The severity of irritation decreased with time, with no irritation noted 72 h after instillation.	4
Saccharomyces Cerevisiae Extract	powdered *Saccharomyces cerevisiae* extract***	NR	100%; 59 mg	3 male New Zealand White rabbits	test substance placed in one eye of each rabbits; examination 1, 24, 48, and 72 h after instillation; 24 h after instillation, 2% fluorescein in water solution instilled to evaluate epithelial damage	Irritation of the conjunctivae, presenting as redness, chemosis, and discharge, was noted in treated eyes; however, this irritation was completely resolved within 48 h for all animals.	2
Yeast Extract (when derived from *Candida oleophila*)	non-cosmetic product containing *Candida oleophila* strain O (as an active ingredient at 57% by weight)***	NR	100%; 100 mg	4 rabbits (sex and strain not stated)	test substance instilled in conjunctive sac of the right eye; animals observed for 15 d	minimally irritating	41

HSCAS = hydrated sodium calcium aluminosilicate; OECD = Organisation for Economic Co-operation and Development test guidelines; PBS = phosphate-buffered saline; t_{50}= duration of exposure resulting in a 50% decrease in MTT conversion; TG = test guideline

*the Irritection® system involved the use of a proprietary solution comprised of both proteins and macromolecules in a well that is covered by a membrane. The test material is applied to the membrane and diffuses into the well. The proteins and macromolecules within the well undergo conformational changes depending on the irritation potential of the test substance that mimic the biomolecular changes that occur when irritants are placed on the skin and eyes. The more turbid the solution becomes, the higher the irritancy level. Irritancy is measured using a spectrophotometer.
**_Saccharomyces cerevisiae_ cell wall contains 24% glucan and 7% mannan
***unknown if test substance is a cosmetic ingredient (e.g., _Candida oleophila_ strain O); however, ingredient relates to INCI ingredient reviewed in this report (Yeast Extract (when derived from _Candida oleophila_)

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: August 7, 2023

SUBJECT: Yeast-Derived Ingredients

Silab. 2023. Qualified Presumption of Safety Status.

Silab, 2023. Sensitization and food use – GRAS data by yeast.

Qualified Presumption of Safety (QPS) status

EFSA (European Food Safety Authority) is an agency of the European Union set up in 2002 to serve as an impartial source of scientific advice to risk managers and to communicate on risks associated with the food chain. This agency collects, appraises and integrates scientific evidence to provide the scientific basis for laws and regulations to protect European consumers from food-related risks. EFSA cooperates with Member States risk assessment organisations, as well as with other EU agencies, international organisations and risk assessors in third countries.

When EFSA receives applications for market authorisations of feed additives, food additives, food enzymes, food flavourings, novel food, and plant protection products, it assesses the safety of the product and/or the micro-organisms involved.

During the pre-assessment, which is the first step that covers safety concerns for humans, animals and the environment, EFSA can trigger a **Qualified Presumption of Safety (QPS) status** evaluation of the micro-organism involved in the substances.
Until the assessment is complete, the micro-organism is considered to be QPS notified.

During the **QPS assessment**, experts from **the EFSA BIOHAZ Panel assess the following key points**: the **taxonomic identity** (1) of the microorganism, the **related body of knowledge** (2) and **potential safety concerns** (3).

(1) Only unambiguously defined biological Taxonomic Units (Tus, =species or family) are considered for inclusion in the QPS list.
(2) The body of knowledge is determined by searching in peer-reviewed articles of the available scientific knowledge indicating a certain degree of exposure of humans and animals through food and feed use (history of use of a TU in the agri-food system or in other sectors, certain aspects of the ecology of the microorganism).
(3) Only TUs that do not represent a hazard to human and animal health and to the environment can be included in the QPS list

This QPS assessment is done at species level (or family level for viruses).
When microorganisms do not meet these previous criteria, they are not considered suitable for QPS status.

- When the QPS status is **attributed** to a microorganism, the applied substance can undergo a **reduced** assessment of the notified dossier from the applicant,
- When the QPS status is **not attributed** to a microorganism, the applied substance must undergo a **full safety assessment for the market authorisation. A "qualification" can be established in** order to exclude the identified hazard, and is evaluated within the full Safety assessment for market authorisation.
  The safety assessment for market authorisation of regulated products is done at a strain level, and includes the assessment of the following aspects :
  - Type and level of exposure of users handling the product
  - Potential allergenicity to microbial residual components
  - Hazards linked to the formulation or other aspects of the processing of those products

In this way, all microorganisms' strains that belong to a taxonomic unit (species of family) that is listed in the QPS list are safe for humans, animals and the environment.
The QPS list can be update with possible additions every 6 months. 111 microorganisms are currently listed, and include 18 yeasts that are the following ones:

Genus	Species	Synonyms commonly used in the feed and food industry/ anamorph (for yeasts)/ previous name
Candida	Limtongozyma cylindracea	Candida cylindracea
Cyberlindnera	Cyberlindnera jadinii	Lindnera jadinii, Pichia jadinii, Hansenula jadinii, Torulopsis utilis, Candida utilis
Debaryomyces	Debaryomyces hansenii	Candida famata
Hanseniaspora	Hanseniaspora uvarum	Kloeckera apiculata
Kluyveromyces	Kluyveromyces lactis	Candida spherica
Kluyveromyces	Kluyveromyces marxianus	Candida kefyr

Komagataella	Komagataella phaffii	
Komagataella	Komagataella pastoris	Pichia pastoris
Ogataea	Ogataea angusta	Pichia angusta
Ogataea	Ogataea polymorpha	Hansenula polymorpha, Candida thermophila
Saccharomyces	Saccharomyces bayanus	
Saccharomyces	Saccharomyces cerevisiae	Saccharomyces boulardii
Saccharomyces	Saccharomyces pastorianus	Saccharomyces carlsbergensis
Schizosaccharomyces	Schizosaccharomyces pombe	
Wickerhamomyces	Wickerhamomyces anomalus	Hansenula anomala, Pichia anomala, Saccharomyces anomalus, Candida pelliculosa
Xanthophyllomyces	Xanthophyllomyces dendrorhous	Phaffia rhodozyma
Yarrowia	Yarrowia lipolytica	Candida lipolytica
Zygosaccharomyces	Zygosaccharomyces rouxii	

List of the microorganisms that have been notified: Microbiological agents as notified to EFSA | Zenodo
QPS list: Updated list of QPS-recommended microorganisms for safety risk assessments carried out by EFSA | Zenodo)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: July 17, 2023

SUBJECT: Galactomyces Ferment Filtrate

Japan Food Research Laboratories. 1980. Acute toxicity test using mice *Galactomyces* ferment filtrate.

Japan Food Research Laboratories. 1980. Mutagenicity test *Galactomyces* ferment filtrate.

Japan Food Research Laboratories. 1980. Eye irritation test *Galactomyces* ferment filtrate.

Ishikawa Clinic. 1980. Results of continuous skin irritation in the human body by *Galactomyces* ferment filtrate.

Anonymous. 1980. Photosensitization test with guinea pigs *Galactomyces* ferment filtrate.

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Test Report

[redacted]

Requester: [redacted]

Test mateiral: *Galactomyces* ferment filtrate

Notes : NA

Test item: Acute toxicity test using mice

The results of the above test requested on November 7, 1979 are as shown in the attachment.

February 16, 1980

Japan Food Research Laboratories

Acute toxicity study of *Galactomyces* ferment filtrate in mice

Summary

An oral acute toxicity study of *Galactomyces* ferment filtrate in mice showed that the LD50 (oral) values of the sample were greater than 6 0000 mg/kg for both males and females.

1. Materials and methods
   (1) Test product
   Galactomyces ferment filtrate
   (2) Route of administration
   oral
   (3) Test animals
   Mouse, ddY-N, male, female, 30-day old
   Starting weight 20-23g, end weight 2 2-29g
   (4) Test Period
   January 28—February 4, 1980
   (5) room temperature
   22—24C
   (6) Method of administration
   Using a gastric sonde, the test sample was administered orally by force-feeding once as it was.
   (7) How to calculate the LD50 value
   By probit method.
   (8) Dosage concentration ratio of specimen
   common ratio 1.20
   (9) Number of animals per test group
   10 males and 10 females

2. Test results

sex	Test group	dose (mg/kg)	Daily Mortality			Mortality rate (%)	LD50 Value
			1 day	2 3 4 5 6	7		
male	1	34730	0/10	———	0/10	0	Above 60000mg/kg
	2	41670	0/10	———	0/10	0	
	3	50000	0/10	———	0/10	0	
	4	60000	0/10	———	0/10	0	
female	1	34730	0/10	———	0/10	0	Above 60000mg/kg
	2	41670	0/10	———	0/10	0	
	3	50000	0/10	———	0/10	0	
	4	60000	0/10	———	0/10	0	

3. Clinical signs

   In each test group, no abnormalities were observed after administration.

4. Autopsy findings

   No abnormalities were observed.

5. Discussion

   Although no deaths were observed in either study group in this study, the LD50 (oral) value of the sample was above 60000mg/kg in both males and females because 60000 mg/kg in the highest treatment group is considered to be a maximum amount that can be administered once orally to mice.
   However, since no abnormalities were observed in the test mice during the test period and all showed normal growth, it seems that the LD50 value is much higher than 60000 mg / kg.

Test location: 821 Yoshikura, Narita-shi, Chiba

Name of testing organization:

Japan Scientific Feeds Association

Scientific Feeds Research Center

Tester name [redacted]

Appendix Table 1 Individual weight of male mice

dose	Mouse No.	weight		dose	Mouse No.	weight	
		At Start (g)	At the end (g)			At the start (g)	At the end (g)
34730 mg/kg	1	20	25	50000mg/kg	21	20	22
	2	20	25		22	20	25
	3	20	26		23	20	25
	4	20	26		24	20	25
	5	22	26		25	22	26
	6	22	26		26	22	26
	7	22	27		27	22	26
	8	23	27		28	23	27
	9	23	28		29	23	28
	10	23	29		30	23	29
	average	21.5	26.5		average	21.5	25.9
41670 mg/kg	11	20	25		31	20	26
	12	20	26		32	20	26
	13	20	26		33	20	26
	14	20	27		34	20	26
	15	22	28		35	22	26
	16	22	26		36	22	26
	17	22	27		37	22	27
	18	23	27		38	23	28
	19	23	28		39	23	28
	20	23	29		40	23	29
	average	21.5	26.9		average	21.5	26.8

Appendix Table 2: Individual weight of female mice

dose	Mouse No.	weight		dose	Mouse No.	weight	
		At Start (g)	At the end (g)			At the start (g)	At the end (g)
34730 mg/kg	101	20	22	50000 mg/kg	121	20	22
	102	20	23		122	20	22
	103	20	23		123	20	22
	104	20	23		124	20	23
	105	20	23		125	20	24
	106	21	22		126	21	22
	107	21	22		127	21	22
	108	21	23		128	21	23
	109	21	25		129	21	25
	110	21	26		130	21	26
	average	20.5	23.2		average	20.5	23.1
41670 mg/kg	111	20	22	60000 mg/kg	131	20	22
	112	20	22		132	20	23
	113	20	22		133	20	23
	114	20	23		134	20	24
	115	20	23		135	20	24
	116	21	23		136	21	22
	117	21	23		137	21	22
	118	21	25		138	21	23
	119	21	26		139	21	23
	120	21	26		140	21	24
	average	20.5	23.5		average	20.5	23.0

Test Report

[redacted]

Requester [redacted]

Name of the sample *Galactomyces* ferment filtrate

Notation —

Test item: Mutagenicity test

The results of the above examination requested on October 22, 1980 are as shown in the attachment.

November 13, 1980

Japan Food Research Laboratories

52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo

Branch office: 3-1 Toyotsu-cho, Suita-shi, Osaka

1. Test Objectives

   In order to investigate the mutation-inducibility of this sample, in accordance with Industrial Safety and Health Law Directive No. 107-2 (Labor Safety Law), using *Escherichia coli* WP2 *uvrA*$^-$ strain and *Salmonella typhimurium* five TA strains, a reverse mutation test including metabolic activation is conducted.

2. Test products

   Sample name: *Galactomyces* ferment filtrate

3. Test method

   1) Test strains

      Salmonella typhimurium TA100, TA1535, TA98, TA1537, TA1538 and *E. coli* WP2 *uvrA*$^-$ strains were used.

      In the test, each bacterium was inoculated in an L-shaped test tube containing 0.5% NaCl addition Nutrient broth (Difco), and cultured at 37C for 16 hours and used for the test.

   2) Preparation of test solution

      The test samples were weighted to make a 100 mg/ml aqueous solution (using sterile distilled water). This was appropriately diluted and used to testing.

   3) Test operation method

      In accordance with the Industrial Safety Law, the test was performed by the pre-incubation method (without and with metabolic activation method).

      a) For S. typhimurium

         0.1ml of Test solution , 0.5ml of S 9 Mix[*1]) or 0.2M Na-phosphate buffer (pH 7.4), and 0.1ml of *S.* typhimurium bacterial suspension were sequentially added to a sterile small test tube, which was shaken in a thermostatic bath for 37C 20 minutes (pre-incubated), and then 2 ml of top agar [*2)] was added to it, It was mixed and uniformly spread on the minimum glucose agar plate medium [*3)] and solidified.

         It was placed in an incubator and cultured at 37C for 48 hours to calculate the number of colonies caused by reverse mutation.

      b) For E. coli

0.1ml of Test solution, 0.5ml of S9Mix*[1]) or buffer solution for E. coli (pH 6.8)[*4)], and 0.1 ml of E.coli suspension were sequentially added to a sterile small test tube, which was shaken in a thermostatic bath for 37C 20 minutes (pre-incubated), then 2 ml of Top Ager [* 2)] is added to it, mixed. It was uniformly spread on the minimum glucose agar plate medium [* 5)] for *E.coli* and solidified.
It was placed in an incubator and cultured at 37C for 48 hours to calculate the number of colonies caused by sudden restoration.

It was confirmed that the bacterial solution, test solution, and S9Mix used in the test were free from bacteria. In addition, a positive control test was performed on the compounds shown in the attached table.

*1) Composition of S9Mix (in 1ml)

S9 draws are divided into	0.1 ml
$MgCl_2$	8 μmole
KCl	33 μmole
G-6-P	5 μmole
NADPH	4 μmole
NADH	4 μmole
Na-phosphate buffer (pH 7.4)	80 μ mole

The S9 fraction was prepared as follows.
Male Wister rats (4 week-old, 100 g) were given a single intraperitoneal dose of PCG (Kanecrol 500) 500 mg/kg, fasted from the evening 4 days after administration, and the liver was removed on the 5th day, Perfusion was performed with a cooled 0.15 M KCl solution. Homogenized with the addition of KCl solution at the rate of 3 ml/g liver and centrifuged at 9 000

x g for 30 mins. The supernatant was used for the experiment.
Note that all operations were performed at 5 C or lower.
The prepared S 9 fraction was cryopreserved at －80C.

*2) Composition of Top Agar

Bacto agar	0.6%
NaCl	0.6%

After heated dissolution, for 5 strains *of Salmonella*, a sterilized 0.75 mM Histidine HCl H2O – 0.75 mM Biotin solution is added by 1/10 volume and mixed.

*3) Composition of minimum glucose agar flat plate medium (per 1 L of medium)

MgSO4 7H2O	0.2g
citric acid	2g
K2HPO4	10g
NaNH4HPO4	3.5g
glucose	20 g
NaCl	5g
Bacto agar	15g

30 ml is poured in a sterile flat plate with a diameter of 100 mm, and solidified.

*4) Composition of buffer for *E.coli* (per 1 L)

MgSO4 7H2O	2g
Citric acid Na 2H2O	10g
(NH4)2SO4	20g
KH2PO4	200g
KOH	45g

*5) Composition of minimum glucose agar flat plate medium for E.coli (per 1L of medium)

MgSO4 7H2O	0.1g

Citric acid Na 2H2O	0.5g
(NH4)2SO4	1g
KH2PO4	10g
KOH	2.25g
glucose	3.2 g
NaCl	4.1g
Nutrient broth (Difco)	0.16g
Bacto agar	15g

40 ml is poured ini a sterile plate with a diameter of 100 mm and solidified.

4. Test results

As shown in Appendix I and II, AF-2, 1-ethyl-2-nitro-3-nitrosoguanidine, 9-aminoacridine, 2-Nitrofluorene used as positive controls showed a marked increase in the number of revert mutant colonies compared to controls. In addition, 2-aminoanthracene was activated by the addition of S9 Mix, inducing a marked reversion mutation. However, in any strains, the test sample showed no increase in the number of revert mutant colonies compared to the control.
From the above, it is concluded that the mutation-inducibility of the test sample under the present experimental conditions is negative.

End of report.

Table I Test Results Table

substance	Test sampleconcentration (μg/plate)	S9Mix	Reverse mutation Colony number/plate					
			Base-paired-substitution type			Frameshift type		
			TA100	TA1535	WP2*uvrA*$^-$	TA98	TA1538	TA1538
Solvent control		-	122 118	17 13	7 11	22 25	20 27	14 17
test sample	10	-	114 101	7 13	12 9	29 28	27 22	12 14
	50	-	117 104	11 11	10 7	27 23	14 15	14 20
	100	-	105 120	12 14	12 10	24 29	25 25	19 21
	500	-	123 120	7 13	7 7	20 29	26 18	23 27
	1000	-	104 104	14 13	13 8	22 24	21 26	19 22
	2500	-	118 134	17 15	12 7	28 21	15 16	23 15
	5000	-	124 117	12 9	4 10	19 25	15 20	17 20
	10000	-	115 136	19 12	12 6	13 16	15 21	16 18
Positive control	Does not require S9Mix	name	AF-2	ENNG	AF-2	AF-2	9-AA	2-NF
		Concentration (μg/plate)	0.01	5	0.01	0.1	80	2
		Number of colonies/plate	602 570	1129 1227	199 194	485 584	227 193	349 277

AF-2:(2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide)

ENNG: 1-ethyl-2-nitro-3-nitrosoguanidine

9-AA: 9-aminoadridine

2-NF: 2-nitrofluorene

TableI I Test Results Table

substance	test sample concentration (μg/plate)	S9Mix	Reverse mutation Colony number/plate					
			Base-paired-substitution type			Frameshift type		
			TA100	TA1535	WP2*uvrA*$^-$	TA98	TA1538	TA1538
Solvent control		+	126 105	14 17	9 7	36 38	18 21	31 35
Test sample	10	+	111 109	11 18	11 8	33 39	10 15	23 23
	50	+	113 114	10 16	10 9	33 28	13 18	36 35
	100	+	103 108	13 16	15 9	45 36	15 15	39 31
	500	+	101 108	19 10	13 12	38 42	8 9	27 30
	1000	+	104 105	14 19	11 11	40 40	13 21	35 41
	2500	+	103 107	13 19	10 13	40 30	16 7	39 44
	5000	+	102 131	10 16	14 11	42 35	13 15	28 26
	10000	+	106 107	21 12	11 8	28 35	12 13	29 30
Positive control	Requires S9Mix	name	2-AA	2-AA	2-AA	2-AA	2-AA	2-AA
		Concentration (μg/plate)	0.5	2	80	0.5	2	0.5
		Number of colonies/plate	255 279	95 75	504 465	249 318	180 238	236 265

2-AA: 2-aminoanthracene

Test Report

[redacted]

Requester [redacted]

Name of the sample *Galactomyces* ferment filtrate

Notation —

Test item: Eye irritation test

The results of the above test requested on November 7, 1979 are as shown in the attachment.

February 16, 1980

Japan Food Research Laboratories

52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo

Branch office: 3-1 Toyotsu-cho, Suita-shi, Osaka

Galactomyces ferment filtrate eye irritation test

Summary

As a result of performing an eye irritation test using the test solution of *Galactomyces* ferment filtrate and the control test solution prepared for this test, no eye irritation was observed.

1. Materials and Methods
    1) Test products
    The test solution with Galactomyces ferment filtrate and the control solution prepared for this test were used.
    2) Service animals
    Three Japanese white rabbits (body weight 2.45~2.78 kg) were put into the study.
    3) Methodology
    0.1 ml of the test solution was instilled in the right eye of the rabbit and 0.1 ml of the empty test solution in the left eye of the rabbit, which was confirmed to have no abnormalities in the eyes in advance, and immediately after instillation, 3, 6, 24, 48 and 72 hours later, both eyes were examined using a slit lamp.
    When instilling, gently pull the lower eyelid of the rabbit to make a sac to instillethe test samples and left it for about 30 seconds so that the test solution blended into the entire eye.
    4) Test period
    1980 January 28 ~ January 31
2. Test results
    At any time of observation, hyperemia or other abnormalities were not observed in the cornea, iris, and conjunctiva of both eyes.
    Therefore, it was determined that the test sample did not have eye irritation.

    Test location 821 Yoshikura, Narita-shi, Chiba
    Name of testing organization: Japan Scientific Feed Association
    Scientific Feed Research Center

Tester name [redacted]

Appendix Table 1 Eye irritation test results

rabbit No	Evaluationtime	Test solution (right eye)				Control solution (left eye)			
		cornea	Iris	conjunctiva		cornea	Iris	conjunctiva	
				hyperaemia	other			hyperaemia	other
1	immediately	—	—	—	—	—	—	—	—
	after	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—
	24	—	—	—	—	—	—	—	—
	48	—	—	—	—	—	—	—	—
	72								
2	immediately	—	—	—	—	—	—	—	—
	after	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—
	24	—	—	—	—	—	—	—	—
	48	—	—	—	—	—	—	—	—
	72								
3	immediately	—	—	—	—	—	—	—	—
	after	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—
	24	—	—	—	—	—	—	—	—
	48	—	—	—	—	—	—	—	—
	72								

Note) - No abnormalities.

± Slightly abnormal.

+ Clearly acknowledge the abnormality.

Results of continuous skin irritation in the human body by Galactomyces ferment filtrate

May 8, 1980

Shin Aoyama Building West Building 3 F Medical Center
1-1-1 Aoyama, Minato-ku, Tokyo
Ishikawa Clinic
Doctor of Medicine [redacted]

Galactomyces ferment filtrate Continuous skin irritation test on the human body

Ishii Clinic
[redacted]

Summary
35 outpatients and 10 female [redacted] were voluntarily selected, and *Galactomyces* ferment filtrate was applied to the facial cheeks continuously for 40 days, in the study between October 1979 and April 1980.
As a result, no abnormal reaction was observed in any cases.

Exam target
A total of 45 people, including voluntarily selected 35 outpatients and similarly selected 10 female [redacted] who consented to participate the *Galactomyces* ferment filtrate continuous application test, were tested.

Test method
10 cm2 sterile gauze containing about 5 ml of *Galactomyces* ferment filtrate solution was applied to the right facial cheek area for 15 minutes (no treatment was done on the left cheek area). The application was performed continuously for 40 days. Since there were few outpatients who could visit to the clinic every day, assessment was done once every three days, and subjects were asked to conduct the same test at home on days when they could not visit to the clinic. 10 [redacted] were tested and assessed every day. (Outpatients usually started trials on Monday and were assessed on Tuesday and Friday.)

Assessment Criteria

No response	-
Slight erythema	±
Obvious erythema	+
Erythema and edema infiltration	++

Conclusion
As shown in the attached test report table, no abnormality was observed in any cases.

Galactomyces ferment filtrate is recognized as being very weakly irritating to human skin.

Patient's Name	age	M Day 1	T Day 2	W Day 3	T Day 4	F Day 5	S Day 6	S Day 7	M Day 8	T Day 9	W Day 10	T Day 11	F Day 12	S Day 13	S Day 14	M Day 15	T Day 16	W Day 17	T Day 18	F Day 19	S Day 20	S Day 21	M Day 22	T Day 23	W Day 24	T Day 25	F Day 26	S Day 27	S Day 28	M Day 29	T Day 30	W Day 31	T Day 32	F Day 33	S Day 34	S Day 35	M Day 36	T Day 37	W Day 38	T Day 39	F Day 40
[redacted]	25	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	34	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	35	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	29	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	40	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	31	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	45	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	36	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	35	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	31	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	33	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	37	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	36	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	31	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	32	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	36	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	35	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	34	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	24	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	32	–	–			–				–			–				–			–				–			–				–			–				–			–

[redacted]	42	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	28	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	26	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	24	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	34	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	26	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
[redacted]	33	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	32	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	33	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	29	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	25	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	26	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	32	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	30	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	39	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	23	–	–			–				–			–				–			–				–			–				–			–				–			–

[redacted]	3 5	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	3 2	–	–			–				–			–				–			–				–			–				–			–				–			–
[redacted]	2 5	–	–			–				–			–				–			–				–			–				–			–				–			–

June 13, 1980

Galactomyces ferment filtrate
Photosensitization test with guinea pigs

We conducted a photosensitization test on guinea pigs of this product, and we would like to report the results.

1. Study period May12, 1980 ~ June 4, 1980

2. Study Location [redacted]

3. Study person in charge [redacted]

4. Test material *Galactomyces* ferment filtrate

5. Animal shelter facility environment

Temperature: 22±1C Humidity: 50±5%.
Total clean air using heat exchanger, Face velocity 20cm/sec
A rack with flushing system was used. Illuminate daily for 12 hours.

6. Breeding environment

Oriental feed RC4: free feeding
Tap water: Free drinking

7. Test method

Healthy Hartley albino guinea pig female weighing 200~250g were purchased from Shizuoka Prefecture Laboratory Animal Cooperative. Prepare two groups of animals (10 animal per one group). One group is for sensitization treatment and another group is for control at the time of induction.
The shoulder hair of each guinea pig is sheared, and then shaved with an electric razor to set a test area of 2 x 4cm 2 .

（1） Intradermally inject 0.1 ml (0.05 ml left and right 2 places) adjuvant (Freund's "complete" adjuvant (manufactured by Difco)) into the test site.

（２） Apply a 20% aqueous solution of sodium lauryl sulfate (JSCI) to produce mild inflammation.

（３） At 24hr after applying a 20% aqueous solution of sodium lauryl sulfate (JSCI), cellophane tape is adhered to the test area and peeled off to remove the stratum corneum. This removal is repeated 7 times.

0.4 ml of the test material is applied to the test area with fingers wearing sterile plastic gloves. At the first hour, each guinea pig is held in a restraint device in an abdominal position and irradiated with long-wavelength ultraviolet rays for 3 hours with a long-wavelength ultraviolet lamp (Toshiba Dermaray II type) from a distance of 10 cm from the test site*. A 3 mm thick glass filter is placed between the lamp and each guinea pig.

Repeat the above operations (2) and (3) 5 times every other day.

As an elicit test, each guinea pig was shaved with a 4 x 4 cm2 electric hair clipper and an electric razor on the central back at the fourth week of the study, and 0.8 ml of the test sample was applied to the back test area with fingers wearing sterile plastic gloves.

After ? hour, each guinea pig is kept in a ventral position retainer and irradiated with ultraviolet light for 1 hour, similar to sensitization treatment. Thereafter, the posterior back is not irradiated with light, but only the induction treatment is performed, and it is blocked from the light.

The response of photosensitization is observed twice at the 24th hour and the 48th hour according to the following criteria. The control group is subjected to the same treatment as the light irradiation induction treatment and the degree of phototoxic reaction is observed. Similarly, light irradiation is not performed, only induction treatment is performed, and it is blocked from light.

8. Criterion a. Erythema formation

No erythema	0
Very mild erythema	1
Distinct erythema	2

Moderate erythema before becoming ferocious	3
from intense erythema to crust formation	4

B. Edema formation

No edema formation	0
Minimal edema	1
Edema is easily noted	2
moderate edema (just 1mm heaving),	3
Intense edema (heaving more than 1mm in all areas),	4

Sensitization is assessed by the difference in positivity rates between the test and control groups.

9. Results

As shown in the appendix
Abnormality was not found in both of the test guinea pigs and the control group.

10. Evaluation

Based on the above results, it is considered that *Galactomyces* ferment filtrate does not pose photosensitization potency.

Test Facility [redacted]

*Wavelength 300 ~ 400 nm
Center wavelength 365 nm
Irradiation energy 1.2 x 10^8 erg/cm2

Appendix

Result

Induction (test) group	Light irradiation section				Non-light irradiation part			
	Post-induction read time				Post-induction read time			
	24 times		48 times		24 times		48 times	
Guinea pig number	erythema	edema	erythema	edema	erythema	edema	erythema	edema
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
total	0	0	0	0	0	0	0	0
			Grand total	0			Grand total	0

Control group	Light irradiation section				Non-light irradiation part			
	Post-induction read time				Post-induction read time			
	24 times		48 times		24 times		48 times	
Guinea pig number	erythema	edema	erythema	edema	erythema	edema	erythema	edema
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0

7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
total	0	0	0	0	0	0	0	0
			Grand total	0			Grand total	0

June 13, 1980

Galactomyces ferment filtrate
Phototoxicity test with rabbits

We have conducted a phototoxicity test using rabbits, and we would like to report the results.

1. Study Period: June 4, 1980 ~ June 7, 1980

2. Study Location [redacted]

3. Study person in Charge [redacted]

4. Test material *Galactomyces* ferment filtrate

5. Animal shelter facility environment

Temperature: 22±1C Humidity: 50±5%.
Total clean air using heat exchanger, Face velocity 20cm/sec
A rack with flushing system was used. Illuminate daily for 12 hours.

6. Breeding environment

Oriental feed RC4: free feeding
Tap water: Free drinking

7. Test method

Three healthy male New Zealand white rabbits weighing 2.0~2.5 kg are used.
The test site is in a telogen phase of the hair cycle. After shearing the hair on the back, the test substance is applied to the area where hair has been removed with a hair remover (containing thioglycolate). The test material application method is to use a 4 x 4 cm2 flannel cloth (confirming to the guaze and cotton wool standard listed in The Japanese Pharmacopeia 9th edition) lined with Blenderm Surgical Tape (3M company) with a sample of 0.8 ml and apply to the skin. It is fixed with

Elatex Expansible Tape (manufactured by Tokyo Eizai Co., Ltd.) and protected by a 1 mm thick rubber belly-band.
After 24 hours, remove the flannel cloth and wipe the test area with gauze containing ethanol and hold the animal in a restraint device in an abdominal position. Immediately cover the test part with a 1 mm thick rubber plate with a hole of 2 cm in diameter, and irradiate long-wavelength ultraviolet rays for 3 hours with a long-wavelength ultraviolet ramp (Toshiba D ermaray II type)*. A 3 mm thick glass filter is placed between the lamp and the test area.
The first observation is made 24hr after ultraviolet irradiation. The second observation is carried out 48 hours after irradiation, and the final evaluation points are obtained from the two observations and the average value of each animal..

8. Criterion a. Erythema formation

No erythema	0
Very mild erythema	1
Distinct erythema	2
Moderate erythema before becoming ferocious	3
from intense erythema to crust formation	4

B. Edema formation

No edema formation	0
Barely noticeable very mild edema	1
Edema is easily observed due to the obvious swelling of the edges of the site.	2
moderate edema (just 1mm heaving),	3
Intense edema (heaving of more than 1mm is observed in all areas),	4

9. Results As shown in the appendix
No abnormalities were found in all three test rabbits.

10. Evaluation on the above results, *Galactomyces* ferment filtrate is considered to be not phototoxic.

Test Facility [redacted]
Section leader [redacted]

*Wavelength 300 ~ 400 nm
Center wavelength 365 nm
Irradiation energy 1.2 x 10^8 erg/cm2

Appendix

Result

Erythema and crusting

		rabbit number		
		1	2	3
UV irradiation	After 24 hours	0	0	0
	After 48 hours	0	0	0
	sum	0	0	0

Edema formation

		rabbit number		
		1	2	3
UV irradiation	After 24 hours	0	0	0
	After 48 hours	0	0	0
	sum	0	0	0

Total 0

Average value 0

Galactomyces ferment filtrate sensitization test using guinea pigs

-According to The Guinea Pig Maximization Test-

Study period October 8, 1980 — October 31, 1980

Location [redacted]

Study person in chage [redacted]

Study director [redacted]

November 4, 1980

Galactomyces ferment filtrate sensitization test
-According to The Guinea Pig Maximization Test-

Summary
sensitization test for *Galactomyces* ferment filtrate was performed by the The Guinea Pig Maximization Test method (Ref. (1) (2)) using paraphenylenediamine as a positive control substance.
As a result of the study, all test animals tested with *Galactomyces* ferment filtrate did not show positive responses of sensitization.
In the positive control group, 9 out of 10 animals had a marked positive reaction by sensitization.

Test Objectives
To investigate the sensitization potency of *Galactomyces* ferment filtrate.

Test materials
Galactomyces ferment filtrate (hereinafter referred to as main test sample)
Positive control substance Paraphenylenediamine
Special-grade reagent purchased from Nakai Chemical Co., Ltd., the purity is 99.5%.
Since paraphenylenediamine is oxidized, it was prepared at the time of use and used for the test. (Hereinafter referred to as a control sample)

Test Animals
Forty six healthy Hartley female guinea pigs, 5wk old, weighing about 300gr purchased from Cary Co., Ltd. were used.
For individual identification, a commercially available black oil-based pen was used.

Breeding environment
5-6 animals were housed in a cages (size 350 x 420 x 200 mm) and fed with Oriental feed RC-4 and tap water.
Temperature: 22±1 C Humidity: 50±5%.
Total fresh air supply (ventilation frequency approx. 10 times/1 hour)
A rack with flushing system is used. It is illuminated by a timer for 12 hours every day.

Test method

The study was conducted according to The Guinea Pig Maximization Test method (Ref. (2)) which was developed by Bertil Magunusson and Albert M. Kligman (Ref. (1)) and recommended by the U.S. Food and Drug Administration (Ref. (3)).

[Preliminary test] Investigate the irritancy of the main test and control samples.

Process 24-hour occlusive application test

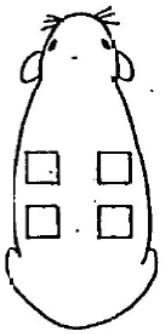


Figure 1

Number of animals: 3 for the main test sample and 3 for the control sample.

Sample preparation:

Main test sample: neat, 50% and 25%, 12.5% aqueous solution

Control sample: 5% and 1%, 0.2%, 0.04% solution

Solvent is 70% ethyl alcohol aqueous solution

Hair removal: As shown in Figure 1, an electric hair clipper and an electric razor are used to remove hair in four places with a width of 5 x 5 cm.

Adhesive plaster:2 x 2 cm Filter paper (Toyo Nthe 1313) lined with 3 x 3 cm Blenderm Surgical Tape (3M Company)

Fabric: 0.3 ml of the sample is applied to the filter paper of adhesive plaster, adhered to the hair-removed skin, and fixed with a 3 x 15 cm Elatex Expansible Tape (manufactured by Tokyo Eizai Co., Ltd.) for 24 hours.

Observations: after 24 hours application, immediately after removing the adhesive plaster, 24 hours and 48 hours after removal.

Criteria: Table 1

Skin reactions of the test area	Grade
No change to the naked eye	0
Mild or scattering erythema	1
Moderate erythema	2
Sever Erythema	3

Result: Table-2

		Animal number	immediately	24 hours	48 hours
Main test sample	neat	1	0	0	0
		2	0	0	0
		3	0	0	0
	50% solution	1	0	0	0
		2	0	0	0
		3	0	0	0
	25% solution	1	0	0	0
		2	0	0	0
		3	0	0	0
	12.5% solution	1	0	0	0
		2	0	0	0
		3	0	0	0
Control sample	5% solution	4			
		5	couldn't judge because it was dyed black.		
		6			
	1% solution	4	0	0	0
		5	0	0	0
		6	0	0	0
	0.2% solution	4	0	0	0
		5	0	0	0
		6	0	0	0
	0.04% solution	4	0	0	0
		5	0	0	0
		6	0	0	0

Evaluation: The main test sample was not irritating even at neat. The moderately irritating concentration of the control sample could not be determined because it was stained black. The maximum concentration without irritation was determined to be at 1%.

[Main test] The outline of the test is shown in Figure 2.

Sensitiziation group

week ([redacted] 1)	(week 2)	(week 3)
↖	↖	↗
Intradermal injection 1. Adjuvant only 2. sample only 3. emulsion of Adjuvant and sample	48-hour occlusive application	24-hour occlusive application (challenge treatment)
Sensitization treatment		

control group for challenge treatment

(1 week)	(2 weeks)	(3 weeks)
		↗
		24-hour occlusive application

Figure 2

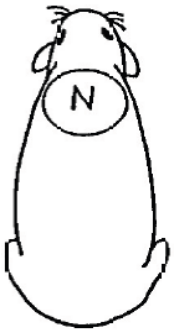


Figure 3

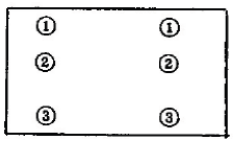


Figure 4

Sensitization group

Number of animals: 10 for the main test sample and 10 for the control sample.

[[Sensitization Treatment-I]] By intradermal injection.

Remove the N part of Figure 3, about 25 cm^2, with an electric hair clipper and an electric razor .

Intradermal injection: intradermal injections are performed in a 4 x 2 cm area in the N part of Fig. 3 (Fig. 4) as following three methods. (Note 1)

At the site ①, 0.05 ml Freund's "Complete" adjuvant (DifcoCompany Made) (hereinafter referred to as FCA as administered intradermally in both the main sample group and the control sample group. (Note 2)

At the site ②, 0.05 ml of the main test sample (neat) and 0.1% control sample aqueous solution is administered intradermally in the main test sample treatment group and the control sample group, respectively.

The sample of the site ③ is as follows.

Main test sample group: As described on page 113 of Reference (1), 5 ml of the main sample was added to 5 ml of FCA, treated with an ultrasonic generator (manufactured by Tommy Seiko Co., Ltd.) for 3 minutes, and 0.05 ml of the water-in-oil emulsion was administered intradermally.

Control sample group: 0.001 gr of control sample was dissolved in 5 ml of saline, added it to 5 ml of F CA, made a water-in-oil emulsion similar to above, and 0.05 ml was administered intradermally.

[[Sensitization Treatment-II]] By 48hr occlusive application.

10% sodium lauryl sulfate aqueous solution application (hereinafter referred to as SLS.): Since the main test sample was unstimulated even at neat (Table 2), SLS was applied to the N part of FIG. 3 6 days after intradermal administration.

Main sample group (Figure 5)

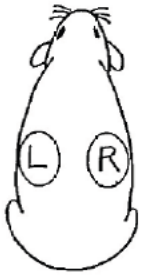
Hair removal: At 7th day of intradermal injection, hair removal is performed as same as Sensitiziation Treatement I.

Application adhesive plaster: 2 x 4 cm filter paper (Toyo No.131) lined with 3 x 5 cm Blenderm Surgical Tape.

Application: Apply 0.6 ml of the sample on the filter paper of the adhesive plaster. the undiluted solution of the main test sample was used for The main test sample group, and 5% Control sample solution (solvent is 70% Ethyl alcohol solution) was used for the control sample group. This adhesive plaster is placed in close contact with the site of Fig. 4, and the fixed with Elatex Expandable Tape as explained above, and Peel off after 48 hours.

[[Challenge Treatment]] 24 Hours occlusive applciation Test

The challenge procedure was carried out on day 21 after the intradermal injection.



Hair removal: Hair removal is performed at the site shown in FIGS. 5 and 6 in the same manner as in the preliminary test.

Control specimen group (Figure 6)

Adhesive plaster: The application method and observation are in accordance with the preliminary test section.

Criteria: According to Table 1 of the preliminary test.

Samples: The main test samples was applied in the L part in the main test sample group, and a 1% solution of the control sample (solvent is 70% ethyl alcohol aqueous solution) was applied in the L part and a 70% ethyl alcohol aqueous solution of the solvent is applied to the R part in the control group.

[Challenge control test] a concentration of non-irritating for the sample for the above challange test has been already determined in the preliminary test, but in order to detect irritation in detail, it is performed on the same day as the challenge test.

Animals: Untreated animals are used, 10 in the main test sample group and 10 in the control sample group.

Samples, hair removal, The adhesive plaster, application method, and observation were the same as the challenge test, and the judgment criteria were as follows Table 1.

Results Table 3 shows the results of the challenge test of the main test sample and Table 4 shows the results of the challenge test of the control sample.

the main test sample (neat)			
Animal number	immediately after	After 24 hours	After 48 hours
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0
16	0	0	0

Table -3

1.0% solution of the control sample (solvent is 70% ethyl alcohol aqueous solution)			
Animal number	immediately after	After 24 hours	After 48 hours
17	0	1	2
18	1	2	2
19	1	2	3
20	0	2	2
21	0	2	2
22	0	1	2
23	1	3	3
24	0	2	2
25	0	1	0
26	0	1	2
70% ethyl alcohol aqueous solution			
Animal number	immediately after	After 24 hours	After 48 hours
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0
21	0	0	0
22	0	0	0
23	0	0	0
24	0	0	0
25	0	0	0
26	0	0	0

Table -4

The results of the challenge controlled test are shown in Table 5.

the main test sample neat			
Animal number	immediately after	After 24 hours	After 48 hours
27	0	0	0
28	0	0	0
29	0	0	0
30	0	0	0
31	0	0	0
32	0	0	0
33	0	0	0
34	0	0	0
35	0	0	0
36	0	0	0
1.0% solution of the control sample (solvent is 70% ethyl alcohol aqueous solution)			
Animal number	immediately after	After 24 hours	After 48 hours
37	0	0	0
38	0	0	0
39	0	0	0
40	0	0	0
41	0	0	0
42	0	0	0
43	0	0	0
44	0	0	0
45	0	0	0
46	0	0	0
70% ethyl alcohol aqueous solution			
Animal number	immediately after	After 24 hours	After 48 hours
37	0	0	0
38	0	0	0
39	0	0	0
40	0	0	0
41	0	0	0
42	0	0	0
43	0	0	0
44	0	0	0
45	0	0	0
46	0	0	0

Table -5

The results of the challenge test and the challenge control test of the main test sample, *Galactomyces* ferment filtrate, showed no change in the naked eye in all animals, and sensitization was not established in all 10 animals.
On the other hand, in the control sample group, 9 animals except for animal #25 had a response of 2 points or more (read after 48 hours), and it was judged that sensitization was established in 9 out of 10. Animal No. 25 showed a response with score 1 at 24hr reading, but it disappeared after 48 hours, so the success sensitiziation was suspicious amd the case was excluded.

Conclusion and discussion: The sensitization rate is calculated by the following formula.

$$\text{Sentiment rate} = \frac{\text{Number of animals sensitized}}{\text{Number of animals tested}} \times 100$$

The sensitization rate of the main test sample was 0%, and the sensitization rate of the control sample was 90%. The sensitization rate of paraphenylenediamine in the results of Fujii, Tanikame et al. in reference (2) was 100%, and the result was almost consistent. In reference (1), sensitization ability is classified according to sensitization rate as follows.

Sensitization rate (%)	strength	classify
0 ～ 8	I	Has a weak sensitizing ability.
9 ～ 28	II	There is a slight sensitization ability.
29 ～ 64	III	There is moderate sensitization.
65 ～ 80	IV	It has a strong sensitizing ability.
81 ～ 100	V	Has extreme sensitizing ability.

Table -6

Paraphenylenediamine has an intensity of V and extreme sensitization.

Galactomyces ferment filtrate is assessed to be a weak sensitizing substance with intensity I, but since the sensitization rate is 0%, it is judged to be very weak or almost non-sensitizing.

Note 1: Intradermal injection is performed as shallowly as possible.
Note 2: FCA is 1.5 ml of Arlacel A (mannide mono oleate) with 8.5 ml of paraffin oil with 5 mg of killed Mycobacterium tuberculosis. Shaken well before use. It was W/O emulsified with an equal amount of saline.

References

（1） Bertil Magnusson & Albert M. Kligmann: Allergic Contact Dermatitis in the guinea pig Identifications of Contact Allergens (1970) CHARLES CTHOMAS PUBLISHER
（2） "New Toxicity and Safety Assessment" SoftScience Inc.
（3） 「Guinea pig maximization test (GPMT) performs “Best over all”」 FDA Report Vol. 1, No.19 September 8, 1980

[redacted]

FINAL REPORT

Study Title

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Test Articles

[redacted]

Facial treatement essence with 92.675% Galactomyces ferment filtrate

Authors

John W. Harbell, Ph.D.
Hans A. Raabe, M.S.
Angela M. Sizemore, B.S.

Study Completion Date

July 14, 2000

Performing Laboratory

Institute for In Vitro Sciences, Inc.
21 Firstfield Road, Suite 220
Gaithersburg, Maryland 20878

[redacted]

Laboratory Project Number

[redacted]

[redacted]

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

SUMMARY TABLE

Facial treatment essence with 92.675% Galactomyces fermenet filtrate	t_{50} (minutes)	
Test Article	**Preliminary**	**Definitive**
[redacted]	> 180 [redacted]	> 240 [redacted]
0.3% Triton X-100	15.4[1]	16.5

1 - The positive control t_{50} value was not within the acceptable range in the preliminary assay. Since the results of the preliminary assay were only used to select appropriate exposure times for the definitive assay, the positive control failure did not affect the outcome of the study.

TABLE OF CONTENTS

[redacted]

STATEMENT OF COMPLIANCE

Facial treatment essence with 92.675% Galactomyces ferment filtrate

The Tissue Equivalent Assay with EpiOcular™ Cultures of the test articles, [redacted], was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the U.S. EPA GLP Standards 40 CFR 160 and 40 CFR 792, the UK GLP Compliance Programme, the Japanese GLP Standard and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test or control articles have not been determined by the testing facility.

The stability of the test or control articles under the test conditions has not been determined by the testing facility and is not included in the final report.

Analyses to determine the uniformity, concentration, or stability of the test or control mixtures, if applicable, were not performed by the testing facility.

John W. Harbell, Ph.D.
Study Director

July 14, 2000
Date

COPY

QUALITY ASSURANCE STATEMENT

Study Title: Tissue Equivalent Assay With Epiocular Cultures

Study Number: [redacted]

Study Director: John W. Harbell, Ph.D.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLP Standards (40 CFR 792 and 40 CFR 160), the UK GLP Compliance Programme, the Japanese GLP Standard and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

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

Inspect on 22 Mar 00, to Study Director 22 Mar 00, to Management 24 Mar 00
Phase: Removal and transfer of tissue - definitive

Inspect on 05 May 00, to Study Director 05 May 00, to Management 05 May 00
Phase: Draft report and data

Inspect on 13 Jul 00, to Study Director 13 Jul 00, to Management 13 Jul 00
Phase: Final report

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

Pamela H. Errico

Pamela H. Errico, M.A., RQAP-GLP
Quality Assurance

July 13, 2000
Date

SIGNATURE PAGE

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Initiation Date:	March 16, 2000
Completion Date:	July 14, 2000
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility:	Institute for In Vitro Sciences, Inc. 21 Firstfield Road, Suite 220 Gaithersburg, Maryland 20878
Archive Location:	Institute for In Vitro Sciences, Inc. Gaithersburg, Maryland 20878
Study Director:	John W. Harbell, Ph.D. July 14, 2000 Date
Laboratory Manager:	Greg Mun, B.A.

COPY

TEST ARTICLE RECEIPT

Facial treatment essence with 92.675% Galactomyces ferment filtrate

IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions[1]
[redacted]	[redacted]	clear, colorless, non-viscous liquid	03/14/00	room temperature
[redacted]				

1 - Protected from exposure to light
2 - One of the two samples received was found to have leaked a small volume during shipment. The leaking sample was not used in this study.

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

[redacted]

INTRODUCTION

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

The purpose of this study was to evaluate the toxicity of the test articles supplied by [redacted] as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to a test article for various exposure times. The laboratory phase of the study was conducted from March 21, 2000 to March 23, 2000 at the Institute for In Vitro Sciences, Inc. After a time range finding assay, the test articles were tested in a definitive assay (four exposure times) to determine the time of exposure to a test article which resulted in the t_{50} endpoint.

---

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

MATERIALS AND METHODS

Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ human cell construct model kit, the solutions were stored as indicated. The EpiOcular™ human cell constructs were stored at 2-8°C until used. An appropriate volume of EpiOcular™ human cell construct assay medium was removed and warmed to approximately 37°C. Nine-tenths ml of assay medium was aliquoted into the wells of 6-well plates. The six-well plates were labeled to indicate test article and exposure time. The samples were inspected for air bubbles between the agarose gel and millicell insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the millicell area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the 6-well plates. The EpiOcular™ human cell constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour. The medium was aspirated and 0.9 ml of fresh medium were added to each assay well below the EpiOcular™ human cell construct. The trays were returned to the incubator until treatment was initiated.

Assessment of Direct Test Article Reduction of MTT

Each test article was added to a 1.0 mg/ml MTT solution in DMEM to assess their ability to directly reduce MTT. Approximately 100 µl of each test article were added to 1 ml of the MTT solution and the mixture was incubated in the dark at room temperature for approximately one hour. If the MTT solution color turned blue/purple, the test article was presumed to have reduced the MTT. Water insoluble test materials may show direct reduction (darkening) only at the interface between the test article and the medium.

Neither test article was observed to directly reduce MTT in the absence of viable tissue, and therefore, the killed-control experiment using freeze-killed tissues was not performed in this study.

Time Range Finding Assay

A time range finding assay was performed to establish an appropriate exposure time range to be used in the definitive assay for each test article. Four exposure times of 10, 30, 60, and 180 minutes were selected for the time range finding assay. One culture was treated per exposure time with 100 µl of the appropriate test article. The negative control (exposure time control)(100 µl of sterile, deionized water (Quality Biological)) was tested for 180 minutes. The positive control (100 µl of 0.3% Triton X-100 (Fisher)) was tested for 15 and 45 minutes.

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with calcium and magnesium-free Dulbecco's Phosphate Buffered Saline (DPBS) and the wash medium was decanted. After rinsing, the tissue was transferred to 5 ml of Assay Medium for a 10 to 20 minute incubation at room temperature to remove any test article absorbed into the tissue. A 1.0 mg/ml solution of MTT in warm Dulbecco's Modified

Eagle's Medium (DMEM) was prepared. Three-tenths ml of MTT (Sigma) reagent were added to wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing. The trays were incubated at 37±1°C for approximately three hours in a humidified atmosphere of 5±1% CO_2 in air.

After the incubation period with MTT solution, the EpiOcular™ cultures were extensively rinsed with DPBS, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 ml of isopropanol. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for two hours at room temperature.

At the end of the extraction period, the liquid within the millicell inserts was decanted into the well from which the millicell insert was taken. The extract solution was mixed and 200 µl were transferred to the appropriate wells of a 96-well plate. The absorbance at 550 nm (OD_{550}) of each well was measured with a Molecular Devices' Vmax plate reader.

In the time range finding assay, the positive control results were not within the acceptable range. However, since the results of the time range finding assay were only used to select appropriate test article exposure times, the positive control failure did not affect the outcome of the study.

Definitive Assay

Based on the results of the time range finding assay, four exposure times were chosen for the definitive assay. The exposure times were chosen such that generally two exposure times were expected to result in survivals lower than 50% and two exposure times were expected to result in survivals greater than 50%. In general, the negative control exposure times were selected to fit the range of the test article or positive control exposure times. The exposure times for the test articles were 60, 120, 180, and 240 minutes. The negative control was tested for 15 and 240 minutes. The positive control was tested for 15 and 45 minutes. The procedures used to conduct the definitive assay were essentially the same as for the time range finding assay with the exception that duplicate cultures were dosed per exposure time.

Presentation of Data

The mean OD_{550} values of the negative control wells, blank control wells, each positive control and each test article well for the various exposure times were calculated. The corrected mean OD_{550} values of the negative control, test article exposure times and the positive control exposure times were determined by subtracting from each the mean OD_{550} values for the blank control. All calculations were performed using IIVS' Laboratory Information Management System. The raw absorbance values were captured, and the following calculations were made:

$$\% \text{ of Control} = \frac{\text{corrected mean } OD_{550} \text{ of Test Article Exposure time}}{\text{corrected mean } OD_{550} \text{ of Negative control}} \times 100$$

[redacted]

RESULTS AND DISCUSSION

Time Range Finding Assay

A time range finding assay was performed, consisting of four exposure times (10, 30, 60 and 180 minutes) for the test articles supplied by [redacted] The time response curves for the test articles are included in Appendix B. Based upon the results of the time range finding assay, four exposure times were selected for each test article for the definitive assay (see Materials and Methods). The t_{50} results for the time range finding assay are reported in Table 1, under "Preliminary".

In the time range finding assay, the positive control t_{50} value was not within the acceptable range. However, since the results of the time range finding assay were only used to select appropriate test article exposure times, the positive control failure did not affect the outcome of the study.

Definitive Assay

facial treatment essence with 92.675% Galactomyces ferment filtrate

Four exposure times were tested in duplicate for each test article. The exposure times for the test articles, [redacted], were 60, 120, 180, and 240 minutes. The negative control was also tested in duplicate for 15 and 240 minutes. Table 1 summarizes the t_{50} results of the definitive EpiOcular™ human cell construct assay for the test articles and the positive control, 0.3% Triton X-100, under "Definitive". The time response curves for each test article as well as the positive control are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (15.5 - 37.0 minutes), and the corrected mean OD_{550} value for the minimum negative control exposure time (1.217) was within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 240 minutes) (1.256), the assay results were accepted. Finally, neither of the test articles was observed to directly reduce MTT in the absence of viable tissue, and therefore, the killed-control experiment using freeze-killed tissues was not performed in this study.

Table 1

Facial treatment essence with 92.675% Galactomyces ferment filtrate	t_{50} (minutes)	
Test Article	**Preliminary**	**Definitive**
[redacted]	> 180 [redacted]	> 240 [redacted]
0.3% Triton X-100	15.4[1]	16.5

1 - The positive control t_{50} value was not within the acceptable range in the preliminary assay. Since the results of the preliminary assay were only used to select appropriate exposure times for the definitive assay, the positive control failure did not affect the outcome of the study.

APPENDIX A

[redacted]

IIVS Study Number: [redacted]

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

1.0 PURPOSE

The purpose of this study is to evaluate the potential toxicity of the test article. In the Tissue Equivalent Assay, stratified human epithelial cell cultures (MatTek EpiOcular™) are exposed to topically applied test articles to evaluate potential ocular toxicity. Cell viability is determined by conversion of 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) in the treated cultures, and is expressed as a percentage relative to untreated (negative control) cultures. The endpoint of the Tissue Equivalent Assay, the t_{50} value, is the time (generally in minutes or hours) of exposure to test article required to reduce cell viability (MTT metabolism) to 50% of negative control levels as calculated from time-response curves.

2.0 SPONSOR

See Protocol Attachment 1

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1 Test Article: See Protocol Attachment 1

3.2 Controls:

Positive:	0.3% Triton X-100
Negative:	Sterile water (or other solvent as appropriate)
	blank control (MTT reading only)

3.3 Determination of Strength, Purity, etc.

3.3.1 The Sponsor will be responsible for determination and documentation of the analytical purity and composition of the test article and the stability and strength of the dosing solutions, as applicable.

3.3.2 The Institute for In Vitro Sciences, Inc. (IIVS) will be responsible for the documentation of the analytical purity and composition of the Triton X-100 used for the stock or working dilution of the positive control. This may be accomplished by maintaining a certificate of analysis from the supplier.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1	Name:	Institute for In Vitro Sciences, Inc.
4.2	Address:	21 Firstfield Road, Suite 220 Gaithersburg, MD 20878
4.3	Study Director:	Hans A. Raabe, M.S.

5.0 TEST SCHEDULE

5.1	Proposed Experimental Initiation Date:	21 March 2000
5.2	Proposed Experimental Completion Date:	30 March 2000
5.3	Proposed Report Date:	28 April 2000

6.0 TEST SYSTEM

The EpiOcular™ human cell construct, provided by the MatTek Corporation, will be used in this study. The use of EpiOcular™ cultures offers several features appropriate for a model for ocular irritation. First, the model is composed of stratified human keratinocytes in a three-dimensional structure. Secondly, test materials can be applied topically to the model so that water insoluble materials may be tested.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The experimental design of this study consists of a time-range-finding assay and a definitive assay. The toxicity of the test article will be evaluated by the exposure time required to reduce cell viability to 50% of controls (t_{50}). Viability will be determined by the succinate dehydrogenase-dependent reduction of MTT in control and test article treated cultures. Data will be presented in the form of relative survival (relative MTT conversion) versus test article exposure time.

One of two exposure time ranges may be used. The standard exposure time range extends up to four hours and is used for most materials to be tested. For extremely mild materials, such as those which might be applied around or in the eyes, a long exposure assay might be used. For the long exposure study, exposure times of up to 24 hours could be used. In general, the standard exposure range will be used, unless the Sponsor specifies an alternative exposure time range or if the study director determines that the class of test articles warrants the use of an alternative exposure time range.

7.1 Media and Reagents

7.1.1 Assay Medium: supplied by MatTek Corporation

7.1.2 EpiOcular Tissue: OCL-200 supplied by MatTek Corporation

7.1.3 Dulbecco's Minimal Essential Medium (DMEM)

7.1.4 3-[4,5 - dimethylthiazol-2-yl] - 2,5 - diphenyltetrazolium bromide (MTT) 10X stock solution: 10 mg/ml MTT in PBS

7.1.5 Dulbecco's Phosphate Buffered Saline (D-PBS)(pH 7.0 ± 0.5)

7.1.6 Extraction Medium: Isopropanol

7.2 Preparation and Delivery of Test Article

Test articles will generally be tested neat. End use concentrations or other forms may be used as directed by the Sponsor. One hundred µl of pipettable substances, such as liquids, gels, creams, and foams, will be applied directly on the tissue so as to cover the upper surface. To aid in filling the pipet for pipettable materials that are viscous, the test article may first be transferred to a syringe. The pipet tip of the positive displacement pipet will be inserted into the dispensing tip of the syringe so that the material can be loaded into the displacement tip under pressure. Simultaneously, the syringe plunger is depressed as the pipet piston is drawn upwards. If air bubbles appear in the pipet tip, the test article should be removed (expelled) and the process repeated until the tip is filled without air bubbles. This method should be used for any materials that can not be easily drawn into the pipet such as gels, (e.g., toothpastes, mascaras, and face creams) and solid test articles which are creamed like lipsticks and antiperspirants/deodorant sticks. A dosing device may be placed over the test article to assure even spreading, if required. Dry powders will be ground with a mortar and pestle and passed through a #40 copper sieve. Thirty mg samples will be measured into glass vials prior to test article treatment. Materials which are too viscous to spread over the tissue will first be spread onto the flat end of a dosing device. The dosing device will be put into the Millicell to bring the test article in contact with the tissue. When the dosing device is used, approximately 30 µl or 30 mg of material will be applied to the dosing device so as to cover the dosing surface. The sample should be spread to form a relatively smooth even layer on the surface of the dosing device to maximize uniform tissue contact. Solids such as lipsticks or antiperspirant/deodorant sticks can be presoftened by creaming a portion in a weigh boat. The softened portion can be transferred to a syringe affixed with a three way stopcock attached to a second syringe. The sample is pushed from syringe to syringe until it is of a consistency which can be pipetted. The exact exposure conditions used for other test article forms will be determined after consultation with the Sponsor and/or the study

director. All exposure conditions will be documented in the study workbook.

The stability of the test article under the actual experimental conditions will not be determined by IIVS.

7.3 Route of Administration

Test article will be administered by topical application to the cell cultures.

7.4 Controls

Two types of control treatments are used in this assay. The negative control cultures (negative control) are treated with sterile water or other solvent. Negative control cultures are dosed and exposed in parallel with the test article and positive control cultures. The exposure times used for the negative controls are selected to address the range of exposure times used for the test article and positive control cultures. Positive control cultures are treated with 0.3% Triton X-100 prepared in sterile water and are dosed and exposed for 15 and 45 minutes.

Time range finding assay: The assay will include a negative, positive control and blank control (plate reading step). Each test group will be tested with at least a single culture. The negative control will be tested in at least one exposure time, which will generally be chosen to address the longest test article or positive control exposure time. Single cultures will be used for each of the two positive control exposure times.

Definitive assay: Generally, at least two negative control exposure times will be used. At least duplicate cultures will be used for each control time. One negative control exposure time will be selected to fit the range of the shortest test article or positive control exposure times (the minimum negative control exposure time will be 15 minutes) while the second negative control exposure time will be selected to match the longest test article or positive control exposure time (whichever is longer, up to 240 minutes). On occasion, the second negative control exposure time may be selected to fit the longest test article exposure time of a test article run concurrently, but from an independent study. For the long exposure assay (exposures of greater than 240 minutes), multiple negative control exposure times may be selected to fit the range of test article exposure times. If all exposure times are less than one hour, a single negative control exposure time may be used. Additional negative control exposure times may be selected at the discretion of the Study Director. Two cultures will be used for each positive control exposure time.

7.5 Receipt of the EpiOcular™ Model

Upon receipt of the EpiOcular™ assay materials, the solutions will be stored as indicated by the manufacturer. The cell cultures will be stored at 2-8°C until used. Cultures should generally be used within 72 hours after they have been placed on agar by the manufacturer.

An appropriate volume of EpiOcular™ assay medium will be removed and warmed to approximately 37°C. Nine tenths (0.9) ml of assay medium will be aliquoted into the wells of 6-well plates. Each culture will be inspected for air bubbles between the agarose gel and Millicell™ insert prior to opening the sealed package. Cultures with air bubbles under greater than 50% of the Millicell™ area will not be used. The 24-well shipping containers will be removed from the plastic bag and the surface disinfected with 70% ethanol. An appropriate number of cultures will be transferred aseptically from the 24-well shipping containers into the 6-well plates. The EpiOcular™ cultures will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour prior to dosing. The medium will be aspirated and 0.9 ml of fresh medium will be added to each assay well below the tissue prior to dosing. Note: The refeeding step may occur at less than 1 hour but the tissue should be allowed at least one hour of incubation to become fully metabolically active before dosing.

7.6 Time-Range-Finding Assay

At least four exposure times will be evaluated for each test article. For the standard assay, the exposure times for the time-range-finding assay will generally be 10, 30, 60 and 180 minutes unless the Sponsor requests other specific exposure times. The maximum exposure time will be 240 minutes. For the long term exposure assay, the exposure times for the time range finding assay will generally be 4, 8, 16, and 24 hours.

Each test article and control exposure time will be tested by treating one culture. One hundred µl of each pipettable test article will be delivered with a positive displacement pipet onto the culture. Powders will be placed directly onto the culture at 30 mg/culture. Those materials which are too viscous to spread on the culture may be spread onto a dosing device. Approximately 30 µl or 30 mg will be applied to the dosing device for each culture. See section 7.2 for precise details. Exposure times of five minutes or greater will be incubated at 37 ± 1°C and 5 ± 1% CO_2 in air.

At the end of the treatment time, the test article will be removed by extensively rinsing both sides of the culture with D-PBS. The process will be performed until the culture appears free of test article. If it is not possible to remove all of the visible test material, this will be noted in the study workbook. After rinsing, the culture will be transferred to 5 ml of Assay Medium for a 10 to 20 minute

incubation at room temperature. This rinse is intended to remove any test article absorbed into the culture.

A 1.0 mg/ml MTT solution will be prepared immediately before use. MTT will be dissolved in warm DMEM and filtered through a 0.45 µm filter to remove undissolved crystals. Alternatively, a 10x stock of MTT prepared in PBS will be thawed and diluted in warm DMEM to produce the 1.0 mg/ml solution. The exact procedure will be documented in the study workbook and the method will be consistent within an assay. Three hundred µl of the MTT solution will be added to each well of a prelabelled 24-well plate. Excess Assay Medium will be removed and then the EpiOcular™ cultures will be transferred to the appropriate wells of the MTT plate. The 24-well plates will be incubated at 37±1°C for approximately 3 hours in a humidified atmosphere of 5±1% CO_2 in air.

After approximately three hours, the EpiOcular™ tissues will be rinsed in D-PBS and transferred to a prelabelled 24-well plate containing 2.0 ml of isopropanol in each well. The plates will be sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time is harvested. If necessary, plates may be stored overnight (or up to 20 hours after the last exposure time is harvested) in the refrigerator prior to extracting the MTT. The plates will then be shaken for 2 hours at room temperature. At the end of the extraction period, the liquid within the Millicell™ inserts will be decanted into the well from which the Millicell™ insert was taken. The extract solution will be mixed and 200 µl transferred to the appropriate wells of a 96-well plate. Two hundred µl of isopropanol will be added to the wells designated as blanks. The absorbance at 550 nm (OD_{550}) of each well will be measured with a Molecular Devices Vmax plate reader.

The range of exposure times for the definitive assay will be chosen to determine the t_{50} (the exposure time to the test article which reduces MTT metabolism by 50%). Based on the results of the time-range-finding assay, two exposure times will be chosen that should result in expected survivals lower than 50%, and two exposure times will be chosen that should result in expected survivals greater than 50%. If a test article fails to cause 50% toxicity in the time-range-finding assay, the maximum exposure time will be 240 minutes, or 24 hours, depending on the assay selected. In some cases, the exposure times for the definitive assay may be selected based on visual observations of the relative MTT reduction in the tissues.

7.7 Assessment of Direct Test Article Reduction of MTT

In some cases, it will be necessary to assess the ability of each test article to directly reduce MTT. A 1.0 mg/ml MTT solution will be prepared in DMEM as described above. Approximately 100 µl (liquid test articles) or 30 mg (solid test articles) will be added to 1 ml of the MTT solution and the mixture incubated in the dark at room temperature for approximately one hour. If the MTT solution color turns blue/purple, the test article is presumed to have reduced the MTT. Water insoluble

test materials may show direct reduction (darkening) only at the interface between the test article and the medium. This test may be required if the MTT cytotoxicity results clearly demonstrate either non-toxic responses at all exposure times, excessive reduction of MTT in treated tissues or an inability to remove the test article from the tissues.

7.8 Definitive Assay

The definitive assay with generally four to five exposure times will be performed exactly like the time-range-finding assay with the exception that cultures will be tested in duplicate for each exposure time. If the test article(s) are found to be non-toxic in the time-range-finding assay, then fewer than four exposure times may be chosen for the definitive assay. Duplicate cultures will generally be tested at each of the positive control exposures. Duplicate cultures will be treated with negative or solvent control for each exposure time (see section 7.4). The determination of the t_{50} will be based upon the results of the definitive assay. At the Study Director's option, a second definitive assay may be performed.

7.9 Presentation of Data

The mean OD_{550} of the exposure time control wells, blank control wells, positive control wells and test article exposure time wells will be calculated. The corrected mean OD_{550} of the exposure time control, test article exposure times, and the positive control will be determined by subtracting the mean OD_{550} of the blank control from their mean OD_{550}s. Generally, all calculations will be performed using IIVS's licensed proprietary Laboratory Information Management System. The raw absorbance values will be captured, and the following calculations made:

$$\% \text{ Of Control} = \frac{\text{corrected mean } OD_{550} \text{ of Test Article Exposure Time}}{\text{corrected mean } OD_{550} \text{ of Control Exposure Time}} \times 100$$

Viability calculations for test articles treated in the long exposure time assay may be performed by comparing the corrected mean OD_{550}s of each test article exposure time to the appropriate exposure time control(s).

Exposure time response curves may be plotted with the % of control on the ordinate and the test article exposure time on the abscissa. Other plot forms may be used as requested by the Sponsor. The t_{50} will be interpolated from each plot. If the shortest test article exposure time shows less then 50% relative survival, the plot will be extended to include the t_0 point which will be given a value of 100%. In this case, the t_{50} will be determined between the t_0 and the shortest exposure time. At the Study Director's option, additional assays may be performed to produce the final t_{50} value.

[redacted]

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The assay will be accepted if the positive control compound, 0.3% Triton X-100, causes a t_{50} within two standard deviations of the historical mean. The historical mean is updated every three months. Since the shortest positive control exposure time is 15 minutes, t_{50} values of less then 15 minutes will be considered unacceptable. The corrected mean OD_{550} value for the minimum negative control exposure time must be within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time for assays up to 240 minutes.

9.0 REPORT

A report of the results of this study will be prepared by the Testing Facility and will accurately describe all methods used for generation and analysis of the data. A separate summary will be prepared reporting the t_{50} values for each assay with each test article as well as the positive control data. A copy of the protocol used for the study and any significant deviation(s) from the protocol and SOPs of the Testing Facility will appear as a part of the final report.

10.0 RECORDS AND ARCHIVES

A separate working notebook will be used to record the materials and procedures used to perform this study. Upon completion of the final report, all raw data and reports will be maintained by IIVS for the time period specified in the Laboratory Services Agreement in effect at the time of completion of the study.

11.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

The regulatory compliance requirements for this study are detailed in the Protocol Attachment 1.

The Quality Assurance Unit will review the study protocol, perform at least one in-process laboratory inspection, and audit the raw data workbook and all reports of the study to assure compliance with the appropriate regulations specified in the Protocol Attachment 1.

12.0 PROTOCOL AMENDMENTS

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

13.0 REFERENCES

MTT Effective Time 50 (ET-50) Protocol, MatTek Corporation

14.0 APPROVAL

(See Sponsor's Protocol Attachment 1)
SPONSOR REPRESENTATIVE

IIVS STUDY DIRECTOR

16 March 2000
DATE

PROTOCOL AMENDMENT I

ORIGINAL

DATE: March 28, 2000

SPONSOR: [redacted]

SPONSOR'S TEST ARTICLE DESIGNATIONS: Facial treatment essence with 92.675% Galactomyces ferment filtrate [redacted]

IIVS STUDY NO: [redacted]

PROTOCOL NO: [redacted]

PROTOCOL TITLE: TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

AMENDMENT:

1) Location: IIVS Study Number, Header page 1

Amendment: Change the following:

"[redacted]" to "[redacted]"

Reason: Typographical error

APPROVAL: [redacted]

AUTHORIZED REPRESENTATIVE DATE

Hans Raab 28 March 2000

STUDY DIRECTOR DATE

PROTOCOL AMENDMENT II

ORIGINAL

DATE: **April 27, 2000**

SPONSOR: [redacted]

SPONSOR'S TEST ARTICLE DESIGNATION: Facial treatment essence with 92.675% Galactomyces ferment filtrate [redacted]

IIVS STUDY NO: [redacted]

PROTOCOL NO: [redacted]

SPONSOR STUDY NO.: [redacted]

PROTOCOL TITLE: **Tissue Equivalent Assay with EpiOcular™ Cultures**

AMENDMENT:

1) Location: § 4.3 Study Director

Amendment: Change Study Director from "Hans A. Raabe, M.S." to "John W. Harbell, Ph.D.

Reason: To provide a transfer of study director responsibilities upon termination of the current study director's employment, effective May 5, 2000.

APPROVAL: [redacted]

AUTHORIZED REPRESENTATIVE DATE

J W Harbell 4/27/00

STUDY DIRECTOR DATE

APPENDIX B

EPIOCULAR BIOASSAY

EXPERIMENT DATE: 21-Mar-00 Study No. [redacted]
TEST MATERIAL: [redacted] Facial treatment essence with 92.675% Galactomyces ferment filtrate
TEST ARTICLE: [redacted]
PRELIMINARY ET50 = > 180 Minutes
CONCENTRATION: 100 %

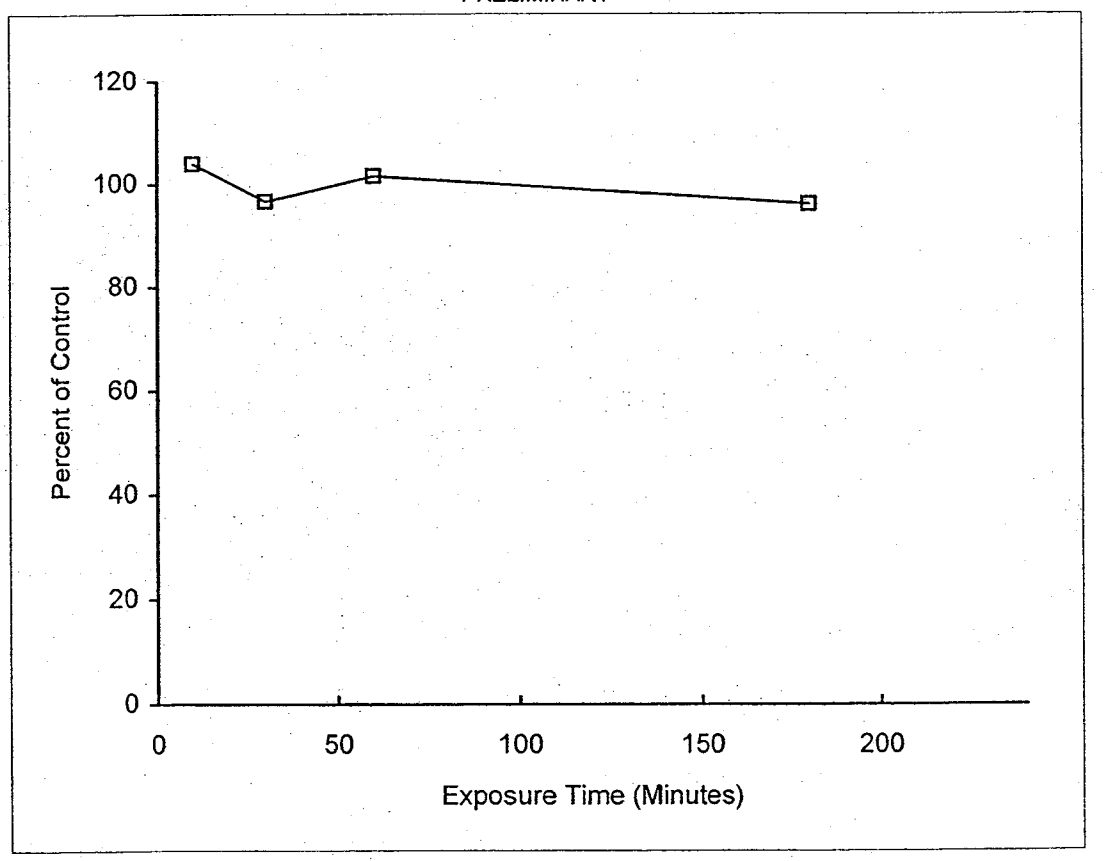
TIME EXPOSURE (Minutes)	PERCENT VIABLE
10	104.0
30	96.6
60	101.6
180	96.1

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	180.0	1	96.1
2	180.0	2	96.1
3	#DIV/0!	3	50
	slope =		#DIV/0!
	y intercept =		#DIV/0!

Facial treatment essence with 92.675% Galactomyces ferment filtrate [redacted]

PRELIMINARY



EPIOCULAR BIOASSAY

EXPERIMENT DATE: 22-Mar-00 Study No. [redacted]
TEST MATERIAL: [redacted] Facial treatment essence with 92.675% Galactomyces ferment filtrate
TEST ARTICLE: [redacted]
TRIAL 1 ET50 = > 240 Minutes
CONCENTRATION: 100 %

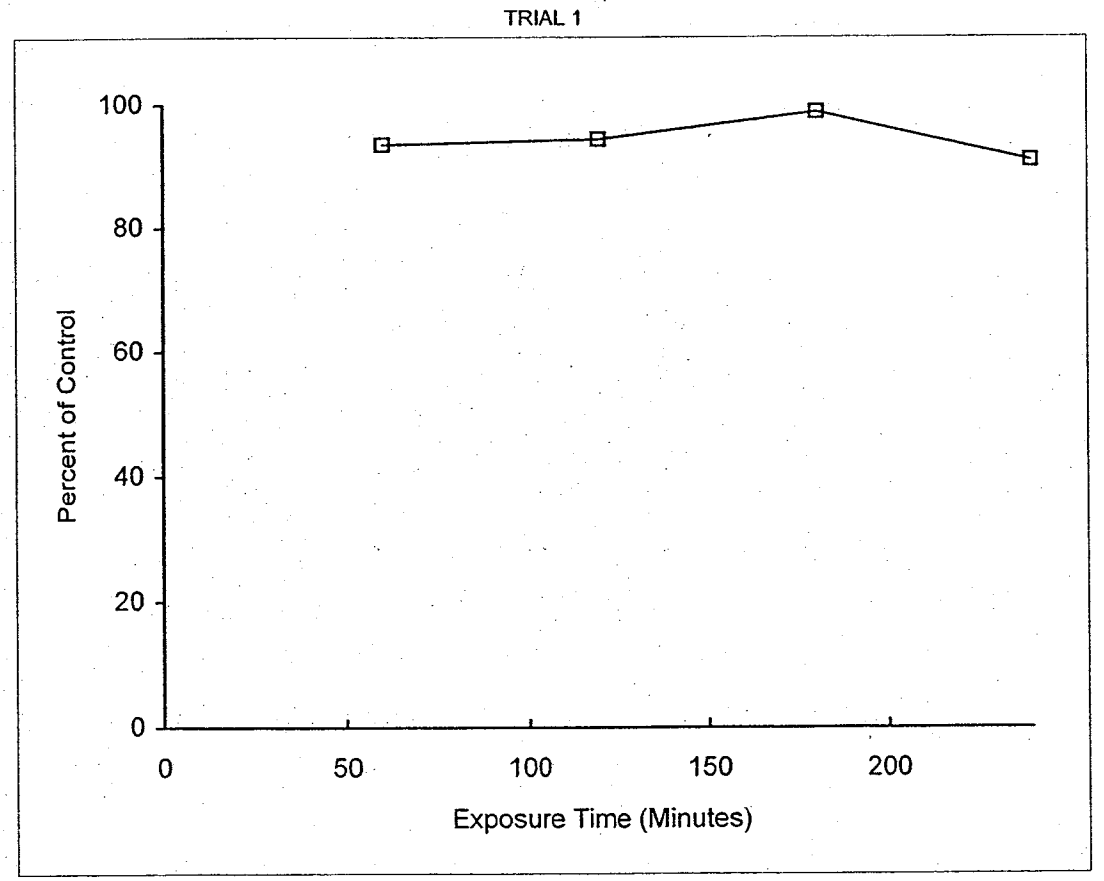
TIME EXPOSURE (Minutes)	PERCENT VIABLE
60	93.5
120	94.4
180	98.8
240	90.9

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	240.0	1	90.9
2	240.0	2	90.9
3	#DIV/0!	3	50
slope =			#DIV/0!
y intercept =			#DIV/0!

Facial treatment essence with 92.675% Galactomyces ferment filtrate

[redacted]



EPIOCULAR BIOASSAY

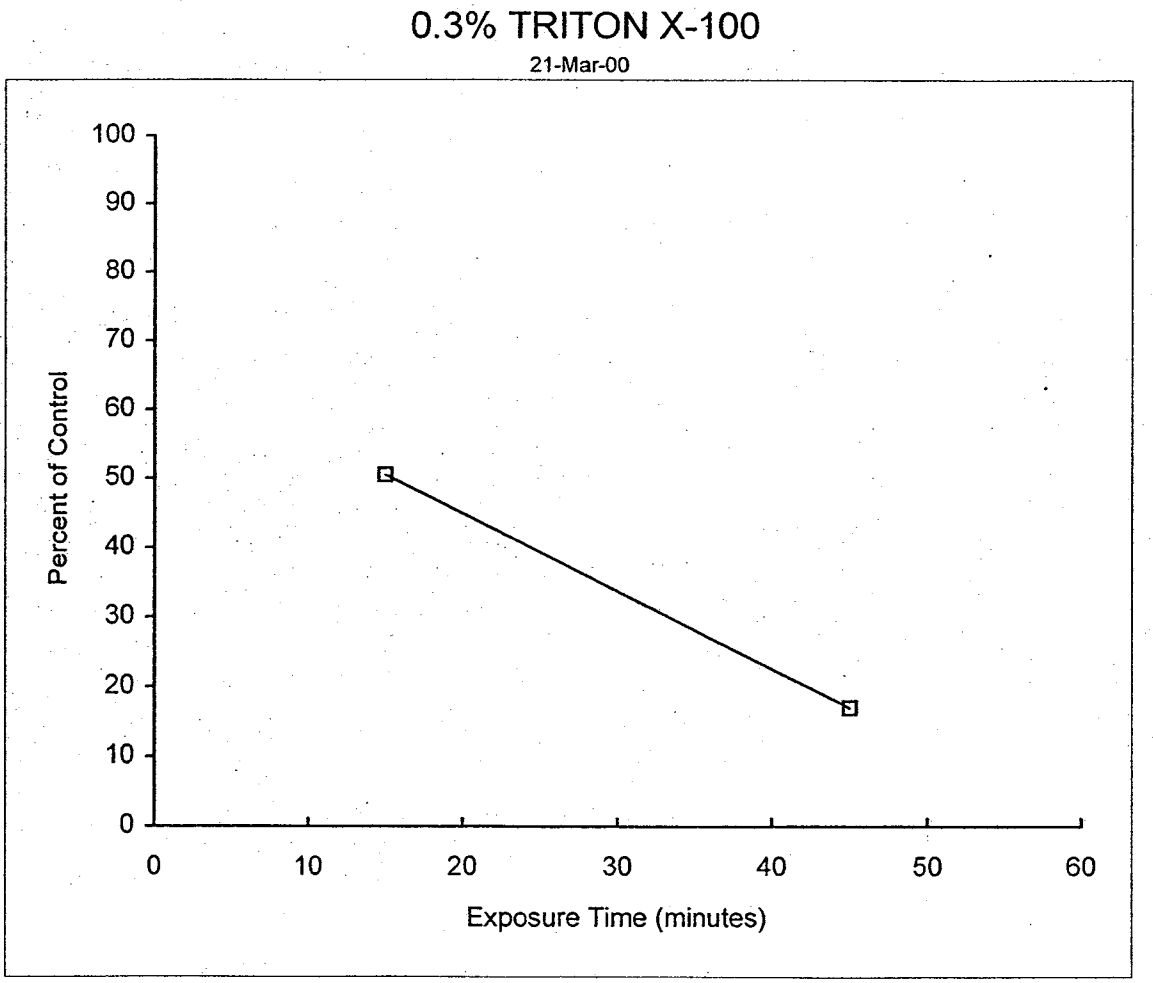
EXPERIMENT DATE: 21-Mar-00
TEST MATERIAL: 0.3% TRITON X-100

ET50 = 15.4 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	50.5
45	17.0

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	50.5
2	45.0	2	17
3	15.447761	3	50
	slope =		-1.116667
	y intercept =		67.25



EPIOCULAR BIOASSAY

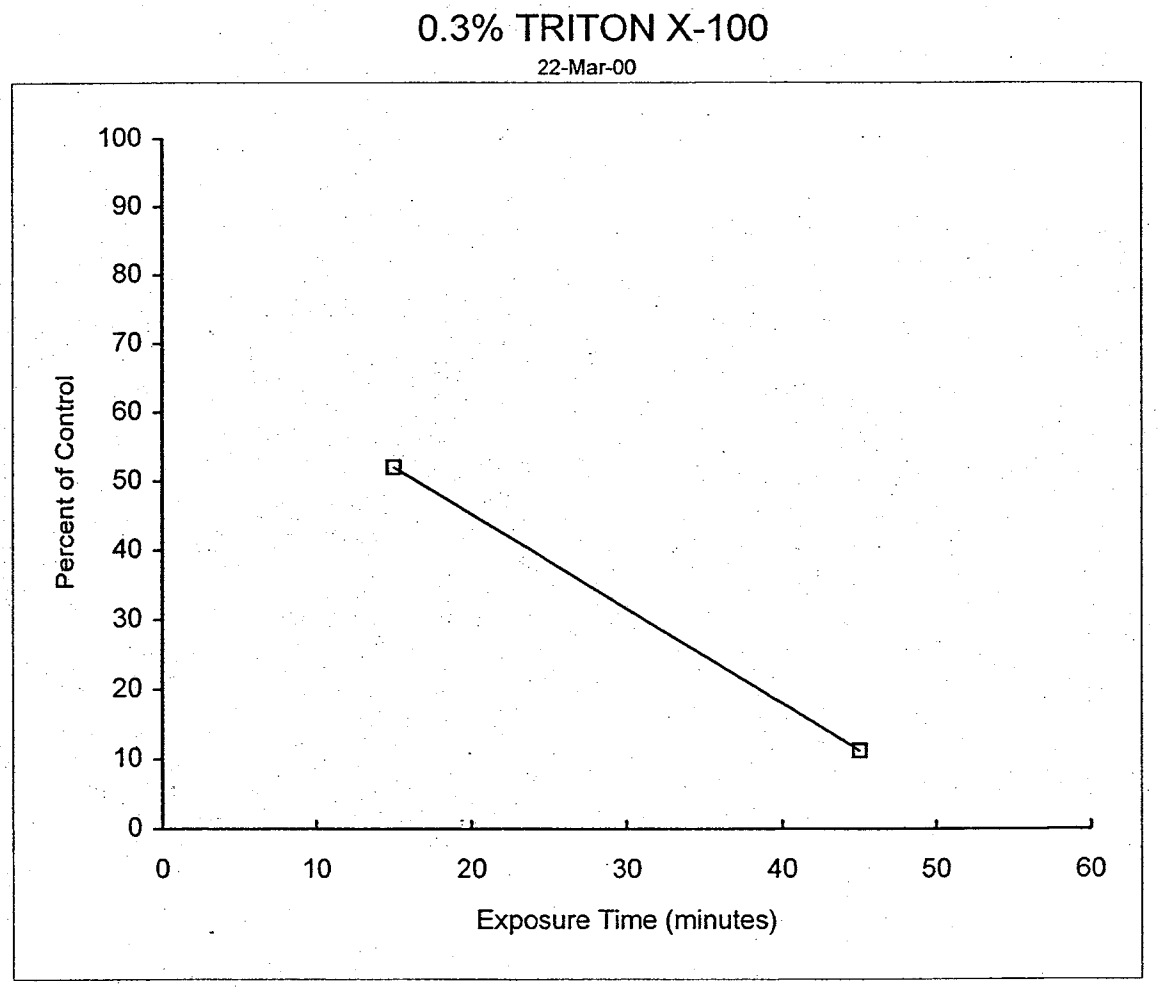
EXPERIMENT DATE: 22-Mar-00
TEST MATERIAL: 0.3% TRITON X-100

ET50 = 16.5 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	52.0
45	11.2

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	52
2	45.0	2	11.2
3	16.470588	3	50
	slope =		-1.36
	y intercept =		72.4



Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: August 16, 2023

SUBJECT: Galactomyces Ferment Filtrate

Anonymous. 2020. 100 Subject human repeat insult patch test for skin irritation and skin sensitization evaluation (test material is a skincare product that contains 1.485% Galactomyces Ferment Filtrate).

[redacted]

100 SUBJECT HUMAN REPEAT INSULT PATCH TEST FOR SKIN IRRITATION AND SKIN SENSITIZATION EVALUATION

Date: September 16, 2020

[redacted]

Sponsor: [redacted]

1.0 Objective: To determine the irritation and sensitization (contact allergy) potential of a test material after repeated application to the skin of human subjects.

2.0 Test Material:

2.1 Test Material Description: test material is a skincare product that contains 1.485% Galactomyces Ferment Filtrate

Date Received: May 29, 2020

Received From: [redacted]

Number Of Test Samples Received: 1

Label On Test Samples: [redacted]

Accession No.: [redacted]

2.2 Handling:

Upon arrival at [redacted] the test material was assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples will be retained for a period of thirty (30) days

[redacted]

beyond submission of final report unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, in which case representative retained samples are kept two (2) years beyond final report submission.

Sample disposition will be conducted in compliance with appropriate federal, state and local ordinances.

3.0 Panel Selection:

3.1 Standards for Inclusion in a Study:

- Individuals who were not currently under a doctor's care.
- Individuals who were free of any dermatological or systemic disorder that would interfere with the results, at the discretion of the Investigator.
- Individuals who were free of any acute or chronic disease that would interfere with or increase the risk of study participation.
- Individuals who completed a preliminary medical history form mandated by BCS and were in general good health.
- Individuals who read, understood and signed an informed consent document relating to the specific type of study.
- Individuals who were able to cooperate with the Investigator and research staff, and were willing to have test materials applied according to the protocol, and complete the full course of the study.

3.2 Standards for Exclusion from a Study:

- Individuals who were under 18 years of age.
- Individuals who were currently under a doctor's care.
- Individuals who were currently taking any medication (topical or systemic) that might mask or interfere with the test results.
- Individuals who had a history of any acute or chronic disease that might interfere with or increase the risk associated with study participation.
- Individuals who were diagnosed with chronic skin allergies.
- Female volunteers who indicated that they were pregnant or nursing.

3.3 Recruitment:

Panel selection was accomplished by advertisements in local periodicals, community bulletin boards, phone

solicitation, electronic media or any combination thereof.

3.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms are available for inspection on the premises of [redacted] only. [Reference 21 CFR Ch. 1 Part 50, Subpart B]

The parties agree to comply with applicable state and federal privacy laws for the use and disclosure of a subject's personal health information by taking reasonable steps to protect the confidentiality of this information. This obligation shall survive the termination or expiration of this Agreement.

4.0 Population Demographics:

Number of subjects enrolled	110
Number of subjects completing study	104
Age Range	18-61
Sex	
Male	32
Female	72
Fitzpatrick Skin Type*	
1 – always burn, does not tan	0
2 – burn easily, tan slightly	0
3 – burn moderately, tan progressively	104
4 – burn a little, always tan	0
5 – rarely burn, tan intensely	0
6 – never burn, tan very intensely	0

*[Agache P., Hubert P.. Measuring the skin. (p. 473, table 48.1) Springer-Verlag Berlin Heidelberg, 2004, (p. 473, table 48.1)]

5.0 Equipment:

Test materials to be tested under occlusive conditions were placed on an 8-millimeter aluminum Finn Chamber® (Epitest Ltd. Oy, Tuusula, Finland) supported on Scanpor® Tape (Norgesplaster A/S, Kristiansand, Norway) or an 8-millimeter filter paper coated aluminum Finn Chamber® AQUA supported on a thin flexible transparent polyurethane rectangular film coated on one side with a medical grade acrylic adhesive, consistent with adhesive used in state-of-the-art hypoallergenic surgical tapes or a 7mm IQ-ULTRA® closed cell system which is made of additive-free polyethylene plastic foam with a filter paper incorporated (It is supplied in units of 10 chambers on a hypoallergenic non woven adhesive tape; the width of the tape is 52mm and the length is 118mm) or other equivalents.

Test materials to be tested under semi-occlusive conditions were placed on a test strip with a Rayon/Polypropylene pad or on a 7.5mm filter paper disc affixed to a strip of hypoallergenic tape (Johnson & Johnson 1 inch First Aid Cloth Tape).

Test materials to be tested in an open patch were applied and rubbed directly onto the back of the subject.

Approximately 0.02-0.05 mL (in case of liquids) and/or 0.02-0.05 gm (in case of solids) of the test material was used for the study. Liquid test material was dispensed on a 7.5mm paper disk, which fit in the Finn Chamber.

6.0 Procedure:

- Subjects were requested to bathe or wash as usual before arrival at the facility.
- Patches containing the test material were then affixed directly to the skin of the intrascapular regions of the back, to the right or left of the midline and subjects were dismissed with instructions not to wet or expose the test area to direct sunlight.
- Patches remained in place for 48 hours after the first application. Subjects were instructed not to remove the patches prior to their 48 hour scheduled visit. Thereafter, subjects were instructed to remove patches 24 hours after application for the remainder of the study.

- This procedure was repeated until a series of nine (9) consecutive, 24-hour exposures had been made three (3) times a week for three (3) consecutive weeks.
- Prior to each reapplication, the test sites evaluated by trained laboratory personnel.
- Following a 10-14 day rest period a retest/challenge dose was applied once to a previously unexposed test site. Test sites were evaluated by trained laboratory personnel 48 and 96 hours after application.
- In the event of an adverse reaction, the area of erythema and edema were measured. Edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin.
- Subjects were instructed to report any delayed reactions that might occur after the final reading.
- Clients will be notified immediately in the case of an adverse reaction and a determination is made as to treatment program if necessary.

7.0 Scoring:

Scoring scale and definition of symbols shown below are based on the scoring scheme according to the International Contact Dermatitis Research Group scoring scale [Rietschel, R.L., Fowler, J.F., Ed., Fisher's Contact Dermatitis (fourth ed.). Baltimore, Williams & Wilkins, 1995] listed below:

0 no reaction (negative)
1 erythema throughout at least ¾ of patch area
2 erythema and induration throughout at least ¾ of patch area
3 erythema, induration and vesicles
4 erythema, induration and bullae

D Site discontinued
Dc Subject discontinued voluntarily
Dcl Subject discontinued per Investigator

NOTE: Clinical evaluations are performed by a [redacted] investigator or designee trained in the clinical evaluation of the skin. Whenever feasible, the same individual will do the scoring of all the subjects throughout the study and will be blinded to the treatment assignments and any previous scores.

8.0 Results:

Accession No.:
Test Material Description: [redacted]

Patch Description: Semi-Occlusive

Subject Information					Induction									Challenge	
No.	Subject ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
1	[redacted]	F	28	3	0	0	0	0	0	0	0	0	0	0	0
2		F	40	3	0	0	0	0	0	0	0	0	0	0	0
3		F	59	3	0	0	0	0	0	0	0	0	0	0	0
4		F	41	3	0	0	0	0	0	0	0	0	0	0	0
5		F	38	3	0	0	0	0	0	0	0	0	0	0	0
6		F	56	3	0	0	0	0	0	0	0	0	0	0	0
7		M	55	3	0	0	0	0	0	0	0	0	0	0	0
8		F	36	3	0	0	0	0	0	0	0	0	0	0	0
9		M	24	3	0	0	0	0	0	0	0	0	0	0	0
10		F	45	3	0	0	0	0	0	0	0	0	0	0	0
11		F	40	3	0	0	0	0	0	0	0	0	0	0	0
12		F	42	3	0	0	0	0	0	0	0	0	0	0	0
13		F	62	3	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl
14		F	44	3	0	0	0	0	0	0	0	0	0	0	0
15		F	34	3	0	0	0	0	0	0	0	0	0	0	0
16		M	40	3	0	0	0	0	0	0	0	0	0	0	0
17		F	47	3	0	0	0	0	0	0	0	0	0	0	0
18		F	25	3	0	0	0	0	0	0	0	0	0	0	0
19		F	53	3	0	0	0	0	0	0	0	0	0	0	0
20		F	25	3	0	0	0	0	0	0	0	0	0	0	0
21		M	28	3	0	0	0	0	0	0	0	0	0	0	0
22		F	40	3	0	0	0	0	0	0	0	0	0	0	0
23		F	47	3	0	0	0	0	0	0	0	0	0	0	0
24		M	34	3	0	0	0	0	0	0	0	0	0	0	0
25		F	24	3	0	0	0	0	0	0	0	0	0	0	0
26		F	31	3	0	0	0	0	0	0	0	0	0	0	0
27		F	29	3	0	0	0	0	0	0	0	0	0	0	0
28		F	25	3	0	0	0	0	0	0	0	0	0	0	0
29		F	24	3	0	0	0	0	0	0	0	0	0	0	0
30		F	27	3	0	0	0	0	0	0	0	0	0	0	0
31		M	56	3	0	0	0	0	0	0	0	0	0	0	0
32		F	47	3	0	0	0	0	0	0	0	0	0	0	0
33		F	58	3	0	0	0	0	0	0	0	0	0	0	0

34	[redacted]	F	43	3	0	0	0	0	0	0	0	0	0	0	0
35		M	30	3	0	0	0	0	0	0	0	0	0	0	0
36		F	46	3	0	0	0	0	0	0	0	0	0	0	0
37		M	45	3	0	0	0	0	0	0	0	0	0	0	0
38		F	30	3	0	0	0	0	0	0	0	0	0	0	0
39		F	22	3	0	0	0	0	0	0	0	0	0	0	0
40		M	22	3	0	0	0	0	0	0	0	0	0	0	0
41		M	38	3	0	0	0	0	0	0	0	0	0	0	0
42		M	41	3	0	0	0	0	0	0	0	0	0	0	0
43		F	43	3	0	0	0	0	0	0	0	0	0	0	0
44		F	37	3	0	0	0	0	0	0	0	0	0	0	0
45		F	31	3	0	0	0	0	0	0	0	0	0	0	0
46		M	37	3	0	0	0	0	0	0	0	0	0	Dc	Dc
47		M	19	3	0	0	0	0	0	0	0	0	0	0	0
48		M	19	3	0	0	0	0	0	0	0	0	0	0	0
49		F	31	3	0	0	0	0	0	0	0	0	0	0	0
50		M	28	3	0	0	0	0	0	0	0	0	0	0	0
51		M	30	3	0	0	0	0	0	0	0	0	0	0	0
52		F	41	3	0	0	0	0	0	0	0	0	0	0	0
53		F	23	3	0	0	0	0	0	0	0	0	0	0	0
54		F	54	3	0	0	0	0	0	0	0	0	0	0	0
55		M	21	3	0	0	0	0	0	0	0	0	0	0	0
56		M	18	3	0	0	0	0	0	0	0	0	0	0	0
57		F	38	3	0	0	0	0	0	0	0	0	0	0	0
58		F	30	3	0	0	0	0	0	0	0	0	0	0	0
59		F	28	3	0	0	0	0	0	0	0	0	0	Dc	Dc
60		F	18	3	0	0	0	0	0	0	0	0	0	0	0
61		F	18	3	0	0	0	0	0	0	0	0	0	0	0
62		F	33	3	0	0	0	0	0	0	0	0	0	0	0
63		F	40	3	0	0	0	0	0	0	0	0	0	0	0
64		F	40	3	0	0	0	0	0	0	0	0	0	0	0
65		F	41	3	0	0	0	0	0	0	0	0	0	0	0
66		F	35	3	0	0	0	0	0	0	0	0	0	0	0
67		F	21	3	0	0	0	0	0	0	0	0	0	0	0
68		F	43	3	0	0	0	0	0	0	0	0	0	0	0
69		M	38	3	0	0	0	0	0	0	0	0	0	0	0
70		F	45	3	0	0	0	0	0	0	0	0	0	0	0
71		M	18	3	0	0	0	0	0	0	0	0	0	0	0
72		M	49	3	0	0	0	0	0	0	0	0	0	0	0
73		F	26	3	0	0	0	0	0	0	0	0	0	0	0
74		F	22	3	0	0	0	0	0	0	0	0	0	0	0

75	[redacted]	M	32	3	0	0	0	0	0	0	0	0	0	0	0
76		F	31	3	0	0	0	0	0	0	0	0	0	0	0
77		F	27	3	0	0	0	0	0	0	0	0	0	0	0
78		M	21	3	0	0	0	0	0	0	0	0	0	0	0
79		M	19	3	0	0	0	0	0	0	0	0	0	0	0
80		F	19	3	0	0	0	0	0	0	0	0	0	0	0
81		F	19	3	0	0	0	0	0	0	0	0	0	0	0
82		M	39	3	0	0	0	0	0	0	0	0	0	0	0
83		M	61	3	0	0	0	0	0	0	0	0	0	0	0
84		F	51	3	0	0	0	0	0	0	0	0	0	0	0
85		M	32	3	0	0	0	0	0	0	0	0	0	0	0
86		M	29	3	0	0	0	0	0	0	0	0	0	0	0
87		F	35	3	0	0	0	0	0	0	0	0	0	0	0
88		M	19	3	0	0	0	0	0	0	0	0	0	0	0
89		F	31	3	0	0	0	0	0	0	0	0	0	0	0
90		F	59	3	0	0	0	0	0	0	0	0	0	Dc	Dc
91		F	42	3	0	0	0	0	0	0	0	0	0	Dc	Dc
92		F	31	3	0	0	0	0	0	0	0	0	0	0	0
93		F	18	3	0	0	0	0	0	0	0	0	0	0	0
94		F	46	3	0	0	0	0	0	0	0	0	0	0	0
95		F	57	3	0	0	0	0	0	0	0	0	0	0	0
96		M	60	3	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl
97		F	19	3	0	0	0	0	0	0	0	0	0	0	0
98		M	39	3	0	0	0	0	0	0	0	0	0	0	0
99		M	25	3	0	0	0	0	0	0	0	0	0	0	0
100		F	39	3	0	0	0	0	0	0	0	0	0	0	0
101		F	54	3	0	0	0	0	0	0	0	0	0	0	0
102		M	51	3	0	0	0	0	0	0	0	0	0	0	0
103		F	18	3	0	0	0	0	0	0	0	0	0	0	0
104		M	29	3	0	0	0	0	0	0	0	0	0	0	0
105		F	30	3	0	0	0	0	0	0	0	0	0	0	0
106		F	22	3	0	0	0	0	0	0	0	0	0	0	0
107		F	23	3	0	0	0	0	0	0	0	0	0	0	0
108		F	28	3	0	0	0	0	0	0	0	0	0	0	0
109		F	18	3	0	0	0	0	0	0	0	0	0	0	0
110		F	42	3	0	0	0	0	0	0	0	0	0	0	0

*Subject dropped due to study criteria

9.0 Evaluation Period:

The study was conducted from July 6, 2020 to September 11, 2020.

10.0 Observations:

No adverse reactions of any kind were reported during the course of this study.

There were three (3) subjects with a Grade 4 reaction, three (3) subjects with a Grade 3 reaction, seven (7) subjects with a Grade 2 reaction, and twenty-two (22) subjects with a Grade 1 reaction to the positive control (2.0% Sodium Lauryl Sulfate Solution). No subjects showed any signs of reaction to the negative control (DI Water).

11.0 Study Archives:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens will be maintained on premises of [redacted] in limited access storage files marked "Archive".

12.0 Conclusions:

Under the conditions of the study, there was no indication of a potential to elicit dermal irritation or sensitization (contact allergy) noted for [redacted]

[redacted]
Clinical Manager

[redacted]
Clinical Quality Assurance Lead

[redacted]

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: June 22, 2023

SUBJECT: Galactomyces Ferment Filtrate

Hill Top Research 1999. Human repeat insult patch test (facial treatment essence with 92.675% Galactomyces Ferment Filtrate).

Institute for In Vitro Sciences Inc. 2000. Tissue Equivalent Assay with Epiocular™ Cultures (facial treatment essence with 92.675% Galactomyces Ferment Filtrate).

HILL TOP RESEARCH, INC.

HUMAN REPEAT INSULT PATCH TEST (HRIPT)

[redacted]

[redacted]

PERFORMED BY:
HILL TOP RESEARCH
3225 N. 75th Street
Scottsdale, Arizona 85251

September 8, 1999

HUMAN REPEAT INSULT PATCH TEST (HRIPT)
HILL TOP PROJECT [redacted]

SAMPLE IDENTIFICATION: [redacted]

Facial treatment essence with 92.675% galactomyces ferment filtrate

CONCENTRATION: Neat
VEHICLE: N/A

SUMMARY:

The test consisted of the following phases:
Induction Exposures: A series of nine patches containing the test substance were applied to the lateral surface of one upper arm. Patches were removed and discarded by the study subject after approximately 24 hours. Each induction patch was applied to the same site, three times per week, for the first three weeks of the study. Subjects who were absent during the three-week, nine-patch induction phase received a make-up induction patch on the first day of the fourth week of the test. Grading of test sites occurred 48 hours after patch application (72 hours after weekend applications).
Challenge Exposures: Prior to patch application subjects were asked whether there had been any change in health status or study exclusion drug use since the beginning of the study. A Challenge patch was applied 12-20 days after the last induction application, to both the original site and a similar alternate site on the opposite side of the body, and again, were worn for 24 hours, then removed and discarded by the study subject. Grading of Challenge sites occurred 48 and 96 hours after patch applications.

One hundred (58 female, 42 male) subjects completed all phases of the study.
Facial treatement essence with 92.675% galactomyces ferment filtrate
Test substance [redacted] did not produce evidence suggestive of a delayed contact hypersensitivity response under the exposure conditions of this test.

[redacted]

Submitted for: **HILL TOP RESEARCH, INC.**

Approved by: Louise B Aust 9-9-99

Louise B. Aust, M.S. Date
Investigator

[redacted]

PURPOSE:	To confirm that a test substance will not produce evidence suggestive of a delayed contact hypersensitivity response under the exposure conditions of the test.
SPONSOR AND MONITOR:	[redacted]
RECORD OF MONITORING VISITS:	This study was not monitored.
INVESTIGATIVE FACILITY:	Hill Top Research, Inc. 3225 N. 75th Street Scottsdale, Arizona 85251
SITE OF INVESTIGATION:	Hill Top Research, Inc. 7506 E. Monterey Way Scottsdale, Arizona Marcos DeNiza High School 6000 Lakeshore Drive Tempe, Arizona
INVESTIGATOR:	Louise B. Aust, M.S.
CONSULTING DERMATOLOGIST:	Harold L. Saferstein, M.D.
STUDY MANAGER:	Marcia Willard, B.S.
STUDY COORDINATORS:	Genevieve Johnson, B.S. Kristin Koppenbrink, B.S.
TEST SCORERS:	Genevieve Johnson, B.S. Kristin Koppenbrink, B.S.
CLINICAL RESEARCH STANDARDS:	This study was conducted according to Good Clinical Practice (GCP) regulations and, as appropriate, followed the principles of Good Laboratory Practice (GLP) regulations.

STUDY DATES:

	Subjects #1-89	Subjects #90-110	Subjects #111-113
Induction:	1/20-2/12/99	1/22-2/12/99	2/10-2/26/99
Challenge:	2/22-2/26/99	2/22-2/26/99	N/A

PROTOCOL: The study protocol (Appendix I) furnished by the study sponsor and approved by the investigator, was followed with the exception of the following deviations. In the opinion of the investigator, the deviations did not compromise the integrity of the study.

DEVIATIONS:

Subject	Application	Deviation from Routine
32, 70	1	Subjects wore patch B for 48 hours. Patch was removed ~15 minutes before scoring.
52	4	Subject wore patch B for 48 hours. Patch was removed immediately before scoring.
2	Make-up	Subject was evaluated approximately 40 hours after patching.
13	Challenge	Subject wore patch B (on the alternate site) for 48 hours. Patch was removed immediately prior to scoring.
49	Challenge	Subject was scored at 48 and 144 hours after patch application.
50	Challenge	Subject was scored at 96 and ~156 hours after patch application.
59, 83, 96	Challenge	Subjects were scored at 48 and 108 hours after patch application.
13	Challenge	Subject was scored at 48 and 156 hours after patch application.

AMENDMENTS: There was one amendment to the protocol (Appendix I).

SUBJECTS:

# Screened but Excluded	16	Appendix II
# Enrolled	112	44 male, 68 female
# Failing to Complete	12	Appendix II
# Completed	100	42 male, 58 female

ADVERSE EVENTS:

- Subject No. 56 had allergies, stuffy nose and head congestion from 2/4/99 through 2/24/99. Subject took Claritin 10mg QD (2/8-2/24/99) and Sulfameth/Trimethoprim 800/160 (2/8-2/17/99). Event was moderate and unrelated to the test articles.
- Subject No. 69 had heavy vaginal bleeding which started on 1/2/99 and was resolved on 2/24/99. Subject was hospitalized and had a hysterectomy on 2/24/99 and was released the same day. Subject took Percoset as needed for pain until 2/27/99 and was also prescribed Vivelle HRT patch. Event was serious, but not related to the test products.

[redacted]

TEST SUBSTANCE

Sample Code	B
Sponsor ID Numbers:	[redacted]
Color:	Transparent
Physical form:	Liquid
Date Received:	December 30, 1998
Initial Weight:	1) 562g 2) 532.8g[1]
Final Weight:	1) 442.2g 2) 261.9g
Concentration:	Neat
Vehicle:	N/A
Dose Preparation:	Applied as provided
Test Substance Preparation:	Test substance was applied to the patch pad ≤30 minutes prior to application.
Storage- Stock Substance:	Room Temperature
Storage- Test Solution:	Room Temperature
Patch Type:	Completely occlusive patch- Webril® nonwoven ~4cm^2 x 1.65mm thick cotton pad, covered and held on all sides with occlusive Blenderm® hypoallergenic tape.
Amount per Patch:	0.2ml
Patch Site:	Upper arm

Facial treatment essence with 92.675% galactomyces ferment filtrate

RESULTS: Test substance [redacted] did not produce evidence suggestive of a delayed contact hypersensitivity response under the exposure conditions of this test.

[1] Jar #1 had a cracked lid upon receipt. Jar #2 leaked during shipping.

[redacted]

QUALITY ASSURANCE STATEMENT

This study was inspected in accordance with the Standard Operating Procedures of the Hill Top Research, Inc. To assure compliance with the study protocol, the Quality Assurance Unit performed an inspection during the conduct of this study and completed an audit of the study records and final report.

Report Reviewed by:

Pems Tebon, B.S. 9-9-99

Pems Tebon, B.S. Date
Auditor, Quality Assurance

[redacted]

APPENDIX I

Total number of pages = 55

Protocol and Amendment

[redacted]

PROTOCOL FOR A HUMAN REPEAT INSULT PATCH TEST (HRIPT)

[redacted]

Investigator: Louise B. Aust

Consulting Dermatologist: Harold L. Saferstein, M.D.

Investigator's Facility and Address: Hill Top Research, Inc.
3225 North 75th Street
Scottsdale, AZ 85251

Site of Investigation: Hill Top Research, Inc.
7506 E. Monterey Way
Scottsdale, AZ 85251

Marcos DeNiza High School
6000 Lakeshore Drive
Tempe, AZ

Sponsor and Address: [redacted]

Sponsor's Monitor/Liaison:

Anticipated Start Date: 1/20/99

Anticipated Completion Date: 2/26/99

Anticipated Report Date: 03/12/1999

Purpose: To confirm that a test substance will not produce evidence suggestive of a delayed contact hypersensitivity response under the exposure conditions of this test.

Route of Administration of Test Substance:

External contact with the skin by means of a repeated patch application procedure.

TABLE OF CONTENTS

I. SPONSOR'S TEST SUBSTANCE INFORMATION

A. Substance Identification and Handling

Facial treatment essence with 92.675% galac

Investigator's Sample Code: B

Product Type: **Skin Lotion**

Color: **Transparent**

Physical Form: **Liquid**

Expiration Date: **April 6, 1999**

Hazards: **Non-hazardous**

Storage-Stock Test Substance:
Temperature : Room Temperature (15- 25 xC)

Storage-Test Solution:
Temperature : Room Temperature (15- 25 xC)

Concentration of Test Solution (w/v, v/v) or Description of Substance (if solid material):

Neat

Vehicle(s): **N/A**

Dose Preparation Frequency: (check one)

[ ] Prepare fresh daily
[ ] Prepare fresh weekly
[X] Apply as provided
[ ] Other _____

Special Instructions for Preparation of Test Substance or Test Solution: (If instructions are not specified, the SOPs of the test facility should be used.)

If applicable, check box.

[ ] Mix/stir non-solid test substance before each withdrawal for dose preparation.
[ ] Stir test solution immediately before application to patches.
[ ] Stir test solution continuously during patch preparation.
[X] Apply test substance/solution or vehicle to patch pad no longer than 30 minutes before application.
[ ] Allow test substance/solution or vehicle to remain on patch pad, open to the air, for at least _____ minutes, but no longer than _____ minutes, before application.

SPONSOR'S TEST SUBSTANCE INFORMATION (cont'd.)

[redacted]

Stock Test Substance/Test Solution Disposition [check box(es) that apply]:

[ ] All unused stock test substance will be returned to the sponsor's sector after the final report has been issued. Include study number and sample code.+
[X] Dispose of unused stock test substance after the final report has been issued.
[ ] All test solutions will be returned to the sponsor's sector at the completion of the study. See attached instructions.+
[ ] Dispose of unused test solutions following use.

+ Return to:

B. Patch to be Used (See page 11, B for patch description)

[X] Completely occlusive patch
[ ] Completely occlusive patch within plastic chamber
[ ] Semi-occlusive patch
[ ] Semi-open patch
[ ] Open application
[ ] Other _____

C. Amount of Substance per Patch

[X] To be determined by contract laboratory* 0.2 ml [redacted]
[ ] _____ ml or gm per patch**
[ ] _____ layers per patch
[ ] _____ square inches or square cm per patch**
[ ] Other _____

* Test substance/solution to be applied to each patch in an amount sufficient to cover/saturate the pad without extruding or extending from it when applied to the skin.

** Write in amount and circle appropriate unit.

D. Patch Location

[ ] Upper arm only (usual site)
[ ] Upper back only (alternate site)
[ ] Lower back only (alternate site)
[X] No preference - to be determined by contract laboratory; may include a mix of arm and back patches (alternate procedure).

[redacted]

SPONSOR'S TEST SUBSTANCE INFORMATION (cont'd)

[redacted]

E. Special Inclusion/Exclusion Criteria:

[X] None
[ ] Special criteria required as specified below:

F. Dermatologist

[X] Dermatologist not required at challenge
[ ] Dermatologist required at challenge
Specify: _____ 48 hrs. _____ 72 hrs. _____ 96 hrs.

G. Safety

Risk Assessment:

This HRIPT is being conducted to confirm the low skin sensitization potential of skin lotion formulation ([redacted] Similar skin lotion formulations have been marketed in Japan over ten years with no significant skin effects reported. Safety testing on these similar formulae have shown no evidence of skin irritation or allergy. Based on the favorable market history and the available safety data on these formulae, as well as the safety data for the ingredients in this formulation, no safety issues are anticipated for the use of [redacted] in this HRIPT study.

Check the appropriate box(es):

[ ] This test substance is regulated by _____
[specify agency(ies)]

_____

[ ] Relevant safety testing has been conducted on this formula/substance.
[X] Relevant safety testing has been conducted on a similar formula/substance.
[ ] IRB approval of this study is required.

H. Irritation Screen

[X] An irritation screen is not required as part of this study.
[ ] An irritation screen should be conducted as part of this study.
[Applicable for studies placed at European contract laboratories only (see Attachment F).]

[redacted]

II. SPONSOR'S STUDY INSTRUCTIONS

A. Number of HRIPT Subjects

A minimum of _100_ subjects are targeted to complete the investigation.

Check box if applicable:

[ ] Enroll a portion of the population (about _____ subjects) as a pilot group. This group will begin the test approximately 3 weeks prior to the main group. Any change in the protocol which results from observations in the pilot group must be approved by the Sponsor.

B. Skin Wiping with Alcohol

[ ] No alcohol wipe prior to patching.

[X] Skin area to be patched will be wiped with a gauze pad saturated with 95% ethanol or 70% isopropyl alcohol before application of the first induction patch and before application of the challenge patches. The skin surface should be dry before the patch(es) is(are) applied.

[ ] Skin area to be patched will be wiped with a gauze pad saturated with 95% ethanol or 70% isopropyl alcohol before application of all induction patches and before application of the challenge patches. The skin should be dry before the patch(es) is (are) applied.

C. Change in Test Solution Concentration

If reactions produced by a test substance concentration indicated on page 4 under "Concentration of Test Solution ...." result in excessive irritation, the concentration may be reduced with verbal followed by written approval from the sponsor. All changes should be documented in the study records and described in the final report.

III. SUBJECT ENROLLMENT

A. Eligibility Criteria

1. Demographic Criteria (check appropriate boxes)

AGE:
- [X] Minimum age based on applicable local laws
- [X] No more than 20% of subjects to be over age 65
- [ ] 10% of subjects may be ages 16-17 if parental informed consent is given

SEX:
- [X] No restrictions
- [ ] Males only
- [ ] Females only

OTHER: [ ] _____

2. Prior Patch Test Participation (check appropriate box)
(This is the minimum of lapsed time since the last exposure in a repeat application or rechallenge patch test.)

- [X] Four weeks
- [ ] Three months
- [ ] Other _____

3. Exclusion Criteria

a. Current use of immunosuppressive drug and/or has received organ transplant

b. Current routine or frequent use of topical or systemic anti-inflammatory drug for a defined medical condition; e.g. ibuprofen, corticosteroid. Maximum acceptable dosage should be determined by written laboratory guidelines.

c. Application within the last two weeks of any anti-inflammatory drug to skin area to be used in testing

d. Clinically significant active dermatitis or skin disease anywhere on the body (excluding facial acne); skin cancer (or a history) at or near sites to be used in testing

e. Insulin-dependent diabetes

f. Asthma requiring daily therapy or other chronic respiratory condition

g. Currently receiving allergy injections, has received a final injection within the last week, or expects to begin injections during the study

h. Treated for malignancy (of any kind) within the last six months

i. Immune deficiency or autoimmune disease

j. Nursing or known to be pregnant

k. Bilateral mastectomy with lymph node removal; mastectomy with lymph node removal within the last year; axillary lymph nodes (both arms) removed for any reason

l. Scars, moles, or other blemishes/abnormalities within the test area which, in the supervisor/designate's judgment, would interfere with grading/assessment of responses

m. Erythema greater than Grade 1 (e.g. due to sunburn) in the test area

n. Has a condition or is taking or has taken a medication which, in the Investigator's judgment, makes the subject ineligible or places the subject at undue risk (If the potential subject is under the care of a physician, approval to participate may be soug from that physician, at the Investigator's discretion and/or in accordance with regulatory requirements.)

o. Is currently participating in another dermal clinical study of any kind.

p. Is participating in any clinical study which, in the judgment of the investigator, could potentially affect responses in either study.

q. Has confirmed sensitization as a result of participation in a dermal clinical study.

r. Is unwilling or unable to give informed consent or to otherwise comply with protocol requirements

4. Documentation of Subject Eligibility

A Medical and Dermatological History Questionnaire is the basis for determining subject eligibility (suggested form is in Attachment A.) When reviewing each Questionnaire, the test supervisor or a designate will further question the potential subject to clarify any answers and add appropriate notations. Circumstances/conditions other than those specified in III.A.3. above could be revealed. The test supervisor/designate may exclude a volunteer for any reason if it is in the individual's best interest to do so. If eligibility is in question, the Investigator will make this decision. The reason for exclusion of any volunteer must be documented on the Questionnaire.

Note regarding Attachment A: A "yes" answer in Sections I. through V. is a basis for exclusion. A "yes" answer in Sections VI. and VII. may be exclusionary and should be discussed with the Investigator if there is any doubt.

B. Informed Consent

Written informed consent conforming to 21 CFR 50.25 will be obtained from each subject prior to enrollment in the study. (A suggested consent statement is in Attachment B). The original signed copy for each subject participating in the study will be retained in the Investigator's study records. Each subject will receive or be offered a copy of his/her signed statement.

C. Subject Identification

During eligibility screening, the subject's initials will be used for identification. Once it is confirmed that all eligibility criteria are met, she/he will be assigned a subject number according to the Investigator's SOP.

IV. STUDY DESCRIPTION

A. Test Substance Sequence

The test substances (or patches) will be applied to the skin in a designated sequence to eliminate position and order bias according to the Investigator's SOP. The sequence may be rotated among the subjects as shown in the following example for four test substances:

1-2-3-4-5-6-7,	2-3-4-5-6-7-1,	3-4-5-6-7-1-2,	4-5-6-7-1-2-3,
5-6-7-1-2-3-4,	6-7-1-2-3-4-5,	7-1-2-3-4-5-6	

The sequence must be documented for each subject and cannot change during the test.

B. Description of Patches

The patch type for a given test substance will be used throughout all phases of the study. The size of the cotton pad will be approximately 2 cm x 2 cm (~ 4 cm^2). When multiple pads are applied to a given subject, the edges of the pads will be at least 2.0 cm apart. For an open application, the area of treated skin will conform to the dimensions for patch pads specified above. The possible patch types in this study are the following:

1. A **completely occlusive patch** consisting of a nonwoven ~ 4 cm^2 x 1.65mm thick cotton pad [e.g. Webril® (Kendall Health Care Products Company)] covered by and held securely to skin on all sides with an occlusive, hypoallergenic tape [e.g. Blenderm® (3M Company)].
2. Same patch as 1. above except that the cotton pad is secured within a **plastic chamber** (e.g. Hill Top chamber).
3. A **semi-occlusive patch** consisting of ***a nonwoven ~ 4 cm^2 x 1.65mm thick cotton pad [e.g. Webril ® (Kendall Health Care Products Company)] covered by and held securely to skin on two opposing sides with a hypoallergenic tape [e.g. Blenderm® (3M Company)].***
4. A **semi-open patch** consisting of ~ 4 cm^2 x 1.65mm thick nonwoven cotton pad (e.g. Webril®) held to the skin on two opposing sides by a strip of hypoallergenic tape (e.g. perforated Blenderm®) (1 x 3 inches; 2.5 x 7.5 cm) with holes over the pad (pad is open on two sides like a Band-Aid®).
5. An **open application** consisting of direct placement of the test material on the skin and covering the site with a loosely woven covering (e.g. cotton gauze) held in place with hypoallergenic tape (e.g. Blenderm®, Dermacil®, Micropore®, Tenderskin®) along two opposing edges.
6. Other _____

C. Description of Patch Locations

Patches may be placed on the lateral surface of the upper arm, on the upper back (in the shoulder blade area), or on the lower back (between the waistline and the lower edge of the shoulder blade). The maximum number of patches that can be applied on the arm is five (5); the maximum number applied on the back is twelve (12). Patches for the induction and challenge phases will be placed in the area indicated under Patch Location, pg. 5 (also see Induction Exposures, pg. 12, and Challenge Exposures, pg. 13). Any deviation from this procedure must be documented and included in the final report.

[To simplify sections D., and E below, the singular form is used (one test substance and one patch), recognizing that multiple test substances and patches are more common.]

D. Patch Application

The site of patch application will be marked according to the ***standard, written practices/procedures of Hill Top Research regarding Study No.*** [redacted] so that the location of the patch pad is clearly indicated for scoring. Gentian violet is suggested for this purpose. To assure good adhesion to skin, the patch may be reinforced with a hypoallergenic tape (e.g., Dermacil®, Micropore®). If extra tape is used, it should be placed in such a way that pressure on all patch pads is equal. Extra tape should not alter designated conditions of test substance exposure; e.g. it should not cover the pad of a semi-occluded or semi-open patch.

E. Induction Exposures

The patch containing the test substance is applied to the lateral surface of one upper arm or to one side of the back (site is specified in Patch Location, pg. 5). Multiple patches are applied in a vertical row on the arm or in a vertical or horizontal row(s) on the back.

The induction site (also called the "original" site) (right or left side of the body) is determined by the ***standard, written practices/procedures of Hill Top Research regarding Study No.*** [redacted] and is noted in the study records. Instances in which an area is not used include interfering conditions such as: large vaccination scar, tattoo, other scar(s), birthmark, mole(s), vitiligo, keloids, or at the subject's request. A patch is not applied at any time to the arm or the side of the back adjacent to a mastectomy site. Use of the opposite side for induction is noted. Any deviations from the above are noted in the final report.

The patch is applied midway between the shoulder and elbow, with the arm in a relaxed position at the side, or in a central location on one side of the back. Areas routinely covered by tight-fitting clothing should be avoided. Each induction patch is applied to the same site (with the aid of the patch application site markings) unless the degree of reaction to the material or the adhesive necessitates relocation. Two alternate locations are adjacent to the original site according to the ***standard, written practices/procedures of Hill Top Research regarding Study No.*** [redacted] The initial placement of the patch should allow for the possibility of use of these sites so that subsequent patches are not placed in awkward positions. Once both adjacent sites have been used during induction, the next patch is returned to the original location, provided this area is clear of any residual response. If a residual reaction is present, the patch is not applied until the site is clear. If repatching at the original location necessitates relocation again, patching of the material will be discontinued until challenge. Any relocation of a patch site or any patch that is not reapplied due to a residual reaction or for any other reason must be documented.

In instances in which only a small portion of the application site shows a response sufficiently intense to require patch movement, the succeeding patch may be adjusted slightly to avoid this area. This is not considered a move to a new site, and the test records do not reflect this action. Instructions regarding patch placement may be given verbally by the test scorer to the person applying the patch.

Each subject is verbally instructed to keep the patch as dry as possible and to remove and discard it after approximately 24 hours. After patch removal, the patch site may be cleansed in a normal manner (no bathing or showering restrictions), but subjects will be cautioned not to irritate the site or to remove the patch application site markings by excessive scrubbing. This information will also be given to each subject in written form on the first test day (also see Subject Instructions, pg. 15).

Induction patches are usually applied on Monday, Wednesday and Friday of the first three weeks of the test. Subjects who are absent once during this three week, nine-patch induction phase usually receive a make-up (MU) induction patch on the fourth Monday of the test. Monday MU applications are usually graded 48 hours later at the MU scoring visit.

F. Challenge Exposures

Prior to applying challenge patches, the subjects should be asked whether there has been any change in health status or study exclusion drug use since the beginning of the study. All changes should be documented. A challenge patch is applied to both the original site and a similar (alternate) site on the opposite side of the body, again to be worn for 24 hours, then removed and discarded by the subject or (optional) by contract laboratory personnel. After patch removal, the patch site may be cleansed in a normal manner (no bathing or showering restrictions), but subjects will be cautioned not to irritate the site or to remove the patch application site markings by excessive scrubbing. The patch on the original site is placed on approximately the same area as the initial induction patch. If a residual reaction from an induction application is severe, or if the individual has an active dermatitis, the subject will not be patched until the Sponsor has been notified. The patch on the alternate site is positioned similarly to that of the first induction patch on the original site. The same interfering conditions noted under Induction Exposures, page 12, also apply to the alternate challenge site. Modification in patch placement must be documented.

Subjects who have had a mastectomy should be challenged on the original site and a naive (alternate) site; e.g. the arm or back on the opposite side of the body away from the mastectomy site. In such a case, the location must be documented.

Challenge patches are applied 12 - 20 days after the last induction application.

G. Grading of Test Sites

An artificial light source, preferably a lamp with a 100 watt incandescent daylight blue bulb is used to illuminate the patch area. For sites on the arm, the arm is held in a relaxed position at the side. Sites will be graded using the grading scale in Attachment C. Any visible response should be palpated to aid in detecting signs of elevation as described in Attachment C. Grading will occur 48 hours after patch application during the induction phase (72 hours after a weekend) and 24 (optional), 48 and 72 and/or 96 hours after challenge applications. (All hours are approximate.) Any deviation will be noted in the final report. A challenge site showing an erythema grade 2 or greater and/or any elevated response at the final regularly scheduled challenge grading will be followed and scored until the response has regressed to an erythema grade 1 or less without signs of elevation. Effort should be made to schedule the first follow-up exam within 24 to 72 hours of the scheduled final challenge evaluation to check for any worsening of the response(s). Every effort should be made to have at least one follow-up exam by laboratory personnel before the subject is dismissed. If the subject is not available for direct follow-up by laboratory personnel, every effort should be made to reach the subject by phone to determine the status of the reaction. See Subject Instructions (pg. 15) regarding the reporting by subjects of delayed reactions or those which increase in intensity after conclusion of the induction or challenge phases. In such a case, the subject will be asked to return to the study site for grading as soon as possible.

Holidays may alter the hours of scoring to some extent; however, there must be two delayed scorings, at least 24 hours apart, after challenge applications. These scores are recorded on suitable grading sheets provided by the Investigator's facility. The score sheets are signed and dated each scoring day by the recorder and scorer. The same person scores the patch sites for each group of subjects for the duration of the study, except in case of illness or other unforeseen absence at which times an alternate trained grader is used. All graders are identified by name in the final report.

H. Photography

Photographs of any responses that occur in this study may be taken, but the Sponsor must be notified of this action. Copies of all photographs should be sent to the Sponsor separate from the study report.

I. Confirmatory Rechallenge

If a test substance produces elevated or other questionable response(s) during the original test, it may, at the Sponsor's discretion, be applied again to the subject(s) in question and to control subjects who are non-reactive study participants or who were not participants in the study. Other related materials may also be applied at this time.

Patches are applied to sites agreed upon by the Investigator and the Sponsor. After 24 hours, patches are removed and discarded either by contract laboratory personnel or by the subject on prior instruction by appropriate test personnel.

The rechallenge application site(s) are graded at least twice at 48 and 72 or 48 and 96 hours after application. If responses at the last scheduled visit are elevated, or erythema is grade 2 or higher, or there is still uncertainty as to the nature of the responses, an additional grading(s) should be conducted. (See G. - Grading of Test Sites; page 13.)

The rechallenge, if required, should be conducted between 4 and 12 weeks after the initial challenge and after any prior reactions have subsided.

Separate informed consent is obtained for this rechallenge, and a copy is offered to the subject. A suggested format for this consent is in Attachment D.

Additional confirmatory testing (e.g. a product use test) may be requested, and, if so, be covered in a separate protocol addendum.

V. CONDUCT OF THE STUDY

A. Subject Attendance

Any subject who is absent more than once during the induction phase or at any time during the challenge week is considered incomplete for data analysis purposes. If a subject is unable to attend the MU grading visit, but all nine induction patches have been applied, the subject is not dropped. This is noted in the MU column on the grading sheet with the designation N9G (see Attachment C). Only subjects who have worn nine induction patches and whose challenge patch sites have been graded twice are included in the final number completing the study.

Holidays, school closings, weather conditions, etc. may cause deviation from the typical schedule specified in this protocol under Induction Exposures, pg. 13 and Challenge Exposures, pg. 13. No deviation should be made which, in the judgment of the Sponsor and the Investigator or the Investigator's designate, would affect the validity of the test. Specific dates of subject participation are given in the final report.

B. Subject Instructions

Each subject will be given written instructions about attendance requirements along with a schedule of study visits. Emergency phone number(s) answered in response to adverse events or study-related questions on a 24-hour basis will be provided. In addition, these instructions will include information about keeping patches as dry as possible, cleansing skin after patch removal, not applying any other product in the patch area (including sunscreens) and precautions about irritation of test sites and preserving markings of patch locations (also see Induction Exposures, pg. 12).

Subjects will be advised to contact the test facility promptly if they observe any increase in a response or the development of a new response after their last induction or challenge visit.

C. Adverse Events

Anticipated or possible adverse events in this study will be visible responses of the skin at or immediately surrounding the site(s) of test substance application. Those most likely to occur will be mild-to-occasional moderate erythema. It is possible that responses will have papules, vesicles, and edema as well as erythema; strong reactions may show evidence of spreading beyond the patch pad area. Hypo- or hyperpigmentation may rarely occur at the site of a strong reaction in an occasional individual and may persist. The skin of some subjects may become irritated by the tape adhesive, and rarely, an allergic reaction to the tape may be seen. Some subjects may occasionally experience itching, burning, or stinging in the test area.

Any unanticipated serious adverse events must be investigated, documented (Attachment E suggested form), and followed to resolution (with documentation) by the Investigator and/or the Consulting Dermatologist to the satisfaction of the Sponsor. Every effort will be made to determine the relationship to the study.

The Sponsor will be notified within one working day of any unanticipated serious or life-threatening adverse event or of any patch reaction graded 2 or higher with an E, P, V, or B. A 24-hour telephone number will be given to each subject for use in reporting adverse events.

Note: Occasionally situations may occur during the study which normally are not considered adverse events but may preclude application of the test substance to the skin area specified in the protocol (e.g. sunburn, insect bites, etc.). The judgment of the test supervisor (in consultation with the Investigator, if necessary) will be utilized as appropriate and the course of action will be documented.

D. Drop-Out; Management and Follow-up

Any subject may voluntarily leave the study at any time. If a subject is eliminated from the study because of failure to meet the attendance requirements specified in the protocol, this is noted according to the ***standard, written practices/procedures of Hill Top Research regarding Study No.*** [redacted] Effort will be made to determine why the subject failed to appear, and the reason will be recorded and included in the final report. If the subject is concerned about a response at a patch site, associates a problem with test participation, dislikes some aspect of the test (such as test-substance odor, tape irritation, etc.), he/she should be encouraged to continue participation if appropriate. Dermatologic consultation, diagnosis, and treatment are offered as needed, and a reasonable attempt is made to resolve the problem to both the Investigator's and subject's satisfaction. There is complete documentation of any such case. If the subject does not continue, grades of the patch sites at the time of drop-out will be obtained if possible.

Appropriate follow-up with a subject is always sought if patch site grades suggest possible sensitization regardless of when the subject is dropped from the study. If a "dropped" subject has shown questionable responses during induction, the subject is asked to participate in the challenge phase of the study.

E. Test Substance Accounting

The test facility will maintain a record of receipt of the test substance(s) and weight(s) upon receipt and following subject dosing.

F. Records of Test Substance Preparation

Records of each preparation of the test substances/solutions (including container weights, quantity of test substance, and other steps taken to prepare the substance for testing) will be maintained. Initials of the individual performing this activity each day will be documented on the record.

G. Documentation of Data

A system for (1) tracking the subject, (2) providing instructions regarding substance placement, (3) documenting patch application on a given day, (4) recording attendance of the subject (5) documenting patch site skin grades, (6) documenting any other relevant subject reactions/activities, and (7) documenting special procedures, such as patch drying time, etc. will be established and appropriate records maintained. Complete reconstruction of the study must be possible from the written records.

All data generated in this study will be recorded in ink and be initialed and dated by the individual responsible for the entry. If subsequent corrections are required, the original entry will be crossed out with a single pen stroke so that it remains clearly legible; the correction will be entered close to it, and initialed and dated by the individual making the correction. The reason for making the correction should be stated near the entry, if possible, or on the data sheet as a referenced footnote.

H. Protocol Modifications/Deviations

If it becomes necessary to make a change in this protocol, the Investigator and the Sponsor will agree to the change before it is implemented. The change and the reason for it will be documented, and a protocol amendment prepared and signed by the Investigator and the Sponsor, preferably before implementation. If a change is necessary on an emergency basis, the Sponsor will be notified as soon as possible after the action has been taken. Documentation will be made as described above.

The Investigator is responsible for any deviation from the protocol and documentation of this deviation in the study records and the final report.

I. Regulatory Compliance

The study should be conducted according to Good Clinical Practice (GCP) regulations and, as appropriate, follow the principles of Good Laboratory Practice (GLP) regulations.

J. Monitoring/Visitors

The Investigator will permit the Sponsor's Monitor(s) to make regular visits during the course of the study. The Investigator will also permit the Monitor to inspect all data collection forms, informed consent statements, test substance inventory records, and other records which are relevant to the protocol. The Monitor will notify the Investigator in advance of the date/time of a monitoring visit. A record of monitoring visits will be included in the final report.

Visitors to the study site who are not directly associated with the conduct of the study are not permitted without the Monitor's concurrence. This includes employees of, or other individuals acting for, the Sponsor. A record of all visitors will be kept and included in the final report.

K. Institutional Review Board (IRB) (if applicable)

If IRB approval is required for this study, the Board will review this protocol, the informed consent statement, and any relevant safety data for the test substance(s). The study will not begin until the IRB has given approval. The Investigator will provide the Monitor with a copy of the IRB approval letter.

Approval procedures for protocol modifications and informing the Board of adverse events, and a final report to the Board will be carried out according to the SOPs of the IRB.

VI. RESULTS

A. Final Report

A separate report for each test substance is issued after all work is completed. Each report will include the following:

- a summary of test results
- identification of study personnel
- identification and concentration of the test substance
- description of patch used including dimension(s) of patch pad or treated skin area
- location and dates of testing
- number of subjects enrolled and completing the study
- subjects who drop from the study and reasons for withdrawal
- any protocol modifications/amendments/deviations
- patches worn <12 hrs., >12 - <23 hrs. and >25 hrs.
- procedures pertaining to test substance/test solution preparation and any special procedures required to prepare/dose test substance/test solution
- unanticipated adverse events and resolution of these events
- complete tabulations of all data including erythema incidence summary
- test substance accounting record
- record of monitoring visits and visitors to the study site
- data interpretation and conclusions developed in conjunction with the Sponsor
- QA statement

A draft report may be required. If so, it will be completed within six (6) weeks of study completion or within a time frame agreed to by the Contract Laboratory and Sponsor.

The final report should be completed within six weeks of study completion or within one month of receiving the Sponsor's comments on the draft report. The final report will be approved and signed by the Investigator. Any consultant(s) providing input into the study design or interpretation should sign for that portion of the report for which he/she is responsible.

B. Retention of Study Records

Data and records resulting from the study will be maintained by the Investigator and the Sponsor in accordance with Good Clinical Practices, any applicable governmental regulations, and Standard Operating Procedures. Contract laboratory records should be maintained for the period of time specified in the Laboratory Services Agreement [redacted]. Appropriate documentation to permit complete reconstruction of the study will be retained by the Investigator.

VII. SIGNATURES

A. Investigator

I have read and understand this protocol and concur with the study design. I agree to participate as the Investigator and to follow the protocol as outlined.

Louise B. Aust _____ Louise B Aust _____ 1-18-1999
Signature Date

B. Sponsor Representative/Monitor

[redacted]

[redacted]

Attachment A

Medical and Dermatological History Questionnaire

Subject Initials: ___/___/___ (F M L) Sex: [ ]M [ ]F Age: _____ Subject #: ___ ___ ___ ___ ___

If you are uncertain about any of these questions, please ask for help.

I. Medical Conditions Now

Do You Currently Have:

	NO	YES	
Psoriasis?			
Eczema?			
Skin cancer (or a history) at or near skin test sites?			
Other skin problems (not including facial acne)?			Describe:
Scars, moles, or other blemishes at or near skin test sites?			
Redness from sunburn or other source at or near skin test sites?			
Asthma requiring daily medication?			
Any other chronic respiratory condition?			Describe:
Diabetes requiring insulin therapy?			
An immune system-related disease (e.g., lupus, thyroiditis)?			

II. Medical Conditions in the Past

Have You Had:

	NO	YES	
An organ transplant?			
A malignancy treated within the last 6 months?			
A mastectomy (one breast removed) within the last year?			If you have ever had a mastectomy, circle side of surgery: Right Left
A bilateral mastectomy (both breasts removed)?			
Axillary lymph nodes removed (from under both arms)?			If removed from one arm only, circle side of surgery: Right Left

III. Medications

Do You Regularly Take or Use Any of the Following:

	NO	YES
An anti-inflammatory drug (for example: aspirin, ibuprofen, hydrocortisone or other steroids)?		
currently?		
on your skin within the last 2 weeks?		
drugs to regulate immune system?		

IV. Allergy Injections

	NO	YES
Are you currently receiving allergy injections?		
Have you received a final injection within the last week?		
Do you expect to begin injections during the study?		

V. Are You:

	NO	YES
Pregnant or nursing (if female)?		
Currently participating in another clinical study of any kind?		

VI. Allergies

Are you allergic to any of the following:

	NO	YES	SPECIFY
detergents/cleaning products?			
cosmetics/perfumes?			
personal cleansing products (soap, shampoo, etc.)?			
skin creams/lotions?			
anti-perspirants/deodorants?			
medicines?			
other materials?			

VII. Miscellaneous Questions

	NO	YES	SPECIFY
Do you regularly use any medication on your skin?			
Do you regularly take any prescription or OTC medication?			
Have you ever participated in a patch test?			Date of last participation:
Confirmed Patch Test Sensitization?			
Are you currently seeing a doctor about:			
allergies?			
skin problems?			
another reason?			
Do you have any other medical condition we should know about?			

Signature of person providing information: _____ **Date:** _____

FOR INTERVIEWER'S USE ONLY (Please review this form with the subject for completeness and accuracy.)

Answers to questions indicate the subject is: [ ] Acceptable
[ ] Not Acceptable - Reason: _____ (use Key for Subject Exclusion)

Comments: _____

Interviewed Signature: _____ Date: _____

[redacted]

[redacted]

[redacted]

Attachment A (cont'd)

Key for Subject Exclusion

01	Psoriasis	14	Immunosuppressive Drug
02	Eczema	15	Allergy Injections
03	Skin Cancer	16	Known Pregnancy/Nursing
04	Other Active Dermatitis	17	Other Dermal Study Participation
05	Skin Abnormality - arms or back	18	Relevant Allergy
06	Sunburn	19	Other Disqualifying Medication
07	Severe Asthmatic	20	Confirmed Patch Test Sensitization
08	Other Chronic Respiratory Condition	21	Patch Test Within Minimum Time Limit
09	Insulin Dependent Diabetic	22	Under an M.D.'s Care at Present
10	Immune System Disease	23	Other Disqualifying Condition
11	Organ Transplant	24	Declined Participation
12	Cancer Treatment	25	Other - Explanation Included in Report
13	Anti-inflammatory Drug		

[redacted]

[redacted]

Suggested Consent for Participation in a Skin Patch Test

We are asking you to participate in a skin patch test of _____ different materials. Examples of these are soaps, detergents, deodorants, shampoos, cosmetics, paper products, drugs or ingredients in these kinds of products.

PURPOSE AND BENEFITS:
We want to learn if any material being considered for use in a marketed product is capable of producing an allergic skin reaction. Participation will not directly benefit you personally other than the compensation you will receive (see below). However, results of the test will aid in the development of products which are safe for millions of people to use.

ELIGIBILITY:
If you would like to participate, we will first ask you to complete a questionnaire about your medical history, allergies, skin problems (such as psoriasis), medications, and participation in previous patch tests. It is possible that you may not be able to participate based on your answers to these questions.

PROCEDURES:
If you are enrolled in the study, we will place _____ cotton pads on _____. The pads are held in place by tape. We will ask you to leave the pads and tape in place for 24 hours. Then you will remove these patches and discard them. You will receive 8 more sets of these patches over a three-week period, and we will examine your _____ before each application. There will also be one examination after the ninth patch. After a rest period of approximately 2 weeks, one set of patches will be applied to _____, worn for 24 hours, and discarded. Two examinations will follow this final application. Overall, there are 13 visits to the test site over a six week period. The first visit should take approximately _____ minutes; all remaining visits should take no longer than _____ minutes. We will give you a schedule (calendar) showing the exact dates/times for each of your visits and whom to contact if you have any questions.

COMPENSATION:
You/your organization will be paid an agreed upon amount for completion of this study. If you are absent more than once during the first 10 visits or you miss any one of the last three visits during the final week, your participation in the test is considered incomplete, and you/your organization will receive prorated compensation based on the visits you have completed up to that time. If you drop out of the study on your own accord for personal reasons or are dismissed for refusal to obey rules or follow directions, compensation to you/your organization will prorated. If, in the judgment of the Investigator/Investigator's designate, it is best to discontinue your participation in the study for other reasons, you/your organization will either be compensated in full or for that portion of the study already completed.

LEAVING THE STUDY:
You may withdraw your consent and discontinue participation in this study at any time without loss of benefits, other than pay, to which you are otherwise entitled.

RISKS AND DISCOMFORTS:
You can expect some skin irritation to occur under one or more of the patch pads or from the tape. The skin may become pink-to-red and temporarily burn or itch. There may be some cracking or peeling of the skin at the patch site. The test supervisor may move a pad to a new site on your _____ or trim the tape to avoid this discomfort. Irritated skin normally improves within one to two days after patch removal.

Attachment B (cont'd)

[redacted]

It is also possible that you may experience an allergic reaction to one or more of the test materials. The reaction looks and feels similar to a poison ivy reaction and normally occurs only in the skin area covered by the pad containing the allergic material. On rare occasions the reaction may spread beyond the pad area. The skin becomes red, itches and often has bumps or blisters on the surface. Swelling typically occurs with this kind of reaction but this is normally confined to the pad area. The skin usually returns to normal in one to two weeks. A reaction may result in lightening or darkening of the skin in the patch area, and this may persist in some individuals, particularly those with dark skin. Because the skin has a "memory" associated with allergic reactions, you can expect similar response if the pad containing the same material is placed in a new skin location. A dermatologist will be available to you for treatment of any skin problem during and associated with the test.

FURTHER TESTING:
We will not identify our test materials to you. However, if we suspect that you are allergic to a material, we may ask you to participate in further testing at a later date. This will help us investigate your reactivity in more detail. It is possible that, once you have experienced this kind of reaction in the patch test, you could experience an allergic skin rash if you contact the same material in a sufficient amount at some time in the future. Additional testing may help us determine if this is likely to occur. If appropriate, we will advise you about any material to avoid currently on the market.

CONFIDENTIALITY:
Any information that is obtained in connection with this study and that can be identified with you will be kept confidential with the study records. However, these records could be examined by the sponsor of the study and by _____
(specify governmental/regulatory agency)

If any information about the study is published or presented, you will be identified by your study identification number only, and not by name or initials.

It is possible that photographs may be taken of skin reactions which develop during the study. If this happens, the photos will be of the area of the reaction only and will not be identified with you by name in a publication or presentation.

QUESTIONS:
You are expected to ask us any questions you may have about the above information. The telephone number to reach us at any time is _____.

You will be given a copy of this form to keep.

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE. YOUR SIGNATURE INDICATES THAT YOU HAVE DECIDED TO PARTICIPATE HAVING READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE.

_____		_____	
Signature of Participant	Date	Signature of Witness	Date
_____		_____	
Signature of Guardian (if applicable)	Date	Test Number/Subject Number	

[redacted]

[redacted]

Attachment C

Scoring Scale and Definition of Symbols Used in Recording Data

ERYTHEMA SCALE: This scale is used only for grading degree of erythema (redness). A score on this scale will be assigned following every application of a test material.

0 No visible erythema

1 Mild erythema (faint pink to definite pink)

2 Moderate erythema (definite redness)

3 Severe erythema (very intense redness)

DESIGNATIONS FOR ELEVATED RESPONSES: Edema, papules, vesicles, and bullae, if present, are graded as independent responses.

E Edema - definite swelling

P Papules - many small, red, solid elevations; surface of reaction has granular feeling.

V Vesicles - small, circumscribed elevations having translucent surfaces so that fluid is visible (blister-like). Vesicles are no larger than 0.5 cm in diameter.

B Bullae - vesicles with a diameter > 0.5 cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.

OTHER RESPONSE CHARACTERISTICS

S Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).

W Weeping - evidence of release of fluid from a vesicular or bullous reaction.

Note: If the presence of edema, papules, vesicles, or spreading beyond the pad area is questionable in the grader's judgment, the letter(s) E, P, V, or S should not be assigned; instead, a separate notation should be made in the study records. Other observations such as glazing, peeling, fissuring, hypo/hyperpigmentation, etc. may be documented separately in the study records at the Investigator's discretion. If any of these is observed in all or nearly all subjects, this should be noted in the final report.

[redacted]

Attachment C (cont'd)

OTHER RECORDING DESIGNATIONS:

A Marked reaction to adhesive (patch relocated).

X Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied.

(Note: Documentation in the records and the final report is made if a patch is not applied for a reason other than a residual reaction.)

L-1 Subject report of lost patch (came off) during first twelve hours of exposure.

L-2 Subject report of lost patch (came off) between 12 and 23 hours of exposure.

(-) Subject absent.

N9G No Ninth Grade. Subject wore nine induction patches but was not present for scoring following ninth application.

CRITERIA FOR MOVING AN INDUCTION PATCH TO A NEW LOCATION: Any erythema grade of 2 or greater with or without a letter E, P, V, or B necessitates relocation of the patch. An erythema grade of 1 with a letter V or B also necessitates relocation. A patch normally is not relocated if the erythema grade is <2 with or without an E or P designation. If more than one test substance is being tested, only the patch pad containing the test substance producing the score warranting the relocation is moved.

DOUBLE GRADE: This is the indication on the scoring sheet that the patch was moved to a new site. The first number (and possibly letters) is the grade for the new site and the second number (and possibly letters) is the grade for the residual reaction at the preceding site. A residual reaction continues to be scored and recorded until it subsides. A residual reaction should be reported at least once in the final report with additional scores reported at the Investigator's discretion. All scores will be made available at the Sponsor's request.

[redacted]

[redacted]

Attachment D

Suggested Consent for Participation in a Follow-up Skin Patch Test

[redacted]

You took part in a skin patch test at _____ _____ weeks ago. Our findings during that test suggest that your skin may have responded allergically to one of the test materials. We are now asking you to participate in a follow-up patch test to help us learn whether or not you are allergic to the material. We also hope to learn about your responses to _____ other related materials. Your participation will help us develop a product which does not contain a material which causes an allergic skin reaction.

This test will last one week and will be similar to the last week of your earlier patch test. We will place _____ cotton pads containing the test materials and held with tape on _____ on _____. You will remove and discard these patches _____ hours later on _____. We will examine your _____ on _____ and _____.

If you are allergic to one or more of the test materials in this test, the reaction you developed in the earlier test will probably occur again to one or more of these materials. You may experience redness, bumps, or blisters, some swelling and itching in areas covered by one or more of the pads. In some instances, a reaction may extend beyond the pad area. The skin usually returns to near normal within one to two weeks. On rare occasions, strong reactions result in lightening or darkening of the skin in the patch area and this may persist in some individuals.

A dermatologist will be available to you for treatment of any skin problem during and associated with the test.

Any information that is obtained in connection with this test and that can be identified with you will be kept confidential with the study records. However, these records could be examined by the sponsor of the study and by _____. Any information that is reported, published,
(specify governmental/regulatory agency)
or presented as data or photographs will only be identified with you by your study identification number and not your name or initials.

A possible benefit to you from participation is learning about a material you should avoid in the future. If we obtain information that indicates you should avoid a material that is available in the marketplace, we will identify it to you. The only other benefit is the compensation you will receive.

Your decision whether or not to participate will not prejudice your future relations with us. If you do decide to participate, you are free to withdraw at any time without prejudice to you.

Please ask us if you have any questions. If you need to contact us at any time, please feel free to use these numbers: _____.

You will be given a copy of this form to keep.

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE. YOUR SIGNATURE INDICATES THAT YOU HAVE DECIDED TO PARTICIPATE HAVING READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE.

_____	_____
Signature of Participant Date	Signature of Witness Date
_____	_____
Signature of Guardian (if applicable) Date	Test Number/Subject Number

[redacted]

Atta_ _ient E

Report of an Adverse Event

Subject Number: ___ ___ ___ ___ ___

Instructions:

1. Always describe the adverse event and record the date of onset. When the event ends, record the ending date, maximum severity, management information, and opinion regarding attributability.
2. If the subject drops out or completes the study before the event ends, check the box marked "continuing."[1]

Description	Date of Onset	Ending Date	Maximum Severity For This Event	Test Materials	Other Action[2]	Event Attributable to Test Participation[4]
	___/___/___ M D Y	___/___/___ M D Y or Continuing[1] [ ]	[ ] Mild [ ] Moderate [ ] Severe	[ ] No Change [ ] Stopped Temporarily [ ] Stopped Permanently	[ ] None [ ] Counteractive Medication[3] [ ] Hospitalized [ ] Other Specify: _____	[ ] Definitely [ ] Probably [ ] Possibly [ ] Not Related [ ] Unknown

1 If the adverse event is continuing, check the box marked "continuing" and follow the adverse event at succeeding visits or as necessary; complete management information if appropriate. At the end of the study or event, complete maximum severity, management information and opinion regarding attributability.

2 All additional actions must be thoroughly documented and accompany study records.

3 All counteractive medication must be documented.

4 To be completed by the Investigator.

Comments or Contributing Factors (use an additional sheet of paper if necessary):

_____ _____ _____ _____

Test Supervisor/Designate Signature [redacted] Date Investigator's Signature Date

[redacted]

Standard Written Practices/Procedures

The standard, written practices/procedures of Hill Top Research regarding Study No. [redacted] are as follows:

1) *Page 12 of 19, Section D, Patch Application:* The site of patch application will be marked with gentian violet surgical marker.

2) *Page 12 of 19, Section E, Induction Exposures, Paragraph 2:* The induction sites shall be the upper right and left arm unless otherwise noted.

3) *Page 12 of 19, Section E, Induction Exposures, Paragraph 3:* The alternate locations are adjacent to the original site, the first move shall be placed to the right of the original site towards the inner arm. The second move shall be placed to the left of the original site towards the outer arm.

4) *Page 16 of 19, Section D, Drop-Out: Management and Follow-up:* If a subject is eliminated from the study because of failure to meet the attendance requirement specified in the protocol, this shall be noted on the "Panelists Failing to Complete Study" form.

Investigator: Louise B. Aust 1-22-99

Louise B. Aust Date

APPENDIX II

Total number of pages = 1

Subjects Screened but Excluded
Subjects Failing to Complete

Subjects Screened but Excluded

Initials	Reason for Exclusion
[redacted]	Schedule conflict
	Accupressure beads in patch area
	Allergic reaction to tape
	Did not agree to use an acceptable means of birth control
	Asthma- took daily medication
	Allergic reaction- anaphylactic shock to Pertussis (DPT)
	Allergy injections
	Schedule conflict
	Schedule conflict
	Schedule conflict
	Schedule conflict
	Allergic reaction to make-up and colognes
	Schedule conflict
	Bumps in patch area
	Under age
	Asthma-daily medication

Subjects Failing to Complete

Subject No.	Date	Reason for Withdrawal
107	1/25/99	Schedule conflict
102	1/25/99	Non-compliance, missed visit
85	1/27/99	Non-compliance, missed 2 visits
110	1/27/99	Non-compliance, missed visit
12	1/29/99	Non-compliance, missed 2 visits
41	2/1/99	Unwilling to continue study due to tape reaction
15	2/1/99	Family emergency
53	2/3/99	Non-compliance, missed 2 visits
69	2/22/99	Medical emergency- See Adverse Events
111	3/1/99	Dropped due to study completion with 100 panelists
112	3/1/99	Dropped due to study completion with 100 panelists
113	3/1/99	Dropped due to study completion with 100 panelists

[redacted]

APPENDIX III

Total number of pages = 9

Tables

Table 1B. Individual reaction scores following the application of test material.

Test Article: [redacted] Facial treatment essence with 92.675% galactomyces ferment filtrate

		Application Number										Challenge			
Subject	Site	1	2	3	4	5	6	7	8	9	MU	O	A	O'	A'
1	O	0	0	0	0	0	0	-	0	0	N9G	0	0	0	0
2	O	0	0	0	0	0	0	-	0	0	0	0	0	0	0
3	O	0	0	0	0	0	0	0	0	0		0	0	0	0
4	O	0	0	0	0	0	0	0	0	0		0	0	0	0
5	O	0	0	0	0	0	0	0	0	0		0	0	0	0
6	O	0	0	0	0	0	0	0	0	0		0	0	0	0
7	O	0	0	0	0	0	0	0	0	0		0	0	0	0
8	O	0	0	0	0	0	0	0	0	0		0	0	0	0
9	O	0	0	0	0	0	0	0	0	0		0	0	0	0
10	O	0	0	0	0	0	0	0	0	0		0	0	0	0
11	O	0	1	0	0	0	0	0	0	0		0	0	0	0
12	O	0	0	-	Dropped										
13	O	0	0	0	0	0	0	0	0	0		0	0	0	0
14	O	0	0	0	0	0	0	0	0	0		0	0	0	0
15	O	0	0	0	0	Dropped									

O = original site; M = first moved site; M1 = second moved site
O = first scoring of original challenge site (48 hours)
O' = second scoring of original challenge site (96 hours)

0 = No visible erythema
1 = Mild erythema (faint pink to definite pink)
2 = Moderate erythema (definite redness)
3 = Severe erythema (very intense redness)

E = Edema - definite swelling
P = Papules - many small, red, solid elevations; surface of reaction has granular feeling
V = Vesicles - small, circumscribed, translucent surfaced elevations (blister-like), less than 0.5cm in diameter.
B = Bullae - vesicles with a diameter > 0.5cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
W = Weeping - evidence of release of fluid from a vesicular or bullous reaction

MU = Make Up
A = first scoring of adjacent challenge site (48 hours)
A' = second scoring of adjacent challenge site (96 hours)

A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
L = Subject report of lost patch.

- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Table 1B. Individual reaction scores following the application of test material.

Test Article: [redacted]

		Application Number										Challenge			
Subject	Site	1	2	3	4	5	6	7	8	9	MU	O	A	O'	A'
16	O	1	1	1	1	1	1	1	1	1		0	0	0	0
17	O	0	0	0	0	0	0	0	0	0		0	0	0	0
18	O	0	0	0	0	0	0	0	0	0		0	0	0	0
19	O	0	0	0	0	0	0	0	0	0		0	0	0	0
20	O	0	0	0	0	0	0	0	0	0		0	0	0	0
21	O	0	0	0	0	0	0	0	0	0		0	0	0	0
22	O	0	0	0	0	0	0	0	0	0		0	0	0	0
23	O	0	0	0	0	0	0	0	0	0		0	0	0	0
24	O	0	0	1	0	0	0	0	0	0		0	0	0	0
25	O	0	0	0	0	0	0	0	0	0		0	0	0	0
26	O	0	0	0	0	0	0	0	0	0		0	1	0	0
27	O	0	0	0	0	0	0	0	0	0		0	0	0	0
28	O	0	0	0	0	0	0	0	-	0	0	0	0	0	0
29	O	0	0	0	0	0	0	0	0	0		0	0	0	0
30	O	0	0	0	0	0	0	0	-	0	0	0	0	0	0

O = original site; M = first moved site; M1 = second moved site
O = first scoring of original challenge site (48 hours)
O' = second scoring of original challenge site (96 hours)

0 = No visible erythema
1 = Mild erythema (faint pink to definite pink)
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P = Papules - many small, red, solid elevations; surface of reaction has granular feeling
V = Vesicles - small, circumscribed, translucent surfaced elevations (blister-like), less than 0.5cm in diameter.
B = Bullae - vesicles with a diameter > 0.5cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
W = Weeping - evidence of release of fluid from a vesicular or bullous reaction

MU = Make Up
A = first scoring of adjacent challenge site (48 hours)
A' = second scoring of adjacent challenge site (96 hours)

A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
L = Subject report of lost patch.

- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Table 1B. Individual reaction scores following the application of test material.

Test Article: [redacted]

		Application Number										Challenge			
Subject	Site	1	2	3	4	5	6	7	8	9	MU	O	A	O'	A'
31	O	0	0	0	0	0	0	0	0	0		0	0	0	0
32	O	0	0	-	0	0	0	0	0	0	0	0	0	0	0
33	O	0	0	0	0	0	0	0	0	0		0	0	0	0
34	O	0	0	0	0	0	0	0	0	0		0	0	0	0
35	O	0	0	0	0	0	0	0	0	0		0	0	0	0
36	O	0	0	0	0	0	0	0	0	0		0	0	0	0
37	O	0	0	0	0	0	0	0	0	0		0	0	0	0
38	O	0	0	0	0	0	0	0	0	0		0	0	0	0
39	O	0	0	0	0	0	0	0	0	0		0	0	0	0
40	O	0	0	0	0	0	0	0	0	0		0	0	0	0
41	O	0	0	0	-	Dropped									
42	O	0	0	0	0	0	0	0	0	0		0	0	0	0
43	O	0	0	0	0	0	0	0	0	0		0	0	0	0
44	O	0	0	0	0	0	0	0	0	0		0	0	0	0
45	O	0	0	0	0	0	0	0	0	0		0	0	0	0

O = original site; M = first moved site; M1 = second moved site
O = first scoring of original challenge site (48 hours)
O' = second scoring of original challenge site (96 hours)

0 = No visible erythema
1 = Mild erythema (faint pink to definite pink)
2 = Moderate erythema (definite redness)
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E = Edema - definite swelling
P = Papules - many small, red, solid elevations; surface of reaction has granular feeling
V = Vesicles - small, circumscribed, translucent surfaced elevations (blister-like), less than 0.5cm in diameter.
B = Bullae - vesicles with a diameter > 0.5cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
W = Weeping - evidence of release of fluid from a vesicular or bullous reaction

MU = Make Up
A = first scoring of adjacent challenge site (48 hours)
A' = second scoring of adjacent challenge site (96 hours)

A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
L = Subject report of lost patch.

- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Table 1B. Individual reaction scores following the application of test material.

Test Article: [redacted]

Subject	Site	Application Number 1	2	3	4	5	6	7	8	9	MU	Challenge O	A	O'	A'
46	O	0	0	0	0	0	0	0	0	0		0	0	0	0
47	O	0	0	0	0	0	0	0	0	0		0	0	0	0
48	O	0	-	0	0	0	0	0	0	0	0	0	1	0	0
49	O	0	0	0	0	0	0	0	0	0		0	0	0	0
50	O	0	0	0	0	0	0	0	0	0		0	0	0	0
51	O	0	0	0	0	0	0	0	0	0		0	0	0	0
52	O	0	0	0	0	0	0	-	0	0	N9G	0	0	0	0
53	O	0	0	0	0	-	Dropped								
54	O	0	0	0	0	0	0	0	0	0		0	0	0	0
55	O	0	0	0	0	0	0	0	0	0		0	0	0	0
56	O	0	0	0	0	0	0	0	0	0		0	0	0	0
57	O	0	0	0	0	0	0	0	0	0		0	0	0	0
58	O	0	0	0	0	0	0	0	0	0		0	0	0	0
59	O	0	0	0	0	0	0	0	-	0	0	0	0	0	0
60	O	Dropped													

O = original site; M = first moved site; M1 = second moved site
O = first scoring of original challenge site (48 hours)
O' = second scoring of original challenge site (96 hours)

0 = No visible erythema
1 = Mild erythema (faint pink to definite pink)
2 = Moderate erythema (definite redness)
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E = Edema - definite swelling
P = Papules - many small, red, solid elevations; surface of reaction has granular feeling
V = Vesicles - small, circumscribed, translucent surfaced elevations (blister-like), less than 0.5cm in diameter.
B = Bullae - vesicles with a diameter > 0.5cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
W = Weeping - evidence of release of fluid from a vesicular or bullous reaction

MU = Make Up
A = first scoring of adjacent challenge site (48 hours)
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A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
L = Subject report of lost patch.

- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Table 1B. Individual reaction scores following the application of test material.
Test Article: [redacted]

Subject	Site	Application Number 1	2	3	4	5	6	7	8	9	MU	Challenge O	A	O'	A'
61	O	0	0	0	0	0	0	0	0	0		0	0	0	0
62	O	0	0	0	0	0	0	0	0	0		0	0	0	0
63	O	0	0	0	0	0	0	0	0	0		0	0	0	0
64	O	0	0	0	0	-	0	0	0	0	0	0	0	0	0
65	O	0	0	0	0	0	0	0	0	0		0	0	0	0
66	O	0	0	0	0	0	0	0	0	0		0	0	0	0
67	O	0	0	0	0	0	0	0	0	0		0	0	0	0
68	O	0	0	0	0	0	0	0	0	0		0	0	0	0
69	O	0	0	0	0	0	0	0	0	0		Dropped			
70	O	0	0	0	0	0	0	0	0	0		0	0	0	0
71	O	0	0	0	0	0	0	0	0	0		0	0	0	0
72	O	0	0	0	0	0	0	0	0	0		0	0	0	0
73	O	0	0	0	0	0	0	0	0	0		0	0	0	0
74	O	0	0	1	0	0	0	0	0	0		0	0	0	0
75	O	0	0	0	0	0	0	0	0	0		0	0	0	0

O = original site; M = first moved site; M1 = second moved site
O = first scoring of original challenge site (48 hours)
O' = second scoring of original challenge site (96 hours)

0 = No visible erythema
1 = Mild erythema (faint pink to definite pink)
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E = Edema - definite swelling
P = Papules - many small, red, solid elevations; surface of reaction has granular feeling
V = Vesicles - small, circumscribed, translucent surfaced elevations (blister-like), less than 0.5cm in diameter.
B = Bullae - vesicles with a diameter > 0.5cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
W = Weeping - evidence of release of fluid from a vesicular or bullous reaction

MU = Make Up
A = first scoring of adjacent challenge site (48 hours)
A' = second scoring of adjacent challenge site (96 hours)

A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
L = Subject report of lost patch.

- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Table 1B. Individual reaction scores following the application of test material.

Test Article: [redacted]

		Application Number										Challenge			
Subject	Site	1	2	3	4	5	6	7	8	9	MU	O	A	O'	A'
76	O	0	0	0	0	0	1	1	0	0		0	0	0	0
77	O	0	0	0	0	0	0	0	0	0		0	0	0	0
78	O	0	0	0	0	0	0	0	0	0		0	0	0	0
79	O	0	0	0	0	0	0	0	0	0		0	0	0	0
80	O	0	0	0	0	-	0	0	0	0	0	0	0	0	0
81	O	0	0	0	0	0	0	0	0	0		0	0	0	0
82	O	0	0	-	0	0	0	0	0	0	0	0	0	0	0
83	O	0	0	0	0	0	0	-	0	0	N9G	0	0	0	0
84	O	0	0	-	0	0	0	0	0	0	0	0	0	0	0
85	O	0	-	Dropped											
86	O	0	0	0	-	0	0	0	0	0	0	0	0	0	0
87	O	0	0	0	0	0	0	0	0	0		0	0	0	0
88	O	0	0	0	0	0	0	0	0	0		0	0	0	0
89	O	0	0	0	0	0	0	0	0	0		0	0	0	0
90	O	0	0	0	0	0	0	0	0	0		0	0	0	0

O = original site; M = first moved site; M1 = second moved site
O = first scoring of original challenge site (48 hours)
O' = second scoring of original challenge site (96 hours)

0 = No visible erythema
1 = Mild erythema (faint pink to definite pink)
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E = Edema - definite swelling
P = Papules - many small, red, solid elevations; surface of reaction has granular feeling
V = Vesicles - small, circumscribed, translucent surfaced elevations (blister-like), less than 0.5cm in diameter.
B = Bullae - vesicles with a diameter > 0.5cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
W = Weeping - evidence of release of fluid from a vesicular or bullous reaction

MU = Make Up
A = first scoring of adjacent challenge site (48 hours)
A' = second scoring of adjacent challenge site (96 hours)

A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
L = Subject report of lost patch.

- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Table 1B. Individual reaction scores following the application of test material.
Test Article: [redacted]

Subject	Site	Application Number 1	2	3	4	5	6	7	8	9	MU	Challenge O	A	O'	A'
91	O	0	0	0	0	0	0	0	0	0		0	0	0	0
92	O	0	0	0	0	0	0	0	0	0		0	0	0	0
93	O	0	0	0	0	0	0	0	0	0		0	0	0	0
94	O	0	0	0	0	0	0	0	0	0		0	0	0	0
95	O	0	0	0	0	0	0	0	0	0		0	0	0	0
96	O	0	0	0	0	0	0	0	0	N9G		0	0	0	0
97	O	0	0	0	0	0	0	0	0	0		0	0	0	0
98	O	0	0	0	0	0	0	0	0	0		0	0	0	0
99	O	0	0	0	0	0	0	0	0	N9G		0	0	0	0
100	O	0	0	0	0	0	0	0	0	0		0	0	0	0
101	O	0	0	0	0	0	0	0	0	1		0	0	0	0
102	O	Dropped													
103	O	0	0	0	0	0	0	0	0	0		0	0	0	0
104	O	0	0	0	0	0	0	0	0	0		0	0	0	0
105	O	0	0	0	0	0	0	0	0	0		0	0	0	0

O = original site; M = first moved site; M1 = second moved site
O = first scoring of original challenge site (48 hours)
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S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
W = Weeping - evidence of release of fluid from a vesicular or bullous reaction

MU = Make Up
A = first scoring of adjacent challenge site (48 hours)
A' = second scoring of adjacent challenge site (96 hours)

A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
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\- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Table 1B. Individual reaction scores following the application of test material.

Test Article: [redacted]

		Application Number										Challenge			
Subject	Site	1	2	3	4	5	6	7	8	9	MU	O	A	O'	A'
106	O	0	0	0	0	0	0	0	0	0		0	0	0	0
107	O	Dropped													
108	O	0	0	0	0	0	0	0	0	0		0	0	0	0
109	O	0	1	1	1	0	0	0	0	0		0	0	0	0
110	O	0	Dropped												
111	O	0	0	0	0	0	0	0	Dropped						
112	O	0	0	0	0	0	0	0	Dropped						
113	O	0	0	0	0	0	0	0	Dropped						

O = original site; M = first moved site; M1 = second moved site
O = first scoring of original challenge site (48 hours)
O' = second scoring of original challenge site (96 hours)

0 = No visible erythema
1 = Mild erythema (faint pink to definite pink)
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E = Edema - definite swelling
P = Papules - many small, red, solid elevations; surface of reaction has granular feeling
V = Vesicles - small, circumscribed, translucent surfaced elevations (blister-like), less than 0.5cm in diameter.
B = Bullae - vesicles with a diameter > 0.5cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
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MU = Make Up
A = first scoring of adjacent challenge site (48 hours)
A' = second scoring of adjacent challenge site (96 hours)

A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
L = Subject report of lost patch.

\- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Table 1B. Summary of reaction scores following the application of test material.

Test Article: [redacted] Facial treatment essence with 92.675% galactomyces ferment filtrate

	Application Number									Challenge				
Scores	1	2	3	4	5	6	7	8	9	MU	O	A	O'	A'
0	109	104	100	103	101	102	98	97	97	11	100	98	100	100
1	1	3	4	2	1	2	2	1	2	0	0	2	0	0
-	0	2	4	2	3	0	4	3	0	0	0	0	0	0
DR	3	4	5	6	8	9	9	12	12	12	13	13	13	13
N9G	0	0	0	0	0	0	0	0	2	3	0	0	0	0

MU = Make Up
O = first scoring of original challenge site (48 hours)
O' = second scoring of original challenge site (96 hours)

0 = No visible erythema
1 = Mild erythema (faint pink to definite pink)
2 = Moderate erythema (definite redness)
3 = Severe erythema (very intense redness)

E = Edema - definite swelling
P = Papules - many small, red, solid elevations; surface of reaction has granular feeling
V = Vesicles - small, circumscribed, translucent surfaced elevations (blister-like), less than 0.5cm in diameter.
B = Bullae - vesicles with a diameter > 0.5cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
S = Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).
W = Weeping - evidence of release of fluid from a vesicular or bullous reaction

A = first scoring of adjacent challenge site (48 hours)
A' = second scoring of adjacent challenge site (96 hours)

A = Marked reaction to adhesive (patch relocated).
X = Succeeding patch not applied and succeeding grade is for residual reaction. At challenge, an "X" denotes that the patch was not applied
L = Subject report of lost patch.

- = Subject absent
DR = Dropped
N9G = No ninth grade. Subject wore nine induction patches but was not present for scoring following ninth application.

Facial treatment essence with 92.675% galactomyces ferment filtrate

[redacted]			PRODUCT CODE : [redacted]				
MF RM Name	MF RM#	[redacted]	[redacted]	Quantity	CTFA Name	CAS#	C. I. #
[redacted] Galactomyces ferment	[redacted]			92.6750	-		

[redacted]

DEC 04 1998

[redacted]

II. **Chemical Characteristics** Facial treatment essence with 92.675% galactomyces ferment filtrate

Physical Appearance:	**Liquid**	Color:	**Transparent**
Melting Point:	**N/A**	Specific Gravity:	**Unknown**
Boiling Point:	**Unknown**	Vapor Pressure:	**Unknown**
pH:	**4.70 - 5.20**	Viscosity:	**Unknown**
Flammable (yes/no):	**No**	Flash Point:	**N/A**
Explosive (yes/no):	**No**	Corrosive (yes/no):	**No**
Solubility:			
Aqueous:	**Soluble**		
Other Solvents:	**N/A**		
Storage Condition:	**Room Temperature**		
Expiration Date:	**April 6, 1999**		

[redacted]

[redacted]

Complete Section 1-9 for all requests. Complete sections 10-11b. (see over) as applicable. Manufacturing/formulating records must be attached with this request for samples tested in safety and clinical studies.

1. Test article name (must be the same as given on accompanying [redacted]:
[redacted] Facial treatment essence with 92.67.5% galactomyces ferment filtrate

2. Manufacturing/formula data: List all ingredients in descending order of predominance. Identify new ingredients with "(n)." Mark any drug actives with (*). For products from the Trade, provide formulation information that is known (at least the Component and Nominal Level and Production Code for Company products from the Trade).
(Use CTFA Name, Full Trade Name or Chemical Name that Uniquely Indicates Material)

Component	Stock Code	Formula Card %	Fraction Active	% Active in Formula	Supplier	CAS#

See the attachment.

2a. Formula Card No. [redacted]

3. Test Article Concentration during Use: N/A %
Amount of Test Article per each use: 0.5 ml

4. Adequate shelf testing (no less than the life of the product as used in this test) in the same container as it is distributed for this test has been completed:
Yes (X) / In Progress ( ) / Is Not Planned To Be Done ( )

5. Notebook ref./Production Code (if applicable): [redacted]
Date made: 07/30/98 / Made by [redacted]
Re-packing date: 10/07/98 / Re-packing site: [redacted]
Sample expiration date: 04/06/99

6. 2 Finished product samples will be retained for Quality Assurance

7. Physical form: Liquid / Color: Transparent / Density: N/A
Solubilities: Water = 100 %, Ethanol = N/A %, Acetone = N/A %,
Other (N/A) = N/A %.
PH = 4.70-5.20 (e.g., 1% aq. soln.).
Storage Conditions: (X)room temperature, ( )refrigerate, ( )freeze, ( )C range.

DEC 04 1998

99 /68 # [redacted] [redacted]

[redacted]

Facial treatment essence with 92.675% galactomyces ferement filtrate

Test article name: [redacted]

8. Work place hazards: None (X) / Identify Hazard ( )

9. Transportation classification: Non-hazardous (X) / Hazardous ( )

10. Characterization, Microbial, and Properties Information for test article. "Analysis" and "Limits" are required For Ship Test, Test Market or Market Clearances. "Date submitted", "Lab Note.#", "Analysis" and "Limits" are required for Bldg. Panel, HPT, MRD, Clinical and other test.

Date Submitted	Lab Note.#	Analysis	Limits	Value	Measured Lab or Data Source	Testing Date Reported
See the Plant Batch record Checklist.					[redacted]	

11. Approvals

a. Microbial Susceptibility Classification Approval (US/Regional)

[redacted]

ITEMS BELOW THIS LINE ARE NOT REQUIRED FOR SHIP TEST, TEST MARKET OR MARKET CLEARANCES.

b. The test substance as made and characterized is a representative example of the intended formation.

Process Development [redacted]

[redacted]

* Or person responsible for placing test if no Products Research person.

c. The test substance is adequately characterized and is acceptable for the testing indicated [redacted] (US/Regional)

[redacted]

d. The test substance production meets GMP and the above "measured values" are reviewed.

GMP-Quality Assurance [redacted]

DISTRIBUTION OF APPROVED FORM:

[redacted]

DEC 04 1998

[redacted]

[redacted]

[redacted]

FINAL REPORT

Study Title

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Test Articles

[redacted]

Facial treatement essence with 92.675% Galactomyces ferment filtrate

Authors

John W. Harbell, Ph.D.
Hans A. Raabe, M.S.
Angela M. Sizemore, B.S.

Study Completion Date

July 14, 2000

Performing Laboratory

Institute for In Vitro Sciences, Inc.
21 Firstfield Road, Suite 220
Gaithersburg, Maryland 20878

[redacted]

Laboratory Project Number

[redacted]

[redacted]

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

SUMMARY TABLE

Facial treatment essence with 92.675% Galactomyces fermenet filtrate	t_{50} (minutes)	
Test Article	**Preliminary**	**Definitive**
[redacted]	> 180 [redacted]	> 240 [redacted]
0.3% Triton X-100	15.4[1]	16.5

1 - The positive control t_{50} value was not within the acceptable range in the preliminary assay. Since the results of the preliminary assay were only used to select appropriate exposure times for the definitive assay, the positive control failure did not affect the outcome of the study.

TABLE OF CONTENTS

[redacted]

STATEMENT OF COMPLIANCE

Facial treatment essence with 92.675% Galactomyces ferment filtrate

The Tissue Equivalent Assay with EpiOcular™ Cultures of the test articles, [redacted], was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the U.S. EPA GLP Standards 40 CFR 160 and 40 CFR 792, the UK GLP Compliance Programme, the Japanese GLP Standard and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test or control articles have not been determined by the testing facility.

The stability of the test or control articles under the test conditions has not been determined by the testing facility and is not included in the final report.

Analyses to determine the uniformity, concentration, or stability of the test or control mixtures, if applicable, were not performed by the testing facility.

John W. Harbell, Ph.D.
Study Director

July 14, 2000
Date

COPY

QUALITY ASSURANCE STATEMENT

Study Title: Tissue Equivalent Assay With Epiocular Cultures

Study Number: [redacted]

Study Director: John W. Harbell, Ph.D.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLP Standards (40 CFR 792 and 40 CFR 160), the UK GLP Compliance Programme, the Japanese GLP Standard and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

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

Inspect on 22 Mar 00, to Study Director 22 Mar 00, to Management 24 Mar 00
Phase: Removal and transfer of tissue - definitive

Inspect on 05 May 00, to Study Director 05 May 00, to Management 05 May 00
Phase: Draft report and data

Inspect on 13 Jul 00, to Study Director 13 Jul 00, to Management 13 Jul 00
Phase: Final report

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

Pamela H. Errico

Pamela H. Errico, M.A., RQAP-GLP
Quality Assurance

July 13, 2000
Date

SIGNATURE PAGE

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Initiation Date:	March 16, 2000
Completion Date:	July 14, 2000
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility:	Institute for In Vitro Sciences, Inc. 21 Firstfield Road, Suite 220 Gaithersburg, Maryland 20878
Archive Location:	Institute for In Vitro Sciences, Inc. Gaithersburg, Maryland 20878
Study Director:	John W. Harbell, Ph.D. July 14, 2000 Date
Laboratory Manager:	Greg Mun, B.A.

COPY

TEST ARTICLE RECEIPT

Facial treatment essence with 92.675% Galactomyces ferment filtrate

IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions[1]
[redacted]	[redacted]	clear, colorless, non-viscous liquid	03/14/00	room temperature
[redacted]				

1 - Protected from exposure to light
2 - One of the two samples received was found to have leaked a small volume during shipment. The leaking sample was not used in this study.

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

[redacted]

INTRODUCTION

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

The purpose of this study was to evaluate the toxicity of the test articles supplied by [redacted] as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to a test article for various exposure times. The laboratory phase of the study was conducted from March 21, 2000 to March 23, 2000 at the Institute for In Vitro Sciences, Inc. After a time range finding assay, the test articles were tested in a definitive assay (four exposure times) to determine the time of exposure to a test article which resulted in the t_{50} endpoint.

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

MATERIALS AND METHODS

Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ human cell construct model kit, the solutions were stored as indicated. The EpiOcular™ human cell constructs were stored at 2-8°C until used. An appropriate volume of EpiOcular™ human cell construct assay medium was removed and warmed to approximately 37°C. Nine-tenths ml of assay medium was aliquoted into the wells of 6-well plates. The six-well plates were labeled to indicate test article and exposure time. The samples were inspected for air bubbles between the agarose gel and millicell insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the millicell area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the 6-well plates. The EpiOcular™ human cell constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour. The medium was aspirated and 0.9 ml of fresh medium were added to each assay well below the EpiOcular™ human cell construct. The trays were returned to the incubator until treatment was initiated.

Assessment of Direct Test Article Reduction of MTT

Each test article was added to a 1.0 mg/ml MTT solution in DMEM to assess their ability to directly reduce MTT. Approximately 100 µl of each test article were added to 1 ml of the MTT solution and the mixture was incubated in the dark at room temperature for approximately one hour. If the MTT solution color turned blue/purple, the test article was presumed to have reduced the MTT. Water insoluble test materials may show direct reduction (darkening) only at the interface between the test article and the medium.

Neither test article was observed to directly reduce MTT in the absence of viable tissue, and therefore, the killed-control experiment using freeze-killed tissues was not performed in this study.

Time Range Finding Assay

A time range finding assay was performed to establish an appropriate exposure time range to be used in the definitive assay for each test article. Four exposure times of 10, 30, 60, and 180 minutes were selected for the time range finding assay. One culture was treated per exposure time with 100 µl of the appropriate test article. The negative control (exposure time control)(100 µl of sterile, deionized water (Quality Biological)) was tested for 180 minutes. The positive control (100 µl of 0.3% Triton X-100 (Fisher)) was tested for 15 and 45 minutes.

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with calcium and magnesium-free Dulbecco's Phosphate Buffered Saline (DPBS) and the wash medium was decanted. After rinsing, the tissue was transferred to 5 ml of Assay Medium for a 10 to 20 minute incubation at room temperature to remove any test article absorbed into the tissue. A 1.0 mg/ml solution of MTT in warm Dulbecco's Modified

Eagle's Medium (DMEM) was prepared. Three-tenths ml of MTT (Sigma) reagent were added to wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing. The trays were incubated at 37±1°C for approximately three hours in a humidified atmosphere of 5±1% CO_2 in air.

After the incubation period with MTT solution, the EpiOcular™ cultures were extensively rinsed with DPBS, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 ml of isopropanol. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for two hours at room temperature.

At the end of the extraction period, the liquid within the millicell inserts was decanted into the well from which the millicell insert was taken. The extract solution was mixed and 200 µl were transferred to the appropriate wells of a 96-well plate. The absorbance at 550 nm (OD_{550}) of each well was measured with a Molecular Devices' Vmax plate reader.

In the time range finding assay, the positive control results were not within the acceptable range. However, since the results of the time range finding assay were only used to select appropriate test article exposure times, the positive control failure did not affect the outcome of the study.

Definitive Assay

Based on the results of the time range finding assay, four exposure times were chosen for the definitive assay. The exposure times were chosen such that generally two exposure times were expected to result in survivals lower than 50% and two exposure times were expected to result in survivals greater than 50%. In general, the negative control exposure times were selected to fit the range of the test article or positive control exposure times. The exposure times for the test articles were 60, 120, 180, and 240 minutes. The negative control was tested for 15 and 240 minutes. The positive control was tested for 15 and 45 minutes. The procedures used to conduct the definitive assay were essentially the same as for the time range finding assay with the exception that duplicate cultures were dosed per exposure time.

Presentation of Data

The mean OD_{550} values of the negative control wells, blank control wells, each positive control and each test article well for the various exposure times were calculated. The corrected mean OD_{550} values of the negative control, test article exposure times and the positive control exposure times were determined by subtracting from each the mean OD_{550} values for the blank control. All calculations were performed using IIVS' Laboratory Information Management System. The raw absorbance values were captured, and the following calculations were made:

$$\% \text{ of Control} = \frac{\text{corrected mean } OD_{550} \text{ of Test Article Exposure time}}{\text{corrected mean } OD_{550} \text{ of Negative control}} \times 100$$

[redacted]

RESULTS AND DISCUSSION

Time Range Finding Assay

A time range finding assay was performed, consisting of four exposure times (10, 30, 60 and 180 minutes) for the test articles supplied by [redacted] The time response curves for the test articles are included in Appendix B. Based upon the results of the time range finding assay, four exposure times were selected for each test article for the definitive assay (see Materials and Methods). The t_{50} results for the time range finding assay are reported in Table 1, under "Preliminary".

In the time range finding assay, the positive control t_{50} value was not within the acceptable range. However, since the results of the time range finding assay were only used to select appropriate test article exposure times, the positive control failure did not affect the outcome of the study.

Definitive Assay

facial treatment essence with 92.675% Galactomyces ferment filtrate

Four exposure times were tested in duplicate for each test article. The exposure times for the test articles, [redacted], were 60, 120, 180, and 240 minutes. The negative control was also tested in duplicate for 15 and 240 minutes. Table 1 summarizes the t_{50} results of the definitive EpiOcular™ human cell construct assay for the test articles and the positive control, 0.3% Triton X-100, under "Definitive". The time response curves for each test article as well as the positive control are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (15.5 - 37.0 minutes), and the corrected mean OD_{550} value for the minimum negative control exposure time (1.217) was within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 240 minutes) (1.256), the assay results were accepted. Finally, neither of the test articles was observed to directly reduce MTT in the absence of viable tissue, and therefore, the killed-control experiment using freeze-killed tissues was not performed in this study.

Table 1

Facial treatment essence with 92.675% Galactomyces ferment filtrate	t_{50} (minutes)	
Test Article	**Preliminary**	**Definitive**
[redacted]	> 180 [redacted]	> 240 [redacted]
0.3% Triton X-100	15.4[1]	16.5

1 - The positive control t_{50} value was not within the acceptable range in the preliminary assay. Since the results of the preliminary assay were only used to select appropriate exposure times for the definitive assay, the positive control failure did not affect the outcome of the study.

APPENDIX A

[redacted]

IIVS Study Number: [redacted]

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

1.0 PURPOSE

The purpose of this study is to evaluate the potential toxicity of the test article. In the Tissue Equivalent Assay, stratified human epithelial cell cultures (MatTek EpiOcular™) are exposed to topically applied test articles to evaluate potential ocular toxicity. Cell viability is determined by conversion of 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) in the treated cultures, and is expressed as a percentage relative to untreated (negative control) cultures. The endpoint of the Tissue Equivalent Assay, the t_{50} value, is the time (generally in minutes or hours) of exposure to test article required to reduce cell viability (MTT metabolism) to 50% of negative control levels as calculated from time-response curves.

2.0 SPONSOR

See Protocol Attachment 1

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1 Test Article: See Protocol Attachment 1

3.2 Controls:

Positive:	0.3% Triton X-100
Negative:	Sterile water (or other solvent as appropriate)
	blank control (MTT reading only)

3.3 Determination of Strength, Purity, etc.

3.3.1 The Sponsor will be responsible for determination and documentation of the analytical purity and composition of the test article and the stability and strength of the dosing solutions, as applicable.

3.3.2 The Institute for In Vitro Sciences, Inc. (IIVS) will be responsible for the documentation of the analytical purity and composition of the Triton X-100 used for the stock or working dilution of the positive control. This may be accomplished by maintaining a certificate of analysis from the supplier.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1	Name:	Institute for In Vitro Sciences, Inc.
4.2	Address:	21 Firstfield Road, Suite 220 Gaithersburg, MD 20878
4.3	Study Director:	Hans A. Raabe, M.S.

5.0 TEST SCHEDULE

5.1	Proposed Experimental Initiation Date:	21 March 2000
5.2	Proposed Experimental Completion Date:	30 March 2000
5.3	Proposed Report Date:	28 April 2000

6.0 TEST SYSTEM

The EpiOcular™ human cell construct, provided by the MatTek Corporation, will be used in this study. The use of EpiOcular™ cultures offers several features appropriate for a model for ocular irritation. First, the model is composed of stratified human keratinocytes in a three-dimensional structure. Secondly, test materials can be applied topically to the model so that water insoluble materials may be tested.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The experimental design of this study consists of a time-range-finding assay and a definitive assay. The toxicity of the test article will be evaluated by the exposure time required to reduce cell viability to 50% of controls (t_{50}). Viability will be determined by the succinate dehydrogenase-dependent reduction of MTT in control and test article treated cultures. Data will be presented in the form of relative survival (relative MTT conversion) versus test article exposure time.

One of two exposure time ranges may be used. The standard exposure time range extends up to four hours and is used for most materials to be tested. For extremely mild materials, such as those which might be applied around or in the eyes, a long exposure assay might be used. For the long exposure study, exposure times of up to 24 hours could be used. In general, the standard exposure range will be used, unless the Sponsor specifies an alternative exposure time range or if the study director determines that the class of test articles warrants the use of an alternative exposure time range.

7.1 Media and Reagents

7.1.1 Assay Medium: supplied by MatTek Corporation

7.1.2 EpiOcular Tissue: OCL-200 supplied by MatTek Corporation

7.1.3 Dulbecco's Minimal Essential Medium (DMEM)

7.1.4 3-[4,5 - dimethylthiazol-2-yl] - 2,5 - diphenyltetrazolium bromide (MTT) 10X stock solution: 10 mg/ml MTT in PBS

7.1.5 Dulbecco's Phosphate Buffered Saline (D-PBS)(pH 7.0 ± 0.5)

7.1.6 Extraction Medium: Isopropanol

7.2 Preparation and Delivery of Test Article

Test articles will generally be tested neat. End use concentrations or other forms may be used as directed by the Sponsor. One hundred µl of pipettable substances, such as liquids, gels, creams, and foams, will be applied directly on the tissue so as to cover the upper surface. To aid in filling the pipet for pipettable materials that are viscous, the test article may first be transferred to a syringe. The pipet tip of the positive displacement pipet will be inserted into the dispensing tip of the syringe so that the material can be loaded into the displacement tip under pressure. Simultaneously, the syringe plunger is depressed as the pipet piston is drawn upwards. If air bubbles appear in the pipet tip, the test article should be removed (expelled) and the process repeated until the tip is filled without air bubbles. This method should be used for any materials that can not be easily drawn into the pipet such as gels, (e.g., toothpastes, mascaras, and face creams) and solid test articles which are creamed like lipsticks and antiperspirants/deodorant sticks. A dosing device may be placed over the test article to assure even spreading, if required. Dry powders will be ground with a mortar and pestle and passed through a #40 copper sieve. Thirty mg samples will be measured into glass vials prior to test article treatment. Materials which are too viscous to spread over the tissue will first be spread onto the flat end of a dosing device. The dosing device will be put into the Millicell to bring the test article in contact with the tissue. When the dosing device is used, approximately 30 µl or 30 mg of material will be applied to the dosing device so as to cover the dosing surface. The sample should be spread to form a relatively smooth even layer on the surface of the dosing device to maximize uniform tissue contact. Solids such as lipsticks or antiperspirant/deodorant sticks can be presoftened by creaming a portion in a weigh boat. The softened portion can be transferred to a syringe affixed with a three way stopcock attached to a second syringe. The sample is pushed from syringe to syringe until it is of a consistency which can be pipetted. The exact exposure conditions used for other test article forms will be determined after consultation with the Sponsor and/or the study

director. All exposure conditions will be documented in the study workbook.

The stability of the test article under the actual experimental conditions will not be determined by IIVS.

7.3 Route of Administration

Test article will be administered by topical application to the cell cultures.

7.4 Controls

Two types of control treatments are used in this assay. The negative control cultures (negative control) are treated with sterile water or other solvent. Negative control cultures are dosed and exposed in parallel with the test article and positive control cultures. The exposure times used for the negative controls are selected to address the range of exposure times used for the test article and positive control cultures. Positive control cultures are treated with 0.3% Triton X-100 prepared in sterile water and are dosed and exposed for 15 and 45 minutes.

Time range finding assay: The assay will include a negative, positive control and blank control (plate reading step). Each test group will be tested with at least a single culture. The negative control will be tested in at least one exposure time, which will generally be chosen to address the longest test article or positive control exposure time. Single cultures will be used for each of the two positive control exposure times.

Definitive assay: Generally, at least two negative control exposure times will be used. At least duplicate cultures will be used for each control time. One negative control exposure time will be selected to fit the range of the shortest test article or positive control exposure times (the minimum negative control exposure time will be 15 minutes) while the second negative control exposure time will be selected to match the longest test article or positive control exposure time (whichever is longer, up to 240 minutes). On occasion, the second negative control exposure time may be selected to fit the longest test article exposure time of a test article run concurrently, but from an independent study. For the long exposure assay (exposures of greater than 240 minutes), multiple negative control exposure times may be selected to fit the range of test article exposure times. If all exposure times are less than one hour, a single negative control exposure time may be used. Additional negative control exposure times may be selected at the discretion of the Study Director. Two cultures will be used for each positive control exposure time.

7.5 Receipt of the EpiOcular™ Model

Upon receipt of the EpiOcular™ assay materials, the solutions will be stored as indicated by the manufacturer. The cell cultures will be stored at 2-8°C until used. Cultures should generally be used within 72 hours after they have been placed on agar by the manufacturer.

An appropriate volume of EpiOcular™ assay medium will be removed and warmed to approximately 37°C. Nine tenths (0.9) ml of assay medium will be aliquoted into the wells of 6-well plates. Each culture will be inspected for air bubbles between the agarose gel and Millicell™ insert prior to opening the sealed package. Cultures with air bubbles under greater than 50% of the Millicell™ area will not be used. The 24-well shipping containers will be removed from the plastic bag and the surface disinfected with 70% ethanol. An appropriate number of cultures will be transferred aseptically from the 24-well shipping containers into the 6-well plates. The EpiOcular™ cultures will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour prior to dosing. The medium will be aspirated and 0.9 ml of fresh medium will be added to each assay well below the tissue prior to dosing. Note: The refeeding step may occur at less than 1 hour but the tissue should be allowed at least one hour of incubation to become fully metabolically active before dosing.

7.6 Time-Range-Finding Assay

At least four exposure times will be evaluated for each test article. For the standard assay, the exposure times for the time-range-finding assay will generally be 10, 30, 60 and 180 minutes unless the Sponsor requests other specific exposure times. The maximum exposure time will be 240 minutes. For the long term exposure assay, the exposure times for the time range finding assay will generally be 4, 8, 16, and 24 hours.

Each test article and control exposure time will be tested by treating one culture. One hundred µl of each pipettable test article will be delivered with a positive displacement pipet onto the culture. Powders will be placed directly onto the culture at 30 mg/culture. Those materials which are too viscous to spread on the culture may be spread onto a dosing device. Approximately 30 µl or 30 mg will be applied to the dosing device for each culture. See section 7.2 for precise details. Exposure times of five minutes or greater will be incubated at 37 ± 1°C and 5 ± 1% CO_2 in air.

At the end of the treatment time, the test article will be removed by extensively rinsing both sides of the culture with D-PBS. The process will be performed until the culture appears free of test article. If it is not possible to remove all of the visible test material, this will be noted in the study workbook. After rinsing, the culture will be transferred to 5 ml of Assay Medium for a 10 to 20 minute

incubation at room temperature. This rinse is intended to remove any test article absorbed into the culture.

A 1.0 mg/ml MTT solution will be prepared immediately before use. MTT will be dissolved in warm DMEM and filtered through a 0.45 µm filter to remove undissolved crystals. Alternatively, a 10x stock of MTT prepared in PBS will be thawed and diluted in warm DMEM to produce the 1.0 mg/ml solution. The exact procedure will be documented in the study workbook and the method will be consistent within an assay. Three hundred µl of the MTT solution will be added to each well of a prelabelled 24-well plate. Excess Assay Medium will be removed and then the EpiOcular™ cultures will be transferred to the appropriate wells of the MTT plate. The 24-well plates will be incubated at 37±1°C for approximately 3 hours in a humidified atmosphere of 5±1% CO_2 in air.

After approximately three hours, the EpiOcular™ tissues will be rinsed in D-PBS and transferred to a prelabelled 24-well plate containing 2.0 ml of isopropanol in each well. The plates will be sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time is harvested. If necessary, plates may be stored overnight (or up to 20 hours after the last exposure time is harvested) in the refrigerator prior to extracting the MTT. The plates will then be shaken for 2 hours at room temperature. At the end of the extraction period, the liquid within the Millicell™ inserts will be decanted into the well from which the Millicell™ insert was taken. The extract solution will be mixed and 200 µl transferred to the appropriate wells of a 96-well plate. Two hundred µl of isopropanol will be added to the wells designated as blanks. The absorbance at 550 nm (OD_{550}) of each well will be measured with a Molecular Devices Vmax plate reader.

The range of exposure times for the definitive assay will be chosen to determine the t_{50} (the exposure time to the test article which reduces MTT metabolism by 50%). Based on the results of the time-range-finding assay, two exposure times will be chosen that should result in expected survivals lower than 50%, and two exposure times will be chosen that should result in expected survivals greater than 50%. If a test article fails to cause 50% toxicity in the time-range-finding assay, the maximum exposure time will be 240 minutes, or 24 hours, depending on the assay selected. In some cases, the exposure times for the definitive assay may be selected based on visual observations of the relative MTT reduction in the tissues.

7.7 Assessment of Direct Test Article Reduction of MTT

In some cases, it will be necessary to assess the ability of each test article to directly reduce MTT. A 1.0 mg/ml MTT solution will be prepared in DMEM as described above. Approximately 100 µl (liquid test articles) or 30 mg (solid test articles) will be added to 1 ml of the MTT solution and the mixture incubated in the dark at room temperature for approximately one hour. If the MTT solution color turns blue/purple, the test article is presumed to have reduced the MTT. Water insoluble

test materials may show direct reduction (darkening) only at the interface between the test article and the medium. This test may be required if the MTT cytotoxicity results clearly demonstrate either non-toxic responses at all exposure times, excessive reduction of MTT in treated tissues or an inability to remove the test article from the tissues.

7.8 Definitive Assay

The definitive assay with generally four to five exposure times will be performed exactly like the time-range-finding assay with the exception that cultures will be tested in duplicate for each exposure time. If the test article(s) are found to be non-toxic in the time-range-finding assay, then fewer than four exposure times may be chosen for the definitive assay. Duplicate cultures will generally be tested at each of the positive control exposures. Duplicate cultures will be treated with negative or solvent control for each exposure time (see section 7.4). The determination of the t_{50} will be based upon the results of the definitive assay. At the Study Director's option, a second definitive assay may be performed.

7.9 Presentation of Data

The mean OD_{550} of the exposure time control wells, blank control wells, positive control wells and test article exposure time wells will be calculated. The corrected mean OD_{550} of the exposure time control, test article exposure times, and the positive control will be determined by subtracting the mean OD_{550} of the blank control from their mean OD_{550}s. Generally, all calculations will be performed using IIVS's licensed proprietary Laboratory Information Management System. The raw absorbance values will be captured, and the following calculations made:

$$\% \text{ Of Control} = \frac{\text{corrected mean } OD_{550} \text{ of Test Article Exposure Time}}{\text{corrected mean } OD_{550} \text{ of Control Exposure Time}} \times 100$$

Viability calculations for test articles treated in the long exposure time assay may be performed by comparing the corrected mean OD_{550}s of each test article exposure time to the appropriate exposure time control(s).

Exposure time response curves may be plotted with the % of control on the ordinate and the test article exposure time on the abscissa. Other plot forms may be used as requested by the Sponsor. The t_{50} will be interpolated from each plot. If the shortest test article exposure time shows less then 50% relative survival, the plot will be extended to include the t_0 point which will be given a value of 100%. In this case, the t_{50} will be determined between the t_0 and the shortest exposure time. At the Study Director's option, additional assays may be performed to produce the final t_{50} value.

[redacted]

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The assay will be accepted if the positive control compound, 0.3% Triton X-100, causes a t_{50} within two standard deviations of the historical mean. The historical mean is updated every three months. Since the shortest positive control exposure time is 15 minutes, t_{50} values of less then 15 minutes will be considered unacceptable. The corrected mean OD_{550} value for the minimum negative control exposure time must be within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time for assays up to 240 minutes.

9.0 REPORT

A report of the results of this study will be prepared by the Testing Facility and will accurately describe all methods used for generation and analysis of the data. A separate summary will be prepared reporting the t_{50} values for each assay with each test article as well as the positive control data. A copy of the protocol used for the study and any significant deviation(s) from the protocol and SOPs of the Testing Facility will appear as a part of the final report.

10.0 RECORDS AND ARCHIVES

A separate working notebook will be used to record the materials and procedures used to perform this study. Upon completion of the final report, all raw data and reports will be maintained by IIVS for the time period specified in the Laboratory Services Agreement in effect at the time of completion of the study.

11.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

The regulatory compliance requirements for this study are detailed in the Protocol Attachment 1.

The Quality Assurance Unit will review the study protocol, perform at least one in-process laboratory inspection, and audit the raw data workbook and all reports of the study to assure compliance with the appropriate regulations specified in the Protocol Attachment 1.

12.0 PROTOCOL AMENDMENTS

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

13.0 REFERENCES

MTT Effective Time 50 (ET-50) Protocol, MatTek Corporation

14.0 APPROVAL

(See Sponsor's Protocol Attachment 1)
SPONSOR REPRESENTATIVE

IIVS STUDY DIRECTOR

16 March 2000
DATE

PROTOCOL AMENDMENT I

ORIGINAL

DATE: March 28, 2000

SPONSOR: [redacted]

SPONSOR'S TEST ARTICLE DESIGNATIONS: Facial treatment essence with 92.675% Galactomyces ferment filtrate [redacted]

IIVS STUDY NO: [redacted]

PROTOCOL NO: [redacted]

PROTOCOL TITLE: TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

AMENDMENT:

1) Location: IIVS Study Number, Header page 1

Amendment: Change the following:

"[redacted]" to "[redacted]"

Reason: Typographical error

APPROVAL: [redacted]

AUTHORIZED REPRESENTATIVE DATE

Hans Raab 28 March 2000

STUDY DIRECTOR DATE

PROTOCOL AMENDMENT II

ORIGINAL

DATE: **April 27, 2000**

SPONSOR: [redacted]

SPONSOR'S TEST ARTICLE DESIGNATION: Facial treatment essence with 92.675% Galactomyces ferment filtrate [redacted]

IIVS STUDY NO: [redacted]

PROTOCOL NO: [redacted]

SPONSOR STUDY NO.: [redacted]

PROTOCOL TITLE: **Tissue Equivalent Assay with EpiOcular™ Cultures**

AMENDMENT:

1) Location: § 4.3 Study Director

Amendment: Change Study Director from "Hans A. Raabe, M.S." to "John W. Harbell, Ph.D.

Reason: To provide a transfer of study director responsibilities upon termination of the current study director's employment, effective May 5, 2000.

APPROVAL: [redacted]

AUTHORIZED REPRESENTATIVE DATE

J W Harbell 4/27/00

STUDY DIRECTOR DATE

APPENDIX B

EPIOCULAR BIOASSAY

EXPERIMENT DATE: 21-Mar-00 Study No. [redacted]
TEST MATERIAL: [redacted] Facial treatment essence with 92.675% Galactomyces ferment filtrate
TEST ARTICLE: [redacted]
PRELIMINARY ET50 = > 180 Minutes
CONCENTRATION: 100 %

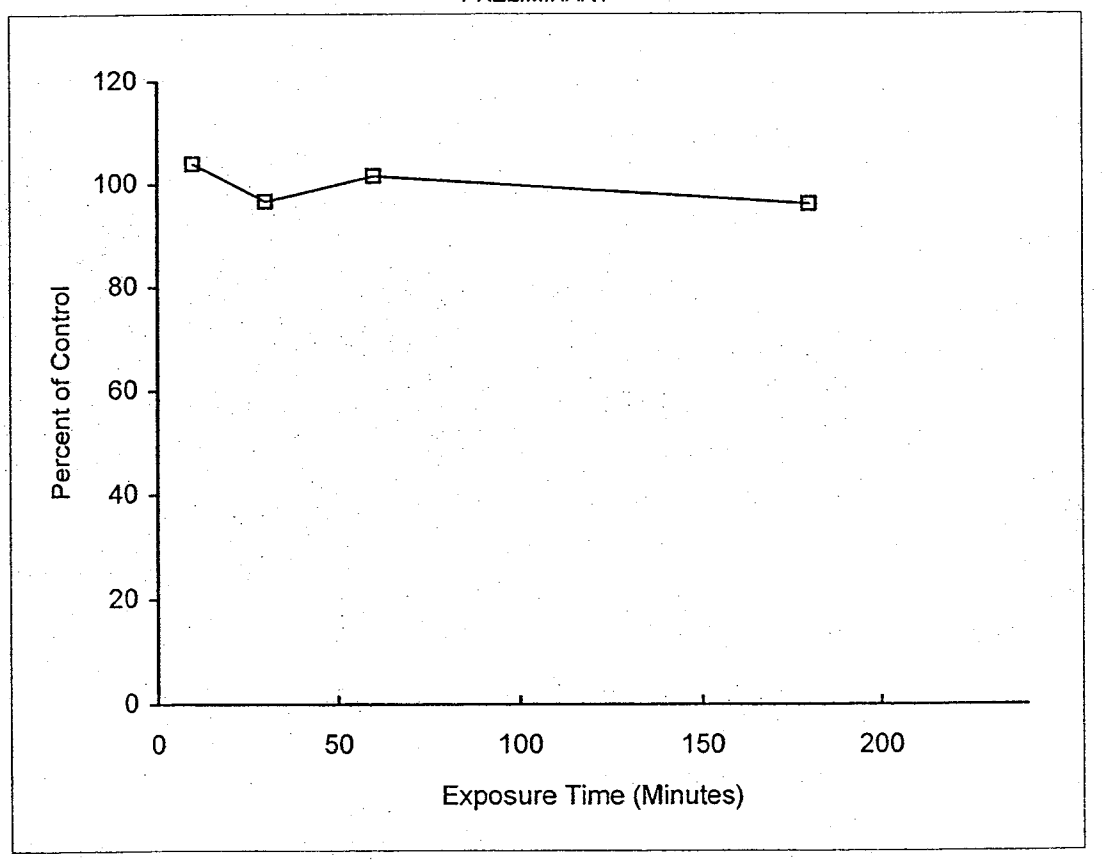
TIME EXPOSURE (Minutes)	PERCENT VIABLE
10	104.0
30	96.6
60	101.6
180	96.1

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	180.0	1	96.1
2	180.0	2	96.1
3	#DIV/0!	3	50
	slope =		#DIV/0!
	y intercept =		#DIV/0!

Facial treatment essence with 92.675% Galactomyces ferment filtrate [redacted]

PRELIMINARY



EPIOCULAR BIOASSAY

EXPERIMENT DATE: 22-Mar-00 Study No. [redacted]
TEST MATERIAL: [redacted] Facial treatment essence with 92.675% Galactomyces ferment filtrate
TEST ARTICLE: [redacted]
TRIAL 1 ET50 = > 240 Minutes
CONCENTRATION: 100 %

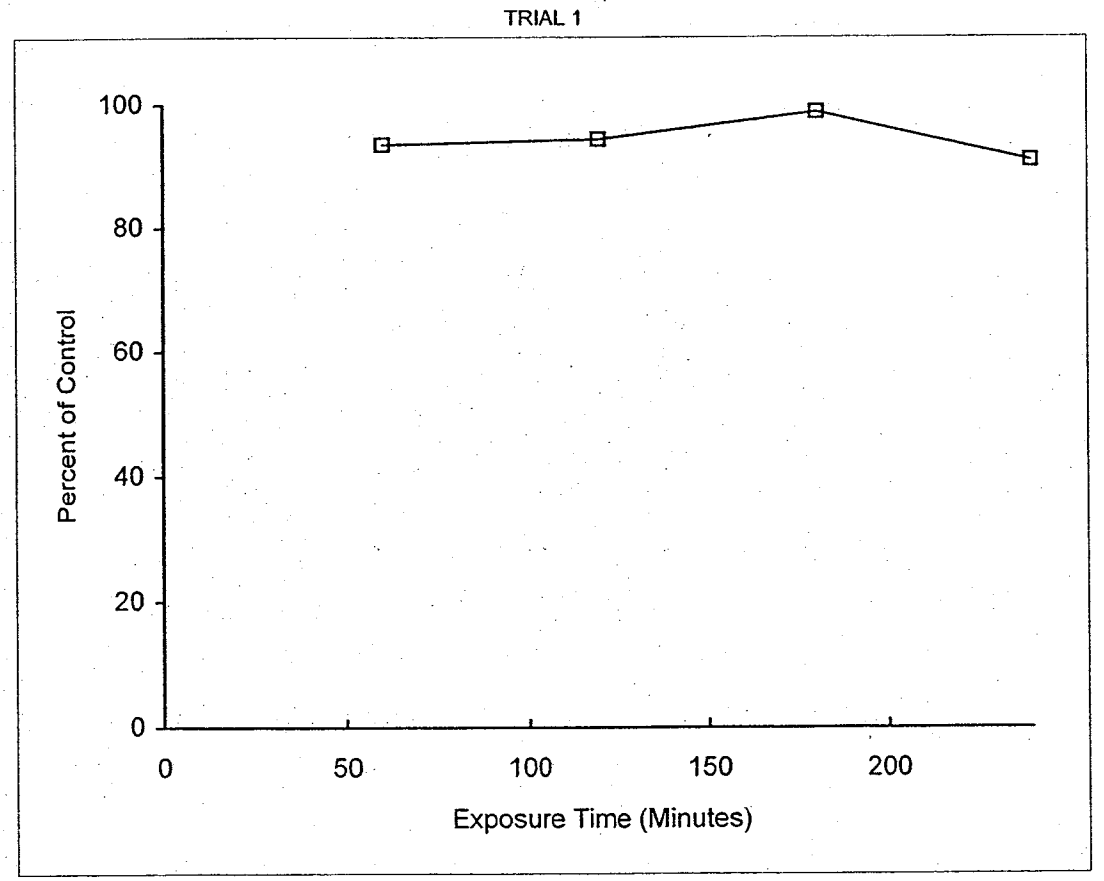
TIME EXPOSURE (Minutes)	PERCENT VIABLE
60	93.5
120	94.4
180	98.8
240	90.9

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	240.0	1	90.9
2	240.0	2	90.9
3	#DIV/0!	3	50
slope =			#DIV/0!
y intercept =			#DIV/0!

Facial treatment essence with 92.675% Galactomyces ferment filtrate

[redacted]



EPIOCULAR BIOASSAY

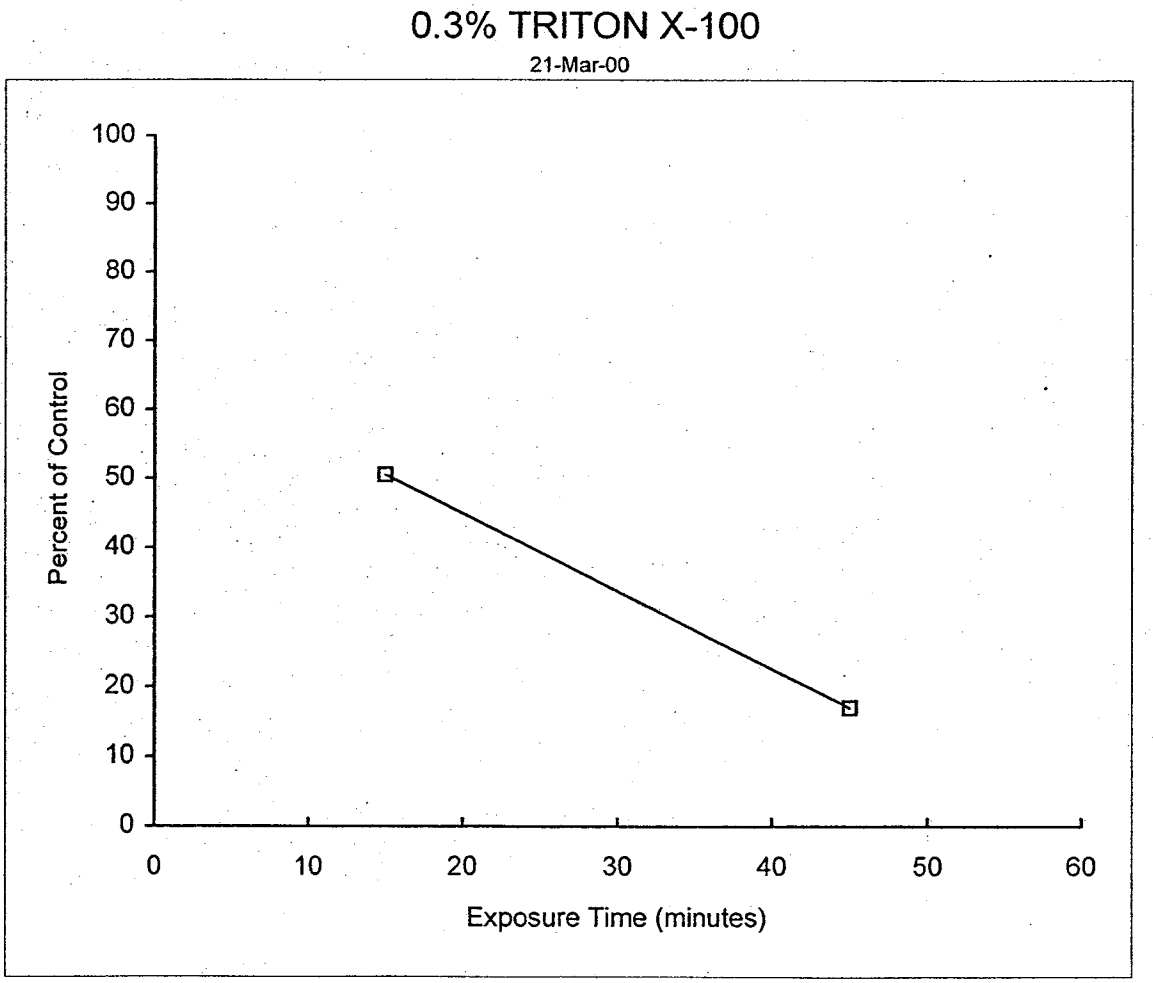
EXPERIMENT DATE: 21-Mar-00
TEST MATERIAL: 0.3% TRITON X-100

ET50 = 15.4 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	50.5
45	17.0

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	50.5
2	45.0	2	17
3	15.447761	3	50
	slope =		-1.116667
	y intercept =		67.25



EPIOCULAR BIOASSAY

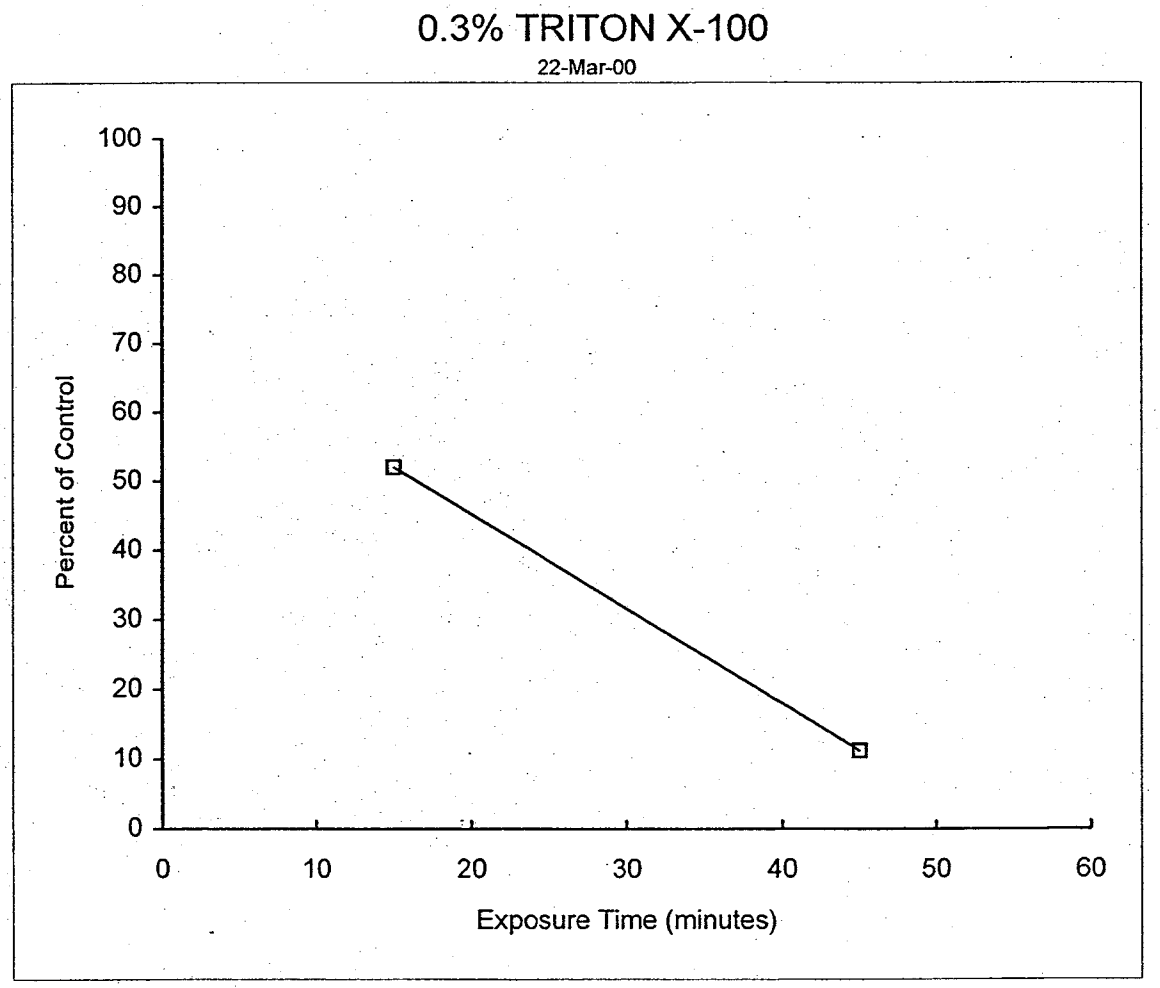
EXPERIMENT DATE: 22-Mar-00
TEST MATERIAL: 0.3% TRITON X-100

ET50 = 16.5 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	52.0
45	11.2

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	52
2	45.0	2	11.2
3	16.470588	3	50
	slope =		-1.36
	y intercept =		72.4



Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: August 22, 2023

SUBJECT: Hydrolyzed Yeast

Anonymous. 2023. Summary of Safety Assessment of Hydrolyzed Yeast.

Anonymous. 2023. In vitro sensitization tests (Extract A= 0.4% Hydrolyzed Yeast; 30% 1,3-BG; 0.08% polysorbate 20; 69.52% water).

Anonymous. 2022. Clinical safety evaluation repeated insult patch test (test article 0.01% extract A [0.00004% Hydrolyzed Yeast]).

August 2023

Summary of Safety Assessment of Hydrolyzed Yeast (Study details attached)

- ✓ Testing sample;
  - ➢ Raw material name; Extract A (Hydrolyzed Yeast; 0.4%, 1,3-BG; 30%, polysorbate 20; 0.08%, water; 69.52%)
  - ➢ Objective substance: Hydrolyzed Yeast in Extract A
  - ➢ Maximum intended use concentration; 1% of Extract A, for facial leave-on product,
- ✓ Dermal sensitization:

  We performed in vitro and human skin sensitization tests of Hydrolyzed Yeast. These tests corresponding to key event (KE) 1, 2 and 3 for skin sensitization AOP (OECD, 2012).
  - ➢ SH test for KE1 (Suzuki *et al.*, 2009; Imai *et al.*, 2021; OECD guidance 256(2016)); Appendix 1
  - ➢ KeratinoSens™ (OECD TG442D (2022)) for KE 2; Appendix 2
  - ➢ human Cell Line Activation Test (h-CLAT, OECD TG442E (2023)) for KE 3; Appendix 3

    As a result, all tests were judged as negative potency of dermal sensitization. And, predicted LLNA EC3 value was calculated using artificial neural network model (OECD guidance 256(2016)). Predicted EC3 value was 61.87% (Appendix 4).

  - ➢ human-repeated insult patch test (hRIPT); Appendix 5

    No skin reaction was observed by the treatment with 0.01% of Extract A (Hydrolyzed Yeast; 0.00004%) under hRIPT condition in 51 subjects.

- ✓ Conclusion

  As a result of the safety Assessment of Extract A including 0.4% of Hydrolyzed Yeast (INCI; Hydrolyzed Yeast), we judged that this ingredient was safe under actual use of condition from the viewpoint of skin sensitization.

- ✓ References:

  - OECD (2012). The adverse outcome pathway for skin sensitisation initiated by covalent binding for proteins. Part 1: Scientific evidence. OECD Environment, Health and Safety Publications Series onTesting and Assessment 168. Paris: OECD Publishing.
  - Suzuki M, Hirota M, Hagino S, Itagaki H, Aiba S. (2009). Evaluation of changes of cell-surface thiols as a new biomarker for in vitro sensitization test. Toxicol. In Vitro 23: 687–696.
  - Imai N, Takeyoshi M, Aizawa S, Tsurumaki M, Kurosawa M, Toyoda A, Sugiyama M, Kasahara

K, Ogata S, Omori T, Hirota M (2021). Improved performance of the SH test as an in vitro skin sensitization test with a new predictive model and decision tree. J Appl Toxicol., 42,1029-1043. doi: 10.1002/jat.4275.

- OECD (2016). Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used Within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation, OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 256.
- OECD (2022). OECD Guideline for testing chemicals, Section 4: Health Effects. Guideline no. 442D. In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method. Paris: OECD Publishing.
- OECD (2023). OECD Guideline for testing chemicals, Section 4: Health Effects. Guideline no. 442E. In Vitro Skin Sensitisation: In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation. OECD Publishing.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

August 2023

In Vitro sensitization tests

(Extract A = 0.4% Hydrolyzed Yeast, 30% 1,3-BG

Appendix 1. Assay procedure and result of SH test 0.08% polysorbate 20, 69.52% Water)

The SH test was performed as described previously with minor modifications (Suzuki *et al.*, 2009; Imai *et al.*, 2021). In brief, THP-1 cells were treated with chemicals for 2 h with test chemicals at eight 1.2-fold serial dilutions starting at 1.2 × the maximum concentration. In this study, the test doses were set as the maximum concentration of 40000 μg/mL because this raw material was not cytotoxic.

After treatment, the cells were washed and incubated with Alexa Fluor 488 C5 maleimide phosphate-buffered saline (PBS) solution for 30 min at 37 °C. They were washed with PBS, and analyzed by flow cytometry. Dead cells were gated out by staining with propidium iodide.

Relative fluorescence intensity (RFI) was calculated by use of the following formula: RFI (% of control) = (mean fluorescence intensity (MFI) of chemical-treated cells/MFI of vehicle control cells) x 100. The judgement criterion was either a decrease of cell-surface thiols to less than 85% (control; 100%) or an increase of cell-surface thiols to more than 115% and the 95% confidence interval for the mean RFI value could not include 100% (equivalent to the control group). The 95% confidence interval was calculated according to the following formula:

95% confidence interval = average ± 1.96 x (standard deviation/$\sqrt{n}$).

If the first three results were consistent, the final judgment was made without calculating the 95% confidence interval. If the first three results were inconsistent, the 95% confidence interval of the mean RFI was calculated (Imai *et al.*, 2021).

The change for RFI value in each chemical was calculated by use of the following formula: Amount of change of average RFI value = |100 – (RFI value)|. The maximum change in each dose was taken as the maximum amount of change (MAC) value.

Results of SH test (Test period; 2017/9/22- 2017/9/27)

	Number of Run / Date / Conc.	1st / 2017/9/22	2nd / 2017/9/25	3rd / 2017/9/27			Average of MFI/RFI/cell viability	SD	95% confidence interval of mean RFI	Amount of change of average RFI value	Judgement
	0(DMSO)	5859	6192	6492			6181	317			

MFI of DNCB (Positive control)	4	4514	4122	5391			4676	650			
MFI of Extract A including 0.4% Hydrolyzed Yeast	Non-treated	6605	6649	6222			6492	235			
	40000	6489	5815	6288			6197	346			
	33333	6513	6580	6868			6654	189			
	27778	6084	6308	6554			6315	235			
	23148	5916	6719	6796			6477	487			
	19290	5874	7276	6393			6514	709			
	16075	6100	7278	7142			6840	644			
	13396	5872	6807	6601			6427	491			
	11163	6269	6648	6853			6590	296			
RFI of DNCB	0(DMSO)	100	100	100			100		—	—	—
	4	77	67	83			61.5	8.1	N.D.	—	Positive
RFI of Extract A including 0.4% Hydrolyzed Yeast	Non-treated	100	100	100			100.0	0	—	0.0	—
	40000	98	87	101			95.6	6.0	N.D.	4.4	Negative
	33333	99	99	110			102.7	18.8	N.D.	2.7	Negative
	27778	92	95	105			97.4	10.2	N.D.	2.6	Negative
	23148	90	101	109			99.9	19.8	N.D.	0.1	Negative
	19290	89	109	103			100.4	8.2	N.D.	0.4	Negative
	16075	92	109	115			105.5	13.3	N.D.	5.5	Negative
	13396	89	102	106			99.1	6.5	N.D.	0.9	Negative

	11163	95	100	110			101.7	14.0	N.D.	1.7	Negative
										5.5	–
Cell viability of DNCB (%)	0(DMSO)	97.2	96.1	95.7			96.3	0.78			
	4	96.7	96.9	95.9			96.5	0.53			
Cell viability of Extract A including 0.4% Hydrolyzed Yeast) (%)	Non-treated	92.9	95.1	97.4			96.2	0.91			
	40000	96.9	96.6	95.2			96.4	1.18			
	33333	97.8	95.7	95.8			96.8	0.76			
	27778	97.6	96.6	96.1			96.8	0.64			
	23148	97.5	96.3	96.5			96.7	0.74			
	19290	97.5	96.4	96.1			96.8	0.35			
	16075	97.1	96.4	96.8			96.0	0.51			
	13396	96.1	95.4	96.4			96.2	1.04			
	11163	95	96.5	97			96.6	0.58			

N.D.: Because the first three results were consistent, the calculations of the 95% confidence interval were not conducted.

conc.: concentration

The SH test which detects changes of cell-surface thiols in chemical-treated THP-1 cells was conducted(N=3). As a result, the test sample (Extract A including 0.4% Hydrolyzed Yeast) did not induced change of cell-surface thiols and was judged as Negative in SH test.

(References)

- ✓ Suzuki M, Hirota M, Hagino S, Itagaki H, Aiba S. 2009. Evaluation of changes of cell-surface thiols as a new biomarker for in vitro sensitization test. Toxicol. In Vitro 23: 687–696.
- ✓ Imai N, Takeyoshi M, Aizawa S, Tsurumaki M, Kurosawa M, Toyoda A, Sugiyama M, Kasahara K, Ogata S, Omori T, Hirota M., 2021. Improved performance

of the SH test as an in vitro skin sensitization test with a new predictive model and decision tree. J Appl Toxicol., 42,1029-1043. doi: 10.1002/jat.4275.

Appendix 2. Assay procedure and result of KeratinoSens™

Procedure of KeratinoSens™ assay

The assays had been performed as described in OECD TG442D(OECD, 2022). Briefly, KeratinoSens™ cells were grown for 24 hours in 96-well plates and then the medium was then replaced with medium containing the test chemical. Each compound was tested at 12 binary dilutions in the range from 0.98 to 2000 μM. Cells were incubated for 48 hours with test chemical, and then luciferase activity and cytotoxicity (MTT assay; Mossmann, 1983) were determined. The gene induction was compared with that of dimethyl sulfoxide controls, and the wells with statistically significant induction over the threshold of 1.5 (i.e., 50% enhanced gene activity) were determined. The EC1.5 values (concentration in μM for induction above these thresholds) were determined by linear extrapolation as described in the OECD guideline (OECD, 2022). In the prediction model, chemicals with significant gene induction (>1.5-fold) at a concentration below 1000 μM and at a concentration at which the cells retain 70% viability in at least two of three repetitions are rated positive.

Results of KeratinoSens™ assay (Test period; 2017/9- 2017/10)

1st Run	Test sample		Extract A including 0.4% Hydrolyzed Yeast											
	Conc.		0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	1000	2000
Luciferase activity	Rep 1-1		465835	479805	485470	474026	533220	524681	510747	537105	464195	447299	404850	338890
	Rep 1-2		408748	453313	473854	470891	456881	426578	506809	470122	474080	471229	444117	366705
	Rep 1-3		543598	462239	502867	487961	575070	493201	495919	470629	494636	457550	484406	377559
	Test sample		Solvent control						Cinnamic aldehyde(Positive control)					Blank
	Conc.		0	0	0	0	0	0	4	*8*	*16*	*32*	*64*	
Luciferase activity	Rep 1-1-		366468	440181	397624	436676	378948	441170	482898	546089	621118	868051	1263346	134
	Rep 1-2		380680	431330	317560	342230	302891	396488	421295	472183	537224	810118	1141488	162
	Rep 1-3		416230	370020	430064	420541	424850	439168	498214	531858	571015	910249	1741852	210

Average of solvent control		Rep 1-1	410178	Rep 1-2	361863	Rep 1-3	416812	-					
Test sample		Extract A including 0.4% Hydrolyzed Yeast											
Conc		0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	1000	2000
Gene Induction Rep 1-1		1.136	1.170	1.184	1.156	1.300	1.279	1.245	1.310	1.132	1.091	0.987	0.826
Rep 1-2		1.130	1.253	1.310	1.301	1.263	1.179	1.401	1.299	1.310	1.302	1.227	1.013
Rep 1-3		1.304	1.109	1.207	1.171	1.380	1.183	1.190	1.129	1.187	1.098	1.162	0.906
Average Gene Induction		1.190	1.177	1.233	1.209	1.314	1.214	1.279	1.246	1.210	1.164	1.126	0.915
Test sample		Solvent control						Cinnamic aldehyde(Positive control)					
Conc.		0	0	0	0	0	0	4	8	16	32	64	EC 1.5
Fold increase Rep 1-1		0.893	1.073	0.969	1.065	0.924	1.076	1.177	1.331	1.514	2.117	3.081	
Rep 1-2		1.052	1.192	0.878	0.946	0.837	1.096	1.164	1.305	1.485	2.239	3.155	
Rep 1-3		0.999	0.888	1.032	1.009	1.019	1.054	1.195	1.276	1.370	2.184	4.181	
Average Gene Induction		0.981	1.051	0.960	1.006	0.927	1.075	1.179	1.304	1.456	2.180	3.472	17.0
Test sample		Extract A including 0.4% Hydrolyzed Yeast											
Conc.		0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	1000	2000
Cell Viability(%)		103.9	104.2	98.2	101.6	100.0	104.8	102.6	105.0	101.9	105.8	103.2	92.7
Test sample		Solvent control						Cinnamic aldehyde(Positive control)					
Conc.		0	0	0	0	0	0	4	8	16	32	64	
Cell Viability(%)		101.1	104.3	96.8	98.8	93.0	105.9	99.8	100.6	105.8	104.0	96.1	

2nd Run	Test sample		Extract A including 0.4% Hydrolyzed Yeast											
	Conc.		0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	1000	2000
Luciferase activity	Rep 2-1		748034	603111	635980	679794	635631	584608	624170	604015	643559	605448	597333	566315
	Rep 2-2		678619	630669	669736	606006	656665	632206	638654	678921	652403	722850	683591	620899
	Rep 2-3		706911	695538	677229	671950	702385	968117	585700	632758	672844	740316	632015	605225
	Test sample		Solvent control						Cinnamic aldehyde(Positive control)					Blank
	Conc.		0	0	0	0	0	0	4	*8*	*16*	*32*	*64*	
Luciferase activity	Rep 2-1		495376	522561	635824	477675	466494	506619	658251	653756	915515	1538783	3358939	434
	Rep 2-2		533649	529814	591501	547156	502260	540834	954508	714964	1062796	1712083	5050863	530
	Rep 2-3		563301	624275	626700	578073	689397	610103	753691	829790	1093025	1586810	3848971	437
Average of solvent control			Rep 1-1	410178	Rep 1-2	310846	Rep 1-3	615308	-					
Test sample			Extract A including 0.4% Hydrolyzed Yeast											
	Conc.		0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	1000	2000
Gene Induction	Rep 2-1		1.446	1.166	1.229	1.314	1.229	1.130	1.206	1.167	1.244	1.170	1.155	1.095
	Rep 2-2		1.255	1.166	1.238	1.121	1.214	1.169	1.181	1.255	1.206	1.337	1.264	1.148
	Rep 2-3		1.149	1.130	1.101	1.092	1.142	1.574	0.952	1.028	1.094	1.203	1.027	0.984
	Average Gene Induction		1.283	1.154	1.190	1.176	1.195	1.291	1.113	1.150	1.181	1.237	1.149	1.075
Test sample			Solvent control						Cinnamic aldehyde(Positive control)					
	Conc.		0	0	0	0	0	0	4	*8*	*16*	*32*	*64*	EC 1.5
Fold increase	Rep 2-1		0.957	1.010	1.229	0.923	0.901	0.979	1.272	1.264	1.770	2.976	6.496	
	Rep 2-2		0.987	0.980	1.094	1.012	0.929	1.000	1.766	1.322	1.966	3.168	9.347	
	Rep 2-3		0.915	1.015	1.019	0.939	1.120	0.992	1.225	1.349	1.777	2.580	6.259	

Average Gene Induction		0.953	1.001	1.114	0.958	0.9834	0.990	1.421	1.312	1.838	2.908	7.368	10.9
Test sample		Extract A including 0.4% Hydrolyzed Yeast											
Conc.		0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	1000	2000
Cell Viability(%)		106.6	86.2	107.3	92.0	89.0	94.8	102.3	85.3	103.2	97.6	84.8	83.6
Test sample		Solvent control						Cinnamic aldehyde(Positive control)					
Conc.		0	0	0	0	0	0	4	8	16	32	64	
Cell Viability(%)		111.8	79.7	82.5	108.5	105.8	111.6	113.9	110.3	113.1	112.9	95.2	

conc.: concentration

The KeratinoSens™ assay was conducted. As a result, the Average Gene Inductions of the test sample (Extract A including 0.4% Hydrolyzed Yeast) were under 1.5 at all of concentrations in independent two runs. The test sample (Extract A including 0.4% Hydrolyzed Yeast) was judged as Negative in KeratinoSens™ and EC1.5 was > 2000μM.

(References)

- ✓ OECD (2022). OECD Guideline for testing chemicals, Section 4: Health Effects. Guideline no. 442D. In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method. Paris: OECD Publishing.
- ✓ Mossmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65, 55–63.

Appendix 3. Assay procedure and result of human cell line activation test(h-CLAT)

The assays had been performed as described in OECD TG442E (OECD, 2023). The test dose was determined based on the test concentration providing a cell viability of 75% (CV75) in the cytotoxicity test. THP-1 cells were plated at 1 × 10^6 cells/mL in a 24-well plate and treated for 24 hours with test chemicals at eight 1.2-fold serial dilutions starting at 1.2 × the CV75. Cells were washed and FcR was blocked. Staining was done with fluorescein isothiocyanate (FITC)-conjugated antihuman CD86 antibody or FITC-conjugated anti-human CD54 antibody at 4 °C for 30 minutes. FITC-labeled mouse IgG1 was used as an isotype control. The cells were washed, and expression of cell surface antigens was analyzed by flow cytometry. The relative fluorescence intensity, calculated according to below formula, was used as an indicator of CD86 and CD54 expression.

Relative fluorescence intensity (RFI, %) = (MFI of chemical–treated cells–MFI of chemical–treated isotype control cells)/
(MFI of vehicle control cells –MFI of vehicle isotype control cells) ×100

The thresholds for CD86 and CD54 expression (EC150 for CD86 and EC200 for CD54) were determined as described previously (Nukada *et al.*, 2012). A minimum induction threshold (MIT) was determined as the smaller of either EC150 or EC200.

Result of h-CLAT assay (Test period; 2017/9/25- 2017/9/28)

1st Run

Exposure date	2017/9/25		MFI (Geo Mean)			corrected MFI		RFI (CD86)		RFI (CD54)		viability		
Analysis date	2017/9/26							Top control		Top control				
Sample	conc. (μg/mL)	Ratio	CD86	CD54	isotype	CD86	CD54	vs medium control	vs solvent control	vs medium control	vs solvent control	CD86	CD54	IgG
control			4.73	3.42	2.37	2.36	1.05	100		100		97.95	98.16	98.17
DMSO	0.20%		4.27	3.68	2.33	1.94	1.35	82	100	128	100	98.19	98.41	98

DNCB	4.0		10.44	10.13	2.42	8.02	7.71		413		571	82.02	82.26	82.4
RPMI1640			4.5	3.45	2.43	2.07	1.02	87	100	97	100	98.28	98.16	97.97
Extract A including 0.4% Hydrolyzed Yeast	1395.4	CV75/1.2^6	4.19	3.49	2.42	1.77	1.07		85		104	98.36	97.97	98.36
	1674.5	CV75/1.2^5	4.41	3.51	2.37	2.04	1.14		98		111	98.03	97.41	97.83
	2009.4	CV75/1.2^4	4	3.42	2.44	1.56	0.98		75		96	97.93	98.57	98.1
	2411.3	CV75/1.2^3	4.45	3.6	2.5	1.95	1.1		94		107	98.11	98.06	97.82
	2893.5	CV75/1.2^2	4.45	3.75	2.54	1.91	1.21		92		118	97.97	98.19	97.81
	3472.2	CV75/1.2	4.26	3.67	2.49	1.77	1.18		85		115	97.43	98.07	97.71
	4166.7	CV75	4	3.8	2.48	1.52	1.32		73		129	98.04	97.82	96.59
	5000.0	CV75 * 1.2	4.19	3.79	2.51	1.68	1.28		81		125	98.31	97.89	97.65

2nd Run

Exposure date Analysis date	2017/9/27 2017/9/28		MFI (Geo Mean)			corrected MFI		RFI (CD86) Top control		RFI (CD54) Top control		viability		
Sample	conc. (μg/mL)	Ratio	CD86	CD54	isotype	CD86	CD54	vs medium control	vs solvent control	vs medium control	vs solvent control	CD86	CD54	IgG
control			4.23	3.56	2.61	1.62	0.95	100		100		98.24	98.38	98.56
DMSO	0.20%		4.47	3.71	2.6	1.87	1.11	115	100	116	100	98.68	98.77	98.58
DNCB	4.0		8.12	6.79	2.49	5.63	4.3		301		387	91.25	90.99	90.91
RPMI1640			4.56	3.69	2.84	1.72	0.85	106	100	89	100	98.31	98.47	98.25
	1395.4	CV75/1.2^6	4.36	3.61	2.66	1.7	0.95		98		111	98.55	98.55	98.63

Extract A including 0.4% Hydrolyzed Yeast	1674.5	CV75/1.2^5	4.24	3.79	2.67	1.57	1.12		91		131	98.54	98.34	98.38
	2009.4	CV75/1.2^4	4.46	3.87	2.79	1.67	1.08		97		127	98.46	98.41	98.61
	2411.3	CV75/1.2^3	4.47	3.7	2.73	1.74	0.97		101		114	98.62	98.49	98.35
	2893.5	CV75/1.2^2	4.26	3.85	2.8	1.46	1.05		84		123	98.53	98.64	98.17
	3472.2	CV75/1.2	3.88	4.09	2.73	1.15	1.36		66		160	98.45	98.4	98.39
	4166.7	CV75	4.15	3.92	2.83	1.32	1.09		76		128	98.19	98.5	98.42
	5000.0	CV75 * 1.2	4.54	4.09	2.8	1.74	1.29		101		151	98.57	98.31	98.48

conc.: concentration

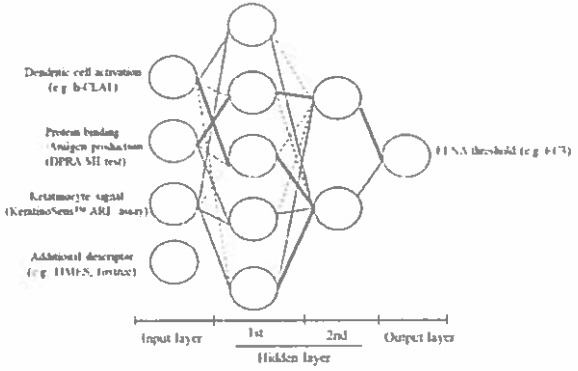
The h-CLAT assay was conducted. As a result, the RFI values of the test sample (Extract A including 0.4% Hydrolyzed Yeast) were under criteria of CD86 and CD54 expressions at all of concentrations in independent two runs. The test sample (Extract A including 0.4% Hydrolyzed Yeast) was judged as Negative in h-CLAT and EC150 for CD86, EC200 for CD54 and MIT were >5000μg/mL. And, CV75 was also >5000 μg/mL because Extract A including 0.4% was not induced cytotoxicity.

(References)

- ✓ OECD (2023). OECD Guideline for testing chemicals, Section 4: Health Effects. Guideline no. 442E. In Vitro Skin Sensitisation: In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation. OECD Publishing.
- ✓ Nukada Y, Ashikaga T, Miyazawa M, Hirota M, Sakaguchi H, Sasa H, Nishiyama N. (2012). Predition of skin sensitization potency of chemicals by human Cell Line Activation Test (h-CLAT) and an attempt at classifying skin sensitization potency. Toxicol. In Vitro 26: 1150–1160.

Appendix 4. LLNA EC3 calculation using artificial neural network model (2017.11.13)

In OECD guidance 256(OECD, 2016), the artificial neural network (ANN) model was introduced as defined approaches for skin sensitization. In the Adverse outcome pathway (AOP) of skin sensitization, molecular initiating event as key event 1, activation of keratinocyte signal as key event 2(KE2) and activation of dendritic cells as key event 3 (KE3) were important factors. In ANN model, the descriptors derided from the assays for KE1, 2 and 3 were set as inputs and the published LLNA EC3 values, which were threshold concentrations in murine local lymph node assay (LLNA; OECD TG429) (OECD, 2010) about 100 chemicals were set as output. The conceptual diagram was shown below (Hirota *et al.*, 2015). The prediction of LLNA EC3 value by ANN model was used in the skin sensitization evaluation of Isothiazolinones published by US EPA (US Environmental Protection Agency, 2020).



In this evaluation of Extract A including 0.4% Hydrolyzed Yeast, The ANN model consisted of descriptors (N=117) derived from h-CLAT, SH test and KeratinoSens™ introduced by OECD GD256 was used for LLNA EC3 prediction. the predicted EC values was calculated as 62.17% by using below descriptors in this ANN model.

	h-CLAT	SH test	KeratinoSens™
	Minimum of MIT and CV75 (μg/mL)	MAC values	EC1.5(μM)
Descriptors	5000	5.5	2000

Predicted EC3 value by ANN model (%)
62.17

(References)

- ✓ OECD (2016). Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used Within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation, OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 256.
- ✓ OECD (2010). OECD Guideline for testing chemicals, Section 4: Health Effects. Guideline no. 429. In Vitro Skin Sensitisation: Local Lymph Node Assay. OECD Publishing.
- ✓ Hirota M, Fukui S, Okamoto K, Kurotani S, Imai N, Fujishiro M, Kyotani D, Kato Y, Kasahara T, Fujita M, Toyoda A, Sekiya D, Watanabe S, Seto H, Takenouchi O, Ashikaga T, Miyazawa M. (2015). Evaluation of combinations of in vitro sensitization test descriptors for the artificial neural network-based risk assessment model of skin sensitization. Journal of Applied Toxicology, 35, 1333–1347.
- ✓ US Environmental Protection Agency, Office of Pesticide Programs, Antimicrobials Division (2020). Hazard Characterization of Isothiazolinones in Support of FIFRA Registration Review.

[redacted]

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

Test article
0.01% Extract A
(0.00004% Hydrolyzed Yeast)

Sponsor

[redacted]

Sponsor Representatives

[redacted]

Clinical Testing Facility

[redacted]

Sponsor Code: [redacted]
Panel No.:
Entry No.:

Date of Final Report

06.24.22

[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

[redacted]
Laboratory Director
Study Director

24 June 2022
Date

[redacted]
Scientific Director
Principal Investigator

6/24/22
Date

[redacted]
Board-Certified Dermatologist
Medical Investigator

6/20/22
Date

[redacted]

QUALITY ASSURANCE STATEMENT

This study ([redacted] Panel No.: [redacted]; [redacted] Entry No.: [redacted]) was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in 21 CFR Part 50 (Protection of Human Subjects – Informed Consent) and the Standard Operating Procedures of [redacted]

For purposes of this clinical study:

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

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

[redacted]
Manager, Quality Assurance

24 June 2022
Date

[redacted]

[redacted] Panel No.:
Entry No.: [redacted]

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[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

1.0 OBJECTIVE

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

2.0 SPONSOR

[redacted]

2.1 Sponsor Representatives

[redacted]

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

[redacted]

4.0 CLINICAL INVESTIGATORS

Study Director:
Principal Investigator:
Medical Investigator:

[redacted]

5.0 STUDY DATES

Study initiation: April 28, 2022

Final evaluation: June 3, 2022

[redacted]

6.0 ETHICS

6.1 Ethical Conduct of the Study

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

6.2 Subject Information and Consent

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

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on March 10, 2022 and identified as follows:

[redacted] Entry No.	Test Article ID	Description
[redacted]	[redacted]	Light Yellow Liquid*

*The test article was volatilized at least 30 minutes, but less than 90 minutes, on the patch prior to application to the skin.

8.0 TEST SUBJECTS

At least 50 male and female subjects ranging in age from 18 to 79 years were to be empanelled for this test. Subject demographics are listed in Table 1.

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

[redacted]

9.0 TEST PROCEDURE

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

9.1 Induction Phase

A sufficient amount of the test article (approximately 0.2 mL) was placed onto a Parke-Davis Readi-Bandage® occlusive patch (approximately 0.05 mL/cm^2 of test material), which was applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

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

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

9.2 Challenge Phase

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

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

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

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

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

9.0 TEST PROCEDURE (CONT'D)

9.3 Data Interpretation

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

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

10.0 RESULTS AND DISCUSSION

(See Table 2 for Individual Scores)

A total of 55 subjects (4 males and 51 females ranging in age from 23 to 78 years) were empanelled for the test procedure. Fifty-one (51/55) subjects satisfactorily completed the test procedure on Test Article: 21-0440-00. Four (4/55) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued subject data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

Induction Phase Summary

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

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

11.0 CONCLUSIONS

Under the conditions of a repeated insult (occlusive) patch test procedure conducted in 51 subjects, Test Article: [redacted] was "Dermatologist-Tested" and was not associated with skin irritation or allergic contact dermatitis in human subjects.

[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 1

SUBJECT DEMOGRAPHICS

Test Article: [redacted]

Subject No.	Initials	Age	Sex	Race	Subject No.	Initials	Age	Sex	Race
1	[redacted]	62	F	BA	29	[redacted]	65	F	WH
2		68	F	HS	30		67	F	WH
3		68	F	WH	31		53	F	HS
4		75	F	WH	32		64	F	WH
5		61	F	WH	33		65	F	WH
6		57	F	WH	34		49	F	WH
7		54	F	HS	35		56	F	WH
8		42	F	HS	36		38	F	HS
9		56	F	HS	37		39	F	BH
10		23	F	WH	38		39	F	HS
11		67	M	BA	39		28	F	HS
12		60	M	BA	40		61	F	WH
13		31	F	WH	41		26	F	WH
14		70	F	HS	42		57	F	WH
15		64	F	WH	43		58	F	WH
16		56	F	AS	44		48	F	WH
17		61	F	WH	45		35	F	WH
18		61	M	WH	46		65	F	WH
19		71	F	WH	47		42	F	BA
20		78	F	HS	48		64	F	BA
21		40	F	HS	49		75	F	WH
22		36	F	AS	50		55	F	WH
23		56	F	WH	51		68	M	WH
24		61	F	WH	52		53	F	HS
25		70	F	WH	53		41	F	HS
26		39	F	WH	54		43	F	WH
27		51	F	WH	55		46	F	HS
28		61	F	WH					

AS = Asian
BA = Black
BH = Black (Hispanic)
HS = Hispanic/Latino
WH = White

Shaded area = Discontinued subject

[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 2

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

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

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

[redacted]

[redacted] Panel No.: [redacted]
Entry No.: [redacted]

TABLE 2 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

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

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

[redacted]

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: June 22, 2023

SUBJECT: Lipomyces Oil and Lipomyces Lipid Bodies

Xylome. 2023. Response to request for dermal data on yeast-derived ingredients (Lipomyces Oil and Lipomyces Lipid Bodies).

Xylome
Renewing tomorrow today

Expert Panel for Cosmetic Ingredient Safety, Request:

Response Prepared by the Scientist at Xylome 06-27-2023.

Summary of Additional Information: YOIL-Cream® and YOIL®

RE: Email Correspondence dated 6-27-2023 from Carol Eisenmann <eisenmannc@personalcarecouncil.org>

Additional Information as Requested:

During the June 2023 meeting, the Expert Panel for Cosmetic Ingredient Safety voted to issue an insufficient data announcement for this report. They requested the following additional information:

1. **Confirmatory dermal sensitization data and data on food use/GRAS status on the yeast species used to derive these ingredients for all ingredients in which this is absent; in lieu of food use/GRAS status data, 28-day dermal toxicity data may be considered.**

Summary of Response:

Xylome has completed technical information meetings with the FDA with respect to our upcoming GRAS notifications for YOIL-Cream ® [INCI Lipomyces lipid bodies] and YOIL® [INCI: Lipomyces Oil] for use in food. As part of the FDA GRAS notification, published toxicology studies are available and a summary has been previously submitted to the INCI expert panel during the INCI application process.

Lipomyces starkeyi is one of the few yeasts that have had the same name since its discovery in 1946 by Prof. Richard Starkey at Rutgers University[1]. Based on the 74-year safe lab history of *Lipomyces starkeyi*, several published assertions have been made for *Lipomyces starkeyi* as a GRAS yeast [2] [3]. Additionally, Xylome's MD medical safety advisor conducted searches of the major medical databases and found no reports of opportunistic infections attributable to *Lipomyces.*

There are no *Lipomyces* yeasts in the products. Additionally, the Xylome production yeast contains no foreign genes and no antibiotic resistance traits as determined by full genomic sequencing and PCR. An evaluation for the absence of any yeast pathogenic traits has been presented to the FDA.

Finally, Xylome conducted a nominal 4-week dermal exposure evaluation with volunteers using YOIL-Cream®, which contains YOIL® within the lipid body cream. *Lipomyces* lipid bodies from Xylome naturally contain 87% *Lipomyces* oil per lipid body. To consider dermal and topical safety, repeated dermal applications were made by human volunteers whose evaluation was done with a total of 579 daily exposures to skin, as a face cream and hand cream for an average period of 27.6 days per volunteer. The conclusion is that the lipid body cream, (Yoil-Cream®) and the Lipomyces oil (YOIL®) within the lipid body cream were well tolerated, and benign in the 579-exposures. This evaluation, 44% of the volunteers participated for more than 30 days of repeated skin applications.

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[1] Starkey RL. Lipid production by a soil yeast. J. Bacteriol. 1946; 5:33-50.
[2] Ragavan, M.L.; Das, N. Optimization of exopolysaccharide production by probiotic yeast Lipomyces starkeyi VIT-MN03 using response surface methodology and its applications. Ann. Microbiol. **2019**, 69, 515–530.
[3] Angel de la Cruz Pech-Canul 1,* , David Ortega 2, Antonio García-Triana 3 , Napoleón González-Silva 4 and Rosa Lidia Solis-Oviedo 5,* A Brief Review of Edible Coating Materials for theMicroencapsulation of Probiotics, Coatings 2020, 10, 197; doi:10.3390/coatings10030197

Personal Care Products Council
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Memorandum

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

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

DATE: July 10, 2023

SUBJECT: Yeast-Derived Ingredients

Active Concepts. 2020. Bacterial reverse mutation test (BiEau® Actif Red Algae contains 49% Phaffia Rhodozyma Extract).

Active Concepts. 2020. Dermal and ocular irritation tests (BiEau® Actif Red Algae contains 49% Phaffia Rhodozyma Extract).

Active Concepts. 2020. OECD TG 442C: In chemico skin sensitization (BiEau® Actif Red Algae contains 49% Phaffia Rhodozyma Extract).

Active Concepts. 2020. OECD TG 442D: In vitro skin sensitization (BiEau® Actif Red Algae contains 49% Phaffia Rhodozyma Extract).

Active Concepts. 2020. Phototoxicity assay analysis (BiEau® Actif Red Algae contains 49% Phaffia Rhodozyma Extract).

Active Concepts. 2020. Cellular viability assay analysis (BiEau® Actif Red Algae contains 49% Phaffia Rhodozyma Extract).

Active Concepts. 2022. Dermal and ocular irritation tests AC NanoVesicular System PS (contains 3% Saccharomyces Cervisiae Extract).

Active Concepts. 2021. Bacterial reverse mutation test AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate).

Active Concepts. 2017. Dermal and ocular irritation tests AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate).

Active Concepts. 2021. OECD TG 442C: In chemical skin sensitization AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate).

Active Concepts. 2021. OECD TG 442D: In vitro skin sensitization AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate).

Active Concepts. 2021. Phototoxicity assay analysis AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate).

Active Concepts. 2022. Dermal and ocular irritation tests AC Dermal Respiratory Factor Powder (contains 98% Saccharomyces Lysate Extract).

Active Concepts. 2013. Cellular viability assay analysis AC Dermal Respiratory Factor (contains 25% Saccharomyces Lysate Extract).

AMA Laboratories, Inc. 2002. 50 Human subject repeat insult open patch test skin irritation/sensitization evaluation (open patch) (C3863 AC Dermal Respiratory Factor contains 25% Saccharomyces Lysate Extract).

Active Concepts. 2017. Dermal and ocular irritation tests AC Liposome Dermal Respiratory Factor (contains 10% Saccharomyces Lysate Extract).

Bacterial Reverse Mutation Test

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Test Article: BiEau® Actif Red Algae
Code Number: 16909
CAS #: 999999-99-4 & N/A

contains 49% Phaffia Rodozyma Extract

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: 5550

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 **BiEau® Actif Red Algae** would cause mutagenic changes in the average number of reveratants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2*uvr*A 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 WP2*uvr*A after Sport Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45^0C 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 37^0C. 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 WP2*uvr*A. 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 *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A 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*uvr*A, 10-60.

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Bacterial Reverse Mutation Test

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*uvr*A 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 *uvr*B gene and the deletion in the *uvr*A 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*uvr*A) 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.

Tester strain	Mutations/Genotypic Relevance
TA98	hisD3052, Dgal chlD bio *uvr*B *rfa* pKM101
TA100	hisG46, Dgal chlD BIO *uvr*B *rfa* pKM101
TA1537	hisC3076, *rfa*, Dgal chlD bio *uvrB*
TA 1535	hisG46, Dgal chlD bio *uvr*B *rfa*
WP2*uvr*A	trpE, *uvr*A

rfa	=	causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
uvrB	=	deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
*uvr*A	=	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 reveratants 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 $2x10^9$/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 µl of culture for each strain and 100 µl of testing solution or vehicle without test material. A 500 µl 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 50µl 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 reveratnts was determined. The mean numbers of revertants of the test plates were compared to the mean number of reverants 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 *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A 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, WP2*uvr*A, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ 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-toxicdose 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 x 10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2 x 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*uvr*A 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 *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A 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*uvr*A, 10-60.

VII. Conclusion

All criteria for a valid study were mete 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*uvr*A.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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Bacterial Reverse Mutation Test

Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	*TA98*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	22	25	24
	1500	29	20	25
	500	23	27	25
	150	22	39	31
	50	17	14	16
	15	19	26	23
	5.0	10	14	12
	1.5	32	39	36
Test Solution w/o S9	5000	21	28	25
	1500	25	27	26
	500	14	16	15
	150	17	28	23
	50	24	32	28
	15	27	19	23
	5.0	25	17	21
	1.5	12	13	13
DI Water w/S9		17	10	14
DI Water w/o S9		58	52	55
2-aminoanthracen w/ S9		144	162	153
2-nitrofluorene w/o S9		120	148	134
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

Bacterial Reverse Mutation Test

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	Concentration µg per Plate	***TA100***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	162	185	174
	1500	155	179	167
	500	206	217	212
	150	172	181	177
	50	215	200	208
	15	144	162	153
	5.0	114	147	131
	1.5	145	161	153
Test Solution w/o S9	5000	158	172	165
	1500	145	169	157
	500	177	215	196
	150	107	120	114
	50	219	242	231
	15	135	127	131
	5.0	120	127	124
	1.5	129	120	125
DI Water w/S9		116	120	118
DI Water w/o S9		107	117	112
2-aminoanthracen w/ S9		618	622	620
Sodium azide w/o S9		559	632	596
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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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*TA1537*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	7	12	10
	1500	16	10	13
	500	22	29	26
	150	15	14	15
	50	19	12	16
	15	10	14	12
	5.0	29	24	27
	1.5	17	21	19
Test Solution w/o S9	5000	22	17	20
	1500	17	29	23
	500	11	17	14
	150	29	26	28
	50	18	34	26
	15	11	19	15
	5.0	10	22	16
	1.5	16	20	18
DI Water w/S9		10	12	11
DI Water w/o S9		28	23	26
2-aminoanthracen w/ S9		390	375	383
2-aminoacridine w/o S9		116	122	119
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**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

Bacterial Reverse Mutation Test

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	Concentration µg per Plate	***TA1535***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	11	21	16
	1500	8	12	10
	500	18	19	19
	150	10	14	12
	50	17	23	20
	15	10	8	9
	5.0	14	18	16
	1.5	12	29	21
Test Solution w/o S9	5000	12	27	20
	1500	18	14	16
	500	7	12	10
	150	19	12	16
	50	17	17	17
	15	14	12	13
	5.0	8	9	9
	1.5	16	15	16
DI Water w/S9		17	21	19
DI Water w/o S9		16	27	22
2-aminoanthracen w/ S9		145	138	142
Sodium azide w/o S9		722	714	718
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**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

Bacterial Reverse Mutation Test

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	Concentration µg per Plate	***WP2uvrA***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	9	17	13
	1500	28	26	27
	500	8	17	13
	150	10	16	13
	50	19	30	25
	15	22	21	22
	5.0	19	25	22
	1.5	27	24	26
Test Solution w/o S9	5000	22	28	25
	1500	34	37	36
	500	22	29	26
	150	26	24	25
	50	27	44	36
	15	40	36	38
	5.0	29	21	25
	1.5	14	13	14
DI Water w/S9		38	42	40
DI Water w/o S9		38	27	33
2-aminoanthracen w/ S9		188	161	175
Methylmethanesulfonate w/o S9		287	315	301
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

Dermal and Ocular Irritation Tests

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Tradename: BiEau® Actif Red Algae (contains 49% Phaffia Rhodozyma Extract)

Code: 16909

CAS #: 999999-99-4 & N/A

Test Request Form #: 5514

Lot #: N190604A

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 **BiEau® Actif Red Algae** 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% CO_2, 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-yl*)], 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.

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Dermal and Ocular Irritation Tests

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% CO_2 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 H_2O
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% CO_2 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% CO_2 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% CO_2, 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% CO_2, 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% CO_2, 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 (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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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 ≤ 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 ≤ 50% for EpiDerm™ or ≤ 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.

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Dermal and Ocular Irritation Tests

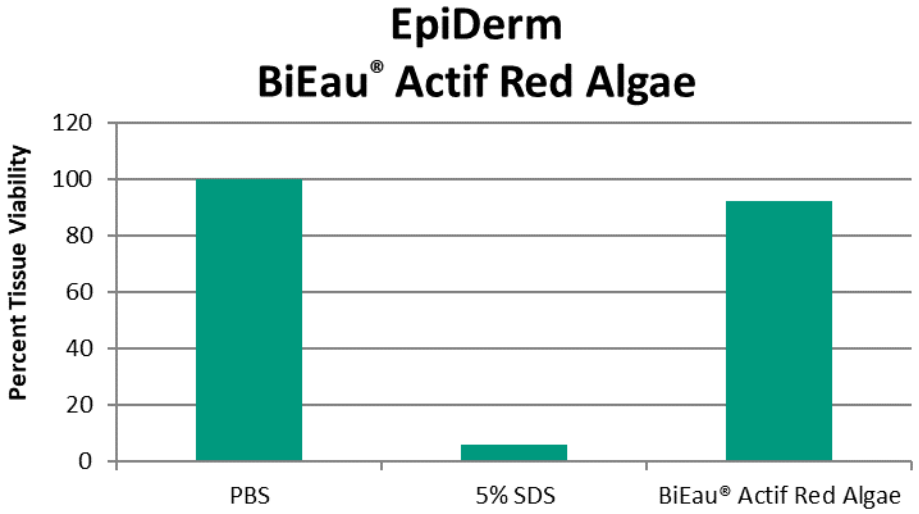

Figure 1: EpiDerm tissue viability

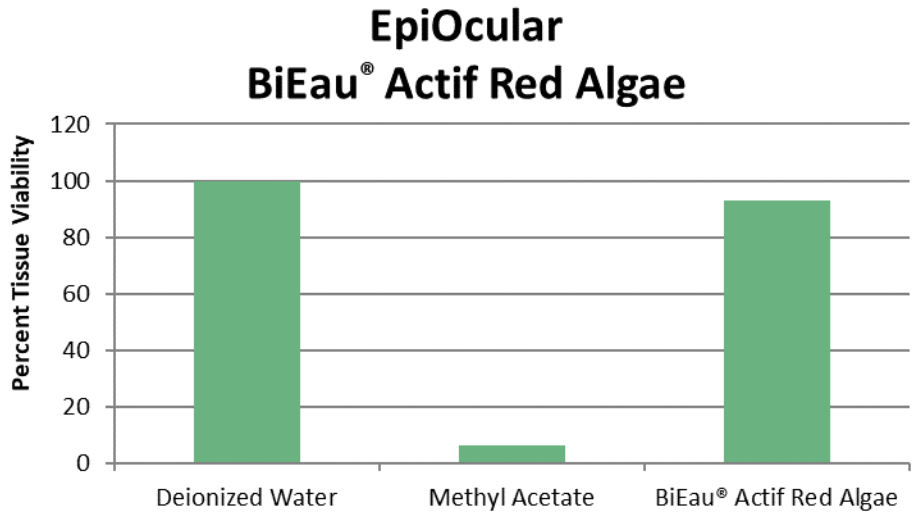

Figure 2: EpiOcular tissue viability

OECD TG 442C: *In Chemico* Skin Sensitization

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Tradename: BiEau® Actif Red Algae (contains 49% Phaffia Rhodozyma Extract)

Code: 16909

CAS #: 999999-99-4 & N/A

Test Request Form #: 5519

Lot #: N190604A

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 contact[1]. 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 dermatitis[2]. 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 **BiEau® Actif Red Algae** 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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OECD TG 442C: *In Chemico* Skin Sensitization

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-RFAA**C**AA-COOH); Lysine peptide (Ac-RFAA**K**AA-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* **BiEau® Actif Red Algae** in Acetonitrile

*For mixtures and multi-constituent substances of known composition such as **BiEau® Actif Red Algae**, 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
• 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)	• 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:

1. The following criteria must be met for a run to be considered valid:
   a. Standard calibration curve should have an $r^2 > 0.99$.
   b. 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.
   c. Mean peptide concentration of reference controls A should be 0.50±0.05mM and the coefficient of variable of the peptide peak areas for reference B and C in acetonitrile should be <15.0%.

2. The following criteria must be met for a test chemical's results to be considered valid:
   a. Maximum standard deviation should be <14.9 for percent cysteine depletion and <11.6 for percent lysine depletion.
   b. Mean peptide concentration of the three reference control C should be 0.50±0.05mM.

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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**
3.00	Minimal Reactivity	Non-sensitizer
3.03	Minimal Reactivity	Non-sensitizer
3.04	Minimal Reactivity	Non-sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.13	Minimal Reactivity	Non-sensitizer
3.10	Minimal Reactivity	Non-sensitizer
3.08	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:

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

Based on HPLC-UV analysis of **BiEau® Actif Red Algae (16909)** 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: BiEau® Actif Red Algae (contains 49% Phaffia Rhodozyma Extract)

Code: 16909

CAS #: 999999-99-4 & N/A

Test Request Form #: 5516

Lot #: N191024C

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 Chemicals[1]. 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 **BiEau® Actif Red Algae** 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

Materials

A. Incubation Conditions:	37°C at 5% CO_2 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 **BiEau® Actif Red Algae** 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% CO_2 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% CO_2. 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 IC_{50} and IC_{30} 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.

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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 EC1.5 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 Imax 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 EC1.5 determining concentration)
3. The $EC_{1.5}$ value is less than 1000 µM (or < 200 µg/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	32.08
DMSO	Non-Sensitizer	No Induction	243.24 µM	0.16
BiEau® Actif Red Algae	Non-Sensitizer	No Induction	> 1000 µM	0.27

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

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OECD TG 442D: *In Vitro* Skin Sensitization

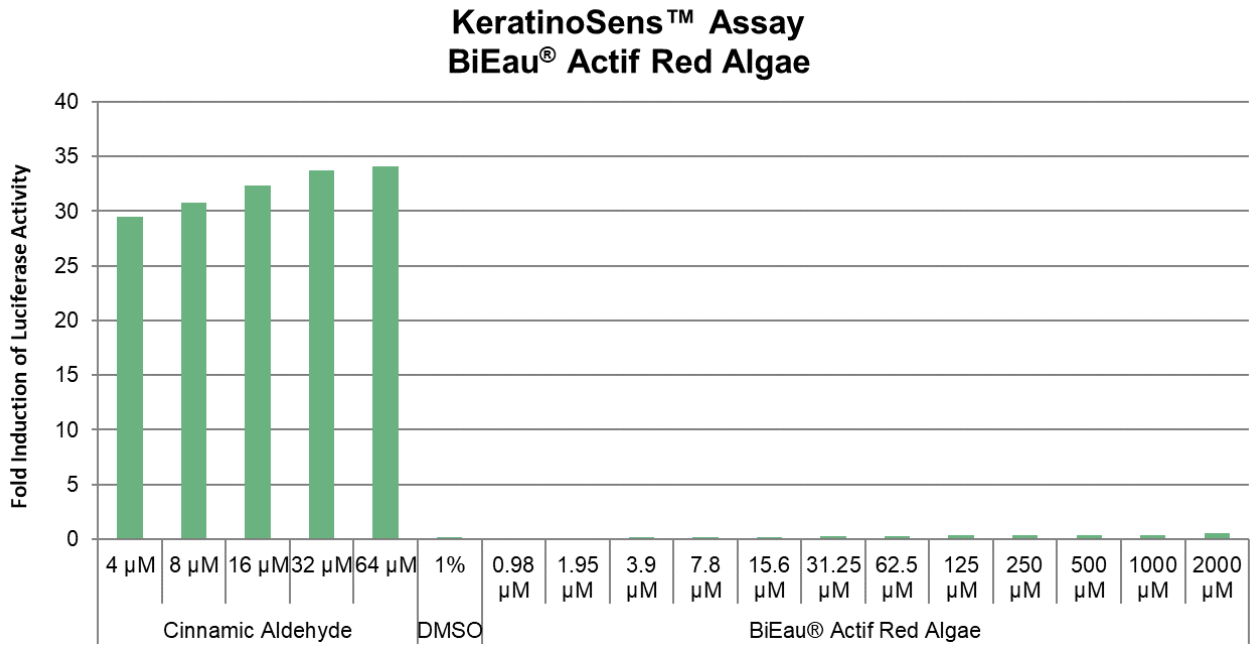

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **BiEau® Actif Red Algae (16909)** 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 **BiEau® Actif Red Algae** can be safely used in cosmetics and personal care products at typical use levels.

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Phototoxicity Assay Analysis

Tradename: BiEau® Actif Red Algae (contains 49% Phaffial Rhodozyma Extract)

Code: 16909

CAS #: 999999-99-4 & N/A

Test Request Form #: 5517

Lot #: N190604A

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 **BiEau® Actif Red Algae** 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% CO_2, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). 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

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_2O
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 chloropromazine, 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/cm^2 is reduced by 20% compared to the non-irradiated control tissues.

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Phototoxicity Assay Analysis

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% CO_2 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% CO_2, 95% RH).

C. Tissue Irradiation

Tissue inserts in their 6-well plates are UVA-irradiated for 60 minutes with 6 J/cm^2 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% CO_2, 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% CO_2, 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 (OD_{570}) 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%.

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Phototoxicity Assay Analysis

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.

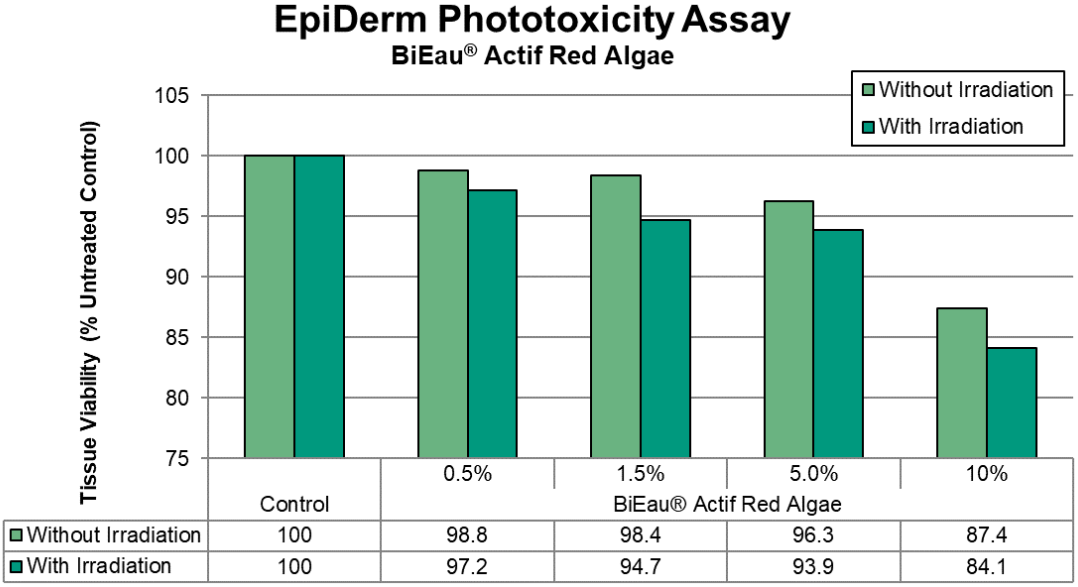
C. Test Validity

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 **BiEau® Actif Red Algae** is not a photoirritant when used at the suggested use levels of 1.0% -10.0%.



	Control	0.5%	1.5%	5.0%	10%
Without Irradiation	100	98.8	98.4	96.3	87.4
With Irradiation	100	97.2	94.7	93.9	84.1

Figure 1: EpiDerm Phototoxicity Graph

Cellular Viability Assay Analysis

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Tradename: BiEau® Actif Red Algae (contains 49% Phaffia Rhodozya Extract)

Code: 16909

CAS #: 999999-99-4 & N/A

Test Request Form #: 5234

Lot #: N190604A

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease in ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **BiEau® Actif Red Algae** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

Cellular Viability Assay Analysis

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Materials

A. Kit:	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D. Cell Line:	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers:	Basal Medium (Fibrolife; LM-0001), 500µg/mL Human Serum Albumins (Fibrolife; LS-1001), 0.6µM Linoleic Acid (Fibrolife; LS-1001), 0.6µg/mL (Fibrolife; LS-1001), 5ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002), 5mg/mL Epidermal Growth Factor (Fibrolife; LS-1003), 30pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003), 7.5mM L-Glutamine (Fibrolife; LS-1006), 1µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007), 50µg/mL Ascorbic Acid (Fibrolife; LS-1005), 5µg/mL Insulin (Fibrolife; LS-1004)
F. Culture Plate:	Falcon flat bottom 96-well tissue culture treated plates
G. Reagents:	PrestoBlue™ reagent (10X)
H. Other:	Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete serum-free media. A 10-fold serial dilution was performed resulting in **BiEau® Actif Red Algae** concentrations of 0.1% and 0.01% in complete serum-free media and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

Cellular Viability Assay Analysis

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Results

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

BiEau® Actif Red Algae did not exhibit negative effects on cell metabolism.

Cellular metabolism results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

$$Percent\ (\%)Change = \frac{MFU_{Control} - MFU_{Sample}}{MFU_{Control}} \times 100$$

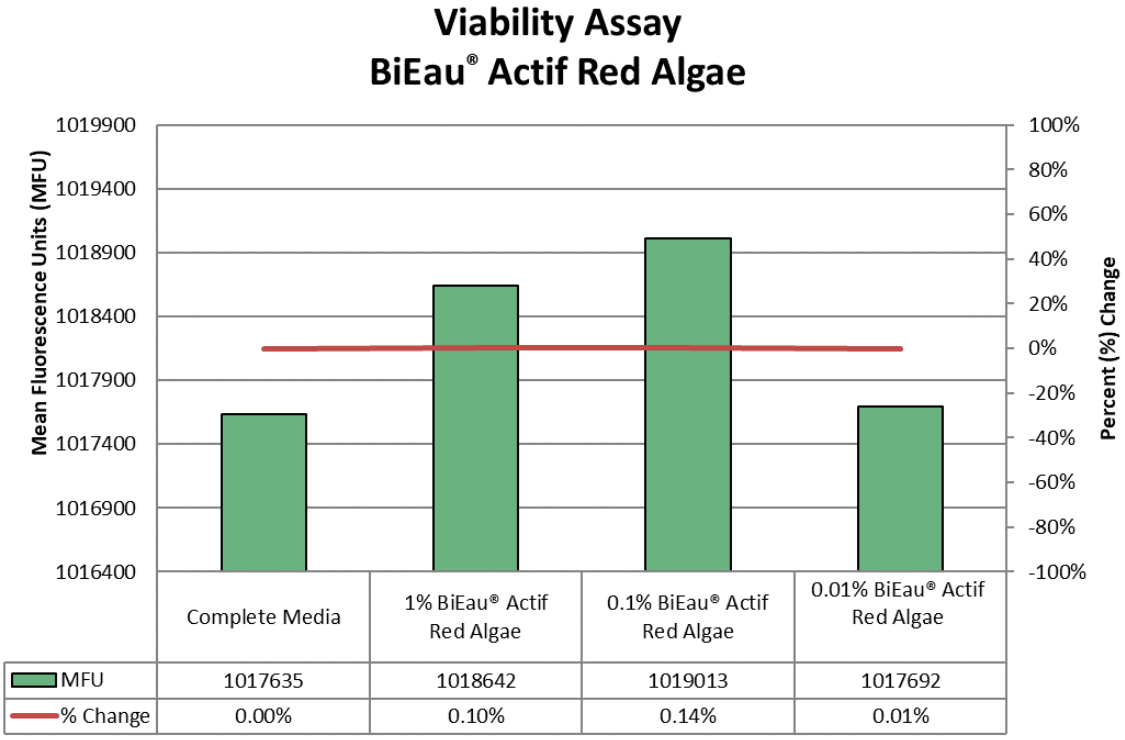

Figure 1: Cellular Metabolism of **BiEau™ Actif Red Algae -**treated fibroblasts

Discussion

In this study, **BiEau® Actif Red Algae (16909)** was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 0.1% and 0.01%, **BiEau® Actif Red Algae**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **BiEau® Actif Red Algae** is not cytotoxic.

Dermal and Ocular Irritation Tests

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Tradename: AC NanoVesicular System P3 (contains 3% Saccharomyces Cerevisiae Extract)

Code: 60051

CAS #: 8002-43-5 & 84604-16-0 & 9007-28-7 & 9004-61-9 & 59-02-9 & 92113-31-0

Test Request Form #: 9650

Lot #: 9126300

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Daniel Shill*

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 **AC NanoVesicular System P3** 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% CO_2, 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-yl*)], 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.

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Dermal and Ocular Irritation Tests

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% CO_2 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 H_2O
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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Dermal and Ocular Irritation Tests

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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% CO_2 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% CO_2 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% CO_2, 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% CO_2, 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% CO_2, 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 (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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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 ≤ 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 ≤ 50% for EpiDerm™ or ≤ 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.

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Dermal and Ocular Irritation Tests

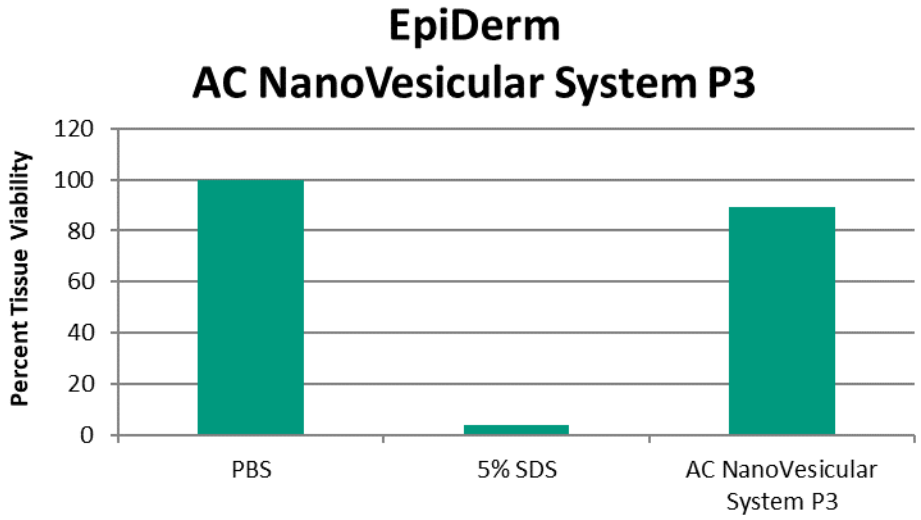

Figure 1: EpiDerm tissue viability

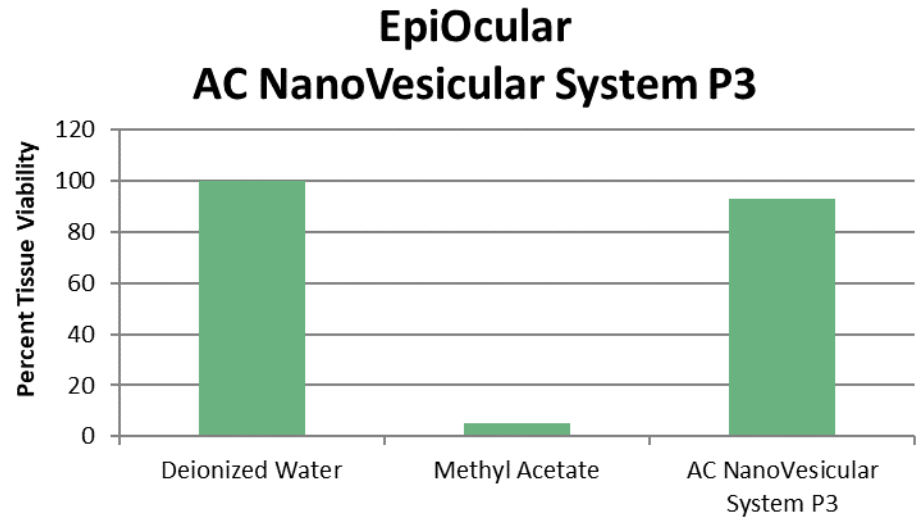

Figure 2: EpiOcular tissue viability

Bacterial Reverse Mutation Test

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Test Article: AC Dermal Respiratory Factor Advanced
Code Number: 20219
CAS #: 7732-18-5 & 8013-01-2
(contains 24.5% Saccharomyces Ferment Lysate Filtrate)

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: 7930

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 **AC Dermal Respiratory Factor Advanced** would cause mutagenic changes in the average number of reveratants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2*uvr*A 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 WP2*uvr*A after Sport Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45^0C 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 37^0C. 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 WP2*uvr*A. 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 *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A 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*uvr*A, 10-60.

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Bacterial Reverse Mutation Test

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*uvr*A 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 *uvr*B gene and the deletion in the *uvr*A 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*uvr*A) 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.

Tester strain	Mutations/Genotypic Relevance
TA98	hisD3052, Dgal chlD bio *uvr*B *rfa* pKM101
TA100	hisG46, Dgal chlD BIO *uvr*B *rfa* pKM101
TA1537	hisC3076, *rfa*, Dgal chlD bio *uvrB*
TA 1535	hisG46, Dgal chlD bio *uvr*B *rfa*
WP2*uvr*A	trpE, *uvr*A

rfa	=	causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
uvrB	=	deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
*uvr*A	=	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 reveratants 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 $2x10^9$/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 µl of culture for each strain and 100 µl of testing solution or vehicle without test material. A 500 µl 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 50µl 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 reveratnts was determined. The mean numbers of revertants of the test plates were compared to the mean number of reverants 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 *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A 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, WP2*uvr*A, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ 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-toxicdose 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 x 10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2 x 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*uvr*A 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 *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A 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*uvr*A, 10-60.

VII. Conclusion

All criteria for a valid study were mete 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*uvr*A.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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Bacterial Reverse Mutation Test

Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	*TA98*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	21	33	27
	1500	24	22	23
	500	20	21	21
	150	18	12	15
	50	17	17	17
	15	11	15	13
	5.0	22	26	24
	1.5	20	23	22
Test Solution w/o S9	5000	30	34	32
	1500	24	29	27
	500	18	19	19
	150	29	32	31
	50	20	20	20
	15	13	13	13
	5.0	22	26	24
	1.5	14	23	19
DI Water w/S9		11	18	14.5
DI Water w/o S9		49	52	51
2-aminoanthracen w/ S9		169	184	177
2-nitrofluorene w/o S9		166	159	163
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	145	123	134
	1500	136	116	126
	500	180	174	177
	150	112	150	131
	50	125	163	144
	15	133	164	149
	5.0	129	175	151
	1.5	120	140	130
Test Solution w/o S9	5000	139	155	147
	1500	132	149	141
	500	162	201	182
	150	144	160	152
	50	205	241	223
	15	162	114	138
	5.0	120	141	131
	1.5	118	107	113
DI Water w/S9		115	107	111
DI Water w/o S9		109	117	112
2-aminoanthracen w/ S9		610	661	636
Sodium azide w/o S9		574	631	603
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	13	15	14
	1500	18	25	22
	500	18	21	20
	150	21	15	18
	50	13	16	15
	15	12	10	11
	5.0	21	21	21
	1.5	18	17	18
Test Solution w/o S9	5000	13	14	14
	1500	10	21	16
	500	17	10	14
	150	19	12	16
	50	14	14	14
	15	15	18	17
	5.0	17	19	18
	1.5	27	12	20
DI Water w/S9		9	13	11
DI Water w/o S9		22	25	24
2-aminoanthracen w/ S9		389	362	376
2-aminoacridine w/o S9		125	116	121
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**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***TA1535***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	23	20	22
	1500	19	18	19
	500	21	15	18
	150	14	16	15
	50	17	26	22
	15	18	25	22
	5.0	12	17	15
	1.5	20	27	24
Test Solution w/o S9	5000	23	31	27
	1500	12	6	9
	500	14	20	17
	150	17	10	14
	50	8	19	14
	15	17	13	15
	5.0	21	17	19
	1.5	24	38	31
DI Water w/S9		23	16	20
DI Water w/o S9		12	10	11
2-aminoanthracen w/ S9		142	162	152
Sodium azide w/o S9		720	738	729
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**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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	Concentration µg per Plate	***WP2uvrA***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	11	13	12
	1500	15	22	19
	500	13	16	15
	150	28	17	23
	50	11	21	16
	15	15	21	18
	5.0	18	20	19
	1.5	17	17	17
Test Solution w/o S9	5000	13	15	14
	1500	22	27	25
	500	7	18	13
	150	24	21	23
	50	18	10	14
	15	19	20	20
	5.0	31	27	29
	1.5	13	7	10
DI Water w/S9		38	30	34
DI Water w/o S9		29	31	30
2-aminoanthracen w/ S9		162	182	172
Methylmethanesulfonate w/o S9		275	281	278
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

Dermal and Ocular Irritation Tests

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Sample: AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate)

Code: 20219

CAS #: 7732-18-5 & 8013-01-2

Test Request Form/Submission #: 228

Lot #: NC120502-A

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Maureen Danaher*

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 **AC Dermal Respiratory Factor Advanced** 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% CO_2, 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-yl*)], 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% CO_2 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 H_2O
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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Dermal and Ocular Irritation Tests

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% CO_2 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% CO_2 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% CO_2, 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% CO_2, 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% CO_2, 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 (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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Dermal and Ocular Irritation Tests

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 ≤ 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 ≤ 50% for EpiDerm™ or ≤ 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.

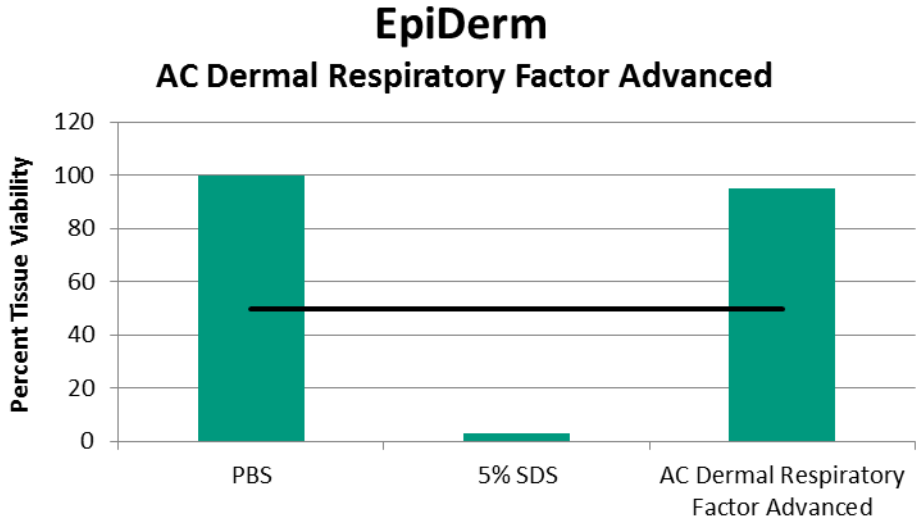

Figure 1: EpiDerm tissue viability

Dermal and Ocular Irritation Tests

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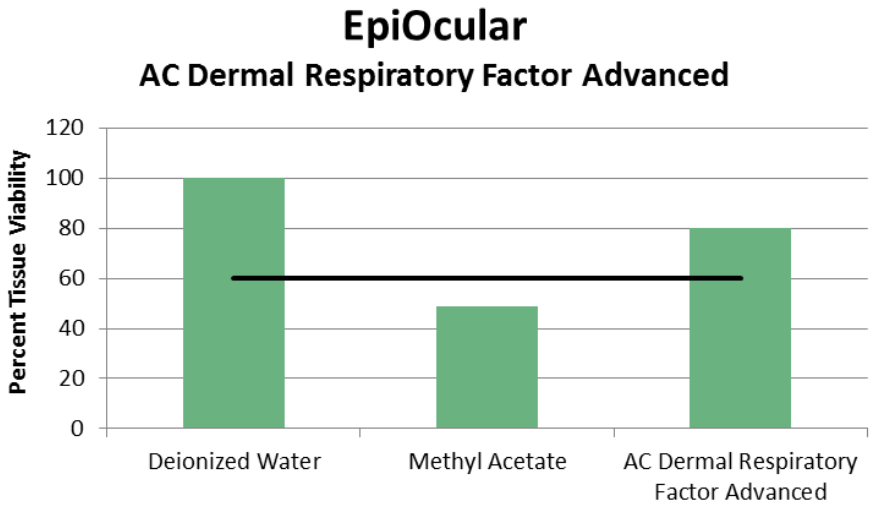

Figure 2: EpiOcular tissue viability

OECD TG 442C: *In Chemico* Skin Sensitization

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Tradename: AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate)

Code: 20219

CAS #: 7732-18-5 & 8013-01-2

Test Request Form #: 7929

Lot #: 7900300

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 contact[1]. 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 dermatitis[2]. 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 **AC Dermal Respiratory Factor Advanced** 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-RFAA**C**AA-COOH); Lysine peptide (Ac-RFAA**K**AA-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* **AC Dermal Respiratory Factor Advanced** in Acetonitrile

*For mixtures and multi-constituent substances of known composition such as **AC Dermal Respiratory Factor Advanced**, 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
• 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)	• 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:

1. The following criteria must be met for a run to be considered valid:
   a. Standard calibration curve should have an $r^2 > 0.99$.
   b. 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.
   c. Mean peptide concentration of reference controls A should be 0.50±0.05mM and the coefficient of variable of the peptide peak areas for reference B and C in acetonitrile should be <15.0%.

2. The following criteria must be met for a test chemical's results to be considered valid:
   a. Maximum standard deviation should be <14.9 for percent cysteine depletion and <11.6 for percent lysine depletion.
   b. Mean peptide concentration of the three reference control C should be 0.50±0.05mM.

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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**
3.23	Minimal Reactivity	Non-sensitizer
3.23	Minimal Reactivity	Non-sensitizer
3.21	Minimal Reactivity	Non-sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.20	Minimal Reactivity	Non-sensitizer
3.19	Minimal Reactivity	Non-sensitizer
3.20	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:

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

Based on HPLC-UV analysis of **AC Dermal Respiratory Factor Advanced (20219)** 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 3.21% 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: AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate)

Code: 20219

CAS #: 7732-18-5 & 8013-01-2

Test Request Form #: 7937

Lot #: 7900300

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 Chemicals[1]. 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 **AC Dermal Respiratory Factor Advanced** 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

Materials

A. Incubation Conditions:	37°C at 5% CO_2 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 **AC Dermal Respiratory Factor Advanced** 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% CO_2 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% CO_2. 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 IC_{50} and IC_{30} 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.

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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 EC1.5 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 Imax 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 EC1.5 determining concentration)
3. The $EC_{1.5}$ value is less than 1000 µM (or < 200 µg/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	32.24
DMSO	Non-Sensitizer	No Induction	243.24 µM	0.16
AC Dermal Respiratory Factor Advanced	Non-Sensitizer	No Induction	> 1000 µM	0.33

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

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OECD TG 442D: *In Vitro* Skin Sensitization

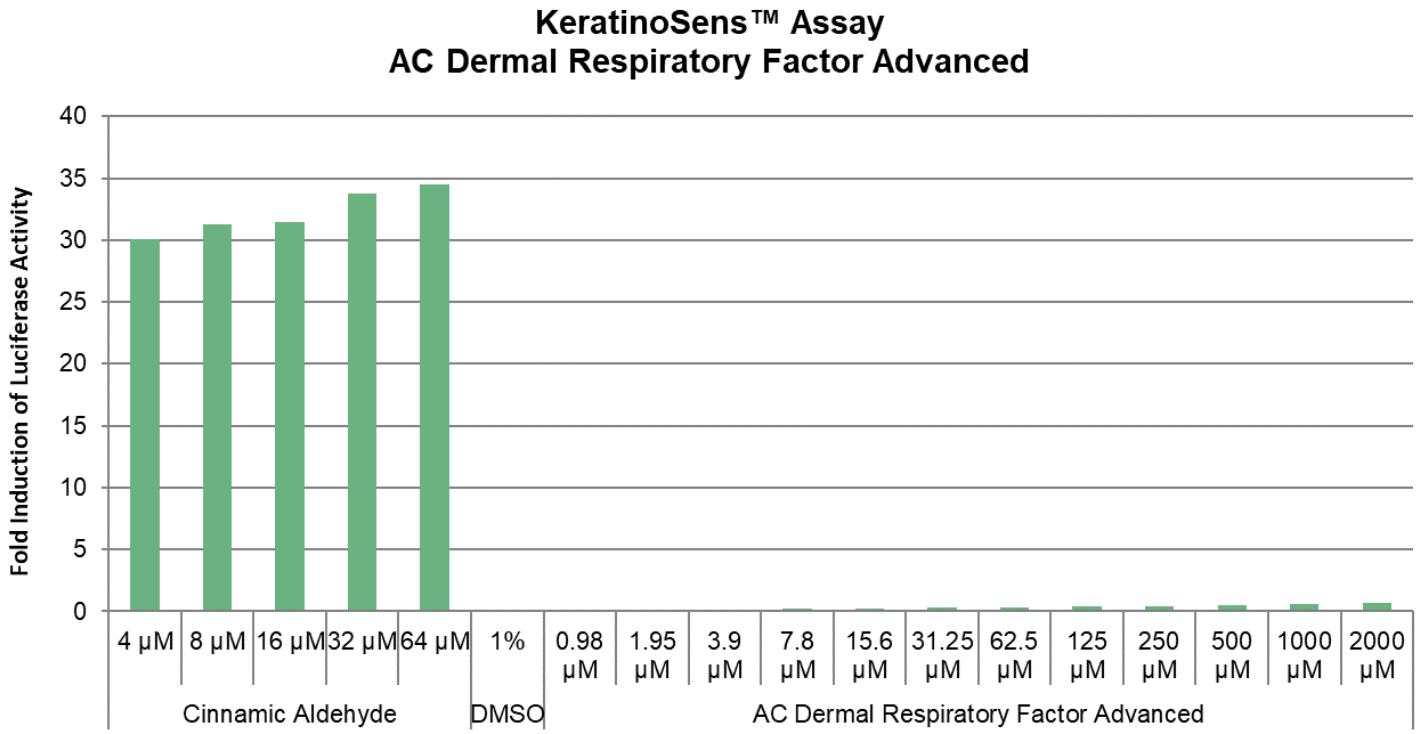

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **AC Dermal Respiratory Factor Advanced (20219)** 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 **AC Dermal Respiratory Factor Advanced** can be safely used in cosmetics and personal care products at typical use levels.

Phototoxicity Assay Analysis

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Tradename: AC Dermal Respiratory Factor Advanced (contains 24.5% Saccharomyces Ferment Lysate Filtrate)

Code: 20219

CAS #: 7732-18-5 & 8013-01-2

Test Request Form #: 7938

Lot #: 7900300

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 **AC Dermal Respiratory Factor Advanced** 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% CO_2, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). 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

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_2O
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 chloropromazine, 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/cm^2 is reduced by 20% compared to the non-irradiated control tissues.

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Phototoxicity Assay Analysis

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% CO_2 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% CO_2, 95% RH).

C. Tissue Irradiation

Tissue inserts in their 6-well plates are UVA-irradiated for 60 minutes with 6 J/cm^2 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% CO_2, 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% CO_2, 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 (OD_{570}) 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%.

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Phototoxicity Assay Analysis

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.

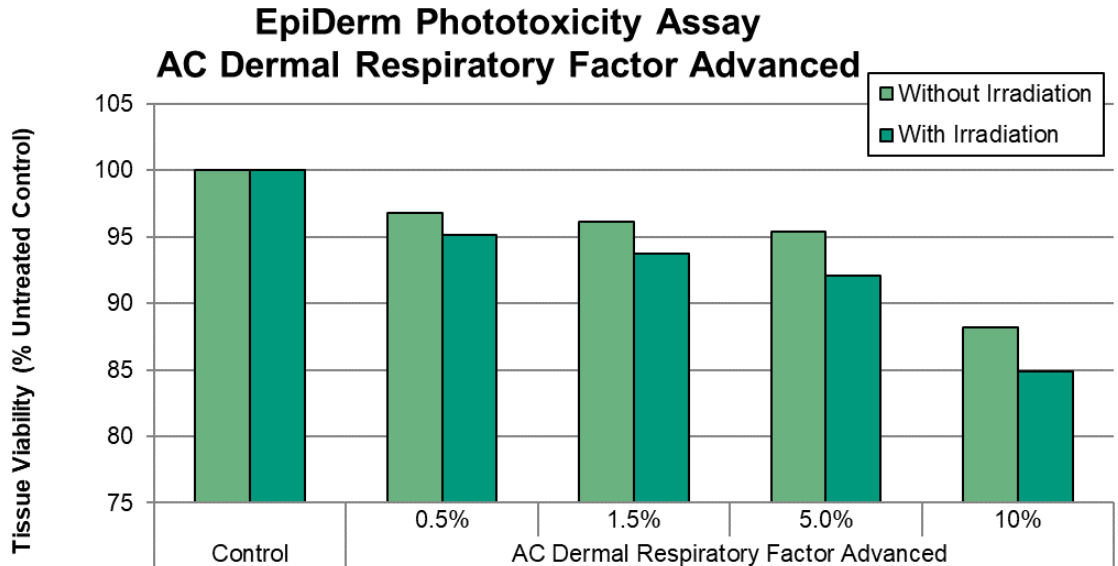
C. Test Validity

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 **AC Dermal Respiratory Factor Advanced** is not a photoirritant when used at the suggested use levels of 0.50 – 1.00%.



	Control	0.5%	1.5%	5.0%	10%
Without Irradiation	100	96.8	96.1	95.4	88.2
With Irradiation	100	95.1	93.7	92.1	84.9

Figure 1: EpiDerm Phototoxicity Graph

Dermal and Ocular Irritation Tests

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Tradename: AC Dermal Respiratory Factor Powder (contains 98% Saccharomyces Lysate Extract)

Code: 20214

CAS #: 8013-01-2

Test Request Form #: 9503

Lot #: N220204B

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Daniel Shill*

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 **AC Dermal Respiratory Factor Powder** 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% CO_2, 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-yl*)], 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.

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Dermal and Ocular Irritation Tests

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% CO_2 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 H_2O
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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Dermal and Ocular Irritation Tests

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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% CO_2 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% CO_2 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% CO_2, 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% CO_2, 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% CO_2, 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 (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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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 ≤ 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 ≤ 50% for EpiDerm™ or ≤ 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.

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Dermal and Ocular Irritation Tests

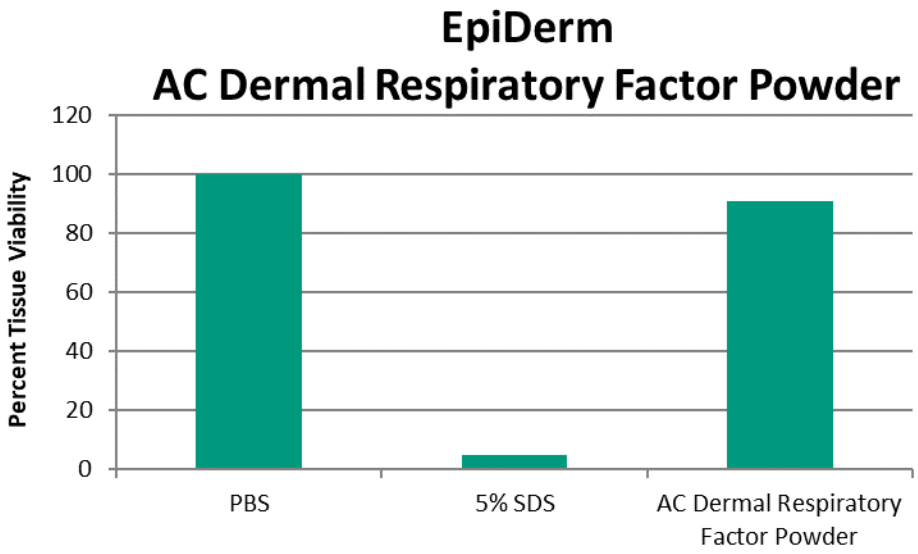

Figure 1: EpiDerm tissue viability

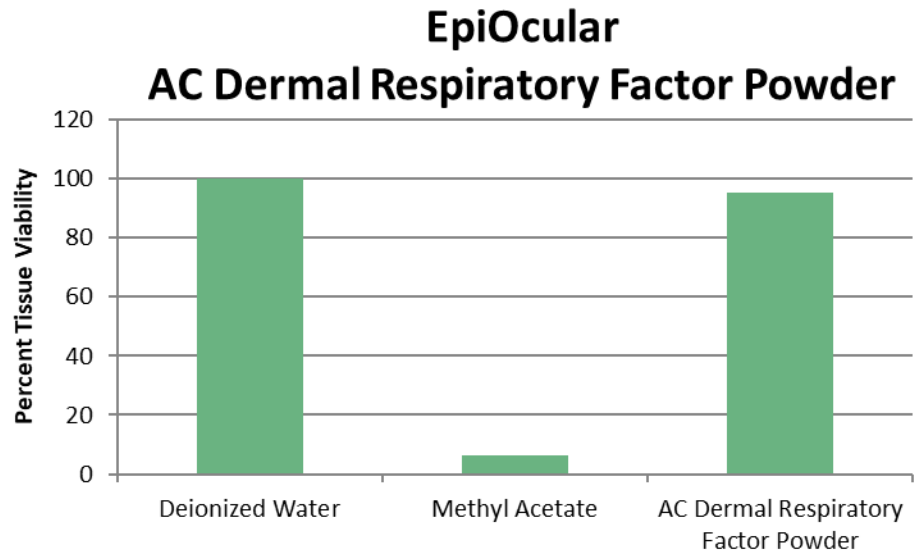

Figure 2: EpiOcular tissue viability

Cellular Viability Assay Analysis

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Tradename: AC Dermal Respiratory Factor (contains 25% Saccharomyces Lysate Extract)

Code: 20211

CAS #: 7732-18-5 & 8013-01-2

Test Request Form #: 626

Lot #: 28813

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease is ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **AC Dermal Respiratory Factor** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

Active Concepts

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Cellular Viability Assay Analysis

Materials

A.	**Kit:**	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
C.	**Equipment:**	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D.	**Cell Line:**	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E.	**Media/Buffers:**	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS)
F.	**Culture Plate:**	Falcon flat bottom 96-well tissue culture treated plates
G.	**Reagents:**	PrestoBlue™ reagent (10X)
H.	**Other:**	Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 10-fold serial dilution was performed resulting in **AC Dermal Respiratory Factor** concentrations of 0.1% and 0.01% in complete DMEM and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

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Cellular Viability Assay Analysis

Results

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

AC Dermal Respiratory Factor did not exhibit significant effects on the cellular metabolism.

Cellular metabolism results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

$$Percent\ (\%)Change = \frac{MFU_{Control} - MFU_{Sample}}{MFU_{Control}} \times 100$$

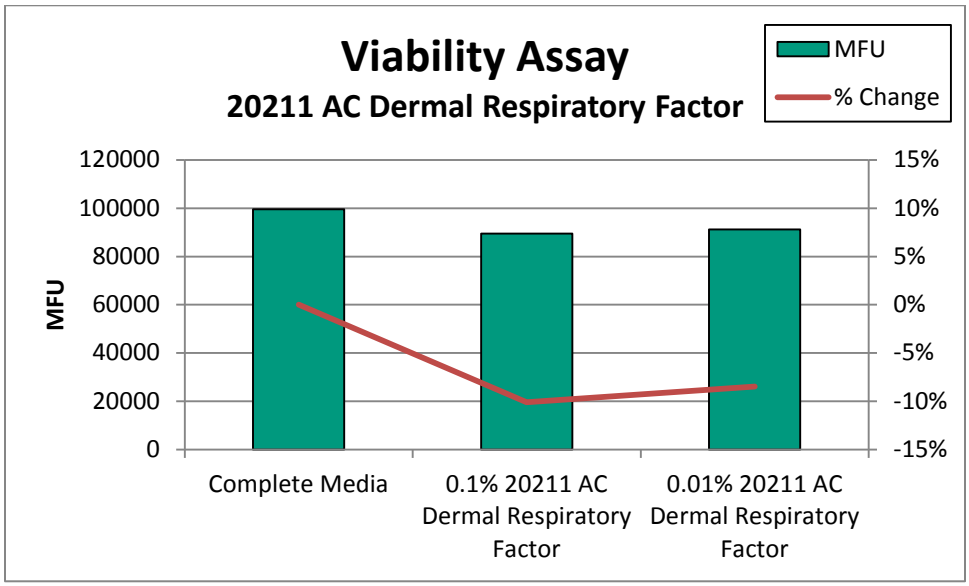

Figure 1: Cellular Metabolism of **AC Dermal Respiratory Factor**-treated fibroblasts expressed in terms of percent of control.

Discussion

In this study, **AC Dermal Respiratory Factor** (code 20211) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of both 0.1% and 0.01% **AC Dermal Respiratory Factor** (code 20211), nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **AC Dermal Respiratory Factor** (code 20211) is not cytotoxic.

AMA LABORATORIES, INC.
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FAX: (845) 638-4872

50 HUMAN SUBJECT REPEAT INSULT OPEN PATCH TEST SKIN IRRITATION/SENSITIZATION EVALUATION (OPEN PATCH)

Date: September 6, 2002

AMA Ref. No.: MS02.RIPT.C3859OP.50.ACTC

Sponsor: Active Concepts
43 Davis Street
South Plainfield, New Jersey 07080

1.0 Objective: Consumer products or raw materials designed for consistent reapplication to areas of the skin may, under proper conditions, prove to be contact sensitizers or irritants in certain individuals. It is the intention of a Repeat Insult Patch Test (RIPT) to provide a basis for evaluation of this irritation/ sensitization potential if such exists.

2.0 Reference: The method is modified to test 50 panelists and not the 200 cited in the reference Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, published by The Association of Food and Drug Officials of The United States. The method also employs nine inductive patchings and not the ten cited in the reference under 'open patch' conditions.

3.0 Test Material:

3.1 Test Material Description:

On July 30, 2002 eight test samples labeled as listed below were received from Active Concepts and assigned AMA Lab Nos. as follows:

Sample Description	AMA Lab No.
AC Dermal Respiratory Factor, Code 20211, Lot SN020726-1	C-3863

Contains 25% Saccharomyces Lysate Extract

3.2 Handling:

Upon arrival at AMA Laboratories, Inc., the test material is assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date and test requested.
Samples are retained for a period of three months beyond submission of final reports unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, representative retained samples are kept two years beyond final report submission.

Sample disposition is conducted in compliance with appropriate federal, state and local ordinances.

3.3 Test Material Evaluation Prerequisite:

Prior to induction of a human test panel, animal toxicology, microbiology and other in-vivo or in-vitro performance spectra may be required to assess the feasibility of commencement as dictated by an Institutional Review Board (IRB) described in Section 4.0.

Sponsor purports that prior to sample submission to AMA the following tests were conducted with no adverse results and that the test data are on file on their premises and have not been made available to AMA personnel:

- CTFA Preservative Efficacy Test or equivalent
- 90 Day Accelerated Stability and Container Compatibility Study

4.0 Institutional Review Board:

Reference: CFR Title 21 Part 56, Subparts A, B, C, and D.
The IRB of AMA Laboratories, Inc., consists of five or more individuals, chosen from within the company for technical expertise and from the local community for lay interaction. The list of IRB members is kept on file at AMA Laboratories, Inc., and is available for inspection during the hours of operation.

5.0 Panel Selection:

5.1 Standards for Inclusion in a Study:

- Individuals who are not currently under a doctor's care.
- Individuals free of any dermatological or systemic disorder which would interfere with the results, at the discretion of the Investigator.
- Individuals free of any acute or chronic disease that might interfere with or increase the risk of study participation.
- Individuals who will complete a preliminary medical history form mandated by AMA Laboratories, Inc. and are in general good health.
- Individuals who will read, understand and sign an informed consent document relating to the specific type of study they are subscribing. Consent forms are kept on file and are available for examination on the premises of AMA Laboratories, Inc. only.
- Individuals able to cooperate with the Investigator and research staff, be willing to have test materials applied according to the protocol, and complete the full course of the study.

5.2 Standards for Exclusion from a Study:

- Individuals under 18 years of age.
- Individuals who are under doctor's care.
- Individuals who are currently taking any medication (topical or systemic) that may mask or interfere with the test results.
- Subjects with a history of any acute or chronic disease that might interfere with or increase the risk of study participation.
- Individuals diagnosed with chronic skin allergies.
- Female volunteers who indicate that they are pregnant or nursing.

5.3 Recruitment:

Panel selection is accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

5.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms, are available for inspection on the premises of AMA Laboratories, Inc. only. Reference 21 CFR Ch. 1 Part 50, Subpart B.

6.0 Population Demographics:

Number of subjects enrolled		54
Number of subjects completing study		50
Age Range		18-72
Sex	Male	10
	Female	44
Race	Caucasian	40
	Hispanic	13
	Asian	1

7.0 Equipment:

- Acculine Surgical Marking Pen (Accu-Line Products, Inc.).
- 1 ml volumetric syringe without a needle and spatula.

8.0 Procedure:

- Subjects are requested to bathe or wash as usual before arrival at the facility.
- As per client request, the test materials were diluted to 10% in distilled water; the test materials , C-3863 and

were diluted to 5% in distilled water; and test material C- was diluted to 2% in distilled water. Dilutions were freshly prepared on each application day.

- 0.2 ml or 0.2 g of the test material is dispensed directly onto a designated area of the panelist's back and allowed to air dry.

- This procedure is repeated until a series of nine consecutive 'open patch' applications have been made for every Monday, Wednesday and Friday for three consecutive weeks.

- In the event of an adverse reaction, the area of erythema and edema is measured. The edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin. Reactions are scored just before applications two through nine and the next test date following application nine. In most instances this is approximately 24 hours after patch removal. Clients are notified immediately in the case of adverse reaction and determination is made as to treatment program if necessary.

- Subjects are then given a 10 - 14 day rest period after which a challenge or retest dose is applied once to a previously unexposed test site. The retest dose is equivalent to any one of the original nine exposures. Reactions are scored 24 and 48 hours after application.

- Comparison is made between the nine inductive responses and the retest dose.

9.0 Results: Please refer to attached Tables.

10.0 Observations: No adverse reactions of any kind were noted during the course of this study.

11.0 Archiving: All original samples, raw data sheets, technicians notebooks, correspondence files, and copies of final reports and remaining specimens are maintained on premises of AMA Laboratories in limited access storage files marked "Archive". A duplicate disk copy of final reports is separately archived in a bank safe deposit vault.

12.0 Conclusion: The test materials listed below when tested under 'open patch' conditions as described herein at the dilutions in distilled water listed, may be considered as **NON-PRIMARY IRRITANTS** and **NON-PRIMARY SENSITIZERS** to the skin according to the reference:

Sample Description	AMA Lab No.
AC Dermal Respiratory Factor, Code 20211, Lot SN020726-1(5%)	C-3863

Shyla Cantor, Ph.D.
Study Director

P. Nou Torres, B.S.
Technician

Lynn Kimiecik, B.S.
Technician

Diana Steixner
Technician

David R. Winne, B.S.
Quality Assurance Supervisor

9/6/02
Date

TABLE 5
SUMMARY OF RESULTS
(OPEN PATCH)

AMA Lab No.: C-3863
Client No.: AC Dermal Respiratory Factor, Code 20211, Lot SN020726-1
Dilution: 5% in distilled water

No.	Subject ID	RACE	SEX	Response									Chall.		Score
				1	2	3	4	5	6	7	8	9	24 HR	48 HR	
1	86 3979	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
2	26 0599	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
3	30 1215	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
4	72 1591	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
5	24 3334	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
6	69 1708	C	F	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A
7	70 3708	C	F	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A
8	41 8535	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
9	50 9640	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
10	46 5842	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
11	62 1627	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
12	50 9087	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
13	36 4212	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
14	46 4213	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
15	26 7211	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
16	44 5435	C	F	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A
17	42 3961	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
18	28 5046	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
19	36 5248	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
20	40 6489	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
21	60 5821	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
22	68 7038	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
23	62 6182	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
24	32 6955	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
25	60 8615	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
26	89 1786	H	M	0	0	0	0	0	0	0	0	0	0	0	0.0
27	34 7962	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
28	40 1260	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
29	50 7621	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
30	66 8570	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
31	66 8507	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
32	62 3596	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0

TABLE 5 (CONT'D)
SUMMARY OF RESULTS
(OPEN PATCH)

AMA Lab No.: C-3863
Client No.: AC Dermal Respiratory Factor, Code 20211, Lot SN020726-1
Dilution: 5% in distilled water

No.	Subject ID	RACE	SEX	Response									Chall.		Score
				1	2	3	4	5	6	7	8	9	24 HR	48 HR	
33	22 4828	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
34	36 9096	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
35	46 7496	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
36	50 4079	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
37	38 9386	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
38	52 6559	A	F	0	0	0	0	0	0	0	0	0	0	0	0.0
39	40 0614	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
40	52 8248	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
41	36 8248	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
42	72 3637	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
43	52 3573	C	M	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A
44	64 3640	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
45	40 6875	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
46	42 5472	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
47	44 5375	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
48	30 5720	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
49	66 9894	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
50	68 8987	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
51	79 3346	H	M	0	0	0	0	0	0	0	0	0	0	0	0.0
52	73 5431	H	M	0	0	0	0	0	0	0	0	0	0	0	0.0
53	40 7089	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
54	49 2301	H	M	0	0	0	0	0	0	0	0	0	0	0	0.0

Evaluation Period:

This study was conducted from July 31, 2002 through September 5, 2002.

Scoring Scale And Definition Of Symbols Shown In Tables:

0 - No evidence of any effect

? - (Barely perceptible) minimal faint (light pink) uniform or spotty erythema

1 - (Mild) pink uniform erythema covering most of contact site

2 - (Moderate) pink\red erythema visibly uniform in entire contact area

3 - (Marked) bright red erythema with accompanying edema, petechiae or papules

4 - (Severe) deep red erythema with vesiculation or weeping with or without edema

D - Patch eliminated due to severe reaction

Dc - Discontinued due to absence of subject on application date

M - Patch applied to an adjacent site after strong test reaction

NA - Score is not calculated for subjects discontinued before challenge

S - Skin stained from pigment in product

T - Tan

NOTE: All technical employees of AMA LABORATORIES, INC. are required to take and pass a visual discrimination examination conducted by a Board Certified Ophthalmologist using the Farnsworth-Munsell 100 Hue Test as published; which determines a person's ability to discern color against a black background. This test was additionally modified to include a flesh tone background more nearly approaching actual use conditions, wherein erythematous skin is graded according to intensity.

Dermal and Ocular Irritation Tests

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Sample: AC Liposome Dermal Respiratory Factor (contains 10% Saccharomyces Lysate Extract)

Code: 60118

CAS #: 7732-18-5 & 8002-43-5 & 8013-01-2

Test Request Form/Submission #: 1225

Lot #: 37487P

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 **AC Liposome Dermal Respiratory Factor** 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% CO_2, 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-yl*)], 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% CO_2 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 H_2O
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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Dermal and Ocular Irritation Tests

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% CO_2 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% CO_2 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% CO_2, 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% CO_2, 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% CO_2, 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 (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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Dermal and Ocular Irritation Tests

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 ≤ 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 ≤ 50% for EpiDerm™ or ≤ 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.

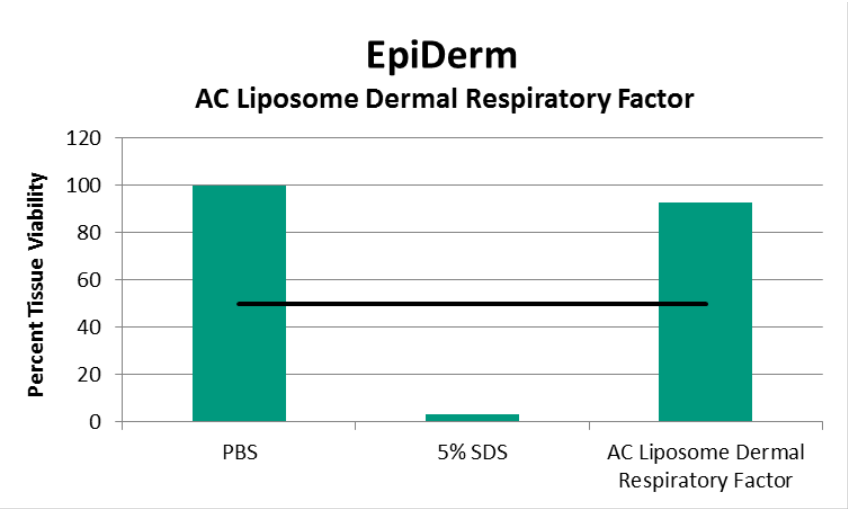

Figure 1: EpiDerm tissue viability

Dermal and Ocular Irritation Tests

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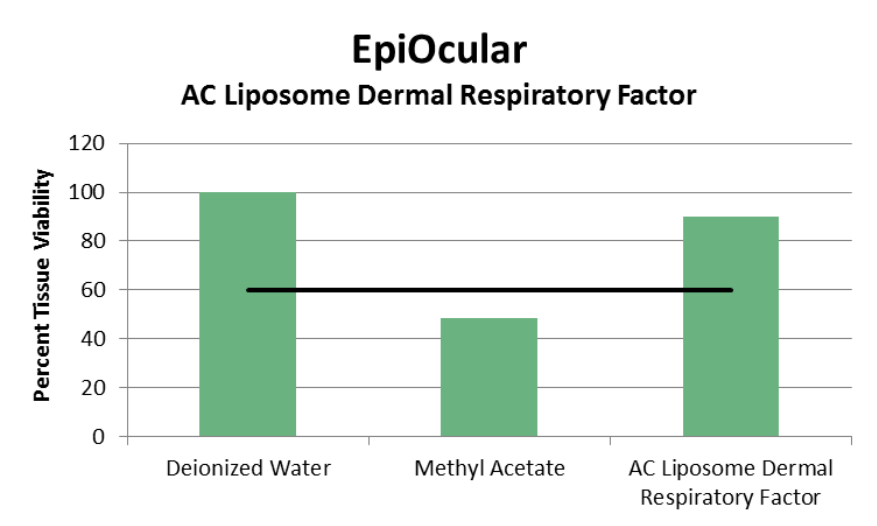

Figure 2: EpiOcular tissue viability

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: September 6, 2023

SUBJECT: Saccharomyces Ferment Lysate Filtrate

BioScreen Testing Services, Inc. 2016. 100 Subject human repeat insult patch test for skin irritation and skin sensitization evaluation (Saccharomyces Ferment Lysate Filtrate tested material contained 2% non-volatile solids in water).

BioScreen® Testing Services, Inc.

3892 Del Amo Boulevard • Torrance, California 90503
(310) 214-0043 • Fax(310) 370-3642
Web Site: www.bioscreen.com • E-Mail: info@bioscreen.com

100 SUBJECT HUMAN REPEAT INSULT PATCH TEST FOR SKIN IRRITATION AND SKIN SENSITIZATION EVALUATION

Saccharomyces Ferment Lysate Filtrate tested material contained 2% non-volatile solids in water

Date: May 11, 2016

BCS Study No.: 16-403A and 16-608A

Sponsor:

1.0 Objective: To determine the irritation and sensitization (contact allergy) potential of a test material after repeated application to the skin of human subjects.

2.0 Test Material:

2.1 Test Material Description:

Date Received: 03/21/2016

Received From:

Number Of Test Samples Received: 1

Label On Test Samples: Skin Serum Prototype

Accession No.: 941777

2.2 Handling:

Upon arrival at BioScreen Clinical Services (BCS) the test material was assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples will be retained for a period of thirty (30) days

beyond submission of final report unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, in which case representative retained samples are kept two (2) years beyond final report submission.

Sample disposition will be conducted in compliance with appropriate federal, state and local ordinances.

3.0 Panel Selection:

3.1 Standards for Inclusion in a Study:

- Individuals who were not currently under a doctor's care.
- Individuals who were free of any dermatological or systemic disorder that would interfere with the results, at the discretion of the Investigator.
- Individuals who were free of any acute or chronic disease that would interfere with or increase the risk of study participation.
- Individuals who completed a preliminary medical history form mandated by BCS and were in general good health.
- Individuals who read, understood and signed an informed consent document relating to the specific type of study.
- Individuals who were able to cooperate with the Investigator and research staff, and were willing to have test materials applied according to the protocol, and complete the full course of the study.

3.2 Standards for Exclusion from a Study:

- Individuals who were under 18 years of age.
- Individuals who were currently under a doctor's care.
- Individuals who were currently taking any medication (topical or systemic) that might mask or interfere with the test results.
- Individuals who had a history of any acute or chronic disease that might interfere with or increase the risk associated with study participation.
- Individuals who were diagnosed with chronic skin allergies.
- Female volunteers who indicated that they were pregnant or nursing.

3.3 Recruitment:

Panel selection was accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

3.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms are available for inspection on the premises of BCS only. [Reference 21 CFR Ch. 1 Part 50, Subpart B]

The parties agree to comply with applicable state and federal privacy laws for the use and disclosure of a subject's personal health information by taking reasonable steps to protect the confidentiality of this information. This obligation shall survive the termination or expiration of this Agreement.

4.0 Population Demographics:

Number of subjects enrolled	113
Number of subjects completing study	105
Age Range	18-64
Sex	
Male	24
Female	81
Fitzpatrick Skin Type*	
1 – always burn, does not tan	0
2 – burn easily, tan slightly	16
3 – burn moderately, tan progressively	66
4 – burn a little, always tan	8
5 – rarely burn, tan intensely	12
6 – never burn, tan very intensely	3

*[Agache P., Hubert P.. Measuring the skin. (p. 473, table 48.1) Springer-Verlag Berlin

Heidelberg, 2004, (p. 473, table 48.1)]

5.0 Equipment:

Test materials to be tested under occlusive conditions were placed on an 8-millimeter aluminum chamber (Finn Chamber, Epitest Ltd Oy, Tuussula, Finland) supported on a sheet of Scanpore® (occlusive) tape (Norgesplaster A/S, Kristiansand, Norway) or a 7mm IQ-ULTRA® closed cell system which is made of additive-free polyethylene plastic foam with a filter paper incorporated (It is supplied in units of 10 chambers on a hypoallergenic non woven adhesive tape; the width of the tape is 52mm and the length is 118mm) or other equivalents.

Test materials to be tested under semi-occlusive conditions were placed on Curad™ sensitive skin bandages or on a 7.5mm filter paper disc affixed to a strip of hypoallergenic tape (Johnson & Johnson 1 inch First Aid Cloth Tape).

Test materials to be tested in an open patch were applied and rubbed directly onto the back of the subject.

Approximately 0.02-0.05 mL (in case of liquids) and/or 0.02-0.05 gm (in case of solids) of the test material was used for the study. Liquid test material was dispensed on a 7.5mm paper disk, which fit in the Finn Chamber.

6.0 Procedure:

- Subjects were requested to bathe or wash as usual before arrival at the facility.
- Patches containing the test material were then affixed directly to the skin of the intrascapular regions of the back, to the right or left of the midline and subjects were dismissed with instructions not to wet or expose the test area to direct sunlight.
- Subjects were instructed to remove the patches approximately 48 hours after the first application and 24 hours thereafter for the remainder of the study.
- This procedure was repeated until a series of nine (9) consecutive, 24-hour exposures had been made three (3) times a week for three (3) consecutive weeks.
- Prior to each reapplication, the test sites were evaluated by trained laboratory personnel.

- Following a 10-14 day rest period a retest/challenge dose was applied once to a previously unexposed test site. Test sites were evaluated by trained laboratory personnel 48 and 96 hours after application.
- In the event of an adverse reaction, the area of erythema and edema were measured. Edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin.
- Subjects were instructed to report any delayed reactions that might occur after the final reading.
- Clients will be notified immediately in the case of an adverse reaction and a determination is made as to treatment program if necessary.

7.0 Scoring:

Scoring scale and definition of symbols shown below are based on the scoring scheme according to the International Contact Dermatitis Research Group scoring scale [Rietschel, R.L., Fowler, J.F., Ed., Fisher's Contact Dermatitis (fourth ed.). Baltimore, Williams & Wilkins, 1995] listed below:

0 no reaction (negative)
1 erythema throughout at least ¾ of patch area
2 erythema and induration throughout at least ¾ of patch area
3 erythema, induration and vesicles
4 erythema, induration and bullae

D Site discontinued
Dc Subject discontinued

NOTE: Clinical evaluations are performed by a BCS investigator or designee trained in the clinical evaluation of the skin. Whenever feasible, the same individual will do the scoring of all the subjects throughout the study and will be blinded to the treatment assignments and any previous scores.

8.0 Results:

Accession No.: 941777
Test Material Description: Skin Serum Prototype - ABC-SC-031716
Patch Description: Occlusive

Subject Information					Induction									Challenge	
No.	ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
1	8776	F	50	2	0	0	0	0	0	0	0	0	0	0	0
2	10884	F	51	5	0	0	0	0	0	0	0	0	0	0	0
3	10940	M	48	2	0	0	0	0	0	0	0	0	0	0	0
4	10982	F	51	3	0	0	0	0	0	0	0	0	0	0	0
5	11166	M	44	4	0	0	0	0	0	0	0	0	0	0	0
6	11202	F	53	4	0	0	0	0	0	0	0	0	0	0	0
7	11668	F	55	2	0	0	0	0	0	0	0	0	0	0	0
8	11733	F	43	4	0	0	0	0	0	0	0	0	0	0	0
9	11822	F	49	4	0	0	0	0	0	0	0	0	0	0	0
10	12107	M	55	3	0	0	0	0	0	0	0	0	0	0	0
11	12243	F	51	2	0	0	0	0	0	0	0	0	0	0	0
12	12602	F	46	3	0	0	0	0	0	0	0	0	0	0	0
13	14415	F	54	2	0	0	0	0	0	0	0	0	0	0	0
14	14675	F	53	3	0	0	0	0	0	Dc	Dc	Dc	Dc	Dc	Dc
15	14690	F	52	2	0	0	0	0	0	0	0	0	0	0	0
16	14809	M	54	5	0	0	0	0	0	0	0	0	0	0	0
17	14827	M	55	2	0	0	0	0	0	0	0	0	0	0	0
18	15586	F	55	4	0	0	0	0	0	0	0	0	0	0	0
19	15623	F	55	3	0	0	0	0	0	0	0	0	0	0	0
20	15754	F	53	6	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
21	15832	F	35	2	0	0	0	0	0	0	0	0	0	0	0
22	15886	M	46	5	0	0	0	0	0	0	0	0	0	0	0
23	15938	M	53	3	0	0	0	0	0	0	0	0	0	0	0
24	16026	F	39	3	0	0	0	0	0	0	0	0	0	0	0
25	16238	M	25	2	0	0	0	0	0	0	0	0	0	0	0
26	16239	M	22	2	0	0	0	0	0	0	0	0	0	0	0
27	16522	F	47	3	0	0	0	0	0	0	0	0	0	0	0
28	17743	M	47	5	0	0	0	0	0	0	0	Dc	Dc	Dc	Dc
29	17854	F	50	4	0	0	0	0	0	0	0	0	0	0	0
30	17866	F	44	2	0	0	0	0	0	0	0	0	0	0	0
31	17925	M	53	2	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
32	18180	F	30	5	0	0	0	0	0	0	0	0	0	0	0
33	19599	M	46	6	0	0	0	0	0	0	0	0	0	0	0

Subject Information					Induction									Challenge	
No.	ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
34	19682	F	49	2	0	0	0	0	0	0	0	0	0	0	0
35	19734	M	46	2	0	0	0	0	0	0	0	0	0	0	0
36	19773	M	43	3	0	0	0	0	0	0	0	0	0	0	0
37	20105	F	53	4	0	0	0	0	0	0	0	0	0	0	0
38	20395	M	32	3	0	0	0	0	0	0	0	0	0	0	0
39	20628	F	49	2	0	0	0	0	0	0	0	0	0	0	0
40	20698	F	49	5	0	0	0	0	0	0	0	0	0	0	0
41	20772	F	51	3	0	0	0	0	0	0	0	0	0	0	0
42	20838	F	61	5	0	0	0	0	0	0	0	0	0	0	0
43	21067	F	21	3	0	0	0	0	0	0	0	0	0	0	0
44	21228	F	61	5	0	0	0	0	0	0	0	0	0	0	0
45	21319	F	24	5	0	0	0	0	0	0	0	0	0	0	0
46	21330	F	37	2	0	0	0	0	0	0	0	0	0	0	0
47	21472	F	36	3	0	0	0	0	0	0	0	0	0	0	0
48	21640	F	54	3	0	0	0	0	0	0	0	0	0	0	0
49	21765	F	30	6	0	0	0	0	0	0	0	0	0	0	0
50	21989	F	27	4	0	0	0	0	0	0	0	0	0	0	0
51	22085	F	39	5	0	0	0	0	0	0	0	0	0	0	0
52	22086	F	51	5	0	0	0	0	0	0	0	0	0	0	0
53	22304	M	36	2	0	0	0	0	0	0	0	0	0	0	0
54	22575	F	34	3	0	0	0	0	0	0	0	0	0	0	0
55	22642	F	46	3	0	0	0	0	0	0	Dc	Dc	Dc	Dc	Dc
56	22895	F	35	3	0	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
57	22974	M	37	5	0	0	0	0	0	0	0	0	0	0	0
58	23025	M	36	5	0	0	0	0	0	0	0	0	0	0	0
59	23059	M	31	6	0	0	0	0	0	0	0	0	0	0	0
60	23064	M	36	3	0	0	0	0	0	0	0	0	0	0	0
61	3000009	F	63	3	0	0	0	0	0	0	0	0	0	0	0
62	3000046	F	52	3	0	0	0	0	0	0	0	0	0	0	0
63	3000096	F	44	3	0	0	0	0	0	0	0	0	0	0	0
64	3000142	F	36	3	0	0	0	0	0	0	0	0	0	0	0
65	3000191	F	37	3	0	0	0	0	0	0	0	0	0	0	0
66	3000222	F	55	3	0	0	0	0	0	0	0	0	0	0	0
67	3000559	F	40	3	0	0	0	0	0	0	0	0	0	0	0
68	3000689	F	38	3	0	0	0	0	0	0	0	0	0	0	0
69	3000707	F	32	3	0	0	0	0	0	0	0	0	0	0	0
70	3000711	F	49	3	0	0	0	0	0	0	0	0	0	0	0
71	3000823	F	60	3	0	0	0	0	0	0	0	0	0	0	0
72	5000027	F	29	3	0	0	0	0	0	0	0	0	0	0	0

Subject Information					Induction									Challenge	
No.	ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
73	5000028	F	39	3	0	0	0	0	0	0	0	0	0	0	0
74	5000029	M	62	3	0	0	0	0	0	0	0	0	0	0	0
75	5000030	M	18	3	0	0	0	0	0	0	0	0	0	0	0
76	5000031	F	53	3	0	0	0	0	0	0	0	0	0	0	0
77	5000032	F	37	3	0	0	0	0	0	0	0	0	0	0	0
78	5000033	F	30	3	0	0	0	0	0	0	0	0	0	0	0
79	5000034	F	35	3	0	0	0	0	0	0	0	0	0	0	0
80	5000035	F	29	3	0	0	0	0	0	0	0	0	0	0	0
81	5000037	F	53	3	0	0	0	0	0	0	0	0	0	0	0
82	5000038	F	26	3	0	0	0	0	0	0	0	0	0	0	0
83	5000039	F	18	3	0	0	0	0	0	0	0	0	0	0	0
84	5000040	F	52	3	0	0	0	0	0	0	0	0	0	0	0
85	5000041	M	60	3	0	0	0	0	0	0	0	0	0	0	0
86	5000042	F	56	3	0	0	0	0	0	0	0	0	0	0	0
87	5000043	F	62	3	0	0	0	0	0	0	0	0	0	0	0
88	5000044	F	50	3	0	0	0	0	0	0	0	0	0	0	0
89	5000045	F	30	3	0	0	0	0	0	0	0	0	0	0	0
90	5000047	F	28	3	0	0	0	0	0	0	0	0	0	0	0
91	5000049	F	57	3	0	0	0	0	0	0	0	0	0	0	0
92	5000051	F	34	3	0	0	0	0	0	0	0	0	0	0	0
93	5000052	F	40	3	0	0	0	0	0	0	0	0	0	0	0
94	5000053	F	25	3	0	0	0	0	0	0	0	0	0	0	0
95	5000054	F	39	3	0	0	0	0	0	0	0	0	0	0	0
96	5000055	F	43	3	0	0	0	0	0	0	0	0	0	0	0
97	5000056	M	49	3	0	0	0	0	0	0	0	0	0	0	0
98	5000057	F	22	3	0	0	0	0	0	0	0	0	0	0	0
99	5000058	F	56	3	0	0	0	0	0	0	0	0	0	0	0
100	5000059	F	49	3	0	0	0	0	0	0	0	0	0	0	0
101	5000060	F	46	3	0	0	0	0	0	0	0	0	0	0	0
102	5000061	F	30	3	0	0	0	0	0	0	0	0	0	Dc	Dc
103	5000063	M	29	3	0	0	0	0	0	0	0	0	0	0	0
104	5000065	F	26	3	0	0	0	0	0	0	0	0	0	0	0
105	5000068	F	64	3	0	0	0	0	0	0	0	0	0	0	0
106	5000069	F	41	3	0	0	0	0	0	0	0	0	0	0	0
107	5000078*	F	37	3	1	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
108	5000079	F	28	3	0	0	0	0	0	0	0	0	0	0	0
109	5000082	F	37	3	0	0	0	0	0	0	0	0	0	0	0
110	5000091	F	45	3	0	0	0	0	0	0	0	0	0	0	0
111	5000092	F	38	3	0	0	0	0	0	0	0	0	0	0	0

Subject Information					Induction									Challenge	
No.	ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
112	5000093	M	28	3	0	0	0	0	0	0	0	0	0	0	0
113	5000104	F	42	3	0	0	0	0	0	0	0	0	0	0	0

**** Subject discontinued due to Primary Investigator determined subject to be hypersensitive. This drop was not related to the test product.***

9.0 Evaluation Period:

The study was conducted from March 28, 2016 to May 6, 2016.

10.0 Observations:

No adverse reactions of any kind were reported during the course of this study.

11.0 Study Archives:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens will be maintained on premises of BCS in limited access storage files marked "Archive".

12.0 Conclusions:

Under conditions of the study, there were no identifiable signs or symptoms of sensitization (contact allergy) noted for Skin Serum Prototype

Mary Fredenberg, MD
Consulting Dermatologist

Jordan DeSantis
Clinical Supervisor

Steve Park
QA Analyst II

Family	Genus	Name	INCI name	Dermal sensitization	additional data	Food use	GRAS Status	QPS status
Ascoideaceae	Ascoidea	Ascoidea rubescens	-	-	-	x	-	-
Debaryomycetaceae	Debaryomyces	Debaryomyces maramus	Yeast extract	-	-	x	-	-
Debaryomycetaceae	Debaryomyces	*Debaryomyces nepalensis*	Yeast extract	-	-	x	-	-
Debaryomycetaceae	Meyerozyma	Meyerozyma caribbica (= Pichia caribbica)	Pichia caribbica ferment	-	-	x	-	-
Debaryomycetaceae	Debaryomyces	Priceomyces carsonii	Yeast extract	-	-	x	-	-
Debaryomycetaceae	Scheffersomyces	Scheffersomyces stipitis	-Yeast extract - Pichia ferment lysate filtrate	no data available for the moment, but will be soon	-	x	-	-
Dipodascaceae	Geotrichum	Geotrichum candidum	- Galactomyces ferment filtrate	-	Geotrichum candidum is a ubiquitous filamentous yeast-like fungus commonly isolated from soil, air, water, milk, silage, plant tissues, digestive tract in humans and other mammals. This species is widely used as adjunct culture in the maturation of cheese. G. candidum infections (mainly pulmonary or bronchopulmonary, but also cutaneous, oral, disseminates) are very rare: fewer than 100 cases reported between 1842 and 2006. Moreover, cases were not all confirmed by repeated isolations and demonstration of the fungus' presence in tissues, a prerequisite to establish a true diagnosis of geotrichosis. Less than 1 case/year of disease was possibly caused by G. candidum and it never included dairy products or foodborne infection. The risk of developing an infection due to G. candidum in connection with its technological use and consumption of dairy products is virtually nil. For these reasons, G. candidum should be proposed for QPS status (Safety assessment of dairy microorganisms: Geotrichum candidum" - Ivannah Pottier, et al)	x	-	-
Dipodascaceae	Dipoascus	Dipodascus fermentans		-	-	x	-	-
Debaryomycetaceae	/	Candida oleophila	Yeast extract	" **No adverse dermal effects have been reported by workers**. " ("Federal Register / Vol. 74, No. 91 / Wednesday, May 13, 2009 / Rules and Regulations" - 40 CFR Part 180, Candida oleophila Strain O; Exemption from the Requirement of a Tolerance)	No pathogenic effects or infections due to Candida oleophila strain O were observed in the infectivity studies studied and no clinical reports of Candida oleophila infection have been published, although various strains of this species are naturally present in foodstuffs such as apples, olives, strawberries, fermented grapes and tomatoes. Acute oral toxicity and pathogenicity study: Groups of rats were given a single oral dose of Candida oleophila strain O at a rate of 2.3-3.8 x 108 colony-forming units (CFU)/animal => no adverse effect => non-toxic. Acute subcutaneous injection toxicity and pathogenicity: groups of rats were injected subcutaneously with Candida oleophila strain O at a dose of 1 to 1.0 x 107 CFU/animal => no adverse effect => non-toxic. Acute pulmonary toxicity and pathogenicity: groups of rats were exposed intratracheally to Candida oleophila strain O at a dose of 1.2-5.2 x 108 CFU/animal => no adverse effect => non-toxic. Genetic toxicity tests: Bacterial reverse mutation test and in vitro genetic mutation test in mammalian cells => no mutagenic potential. ("Federal Register / Vol. 74, No. 91 / Wednesday, May 13, 2009 / Rules and Regulations" - 40 CFR Part 180, Candida oleophila Strain O; Exemption from the Requirement of a Tolerance)	x	-	notified for QPS Status
Debaryomycetaceae	/	Candida saitoana	- Yeast extract - Hydrolyzed candida saitoana	Patch test: non irritant HRIPT : non sensitizing	-	x	-	-
Dipodascaceae	Yarrowia	*Yarrowia lipolytica*	- Yeast extract - Yarrowia lipolytica extract - Yarrowia lipolytica ferment lysate - Yarrowia lipolytica Oil	-	-	x	GRAS notices	**QPS status**
Endomycetaceae	Endomyces	Endomyces decipiens		-	-	not direct food use : Endomyces decipiens is studied for its potency as a biocontrol agent of ochratoxin a-producing fungi and its effect on arabica coffee taste (1) Geotrichum decipiens are found in the basidiocarps of Armillaria fungi species (2) Bibliographical data : (1) "Potency of yeast as a biocontrol agent of ochratoxin a-producing fungi and its effect on arabica coffee taste" - Okky S. Dharmaputra, et al (2) Book "The yeasts, Fifth edition" 27.3 Dipodascus Armillariae W. Gams (1983) - G. Sybren de Hoog, Maudy Th. Smith	-	-
Metschnikowiaceae	Metschnikowia	*Metschnikowia agaves*	- Hydrolyzed Metschnikowia agaves extract - Metschnikowia agaves extract - Yeast extract	Patch test : non irritant HRIPT: non sensitizing	-	x	-	-

Metschnikowiaceae	Metschnikowia	*Metschnikowia gruessii*	Yeast extract	-	-	not direct food use data : Persimmon (Diospyros kaki Thumb.); a Korean medicinal plant, is inhabitated by Metschnikowia gruessi, which is mainly found in the nectar. (1) M. gruessi is a cosmopolitan yeast species that inhabits floral nectar.(2) M. gruessi is a members of a yeast clade that is adapted to a wide variety of habitats, including flowers and their pollinators. (3) Insects, in particular bees, are the vectors distributing yeasts among flowers. Moreover, morphological adaptation of Metschnikowia gruessii to its dispersal by bumblebees (Bombus sp.) and honeybees (Apis mellifera) is described. M. gruessii may over-winters inside bumblebees and is inoculated into floral nectars in early spring. M. gruessi has been isolated from various plant families (Asteraceae, Berberidaceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Dipsacaceae, Ericaceae, Fabaceae, Hydrophyllaceae, Lamiaceae, Liliaceae, Onagraceae, Ranunculaceae, Rosaceae, Scrophulariaceae, Solanaceae). The isolation substrates were mostly Nectar, and Bombus spec (surface and proboscis), but also in Honey pots (from Bumblebee honey). (4) Bibliographical data : (1) "Characterization of a novel yeast species Metschnikowia persimmonesis KCTC 12991BP (KIOM G15050 type strain) isolated from a medicinal plant, Korean persimmon calyx (Diospyros kaki Thumb)" - Young Min Kang, et al (2) Myco Cosm - https://mycocosm.jgi.doe.gov/Metgru1/Metgru1.home.html (3) "Nectar yeasts of the Metschnikowia clade are highly susceptible to azole antifungals widely used in medicine and agriculture" - Sergio Alvarez-Perez, et al (4) "Ecology of yeasts in plant–bumblebee mutualism in Central Europe" - Michael Brysch-Herzberg	-	-
Metschnikowiaceae	Metschnikowia	*Metschnikowia koreensis*	Yeast extract	-	-	x	-	-
Metschnikowiaceae	Metschnikowia	*Metschnikowia pulcherrima*	Yeast extract	-	-	x	"Metschnikowia pulcherrima DANMET-A and M. fructicola DANMET-B" GRAS notice (2021) (FDA's letter still pending)	-
Metschnikowiaceae	Metschnikowia	*Metschnikowia reukaufii*	- Yeast extract - Hydrolyzed Metschnikowia reukaufii extract - Metschnikowia reukaufii lysate extract	Patch test: non irritant KeratinoSens (OCDE 442D) : no sensitizing potential HRIPT: non sensitizing	-	x	-	-
Metschnikowiaceae	Metschnikowia	*Metschnikowia rubicola*		-	-	x	-	-
Metschnikowiaceae	Metschnikowia	*Metschnikowia viticola*	*-Metschnikowia viticola extract*	-	-	x	-	-
Phaffomycetaceae	Wickerhamomyces	Wickerhamomyces anomalus	- Yeast extract - Pichia anomala extract	Patch test: non irritant HRIPT : non irritant and non sensitizing	-	x	-	QPS status
Phaffomycetaceae	Barnettozyma	Barnettozyma populi (=Pichia populi)	- Yeast extract - Pichia Ferment lysate filtrate (from Pichia populi or from Pichia stipitis)	-		not a direct food use : As a part of the research projet "Technologies to Improve Conversion of Biomass-Derived Sugars to Bioproducts", it has been found that Barnettozyma populi is capable of a great production of arabitolfree xylitol from a mixture of xylose and arabinose. This culture strategy will greatly benefit xylitol production from hemicellulosic hydrolysates, which often contain glucose ("Production of xylitol from mixed sugars of xylose and arabinose without co-producing arabitol" - Badal C. Saha, et al "Optimization of xylitol production from xylose by a novel arabitol limited co-producing Barnettozyma populi NRRL Y-12728" - Badal C. Saha, et al)	-	-
Phaffomycetaceae	Komagataella	Komagataella pastoris (= Pichia pastoris)	- Pichia pastoris Ferment filtrate - Pichia ferment extract filtrate	-		x	GRAS notices	QPS Status
Pichiaceae	Ogataea	Ogataea siamensis	Yeast extract	Keratinosens (OCDE 442D) : no sensitizing potential Patch test : non irritant HRIPT: non irritant and non sensitizing	-	x	-	-
Pichiaceae	Ogataea	Ogataea minuta	- Yeast extract - Pichia minuta extract	Patch test: non irritant HRIPT: non sensitizing Keratinosens (OCDE 442D) : no sensitizing potential SENS-IS : negative U-SENS (OCDE 442E) : negative	Acute toxicity 3T3 NRU (OCDE 129) : LD50 > 2000 mg/kg In vitro mammalian cell micronucleus test on TK6 lymphoblastoid human cells Screening assay performed in Micromethod : negative AMES (OECD 471) : negative BCOP (OCDE 437) : not requiring classification for eye irritation or serious eye	x	-	-
Pichiaceae	Ogataea	Ogataea naganishii	Yeast extract	Keratinosens (OCDE 442D) : no sensitizing potential SENS-IS : negative H-CLAT (OCDE 442E) : negative	Acute toxicity 3T3 NRU (OECD 129) : LD50 > 2000 mg/kg AMES (OCDE 471): negative In vitro micronucleus test in L5178Y TK+/- mouse lymphoma cells /screening test : negative 3T3 NRU (OCDE 129) : non phototoxic BCOP (OCDE 437) : not requiring classification for eye irritation or serious eye damage NRR: negligible cytotoxicity	Not a food use : O. naganishii has been isolated from plant exudates ("Early Ongoing Speciation of Ogataea uvarum Sp. Nov. Within the Grape Ecosystem Revealed by the Internal Variability Among the rDNA Operon Repeats" - Luca Roscini, et al)	-	-
Pichiaceae	Pichia	*Pichia heedii*	- Yeast extract - Pichia heedi extract	Patch test: non irritant HRPIT: non irritant and non sensitizing	Acute toxicity, 3T3 NRU (OECD 129) : LD50 > 2000 mg/kg	not a direct food use : Pichia heedii has been isolated from the soft-rot of Lophocereus schottii and from Drosophila pachea, which utilizes L. schottii as a host plant. P. heedii has the ability to assimilate D-xylose. P. heedii has also been isolated from Carnegiea gigantea, Pachycereus pringlei, Machaerocereus gummosus (des cactus) and Drosophilia nigrospiracula. ("Pichia heedii, a New Species of Yeast Indigenous to Necrotic Cacti in the North American Sonoran Desert" - H. J. PHAFF, W. T. STARMER, et al)	-	-
Pichiaceae	Pichia	Pichia membranifaciens		-	-	x	-	-
Saccharomycetaceae	Eremothecium	*Eremothecium ashbyii*		-	-	x	GRAS	notified for QPS Status
Saccharomycetaceae	Kluyveromyces	Kluyveromyces lactis	- Hydrolyzed Kluyveromyces extract - Kluyveromyces extract	-	-	x	GRAS	QPS status
Saccharomycetaceae	Kluyveromyces	Kluyveromyces marxianus		-	-	x	GRAS	QPS status
Saccharomycetaceae	Saccharomyces	Saccharomyces cerevisiae	- Yeast extract - Saccharomyces Cerevisiae extract	OCDE 406 : non sensitizing	OCDE 401 : non toxic OCDE 404 : non irritant OCDE 405: non irritant OCDE 471 : non mutagenic	x	GRAS Status	QPS status

Saccharomycetaceae	Torulaspora	*Torulaspora delbrueckii*	-Yeast extract - Hydrolyzed Torulaspora delbrueckii extract - Torulaspora delbrueckii ferment	-	-	x	-	-
Saccharomycetaceae	Zygosaccharomyces	*Zygosaccharomyces rouxii*	Yeast extract	-		x	-	QPS status
Saccharomycetales incertae sedis	Starmerella	Starmerella magnoliae	Yeast extract	Keratinosens (OCDE 442D) : no sensitizing potential SENS-IS assay : negative	/	x	-	-
Saccharomycetales incertae sedis	Starmerella	Starmerella bombicola (= candida bombicola)	Hydrolyzed Candida bombicola extract	-	-	x	-	-
Saccharomycodaceae	Hanseniaspora	*Hanseniaspora opuntiae*		-	-	x	-	-
Saccharomycopsidaceae	Saccharomycopsis	Saccharomycopsis fibuligera		-	-	x	-	-
Trichomonascaceae	Wickerhamiella	Wickerhamiella azyma	Yeast extract	-	-	x	-	-

Family	Genus	Name	INCI name	Food use
Ascoideaceae	Ascoidea	Ascoidea rubescens	-	Diganta Narzary, Nitesh Boro et al. (2021), Community structure and metabolic potentials of the traditional rice beer starter 'emao'
Debaryomycetaceae	Debaryomyces	Debaryomyces maramus	Yeast extract	Debaryomyces maramus and C. famata (the anamorphic form of D. hansenii) were isolated throughout the processing of Parma ham and other traditional Greek and Spanish dry-cured products (1) Strains of D. hansenii, Debaryomyces maramus, Hyphopichia burtonii, Penicillium chrysogenum, and Penicillium sp. have been used as starters in the manufacturing of South European dry-cured hams (2) Debaromyces maramus is part of the microbial ecology of probiotic dry-fermented sausages (3) Dépollution des sols : La dégradation des PCB par les champignons levuriformes a été mise en évidence, notamment par Debaryomyces maramus en consortium avec d'autres levures. Ainsi, l'utilisation de levures pour remédier les sols ou les sédiments pollués présente un intérêt (4) Bibliographical data (1) "Biocontrol of Penicillium nordicum Growth and Ochratoxin A Production by Native Yeasts of Dry Cured Ham" - Roberta Virgili, Nicoletta Simoncini, et al (2) "Role of Starter Cultures on the Safety of Fermented Meat Products" - Marta Laranjo, Maria Eduarda Potes, and Miguel Elias (3) "Effective survival of immobilized Lactobacillus casei during ripening and heat treatment of probiotic dry-fermented sausages and investigation of the microbial dynamics" - Marianthi Sidira, Athanasios Karapetsas, et al (4) "Les polychlorobiphényles : enjeux environnementaux et sanitaires, et mycoremédiation" - Paul Cornu
Debaryomycetaceae	Debaryomyces	*Debaryomyces nepalensis*	Yeast extract	Debaryomyces nepalensis is a halotolerant strain of yeast. Halotolerant strains are of considerable biotechnological significance including production of compatible solutes (such as glycerol, trehalose, etc.), haloenzymes, alcoholic beverages, and biological waste treatment (1) D. nepalensis treatment could not only maintain storage quality of mango fruit, but also decrease the decay incidence to anthracnose disease. The results of the study indicated that D. nepalensis has great potential for development of commercial formulations to control postharvest pathogens of mango fruit. (2) D. nepalensis is a promising strain for ecofriendly xylitol production as it exhibits broad specificity to lignocellulose substrates, fermentation of mixed sugars and (ii) tolerance towards lignocellulosic inhibitors making the process more economical. (3) An optimization was done for production of pectic lyases (PL and PGL) by yeast Debaryomyces nepalensis. This could be an alternative to fungal pectolytic enzymes. (4) Debaryomyces nepalensis is a biocontrol yeast. (5) D. nepalensis has been isolated from persimmon fruit, passion fruit, avocado and cape gooseberry. D. nepalensis showed a specific capacity to produce interesting flavors with potential interest for applications in food industry. (6) Bibliographical data: (1) "Growth of Halotolerant Food Spoiling Yeast Debaryomyces nepalensis NCYC 3413 Under the Influence of pH and Salt" - Sawan Kumar, Pradeep Lal & Sathyanarayana N. Gummadi (2) "Biocontrol of Postharvest Anthracnose of Mango Fruit with Debaryomyces Nepalensis and Effects on Storage Quality and Postharvest Physiology" - Shanshan Luo, Bin Wan, Shuhan Feng, Yuanzhi Shao (3) "Bioconversion of Non-Detoxified Hemicellulose Hydrolysates to Xylitol by Halotolerant Yeast Debaryomyces nepalensis NCYC 3413" - Bhaskar Paidimuddala and Sathyanarayana N Gummadi (4) "Enhanced Production of Pectin Lyase and Pectate Lyase by Debaryomyces nepalensis in Submerged Fermentation by Statistical Methods" - Sathyanarayana N. Gummadi and D. Sunil Kumar (5) "Debaryomyces hansenii Strains Isolated From Danish Cheese Brines Act as Biocontrol Agents to Inhibit Germination and Growth of Contaminating Molds" - Chuchu Huang, Ling Zhang, et al (6) "A comparative study on the potential of epiphytic yeasts isolated from tropical fruits to produce flavoring compounds" - Eric Grondin, Alain Shum Cheong Sing, et al

Debaryomycetaceae	Meyerozyma	Meyerozyma caribbica (= Pichia caribbica)	Pichia caribbica ferment	Kombucha tea culture is a symbiosis of AAB and yeasts, including Meyerozyma caribbica. (1) Meyerozyma caribbica has been isolated from Brazilian fermented table olives (2) M. caribbica is a promising fermenter for alcoholic beverages due to its osmotolerance and high ethanol yield (3) The capability of Meyerozyma caribbica to produce malic acid using glucose and cassava pulp has been reported. Malic acid has many applications as an acidulant and flavor enhancer, which was widely used in the beverage and food industry and used in metal cleaning, fabric dying, watertreatment, textile finishing, agriculture and pharmaceuticals (4) Bibliographical data: (1) "Kombucha Tea: A Promising Fermented Functional Beverage" - Gülşah Özcan Sinir et al (2) "Consumer's acceptability and health consciousness of probiotic and prebiotic of non-dairy products" - Fernanda Cosme, Antonio Ines, Alice Vilela (3)"Yeasts with Fermentative Potential Associated with Fruits of Camu-Camu (Myrciaria dubia, Kunth) from North of Brazilian Amazon" - Ítalo Thiago Silveira Rocha Matos et al (4) "Newly isolated malic acid fermenting yeast Meyerozyma caribbica AY 33-1 for bioconversion of glucose and cassava pulp" - Thannapat Rattanapatpokin, Chakrit Tachaapikoon, et al
Debaryomycetaceae	Debaryomyces	Priceomyces carsonii	Yeast extract	Patricia Lappe-Oliveras , Ruben Moreno-Terrazas et al., (2008), "Yeasts associated with the production of Mexican alcoholic nondistilled and distilled Agave beverages" Debaryomyces carsonii has been found in a variety of processed foods such as fruit juices and soft drinks, wine, beer, sugary products, bakery products, dairy products and meat or processed meats. The presence of Debaryomyces species in foods usually has no detrimental effects and in some cases is beneficial to the food. For example, it has been isolated from Alpechin (energy drink ?) and Wine. ("Sequence-based identification of species belonging to the genus Debaryomyces" - Patricia Martorell, M. Teresa Fernandez-Espinar, et al)
Debaryomycetaceae	Scheffersomyces	Scheffersomyces stipitis	-Yeast extract - Pichia ferment lysate filtrate	not a direct food use: Pichia stipitis is capable of fermenting glucose, xylose, galactose, and cellobiose under anaerobic conditions. Pichia stipitis has the highest native capacity of xylose fermentation among known microbes (1) P. stipitis has pentose-fermenting capabilities, and is one of the most studied yeasts regarding its biochemistry of xylose pathway and xylose conversion into ethanol (2) Pichia stipitis is a promising species for production of bioethanol (3) Pichia stipitis is largely used in cellulosic ethanol industry (4) Pichia stipitis is a lignocellulose-bioconverting and xylose-fermenting yeast (5) Bibliographical data : (1) "Ethanol Production from Biomass" - Haruki Ishizaki, et al (2) "Strategies on simultaneous fermentation of pentose and hexose to bioethanol" - Man Zhou, Xin Lü, in (3) "Potentials of postharvest rice crop residues as a source of biofuel" - Pratyush Kumar Das, et al (3) "Current Bioenergy Researches" - Naveen Kumar Mekala, et al (4) "Genome sequence of the lignocellulose-bioconverting and xylose-fermenting yeast Pichia stipitis" - Thomas W Jeffries, et al

Dipodascaceae	Geotrichum	Geotrichum candidum	- Galactomyces ferment filtrate	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "Food fermentations: Microorganisms with technological beneficial use" Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 Geotrichum candidum is a key agent in the ripening of cheese. It is widely used to obtain mottled white skin, grown on the surface of a soft cheese (1) Geotrichum candidum is used as a culture for cheesemaking and in some traditional fermented milks. The development of G. candidum is typical for many mold-ripened, smear-ripened, and acid-coagulated cheeses. Geotrichum candidum contributes to the characteristic appearance, taste, and aroma of these cheeses (2) G. candidum is listed in the permitted ingredients of the Standard for Coulommiers and of the Standard for Camembert of FAO/WHO Codex Alimentarius (3) Bibliographical data : (1) "Diversity of Geotrichum candidum strains isolated from traditional cheesemaking fabrications in France" - N Marcellino, et al (2) "Yeasts and Molds | Geotrichum candidum" - F. Eliskases-Lechner, et al (3) Standard for Coulommiers and of the Standard for Camembert of FAO/WHO Codex Alimentarius
Dipodascaceae	Dipoascus	Dipodascus fermentans		not direct food use: Geotrichum fermentans is a species that may reduce mycotoxins in wheat (feed wheat, for food and agriculture industry) (1) Pretreated rice straw hydrolysate as a substrate for microbial lipid production by Geotrichum fermentans, also known as Trichosporon fermentans, was evaluated (2) An optimization for efficient sugar conversion has not yet been achieved. Identification of oleaginous yeasts capable of simultaneous glucose and xylose conversion is thus critical when seeking to improve lipid production efficiency. Efforts have been made to find such yeast. For example, Geotrichum fermentans (formerly Trichosporon fermentans) engage in simultaneous glucose and xylose consumption from detoxified lignocellulosic hydrolysates. => it has been found that detoxified rice straw hydrolysate could be used for lipid production by G. fermentans.(3) The cultivation of G. fermentans in wheat flour and composite fodder for sucker pigs enables a significant diminution of the mycotoxins amount in these feed cereals => this yeast strain may be a promising mean to reduce or prevent the adverse effects of mycotoxins on animal health and production safety (4) Bibliographical data : (1) "The influence of different species of yeast on mycotoxins concentrations in wheat" - Gintarė VAIČIULIENĖ, et al (2) "Mechanistic insights into the effect of imidazolium ionic liquid on lipid production by Geotrichum fermentans" - Li-Ping Liu, et al (3) "Lipid production via simultaneous conversion of glucose and xylose by a novel yeast, Cystobasidium iriomotense" - Ayumi Tanimura, et al (4) "Toxin-producing fungi on feed grains and application of yeasts for their detoxification" - J. Repeckiene, et al
Debaryomycetaceae	/	Candida oleophila	Yeast extract	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 As part of the exploration of alternatives for reducing ethanol in wines, the fermentation capacity of C. oleophila in monoculture and in sequential fermentations to produce Chilean Sauvignon Blanc wine was studied. (1) Candida oleophila has been identified as naturally present in rose apples and passion fruit in Madagascar. (2) Candida oleophila Strain O is found naturally on plant tissues (fruit, flowers, wood) and in water. This yeast was originally isolated from golden apples and is intended to be used as an antagonist to control fungal pathogens, grey rot (Botrytis cinerea) and blue rot (Penicillium expansum), which cause post-harvest rot on apples and pears. Based on the required toxicity and pathogenicity testing, no risk to human health is expected when the product containing Candida oleophila strain O is used according to label directions (US EPA). (⚠ This source also indicates that incidents of respiratory hypersensitivity have been reported by workers not wearing personal protective equipment during production involving large quantities of C. Oleophila) In 2009, the Environmental Protection Agency established a permanent exemption from the requirement for a tolerance for residues of the microbial pesticide C. oleophila strain O on apples and pears when applied/used as a post-harvest biofungicide.(3)

Debaryomycetaceae	/	Candida saitoana	- Yeast extract - Hydrolyzed candida saitoana	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 "Candida Saitoana is widely investigated for its biocontrol properties. Indeed, the fungicidal properties of Candida Saitoana, by means of its chitinases, are effective against several microorganisms, such as B. cinerea. (1) As a matter of fact, biocontrol activity of Candida Saitoana and its interaction with Botrytis Cinerea in apple wounds have been demonstrated. (2) Candida Saitoana is an antagonist microorganism that is also able to protect potatoes from Phytophthora infestans' contamination. (3) Moreover, efficacy of the combination of Candida Saitoana and 0.2% 2-deoxy-D-glucose as a treatment for the biological control of postharvest diseases of apple and citrus fruits has been determined and showed positive results. (4) Furthermore, Candida Saitoana is commonly used in animal feeds as a probiotic additive. (5) Besides, according to Bio-Pesticides DataBase of the University of Hetforshire, no adverse effects are identified or expected in products containing Candida saitoana biopesticides. (6)" Bibliographical data : (1) Swiontek Brzezinska M, Jankiewicz U, Burkowska A, Walczak M. Chitinolytic Microorganisms and Their Possible Application in Environmental Protection. Curr Microbiol. 1 janv 2014;68(1):71-81. (2) El-Ghaouth A, Wilson CL, Wisniewski M. Ultrastructural and Cytochemical Aspects of the Biological Control of Botrytis cinerea by Candida saitoana in Apple Fruit. Phytopathology. avr 1998;88(4):282-91. (3) Hadwiger LA, McDonel H, Glawe D. Wild Yeast Strains as Prospective Candidates to Induce Resistance Against Potato Late Blight (Phytophthora infestans). Am J Potato Res. 1 juin 2015;92(3):379-86. (4) Control of Decay of Apple and Citrus Fruits in Semicommercial Tests with Candida saitoana and 2-Deoxy-d-glucose | Elsevier Enhanced Reader (5) Bovill R, Bew J, Robinson S. Comparison of selective media for the recovery and enumeration of probiotic yeasts from animal feed. International Journal of Food Microbiology. 20 juill 2001;67(1):55-61. (6) Lewis KA, Tzilivakis J, Warner DJ, Green A. International database for pesticide risk assessments and management. Human and Ecological Risk Assessment: An International Journal. 18 mai 2016;22(4):1050-64.
Dipodascaceae	Yarrowia	*Yarrowia lipolytica*	- Yeast extract - Yarrowia lipolytica extract - Yarrowia lipolytica ferment lysate - Yarrowia lipolytica Oil	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022
Endomycetaceae	Endomyces	Endomyces decipiens		not direct food use : Endomyces decipiens is studied for its potency as a biocontrol agent of ochratoxin a-producing fungi and its effect on arabica coffee taste (1) Geotrichum decipiens are found in the basidiocarps of Armillaria fungi species (2) Bibliographical data : (1) "Potency of yeast as a biocontrol agent of ochratoxin a-producing fungi and its effect on arabica coffee taste" - Okky S. Dharmaputra, et al (2) Book "The yeasts, Fifth edition" 27.3 Dipodascus Armillariae W. Gams (1983) - G. Sybren de Hoog, Maudy Th. Smith
Metschnikowiaceae	Metschnikowia	*Metschnikowia agaves*	- Hydrolyzed Metschnikowia agaves extract - Metschnikowia agaves extract - Yeast extract	Lin, Xue; Hu, Xiaoping; Wang, Qingke; Li, Congfa, (2020), "Improved flavor profiles of red pitaya (Hylocereus lemairei) wine by controlling the inoculations of Saccharomyces bayanus and Metschnikowia agaves and the fermentation temperature" Ten strains of M. agaves were isolated from basal leaf necroses of agave plants (Agave tequilana var. azul) used in the production of tequila. (1) Fresh agaves contains Metschnikowia agaveae. M. agaveae is part of the yeast communities identified in tequila fermentation. Major components of the yeasts of agave roots, including M. agaveae, share the common characteristics of being moderately fermentative and of utilizing a relatively large number of carbon compounds (2) M. agaves is being studied as potential anti-aging agents in cosmetics. (pas alimentarité, mais cosmétiques) (3) Bibliographical data : (1) "Metschnikowia agaveae sp.nov., a heterothallic haploid yeast from blue agave" - Marc André Lachance (2) "Yeast communities in a natural tequila fermentation" - Marc André Lachance (3) "Development of a Transformation Method for Metschnikowia borealis and other CUG-Serine Yeasts" - Zachary B. Gordon

Metschnikowiaceae	Metschnikowia	*Metschnikowia gruessii*	Yeast extract	*not direct food use data :* *Persimmon (Diospyros kaki Thumb.); a Korean medicinal plant, is inhabitated by Metschnikowia gruessi, which is mainly found in the nectar. (1)* *M. gruessi is a cosmopolitan yeast species that inhabits floral nectar.(2)* *M. gruessi is a members of a yeast clade that is adapted to a wide variety of habitats, including flowers and their pollinators. (3)* *Insects, in particular bees, are the vectors distributing yeasts among flowers. Moreover, morphological adaptation of Metschnikowia gruessii to its dispersal by bumblebees (Bombus sp.) and honeybees (Apis mellifera) is described. M. gruessii may over-winters inside bumblebees and is inoculated into floral nectars in early spring. M. gruessi has been isolated from various plant families (Asteraceae, Berberidaceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Dipsacaceae, Ericaceae, Fabaceae, Hydrophyllaceae, Lamiaceae, Liliaceae, Onagraceae, Ranunculaceae, Rosaceae, Scrophulariaceae, Solanaceae). The isolation substrates were mostly Nectar, and Bombus spec (surface and proboscis), but also in Honey pots (from Bumblebee honey). (4)* *Bibliographical data :* *(1) "Characterization of a novel yeast species Metschnikowia persimmonesis KCTC 12991BP (KIOM G15050 type strain) isolated from a medicinal plant, Korean persimmon calyx (Diospyros kaki Thumb)" - Young Min Kang, et al* *(2) Myco Cosm - https://mycocosm.jgi.doe.gov/Metgru1/Metgru1.home.html* *(3) "Nectar yeasts of the Metschnikowia clade are highly susceptible to azole antifungals widely used in medicine and agriculture" - Sergio Alvarez-Perez, et al* *(4) "Ecology of yeasts in plant–bumblebee mutualism in Central Europe" - Michael Brysch-Herzberg*
Metschnikowiaceae	Metschnikowia	*Metschnikowia koreensis*	Yeast extract	Used for beer fermentation (https://www.rolling-beers.fr/fr/the-yeast-bay/4014-metschnikowia-reukaufii.html) Metschnikowia reufkaufii is used for its ability to act on the skin microbiota and in particular on the microbiota of mature skin. Phylogenically, this species is very close to Metschnikowia koreensis. Other Metschnikowia species are used in the food industry, particularly in beer and cheese fermentation. Among the non-Saccharomyces wine yeasts, Metschnikowia is one of the most studied genera due to its widespread presence and impact in winemaking, and has been found in vine phyllospheres, fruit flies, grapes and wine fermentations as part of the resident microbiota of wineries and winemaking equipment. The versatility that allows some Metschnikowia species to be used in winemaking is based on an ability to grow in combination with other yeast species, such as S. cerevisiae, during the early stages of wine fermentation, thereby modulating the synthesis of secondary metabolites during fermentation to improve the sensory profile of the wine. Metschnikowia has moderate fermentation power, some interesting enzymatic activities involving aroma and colour precursors, and potential antimicrobial activity against yeasts and spoilage fungi, making this yeast an interesting tool for improving wine quality. The properties were mainly determined from studies on Metschnikowia pulcherrima wine strains. Bibliographical data : WO2019149754 EXTRAIT DE METSCHNIKOWIA REUKAUFII ET UTILISATION EN COSMETIQUE (wipo.int) Mendonça-Hagler, L. C.; Hagler, A. N.; Kurtzman, C. P. Phylogeny of Metschnikowia Species Estimated from Partial RRNA Sequences. Int. J. Syst. Bacteriol. 1993, 43 (2), 368–373 Vicente, J.; Ruiz, J.; Belda, I.; Benito-Vázquez, I.; Marquina, D.; Calderón, F.; Santos, A.; Benito, S. The Genus Metschnikowia in Enology. Microorganisms 2020, 8 (7), 1038
Metschnikowiaceae	Metschnikowia	*Metschnikowia pulcherrima*	Yeast extract	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 M. pulcherrima exhibits a broad biotechnological potential for application in various industrial processes (1) D-Arabitol was produced by using Metschnikowia pulcherrima. (2) M. pulcherrima is naturally present on the apples, and would be interesting for production of ice cider. (3) Strains belonging to M. pulcherrima clade exhibit a broad spectrum of enzymatic activities and antimicrobial potential, and have been proposed as non-saccharomyces starter cultures for winemaking. (4) Bibliographical data : (1) "Finding a correct species assignment for a Metschnikowia strain: insights from the genome sequencing of strain DBT012" - Eleonora Troiano, et al "Survey of arthropod assemblages responding to live yeasts in an organic apple orchard" - Stefanos S. Andreadis, et al (2) EUROPEAN PATENT APPLICATION - 03.01.2001 - https://patentimages.storage.googleapis.com/30/fe/a0/73439b09fb53c3/EP1065276A1.pdf (3) "Yeasts in Brännland Cider's spontaneously fermented ice cider" - Ella Råhlén and Daniel Eriksson (4) "Expanding the biotechnological potential of Metschikowia pulcherrima/frucicola clade for wine-related applications" - E. Troiano, et al

Metschnikowiaceae	Metschnikowia	*Metschnikowia reukaufii*	- Yeast extract - Hydrolyzed Metschnikowia reukaufii extract - Metschnikowia reukaufii lysate extract	Used for beer fermentation (https://www.rolling-beers.fr/fr/the-yeast-bay/4014-metschnikowia-reukaufii.html) Metschnikowia reufkaufii is used for its ability to act on the skin microbiota and in particular on the microbiota of mature skin. Phylogenically, this species is very close to Metschnikowia koreensis. Other Metschnikowia species are used in the food industry, particularly in beer and cheese fermentation. Among the non-Saccharomyces wine yeasts, Metschnikowia is one of the most studied genera due to its widespread presence and impact in winemaking, and has been found in vine phyllospheres, fruit flies, grapes and wine fermentations as part of the resident microbiota of wineries and winemaking equipment. The versatility that allows some Metschnikowia species to be used in winemaking is based on an ability to grow in combination with other yeast species, such as S. cerevisiae, during the early stages of wine fermentation, thereby modulating the synthesis of secondary metabolites during fermentation to improve the sensory profile of the wine. Metschnikowia has moderate fermentation power, some interesting enzymatic activities involving aroma and colour precursors, and potential antimicrobial activity against yeasts and spoilage fungi, making this yeast an interesting tool for improving wine quality. The properties were mainly determined from studies on Metschnikowia pulcherrima wine strains. Bibliographical data : WO2019149754 EXTRAIT DE METSCHNIKOWIA REUKAUFII ET UTILISATION EN COSMETIQUE (wipo.int) Mendonça-Hagler, L. C.; Hagler, A. N.; Kurtzman, C. P. Phylogeny of Metschnikowia Species Estimated from Partial RRNA Sequences. Int. J. Syst. Bacteriol. 1993, 43 (2), 368–373 Vicente, J.; Ruiz, J.; Belda, I.; Benito-Vázquez, I.; Marquina, D.; Calderón, F.; Santos, A.; Benito, S. The Genus Metschnikowia in Enology. Microorganisms 2020, 8 (7), 1038
Metschnikowiaceae	Metschnikowia	*Metschnikowia rubicola*		Different yeasts are naturally present on the apples, including M. rubicola, which could be used for production of ice cider. ("Yeasts in Brännland Cider's spontaneously fermented ice cider" - Ella Råhlén and Daniel Eriksson)
Metschnikowiaceae	Metschnikowia	*Metschnikowia viticola*	*-Metschnikowia viticola extract*	M. viticola is part of the most species naturally found in wine environments with well-established antimicrobial activities(1) M. viticola has been isolated from grapes grown in Hungary (2) M. viticola has been isolated grom grape barrie. It is a species present in chardonnay and pinot noir grape varieties (3) M. viticola has been indigenously isolated in Denmark, and was used in sequential fermentations with S. cerevisiae on three cool-climate grape cultivars, which resulted in richer berry and fruity flavours in wines. (4) Bibliographical data : (1) "Metschnikowia pulcherrima as biocontrol agent and wine aroma enhancer in combination with a native Saccharomyces cerevisiae" - Laura Canonico, et al (2) "Metschnikowia viticola sp. nov., a new yeast species from grape" - Gabor Peter, et al (3) "Diversity of indigenous microflora of vineyards from Burgundy and Rhone Valley" - Mohand Sadoudi, et al (4) "Effect of sequential fermentations and grape cultivars on volatile compounds and sensory profiles of Danish wines" - Jing Liu, et al Javier Vicente, Javier Ruiz, (2020), "The Genus Metschnikowia in Enology", Microorganisms"
Phaffomycetaceae	Wickerhamomyces	Wickerhamomyces anomalus	- Yeast extract - Pichia anomala extract	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 The yeast Pichia anomala has been isolated from a variety of habitats, both man-made and natural. It is frequently found in fermented drinks and foods where it plays a role in the spontaneous fermentation. Pichia anomala is frequently associated with food and feed products, either as a production organism or as a spoilage yeast. It belongs to the nonSaccharomyces wine yeasts and contributes to the wine aroma by the production of volatile compounds (1) Pichia anomala SKM-T was used as a starter for making white pan bread to extend the shelf-life and to improve flavor properties.(2) Bibliographical data: (1) "Biotechnology, physiologyand genetics of the yeast Pichia anomala" - Volkmar Passoth, et al (2) "Production of white pan bread leavened by Pichia anomala SKM-T" - Eun Kyoung Mo, et al
Phaffomycetaceae	Barnettozyma	Barnettozyma populi (=Pichia populi)	- Yeast extract - Pichia Ferment lysate filtrate (from Pichia populi or from Pichia stipitis)	not a direct food use : As a part of the research projet "Technologies to Improve Conversion of Biomass-Derived Sugars to Bioproducts", it has been found that Barnettozyma populi is capable of a great production of arabitolfree xylitol from a mixture of xylose and arabinose. This culture strategy will greatly benefit xylitol production from hemicellulosic hydrolysates, which often contain glucose ("Production of xylitol from mixed sugars of xylose and arabinose without co-producing arabitol" - Badal C. Saha, et al "Optimization of xylitol production from xylose by a novel arabitol limited co-producing Barnettozyma populi NRRL Y-12728" - Badal C. Saha, et al)
Phaffomycetaceae	Komagataella	Komagataella pastoris (= Pichia pastoris)	- Pichia pastoris Ferment filtrate - Pichia ferment extract filtrate	Sebastian C Spohner, Hagen Müller et al. (2015), "Expression of enzymes for the usage in food and feed industry with Pichia pastoris", J Biotechnol

Pichiaceae	Ogataea	Ogataea siamensis	Yeast extract	Pichia spp. (Pichia siamensis is a synonym for Ogataea siamensis) is one of the yeasts found in pulque, according to several studies carried out between 1993 and 2008. Pulque is a pre-Hispanic drink obtained by spontaneous fermentation of the sweet juice harvested from a cavity dug in the centre of various species of agave. The drink was consumed in Mexico mainly during Aztec religious ceremonies. (Patricia Lappe-Oliveras, Rubén Moreno-Terrazas et al. (2008), "Yeasts associated with the production of Mexican alcoholic nondistilled and distilled Agave beverages",FEMS Yeast Research)
Pichiaceae	Ogataea	Ogataea minuta	- Yeast extract - Pichia minuta extract	Yue Suna,Yanlin Liu, (2014), "Investigating of yeast species in wine fermentation using terminal restriction fragment length polymorphism method", Food microbiology not a direct Food use : Pichia minuta has been isolated from olive trees cultures (Cacerena cultivar) ("Plant-Based Fermented Food and Beverage Technology, Second Edition" - Y. H. Hui, E. Özgül Evranuz)
Pichiaceae	Ogataea	Ogataea naganishii	Yeast extract	Not a food use : O. naganishii has been isolated from plant exudates ("Early Ongoing Speciation of Ogataea uvarum Sp. Nov. Within the Grape Ecosystem Revealed by the Internal Variability Among the rDNA Operon Repeats" - Luca Roscini, et al)
Pichiaceae	Pichia	*Pichia heedii*	- Yeast extract - Pichia heedi extract	not a direct food use : Pichia heedii has been isolated from the soft-rot of Lophocereus schottii and from Drosophila pachea, which utilizes L. schottii as a host plant. P. heedii has the ability to assimilate D-xylose. P. heedii has also been isolated from Carnegiea gigantea, Pachycereus pringlei, Machaerocereus gummosus (des cactus) and Drosophilia nigrospiracula. ("Pichia heedii, a New Species of Yeast Indigenous to Necrotic Cacti in the North American Sonoran Desert" - H. J. PHAFF, W. T. STARMER, et al)
Pichiaceae	Pichia	Pichia membranifaciens		Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 Pichia membranifaciens is naturally present on apples. (1) P. membranifaciens is one of the yeast species most frequently isolated from acid-curd cheeses (quark, Gervais, cottage cheese, cream cheese). (2) P. membranifaciens is a common brewery contaminant, including in whisky fermentations. (3) Pichia membranifaciens is commonly found in fermented beverages. (4) P. membranificiens can be found in minced and ground meats. (5) Pichia membranifaciens is a yeast that is commonly found on various crops and plants and is known to be involved in alcohol fermentation. P. membranifaciens prevents food spoilage and contamination with its capability to kill competitor microbes that spoil commercial crops and fermented liquids like wine. Due to its osmotolerance, its killer activity towards other yeasts and microbes it competes with, along with its fermentation capabilities, it has recently garnered interest in the scientific community as a potential non-chemical fungicide. Additionally, the yeast's ability to form bioflocculants in wastewater and capability to ferment sugars into ethanol at a high capacity are currently being investigated as potential affordable and sustainable biological solutions to the global water and energy crisis, (6) Bibliographical data: (1) "Yeasts in Brännland Cider's spontaneously fermented ice cider" - Ella Råhlén and Daniel Eriksson (2) "YEASTS AND MOLDS | Yeasts in Milk and Dairy Products" - N.R. BüchlH. Seiler (3) "Contamination: Bacteria and wild yeasts in whisky fermentation" - Nicholas R. Wilson (4) "Chapter 57 - Pichia E.C. Hansen (1904)" - Cletus P. Kurtzman (5) "Volume 1 The Yeasts (Fifth Edition), 2011 - Chapter 5" - Graham H. Fleet (6) Microbe Wiki https://microbewiki.kenyon.edu/index.php/Pichia_membranifaciens

Saccharomycetaceae	Eremothecium	*Eremothecium ashbyii*		Safety and efficacy of the feed additive consisting of Vitamin B/Riboflavin produced by Eremothecium ashbyi CCTCCM 2019833 for all animal species (Hubei Guangji Pharmaceutical Co., Ltd) , EFSA The first microbial riboflavin production processes developed in the 1940s used Eremothecium ashbyi as a production strain. (1) Riboflavin (water-soluble B2 vitamin complex) is a natural yellow food colourant produced using the fungi Eremothecium ashbyii. it often gives the yellow to orange colour of vitamin supplements, and is often included (in this case not as colourant but as vitamin) in fortified cereals and dairy products (breakfast cereals, pastas, sauces, processed cheese, fruit drinks, milks, energy drinks, and baby foods and formula). The synthetic vitamin is approved for use as a food colourant in the United States (Code of Federal Regulations, 2011) and the EU. The latter also accepts usage of riboflavin-5′-phosphate as a food colourant (E101a), but the United States does not. (2) Eremothecium ashbyi is a pathogen of cotton and citrus, and occurs in warmer parts of the northern and southern hemispheres where these crops are normally grown. Eremothecium ashbyi is often used for the industrial production of riboflavin.(3) E. ashbyi has a long history of causing cotton boll rot in various species of Gossypium, and cankers on citrus fruit.(4) Bibliographical data : (1) "Cofactors - 7.02 - Riboflavin Biosynthesis" - Hans-Peter Hohmann, et al (2) "Food colour additives of natural origin" - K. Solymosi, et al (3) Book "The Yeasts, Fifth edition, 2011 - Chapter 30 - Eremothecium Borzi emend. Kurtzman (1995)" - Cletus P. Kurtzman, et al (4) Book "The Yeasts, Fifth edition, 2011 - Chapter 4 - Agriculturally Important Yeasts: Biological Control of Field and Postharvest Diseases Using Yeast Antagonists, and Yeasts as Pathogens of Plants" - David A. Schisler, et al
Saccharomycetaceae	Kluyveromyces	Kluyveromyces lactis	- Hydrolyzed Kluyveromyces extract - Kluyveromyces extract	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 Kluyveromyces marxianus and K. lactis (Kluyveromyces spp.) are the only lactose-fermenting species regularly found in milk and dairy products. Their main role in dairy products is lactose metabolism, but they also possess weak proteolytic and lipolytic activities. (Book "Encyclopedia of Dairy Sciences (second edition) - Yeasts and Molds | Kluyveromyces spp." - C. Belloch)
Saccharomycetaceae	Kluyveromyces	Kluyveromyces marxianus	-	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022
Saccharomycetaceae	Saccharomyces	Saccharomyces cerevisiae	- Yeast extract - Saccharomyces Cerevisiae extract	"Saccharomyces cerevisiae and its industrial applications" - Maria Parapouli, et al Code of Federal Regulation, Title 21, Chapter 1, Subpart B, Part 172
Saccharomycetaceae	Torulaspora	*Torulaspora delbrueckii*	-Yeast extract - Hydrolyzed Torulaspora delbrueckii extract - Torulaspora delbrueckii ferment	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022
Saccharomycetaceae	Zygosaccharomyces	*Zygosaccharomyces rouxii*	Yeast extract	Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use**" Listed in the bulletin of the IDF (**International Dairy Federation**), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 European Commission. Opinion on mannitol manufactured by fermentation. 1999. Rojo M C et al. Incidence of osmophilic yeasts and Zygosaccharomyces rouxii during the production of concentrate grape juices. Food Microbiology. 64 : 7-14. 2017. Escott C et al. Zygosaccharomyces rouxii : Control Strategies and Applications in Food and Winemaking. Fermentation. 2018.

Saccharomycetales incertae sedis	Starmerella	Starmerella magnoliae	Yeast extract	C. magnoliae has been isolated from lime honey from Poland for value-added chemical synthesis. It has been established. C. magnoliae is able to produce kynurenic acid, erythritol, mannitol, and citric acid in different concentrations growing on fructose or technical glycerol. Until now, the best producer of mannitol described in the literature has been C. magnoliae growing in a fed-batch culture. => it has been proven that natural products such as lime honey can be an excellent source of wild-type yeasts with valuable production properties (1) Past studies have singled out Starmerella (Candida) magnoliae as the most common yeast species in honey bee-stored bee bread (bee-bread = pollen stocké = source principale de protéines de la colonie d'abeilles => le pollen est vendu en alimentaire). Starmerella species are ecological specialists with potential biotechnological value. Four samples of unripened honey yielded counts of S. magnoliae. In honey bees, S. magnoliae was one of the most frequently isolated species from honey stomachs and the intestines of almost 200 pollen foragers (2) Candida magnoliae isolated from honeycomb is an industrially important yeast with high erythritol-producing ability. Erythritol has been used as functional sugar substitute for various foods (3) The potential of C. magnoliae to produce low-alcoholic and low-caloric fermented beverages has been evaluated and proven successful as Fruit juice fermentations with C.magnoliae PYCC 2903 revealed a potential for the production of beverages with interesting sensorial properties. C.magnoliae is able to produce sugar alcohols that are also found naturally in fruits and vegetables in small quantities.(4) Biobliographical data : (1) "Honey's Yeast—New Source of Valuable Species for Industrial Applications" - Ziuzia, Patrycja; Janiec, Zuzanna, et al (2) "Specialisation of Yeast Genera in Different Phases of Bee Bread Maturation" - Roxane Detry,et al (3) "Selective Utilization of Fructose to Glucose by Candida magnoliae , an Erythritol Producer" - Ji-Hee Yu, Dae-Hee Lee, et al (4) "Fermentation of fruit juices by the osmotolerant yeast Candida magnoliae - Dissertation for the degree of Master in Biotechnology" - Andreia Sofia Soares de Medeiros
Saccharomycetales incertae sedis	Starmerella	Starmerella bombicola (= candida bombicola)	Hydrolyzed Candida bombicola extract	Starmerella bombicola is one of the most important microbial producers of biosurfactants : sophorolipids, with application potential in food, pharmaceutical, cosmetic and cleaning industries (1) The Sophorolipids produced by S. bombicola are stable molecules that show promising action for the potential replacement of pesticides in the field and the post-harvest process against the main tomato phytopathogens (2) Starmerella bombicola is used to produce sophorolipids (SLs), which are commercially available biosurfactants (3) Starmerella bombicola (formerly Candida stellata) was evaluated for ethanol reduction in wine in a static condition and in a immobilized form with promising results (4) Bibliographical data : (1) "Starmerella bombicola, an industrially relevant, yet fundamentally underexplored yeast" - Marilyn De Graeve, et al (2) "Antimicrobial activity of sophorolipids produced by Starmerella bombicola against phytopathogens from cherry tomato" - Talita de O Caretta, et al (3) "Optimal preparation of food waste to increase its utility for sophorolipid production by Starmerella bombicola" - Ming Ho To, et al (4) "Sequential Fermentation with Selected Immobilized Non-Saccharomyces Yeast for Reduction of Ethanol Content in Wine" - Laura Canonico, et al

Saccharomycodaceae	Hanseniaspora	*Hanseniaspora opuntiae*		Nuno Bourbon-Melo, Margarida Palma, (2021), "Use of Hanseniaspora guilliermondii and Hanseniaspora opuntiae to enhance the aromatic profile of beer in mixed-culture fermentation with Saccharomyces cerevisiae", Food Microbiol An organoleptic evaluation of single-culture fermentations has been performed by Hanseniaspora opuntiae, which has been selected. H. opuntiae has an important contribution to winemaking. H. opuntiae is studied for its potential to favourably enhance the aroma profile of beer. (1) Hanseniaspora opuntiae is a commonly found yeast species in naturally fermenting cocoa pulp-bean mass(2) Hanseniaspora opuntiae is an apiculate yeast normally found on the skins of ripe grapes and at the beginning of alcoholic fermentation. Several studies have reported that this species can provide interesting sensory characteristics to wine by contributing high levels of acetate esters and can increase the mouthfeel and body of wines. H. opuntiae is studied to improve the sensory profile of Albillo Mayor white wines (3) Hanseniaspora opuntiae IST408 was selected to undergo mixed beer fermentations with the commercial strain S. cerevisiae US-05, either inoculated simultaneously or sequentially (4) Bibliographical data : (1) "Use of Hanseniaspora guilliermondii and Hanseniaspora opuntiae to enhance the aromatic profile of beer in mixed-culture fermentation with Saccharomyces cerevisiae" - Nuno Bourbon-Melo (2) "An in-depth multiphasic analysis of the chocolate production chain, from bean to bar, demonstrates the superiority of Saccharomyces cerevisiae over Hanseniaspora opuntiae as functional starter culture during cocoa fermentation" - Cristian Diaz-Munoz, et al (3) "Improving Aroma Complexity with Hanseniaspora spp.: Terpenes, Acetate Esters, and Safranal" - Juan Manuel del Fresno, et al (4) "Use of Hanseniaspora opuntiae in co-fermentation with Saccharomyces cerevisiae to enhance the aromatic profile of craft beer" - Miguel Pinto Rocha
Saccharomycopsidaceae	Saccharomycopsis	Saccharomycopsis fibuligera		Zai-Bin Xie, Kai-Zheng Zhang (2021), "Saccharomycopsis fibuligera in liquor production: A review", European Food Research and Technology Saccharomycopsis fibuligera has been isolated worldwide from high starch substrates, and from studies of the microbiota of cereal-based fermented foods and beverages. Starch hydrolysis is performed by S. fibuligera, and this discovery formed the basis of the Swedish Symba yeast process in which a mixed culture of S. fibuligera and Candida utilis was used to break down potato processing wastes to produce yeast cells for cattle feed. S. fibuligera presents interest in production of α-amylase and glucoamylase. Saccharomycopsis fibuligera is found worldwide in starchy substrates and is the major amylolytic yeast in indigenous food fermentations using rice and cassava. (1) S. fibuligera is predominant in the six brands of Huangjiu (vin jaune chinois, le plus ancien) starter, suggesting that it is crucial for the production performance of Huangjiu starter. S. fibuligera is the main producer of wine aroma and can generate amylase. It has been isolated from Guangxi rice wine (chinois) starter. (2) Saccharomycopsis fibuligera KJJ81 isolated from nuruk (ingrédient traditionnel coréen pour débuter un processus de fermentation alcoolique) is an amylolytic yeast that is widely used as a microbial starter in various fermented foods. (3) The yeast species Saccharomycopsis fibuligera is known to produce exceptionally pleasant plum and berry flavors during brewer's wort fermentation. (4) Bibliographical data : (1) "The yeasts (Fifth edition) - Chapter 63 - Saccharomycopsis Schiönning (1903)" - Cletus P. Kurtzman, et al (2) "Saccharomycopsis fbuligera in liquor production: A review" - Zai-Bin Xie, et al (3) "Bioformation of Volatile and Nonvolatile Metabolites by Saccharomycopsis fibuligera KJJ81 Cultivated under Different Conditions—Carbon Sources and Cultivation Times" - Sang Mi Lee, et al (4) "Beer fermentation performance and sugar uptake of Saccharomycopsis fibuligera–A novel option for low-alcohol beer" - Yvonne Methner, et al
Trichomonascaceae	Wickerhamiella	Wickerhamiella azyma	Yeast extract	Wickerhamiella azyma has been found in tea flowers collected from Nan and Phrae provinces(1) Candida azyma appears to be present on grapes, in fermented beverages and in drosophilia (3) C. azyma was isolated from Bangalore blue and Cabernet varieties (variétés de raisins) grown in different localities. The association of C. azyma with sugarcane (canne à sucre) phylloplane (phylloplane = surface d'une feuille, considéré comme habitat notament pour les µ-org) is known (3) Bibliographical data : (1) Three new yeast species from flowers of Camellia sinensis var. assamica collected in Northern Thailand and their tannin tolerance characterization" - Apinun Kanpiengjai, et al (2) "Ecology and diversity of yeasts in fermented food ecosystems" - Thibault Nidelet, et al (3) "Natural yeast flora of different varieties of grapes used for wine making in India" - Pradnya Chavan, et al

Concentration of Use by FDA Product Category – Yeast Additions*

Hydrolyzed Saccharomyces Cell Wall
Saccharomyces Ferment Extract
Saccharomyces Ferment Extract Lysate Filtrate

Ingredient	Product Category	Maximum Concentration of Use
Saccharomyces Ferment Extract Lysate Filtrate	Moisturizing Products Not spray	 0.25%

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

Information collected in 2023
Table prepared: July 5, 2023

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: October 27, 2023

SUBJECT: Saccharomyces Ferment Lysate Filtrate, Saccharomyces Lysate Extract and Yeast Extract

Anonymous. 2004. Clinical safety evaluation repeated insult patch test (cream (tested as provided) contains 0.0135% Saccharomyces Ferment Lysate Filtrate).

Anonymous. 2008. Clinical safety evaluation repeated insult patch test (cream (tested as provided) contains 0.028% Saccharomyces Lysate Extract).

Anonymous. 2005. Clinical safety evaluation repeated insult patch test (lotion (tested as provided) contains 0.0045% Yeast Extract).

[redacted]

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

cream (tested as provided) contains 0.0135% Saccharomyces Ferment Lysate Filtrate

Sponsor

[redacted]

Sponsor Representatives

[redacted]

Clinical Testing Facility

[redacted]

Sponsor Code: [redacted]
Panel No.:
Entry No.:

Date of Final Report

3-5-04

[redacted]

[redacted] Panel No.:
Entry No.: [redacted]

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

[redacted]

Study Director

3/3/2004
Date

[redacted]

Scientific Director
Principal Investigator

2 March 2004
Date

[redacted]

Board-Certified Dermatologist
Medical Investigator

3/4/04
Date

[redacted]

QUALITY ASSURANCE STATEMENT

This study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in CFR Title 21, Parts 50, 56 and 312 and/or the Declaration of Helsinki, as appropriate.

For purposes of this clinical study:

__X__ Informed Consent was obtained.

_____ Informed Consent was not obtained.

__X__ An IRB review was not required.

_____ An IRB review was conducted and approval to conduct the proposed clinical research was granted.

This study report has been reviewed to assure that it correctly describes the methods of testing and that the reported results accurately reflect the data obtained during the clinical study ([redacted] Panel No.: [redacted]; [redacted] Entry No.: [redacted]).

[redacted]
Manager, Quality Assurance

05 Mar 2004
Date

[redacted]

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[redacted] Panel No.:
Entry No.: [redacted]

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

1.0 OBJECTIVE

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

2.0 SPONSOR

[redacted]

2.1 Sponsor Representatives

[redacted]

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

[redacted]

4.0 CLINICAL INVESTIGATORS

Study Director:
Principal Investigator:
Medical Investigator:

[redacted]

5.0 STUDY DATES

Study initiation: January 7, 2004

Final evaluation: February 12, 2004

[redacted]

6.0 ETHICS

6.1 Ethical Conduct of the Study

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

6.2 Subject Information and Consent

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

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on December 22, 2003 and identified as follows:

[redacted] Entry No.	Test Article I.D.	Physical Description
[redacted]	[redacted]	White Cream

8.0 TEST SUBJECTS

A total of 55 subjects, 9 males and 46 females ranging in age from 21 to 69 years, were empaneled for this test. Subject demographics are listed in Table 1.

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

9.0 TEST PROCEDURE

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

9.1 Induction Phase (patch size 2 cm^2)

A sufficient amount of the test article (an amount to adequately cover the surface of the patch unit-approximately 0.1 g - 0.15 g) was placed onto a Parke-Davis Readi-Bandage® occlusive patch and applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

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

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

9.2 Challenge Phase

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

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

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

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

[redacted]

10.0 RESULTS AND DISCUSSION

(See Table 2 for Individual Scores)

Fifty-two (52/55) subjects satisfactorily completed the test procedure on Test Article: # BOP-0302. Three (3/55) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued panelist data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

Transient, barely perceptible (+) patch test responses were observed on one (1/52) test panelist (Subject No. 10) during the Induction phase of the study. These responses are judged to be non-specific in nature and are not indicative of clinically significant irritation.

There were no responses on any subject during the Challenge phase.

11.0 CONCLUSIONS

Under the conditions of a repeated insult (occlusive) patch test procedure, Test Article: # BOP-0302 was "Dermatologist-Tested" and did not induce clinically significant skin irritation nor show any evidence of induced allergic contact dermatitis in human subjects.

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 1

SUBJECT DEMOGRAPHICS

REPEATED INSULT PATCH TEST – OCCLUSIVE

Test Article: [redacted]

Subject No.	Initials	Sex	Age	Race	Subject No.	Initials	Sex	Age	Race
1	[redacted]	F	45	HS	28	[redacted]	F	22	CE
2		M	45	CE	29		F	55	CE
3		F	50	CE	30		M	28	CE
4		F	43	CE	31		F	46	CE
5		F	60	CE	32		F	49	CE
6		F	62	BA	33		F	52	CE
7		F	56	CE	34		F	48	CE
8		F	42	CE	35		F	51	CE
9		F	56	HS	36		F	44	CE
10		F	59	CE	37		F	41	CE
11		F	49	CE	38		F	46	CE
12		M	37	CE	39		F	48	HS
13		F	54	CE	40		M	53	HS
14		F	56	CE	41		M	43	HS
15		M	69	HS	42		M	61	CE
16		F	66	HS	43		F	21	CE
17		F	43	CE	44		F	55	CE
18		F	43	CE	45		F	50	CE
19		F	68	CE	46		F	45	CE
20		F	61	CE	47		F	43	CE
21		F	49	CE	48		F	53	CE
22		F	50	CE	49		F	38	CE
23		F	53	CE	50		F	51	CE
24		M	44	CE	51		F	49	CE
25		F	63	CE	52		F	49	CE
26		F	35	CE	53		M	62	CE
27		F	65	CE	54		F	53	CE
					55		F	35	CE

BA = Black (African-American)
CE = White (Non-Hispanic)
HS = Hispanic

Shaded Area = Discontinued Subject

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 2

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

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

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

[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 2 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

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

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
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)

[redacted]

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

cream (tested as provided) contains 0.028% Saccharomyces Lysate Extract

Sponsor

[redacted]

Sponsor Representatives

[redacted]

Clinical Testing Facility

[redacted]

Sponsor Code: [redacted]
Panel No.:
Entry No.:

Date of Final Report

5/2/08

[redacted]

[redacted] Panel No.:
Entry No.: [redacted]

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

[redacted]
Laboratory Manager
Study Director

1 May 2008
Date

[redacted]
Scientific Director
Principal Investigator

1 May 2008
Date

[redacted]
Board-Certified Dermatologist
Medical Investigator

Date

[redacted]

QUALITY ASSURANCE STATEMENT

This study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in CFR Title 21, Parts 50, 56 and 312 and/or the Declaration of Helsinki, as appropriate.

For purposes of this clinical study:

__X__ Informed Consent was obtained.

_____ Informed Consent was not obtained.

__X__ An IRB review was not required.

_____ An IRB review was conducted and approval to conduct the proposed clinical research was granted.

This study report has been reviewed to assure that it correctly describes the methods of testing and that the reported results accurately reflect the data obtained during the clinical study ([redacted] Panel No.: [redacted]; [redacted] Entry No.: [redacted]).

[redacted]
Manager, Quality Assurance

02 May 2008
Date

[redacted]

[redacted] Panel No.:
Entry No.: [redacted]

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[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

1.0 OBJECTIVE

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

2.0 SPONSOR

[redacted]

2.1 Sponsor Representatives

[redacted]

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

[redacted]

4.0 CLINICAL INVESTIGATORS

Principal Investigator:
Medical Investigator:
Study Director:

[redacted]

5.0 STUDY DATES

Study initiation: March 17, 2008

Final evaluation: April 25, 2008

[redacted]

6.0 ETHICS

6.1 Ethical Conduct of the Study

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

6.2 Subject Information and Consent

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

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on March 10, 2008 and identified as follows:

[redacted] Entry No.	Test Article I.D.	Description
[redacted]	[redacted]	White Cream*

*The test article was volatilized at least 30 minutes, but less than 90 minutes, on the patch prior to application to the skin.

8.0 TEST SUBJECTS

A total of 55 subjects, 12 males and 43 females ranging in age from 18 to 71 years, were empaneled for this test. Subject demographics are listed in Table 1.

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

9.0 TEST PROCEDURE

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

9.1 Induction Phase

(patch size 2 cm^2)

A sufficient amount of the test article (approximately 0.1 g – 0.15 g) was placed onto a Parke-Davis Readi-Bandage® occlusive patch, which was applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

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

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

9.2 Challenge Phase

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

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

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

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

10.0 RESULTS AND DISCUSSION

(See Table 2 for Individual Scores)

Fifty (50/55) subjects satisfactorily completed the test procedure on Test Article: [redacted]. Five (5/55) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued panelist data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

Induction Phase Summary

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

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

11.0 CONCLUSIONS

Under the conditions of a repeated insult (occlusive) patch test procedure conducted in 50 subjects, Test Article: # SMN-0701 was "Dermatologist-Tested" and was not associated with skin irritation or allergic contact dermatitis in human subjects.

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 1

SUBJECT DEMOGRAPHICS

Test Article: [redacted]

Subject No.	Initials	Age	Sex	Race	Subject No.	Initials	Age	Sex	Race
1	[redacted]	56	F	CA	29	[redacted]	57	F	CA
2		45	F	CA	30		23	F	CA
3		30	F	HS	31		23	F	HS
4		62	F	CA	32		27	M	CA
5		54	M	CA	33		55	M	CA
6		53	F	CA	34		39	F	CA
7		42	F	CA	35		35	F	CA
8		53	F	CA	36		52	F	CA
9		64	F	CA	37		64	F	CA
10		49	M	CA	38		28	F	HS
11		62	F	CA	39		48	F	CA
12		58	F	CA	40		45	F	CA
13		58	F	OT	41		51	F	AS
14		43	F	CA	42		31	F	CA
15		55	F	CA	43		41	M	CA
16		34	F	HS	44		59	M	CA
17		52	F	CA	45		20	F	HS
18		50	F	CA	46		69	M	CA
19		67	M	CA	47		71	F	CA
20		42	F	HS	48		53	F	CA
21		69	F	CA	49		54	M	CA
22		47	M	CA	50		69	F	CA
23		57	F	CA	51		55	F	CA
24		55	F	CA	52		22	F	CA
25		18	F	AS	53		65	F	CA
26		52	M	CA	54		64	F	HS
27		31	F	CA	55		37	F	HS
28		43	M	CA					

AS = Asian or Pacific Islander
CA = Caucasian
HS = Hispanic
OT = Other

TABLE 2

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

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

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

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 2 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

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

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

[redacted]

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

lotion (tested as provided) contains 0.0045% Yeast Extract

Sponsor

[redacted]

Sponsor Representatives

[redacted]

Clinical Testing Facility

[redacted]

Sponsor Code: [redacted]
Panel No.:
Entry No.: [redacted]

Date of Final Report

1-14-05

[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

[redacted]
Study Director

10 Jan 2005
Date

[redacted]
Scientific Director
Principal Investigator

10 Jan 05
Date

[redacted]
Board-Certified Dermatologist
Medical Investigator

1/7/05
Date

[redacted]

QUALITY ASSURANCE STATEMENT

This study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in CFR Title 21, Parts 50, 56 and 312 and/or the Declaration of Helsinki, as appropriate.

For purposes of this clinical study:

__X__ Informed Consent was obtained.

_____ Informed Consent was not obtained.

__X__ An IRB review was not required.

_____ An IRB review was conducted and approval to conduct the proposed clinical research was granted.

This study report has been reviewed to assure that it correctly describes the methods of testing and that the reported results accurately reflect the data obtained during the clinical study ([redacted] Panel No.: [redacted]; [redacted] Entry No.: [redacted]).

[redacted]
Manager, Quality Assurance

11 Jan 2005
Date

[redacted]

TABLE OF CONTENTS

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

1.0 OBJECTIVE

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

2.0 SPONSOR

[redacted]

2.1 Sponsor Representatives

[redacted]

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

[redacted]

4.0 CLINICAL INVESTIGATORS

Study Director:
Principal Investigator:
Medical Investigator:

[redacted]

5.0 STUDY DATES

Study initiation: November 3, 2004

Final evaluation: December 9, 2004

[redacted]

6.0 ETHICS

6.1 Ethical Conduct of the Study

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

6.2 Subject Information and Consent

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

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on October 18, 2004 and identified as follows:

[redacted] Entry No.	Test Article I.D.	Physical Description
[redacted]	[redacted]	Light Green Transparent Viscous Liquid*

*The test article was volatilized at least 30 minutes, but less than 90 minutes on the patch prior to application to the skin.

8.0 TEST SUBJECTS

A total of 55 subjects, 13 males and 42 females ranging in age from 18 to 68 years, were empaneled for this test. Subject demographics are listed in Table 1.

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

9.0 TEST PROCEDURE

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

9.1 Induction Phase

(patch size 2 cm^2)

A sufficient amount of the test article (approximately 0.2 mL) was placed onto a Parke-Davis Readi-Bandage® occlusive patch and applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

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

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

9.2 Challenge Phase

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

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

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

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

10.0 RESULTS AND DISCUSSION

(See Table 2 for Individual Scores)

Fifty-two (52/55) subjects satisfactorily completed the test procedure on Test Article: [redacted] Three (3/55) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued panelist data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

There was no skin reactivity on any subject at any time during the study.

11.0 CONCLUSIONS

Under the conditions of a repeated insult (occlusive) patch test procedure, Test Article: [redacted] was "Dermatologist-Tested" and did not induce skin irritation nor show any evidence of induced allergic contact dermatitis in human subjects.

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 1

SUBJECT DEMOGRAPHICS

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

Subject No.	Initials	Sex	Age	Race	Subject No.	Initials	Sex	Age	Race
1	[redacted]	M	18	BA	29	[redacted]	F	44	HS
2		M	65	BA	30		M	30	CA
3		M	46	CA	31		F	42	CA
4		F	47	CA	32		F	66	CA
5		M	43	CA	33		F	53	CA
6		F	57	CA	34		M	40	HS
7		F	37	CA	35		F	48	CA
8		M	18	BA	36		F	59	CA
9		F	39	CA	37		F	39	HS
10		F	63	CA	38		M	42	HS
11		F	59	CA	39		F	45	CA
12		F	48	CA	40		F	49	CA
13		M	49	CA	41		F	19	HS
14		F	35	CA	42		F	56	CA
15		M	36	CA	43		F	38	CA
16		M	68	CA	44		M	55	HS
17		F	63	CA	45		F	27	BA
18		F	68	CA	46		F	45	CA
19		F	65	CA	47		F	39	CA
20		F	18	CA	48		F	43	BA
21		F	22	CA	49		F	33	HS
22		F	67	CA	50		F	47	CA
23		F	68	CA	51		F	47	CA
24		F	59	CA	52		F	46	CA
25		F	48	CA	53		F	68	CA
26		F	51	CA	54		F	40	CA
27		M	50	CA	55		F	44	HS
28		F	45	CA					

AS = Asian or Pacific Islander
BA = Black/African-American
CA = Caucasian
HS = Hispanic

Shaded area = Discontinued subject

[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 2

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

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

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

[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

TABLE 2 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

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

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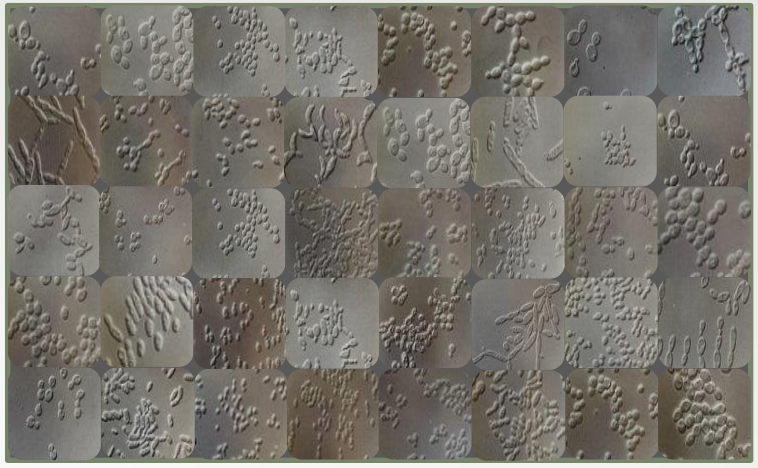
4 = Severe (Deep red erythema with/without vesiculation or weeping)

Yeast-derived Cosmetic Ingredients

September 26 – 27th, 2022

Audrey POKRZYWA
Sylvain MAZALREY
(SILAB)



YEASTS : characteristics & identification (third edition)

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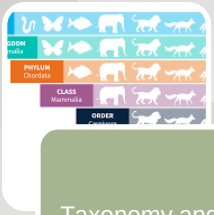
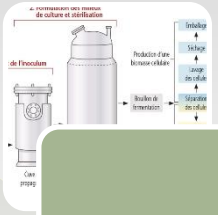
Sourcing
Origin

Safety measures and Identification

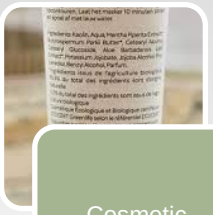


Taxonomy and INCI names



Process

Characterization
Toxicological data

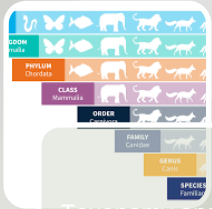


Cosmetic ingredient

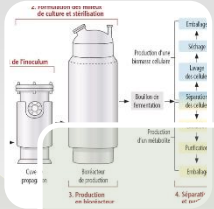
Sourcing

Sourcing

Safety measures and Identification



Taxonomy and INCI names



Process

Characterization

Toxicological data

Cosmetic ingredient

Origin of the strain

- The strains can be sourced from :
  - **Official collection** : ATCC (American Type Culture Collection), CBS (Westerdijk Fungal Bio Diversity Institute) , DSMZ (German Collection of Microorganisms and Cell Cultures), MUCL (Belgian Coordinated Collections of Microorganisms),…

ATCC

WESTERDIJK FUNGAL BIO DIVERSITY INSTITUTE

DSMZ Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH

BCCM BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS

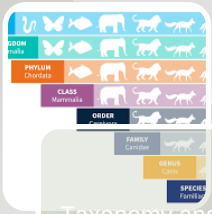
  - **Custom collection** : partnerships with International Centers for Microbial Resources, for example : CIRM dedicated to yeasts in Montpellier (France)

Cirm

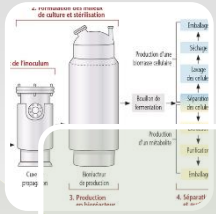
Safety measures, Identification

Sourcing

Safety measures and Identification



Taxonomy and INCI names



Process

Characterization
Toxicological data

Cosmetic ingredient

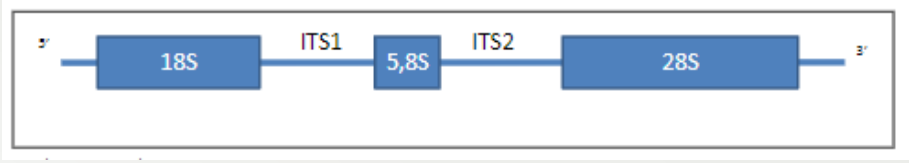
Strain identification

- To be sure to work on the right strain, it is imperative to confirm its taxonomic identification

The best way is by r-28S DNA sequencing and ITS

Principle: Amplification and sequencing of a portion of 28S rRNA encoding the 60S ribosomal subunit. The Internal Transcribed Space (ITS) is a region located on the genomic DNA of eukaryotes between the 28S rRNA and 18S rRNA coding genes. It is composed of three sub-regions: ITS1, ITS2 and the 5.8S gene.

The variability of the ITS seems to favour the identification of the genus and species of fungal populations.

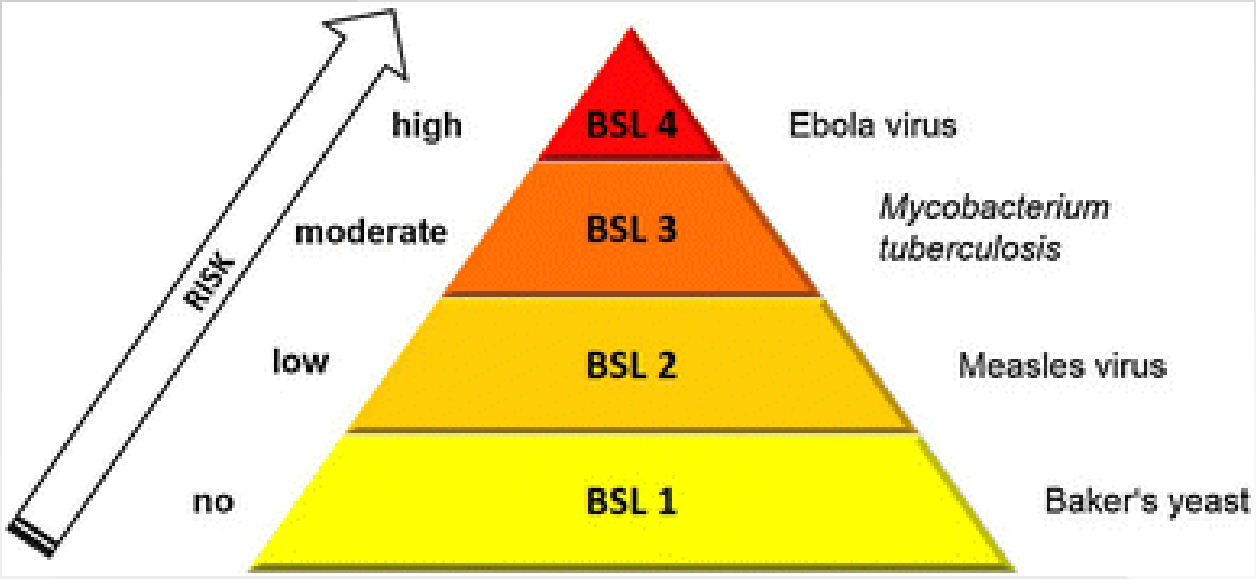


Strain Identification

Biosafety Level	BSL-1	BSL-2	BSL-3	BSL-4
Description	· No Containment · Defined organisms · Unlikely to cause disease	· Containment · Moderate Risk · Disease of varying severity	· High Containment · Aerosol Transmission · Serious/Potentially lethal disease	· Max Containment · "Exotic," High-Risk Agents · Life-threatening disease
Sample Organisms	E.Coli	Influenza, HIV, Lyme Disease	Tuberculosis	Ebola Virus
Pathogen Type	Agents that present minimal potential hazard to personnel & the environment.	Agents associated with human disease & pose moderate hazards to personnel & the environment.	Indigenous or exotic agents, agents that present a potential for aerosol trans-mission, & agents causing serious or potentially lethal disease.	Dangerous & exotic agents that pose a high risk of aerosol-transmitted lab-oratory infections & life-threatening disease.
Autoclave Requirements	None	None	Pass-thru autoclave with Bioseal required in laboratory room.	Pass-thru autoclave with Bioseal required in laboratory room.

Strain Identification

BSL-1 (Biosafety Level One) : this level is defined by the American Centers for Disease Control and Prevention (CDC)



We highly recommend only the use of BSL-1 to manufacture Cosmetic ingredients

Strain Identification

Table 1. ***Classification of infective microorganisms by risk group***

Risk Group 1 *(no or low individual and community risk)*
A microorganism that is unlikely to cause human or animal disease.

Risk Group 2 *(moderate individual risk, low community risk)*
A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 *(high individual risk, low community risk)*
A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 *(high individual and community risk)*
A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Table 2. ***Relation of risk groups to biosafety levels, practices and equipment***

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

World Health Organization

Laboratory safety manual (Third edition)

Safety measures

Collection strain

Culture

Primary stock

verification of the absence of contaminants
yeast count
regrowth check

Stock validation

Securing primary stock at -80°C

One cryotube of Primary stock

culture

Secondary stock

verification of the absence of contaminants
yeast count
regrowth check

Stock validation

Securing secondary stock at -80°C

The freezers at -80°C should be under reinforced surveillance

Stocks must be placed in different freezers at -80°C

Business Continuity Plan

Taxonomy and INCI names

Sourcing

Safety measures and Identification

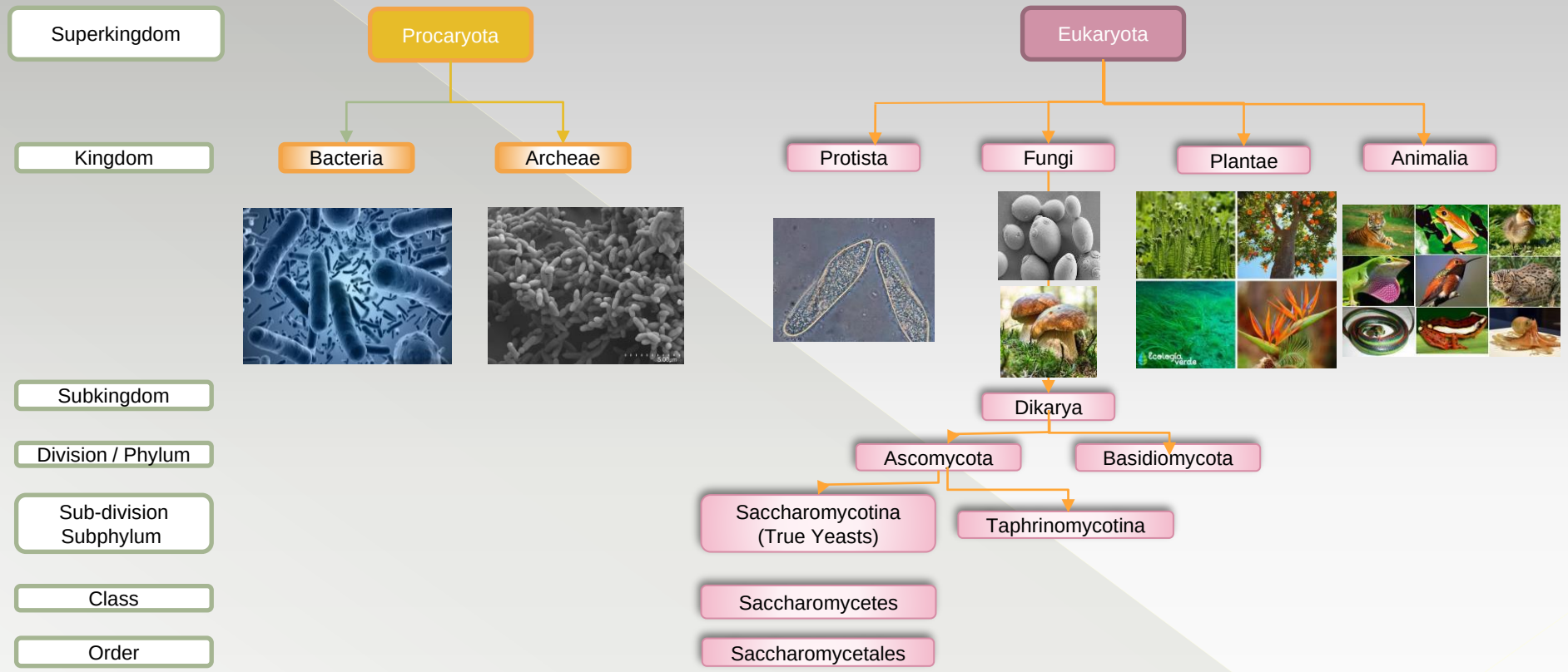
Taxonomy and INCI names

Process

Characterization
Toxicological data

Cosmetic ingredient

Taxonomy



Taxonomy - Definitions

Basionym : the originally described name, attached to the type material and species description

Homotypic synonym : names generated after the basionym (e.g. by moving it to a different genus) but sharing the same type

Heterotypic synonym : names with a different basionym and type from those mentioned above

Source : NCBI Taxonomy: a comprehensive update on curation, resources and tools

INCI names

- The purpose of this report is to be exhaustive regarding all INCI names used (at least declared) in cosmetic products

- That is why, the choice of INCI names to study is based on :

  - the review of all yeast-related INCI names in the **PCPC dictionary**

  - INCI names referenced in the VCRP (Voluntary Cosmetic Registration Program) (2022)

Identification

- The objective is to check their compliance with the PCPC definition of YEAST:

  Yeast is a class of microorganisms (Saccharomycetes) characterized by their lack of photosynthetic ability, existence as unicellular or simple irregular filaments, and reproduction by budding or direct division

- And to study yeasts that belong to all families of the Saccharomycetes class in order to guarantee the completeness of our study

Identification – INCI names

(1/6) – July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
Saccharomycetes	Saccharomycetales	Ascoideaceae	Ascoidea	Ascoidea rubescens	-
		Debaryomycetaceae	Kurtzmaniella	Candida oleophila => Undergoing modification	-
			-	Candida saitoana => Undergoing modification	Hydrolyzed Candida Saitoana Extract
			Debaryomyces	Debaryomyces maramus	-
			Debaryomyces	Debaryomyces nepalensis	-
			Meyerozyma	Meyerozyma caribbica Basionym: Pichia caribbica	Pichia Caribbica Ferment
			Debaryomyces	Priceomyces carsonii Homotypic synonym : Debaryomyces carsonii Basionym: Pichia carsonii	-
			Scheffersomyces	Scheffersomyces stipitis Basionym :Pichia stipitis Homotypic synonym : Yamadazyma stipitis	Pichia Ferment Lysate Filtrate

Source: NCBI : National Center for Biotechnology Information

Identification – INCI names

(2/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
Saccharomycetes	Saccharomycetales	Dipodascaceae	Geotrichum	Geotrichum candidum Basionym: Endomyces geotrichium Heterotypic basionym : Galactomyces candidus	Galactomyces Ferment Filtrate
			Dipoascus	Dipodascus fermentans Basionym: Trichosporon fermentans Homotypic synonym: Galactomyces fermentans	Galactomyces Ferment Filtrate
			Yarrowia	Yarrowia lipolytica Basionym : Endomycopsis lipolytica Heterotypic synonym : Mycotorula lipolytica	Yarrowia Lipolytica Extract Yarrowia Lipolytica Ferment Lysate Yarrowia Lipolytica Oil
		Endomycetaceae	Endomyces	Endomyces decipiens	-

Identification – INCI names

(3/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
Saccharomycetes	Saccharomycetales	Metschnikowiaceae	Metschnikowia	Metschnikowia agaves	Hydrolyzed Metschnikowia Agaves Extract Metschnikowia Agaves Polysaccharides Metschnikowia Agaves Extract
			Metschnikowia	Metschnikowia bicuspidata Basionym: Monospora bicuspidata	-
			Metschnikowia	Metschnikowia gruessii	-
			Metschnikowia	Metschnikowia hawaiiensis	-
			Metschnikowia	Metschnikowia henanensis	Metschnikowia Henanensis Extract
			Metschnikowia	Metschnikowia hibisci	-
			Metschnikowia	Metschnikowia koreensis	-
			Metschnikowia	Metschnikowia lunata	-
			Metschnikowia	Metschnikowia pulcherrima Heterotypic synonym: Candida pulcherrima	-
			Metschnikowia	Metschnikowia reukaufii Heterotypic synonym: Candida reukaufii	Hydrolyzed Metschnikowia Reukaufii Extract Metschnikowia Reukaufii Lysate Extract
			Metschnikowia	Metschnikowia rubicola	-
			Metschnikowia	Metschnikowia shanxiensis	Hydrolyzed Metschnikowia Shanxiensis Extract
			Metschnikowia	Metschnikowia viticola	Metschnikowia Viticola Extract

Identification – INCI names

(4/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
Saccharomycetes	Saccharomycetales	Phaffomycetaceae	Wickerhamomyces	Wickerhamomyces alni Homotypic synonym : Pichia alni	
			Barnettozyma	Barnettozyma populi Basionym : Hansenula populi Homotypic synonym : Pichia populi	Pichia Ferment Lysate Filtrate
			Komagataella	Komagataella pastoris Basionym : Zygosaccharomyces pastoris Homotypic synonym : Pichia pastoris	Pichia Ferment Extract FIltrate Pichia Pastoris Ferment FIltrate Pichia Ferment Lysate Filtrate
			Wickerhamomyces	Wickerhamomyces anomalus Basionym: Saccharomyces anomalus Homotypic synonym : Pichia anomala	Pichia Anomala Extract
		Pichiaceae	Ogataea	Ogataea minuta Basionym : Hansenula minuta Homotypic synonym : Pichia minuta	Pichia Minuta Extract
			Ogataea	Ogataea naganishii Basionym : Pichia naganishii	-
			Ogataea	Ogataea siamensis Basionym: Pichia siamensis	-
			Pichia	Pichia heedii	Pichia Heedii Extract
			Pichia	Pichia membranifaciens Basionym : Saccharomyces menbranifaciens	-
			Pichia	Pichia	Pichia Extract

Identification – INCI names

(5/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
Saccharomycetes	Saccharomycetales	Saccharomycetaceae	Eremothecium	Eremothecium ashbyii	-
			Kluyveromyces	Kluyveromyces lactis Basionym: Torulapora lactis Homotypic synonym: Saccharomyces lactis	Kluyveromyces Extract
			Kluyveromyces	Kluyveromyces marxianus Basionym : Saccharomyces marxianus Heterotypic synonym : Kluyveromyces fragilis Homotypic synonym : Dekkeromyces marxianus	Hydrolyzed Kluyveromyces Extract
			Saccharomyces	Saccharomyces cerevisiae	Saccharomyces Cerevisiae Extract
			Saccharomyces	Saccharomyces sp.	Saccharomyces Saccharomyces Extract Saccharomyces Ferment Filtrate Saccharomyces Ferment Lysate Filtrate Saccharomyces Ferment Saccharomyces Lysate Extract Filtrate Saccharomyces Lysate Extract Saccharomyces Lysate Saccharomyces Polypeptides Saccharomyces
			Torulaspora	Torulaspora delbrueckii Basionym: Saccharomyces delbrueckii	Torulaspora Delbrueckii Extract Torulaspora Delbrueckii Ferment Hydrolyzed Torulaspora Delbruekii Extract

Identification – INCI names

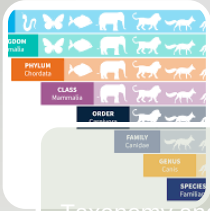
(6/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
Saccharomycetes	Saccharomycetales	Saccharomycetaceae	Zygosaccharomyces	Zygosaccharomyces rouxii Basionym : Saccharomyces rouxii	-
		Saccharomycetales incertae sedis	Starmerella	Starmerella magnoliae Basionym: Torulaspis magnoliae Homotypic synonym: Candida magnoliae	-
		Saccharomycetales incertae sedis	Starmerella	Starmerella bombicola Heterotypic synonym : Candida bombicola	Hydrolyzed Candida Bombicola Extract
		Saccharomycodaceae	Hanseniaspora	Hanseniaspora opuntiae	-
		Saccharomycopsidaceae	Saccharomycopsis	Saccharomycopsis fibuligera	-
		Trichomonascaceae	Wickerhamiella	Wickerhamiella azyma Current name : Candida azyma ; Basionym : Torulopsis azyma	-

Process

Sourcing

Safety measures and Identification



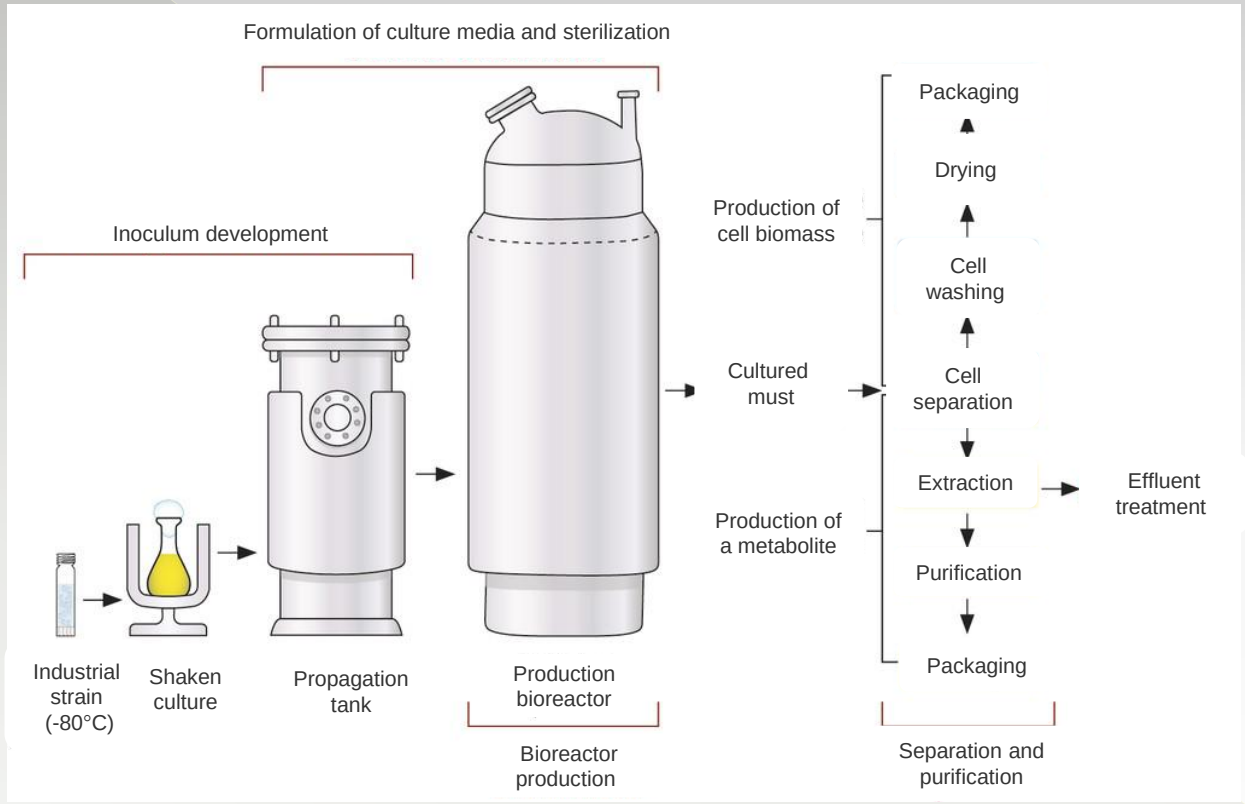
Taxonomy and INCI names

Process

Characterization
Toxicological data

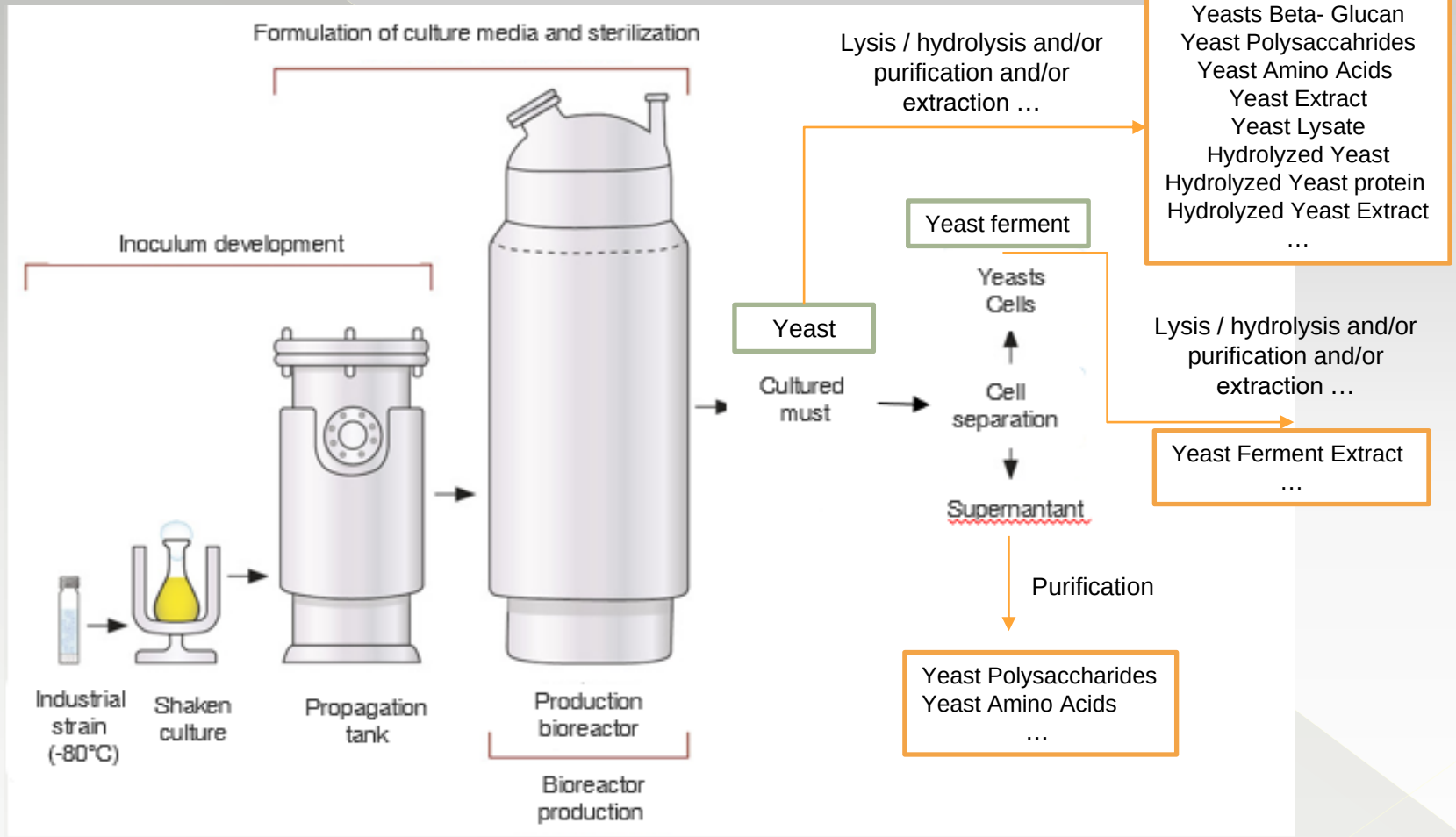
Cosmetic ingredient

Process => culture of Yeast



Technologies des bioprocédés industriels, 2e édition, Louis Tessier

INCI names linked to Manufacturing process



Identification - INCI names

Hydrolyzed Candida Saitoana Extract
Pichia Caribbica Ferment
Pichia Ferment Lysate Filtrate
Galactomyces Ferment Filtrate
Yarrowia Lipolytica Extract
Yarrowia Lipolytica Ferment Lysate
Yarrowia Lipolytica Oil
-Hydrolyzed Metschnikowia Agaves Extract
Metschnikowia Agaves Polysaccharides
Metschnikowia Agaves Extract
Metschnikowia Henanensis Extract
Hydrolyzed Metschnikowia Reukaufii Extract
Metschnikowia Reukaufii Lysate Extract
Hydrolyzed Metschnikowia Shanxiensis Extract
Metschnikowia Viticola Extract
Pichia Anomala Extract
Pichia Ferment Extract FIltrate
Pichia Pastoris Ferment FIltrate
Pichia Ferment Lysate Filtrate
Pichia Heedii Extract
Pichia Extract
Hydrolyzed Kluyveromyces Extract
Kluyveromyces Extract
Saccharomyces Cerevisiae Extract
Saccharomyces Extract
Saccharomyces
Saccharomyces Ferment Filtrate
Saccharomyces Ferment Lysate Filtrate
Saccharomyces Ferment
Saccharomyces Lysate Extract Filtrate
Saccharomyces Lysate Extract
Saccharomyces Lysate
Saccharomyces Polypeptides
Saccharomyces
Saccharomyces Extract
Saccharomyces
Saccharomyces Ferment Filtrate
Saccharomyces Ferment Lysate Filtrate
Saccharomyces Ferment
Saccharomyces Lysate Extract Filtrate
Saccharomyces Lysate Extract
Saccharomyces Lysate
Saccharomyces Polypeptides
SaccharomycesTorulaspora Delbrueckii Extract
Torulaspora Delbrueckii Ferment
Hydrolyzed Torulaspora Delbruekii Extract
Hydrolyzed Candida Bombicola Extract
...

→

Hydrolyzed Yeast
Hydrolyzed Yeast Extract
Hydrolyzed Yeast Protein
Yeast Amino Acids
Yeast Extract
Yeast Ferment Extract
Yeast Beta-Glucan
Yeast Polysaccharides
...

→

Yeast

Genenic
INCI name

Saccharomycetales

Order

Saccharomycetes

Class

Ascoideaceae
Debaryomycetaceae
Dipodascaceae
Endomycetaceae
Metschnikowiaceae
Phaffomycetaceae
Pichiaceae
Saccharomycetaceae
Saccharomycetales incertae sedis
Saccharomycodaceae
Saccharomycopsidaceae
Trichomonascaceae

Families

INCI names – Conclusion

- All families belonging to the « Saccharomycetales » order from «Saccharomycetes » class are reviewed in this report
- From a taxonomy point of view, all yeast-related INCI names can be grouped into one generic INCI name : YEAST
- From a manufacturing process point of view : all yeast-related INCI names can be grouped into one generic INCI name: YEAST
- Great advantage of having only one generic INCI name : Yeast (in accordance with PCPC definition) rather than a multitude of INCI names with the scientific name which may regularly change due to taxonomy evolution

Process => media

- A generical culture media can be used for yeast growth; for example, a synthetic culture medium which allows very good repeatability because it is a standardized medium

Base medium	Quantity (g/L)
Ammonium sulphate	Confidential data
Potassium phosphate	
Sodium phosphate di basic	
Magnesium sulphate	
L-glutamic acid	
Sucrose or glucose or molasses	
Antifoam	
Ammoniac	

Oligo-elements	Quantity (mg/L)
EDTA	Confidential data
ZnSO4. 7H2O	
MnCl2. 4H2O	
CuSO4. 5H2O	
Na2MoO4. 2H2O	
CaCl2. 2H2O	
FeSO4. 7H2O	
KI	

Vitamins	Quantity (mg/L)
Biotin	Confidential data
Pantothenic acid	
Nicotinic acid	
Myo-inositol	
Thiamine-HCl	
Pyridoxine-HCl	
Para-amino-benzoic acid	

Process => Absence of impurities

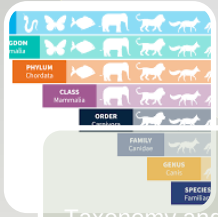
Once the protocol has been established in R&D, this process is always applied in the same way to avoid any contamination / impurities or alteration (reproductible):

Revivification of the strain from the same secondary stock	The same inoculation rate is applied
The same culture medium is always used (preference for a standardized synthetic medium)	The chain of seeding and cultivation is always done over the same period
The sugar used is always the same and in the same quantity (glucose, sucrose or molasses)	The sugar supply is always the same
PH, temperature, oxygen, pressure, aeration are regulated	Many controls during culture : microscope, Optic Density, microbiology to ensure there is no contamination
Cleaning in place is systematically controlled	=> All these controls allow : controlled, repeatable and reproducible culture

Characterization and Toxicological data

Sourcing

Safety measures and Identification



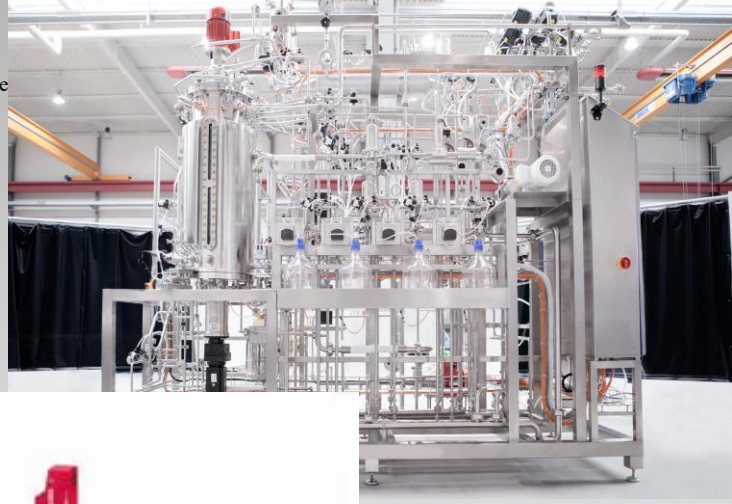
Taxonomy and INCI names

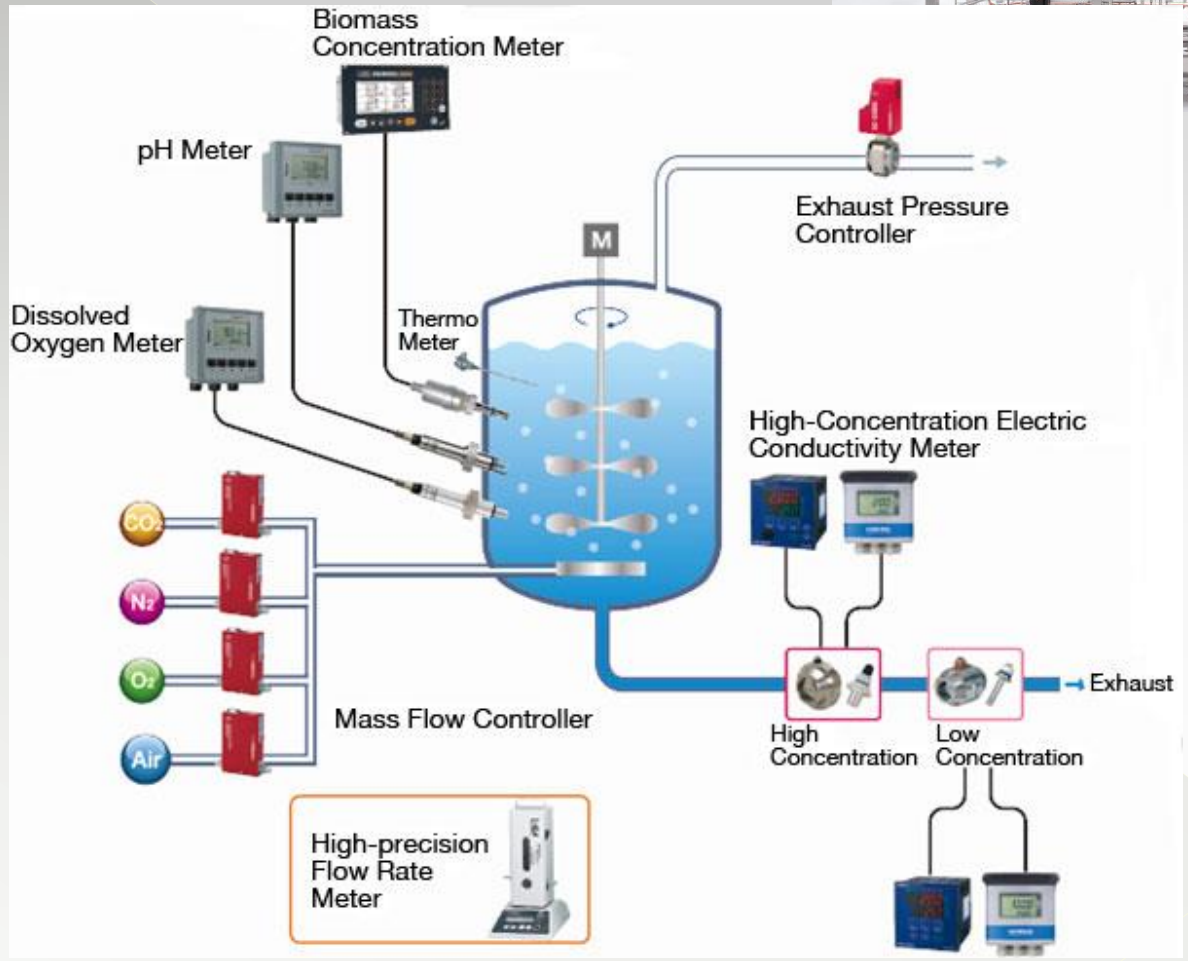
Process

Characterization Toxicological data

Cosmetic ingredient

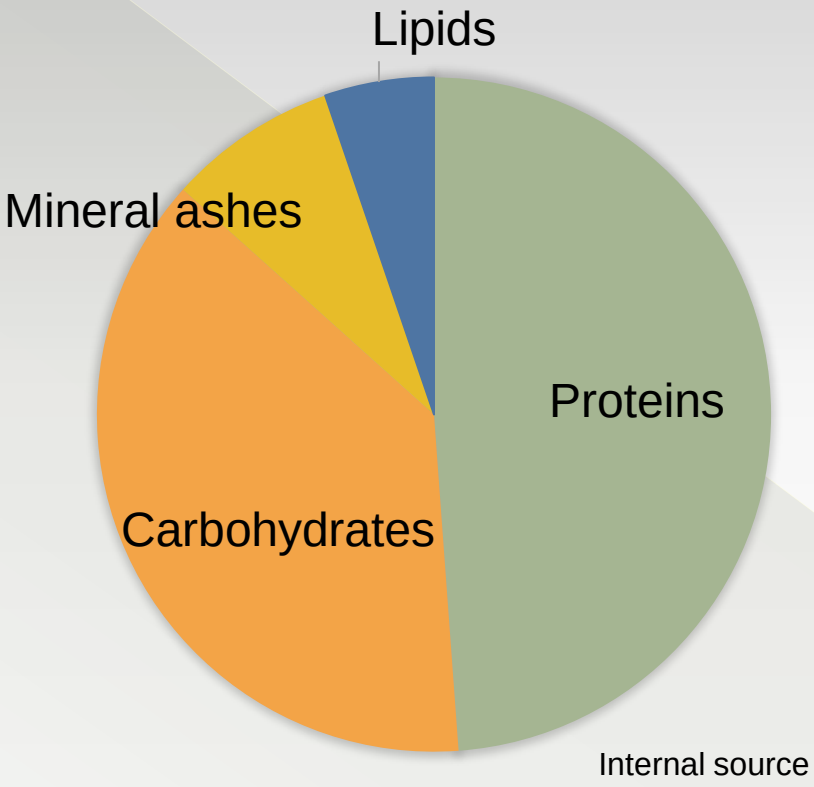
Controlled parameters





Analytical Characterization

Yeasts are always analytically characterized, in general :



Bibliographical characterization

Pathogenic Yeasts are well identified, 5 strains from Saccharomycetes class :

Hazard 2

In Europe :

Candida albicans, Candida dubliensis, Candida glabrata, Candida parapsilosis, Candida tropicalis

In USA :

Candida auris (antibiotic resistance)

According to :

- Directive (EU) 2019/1833 of the commission of the October 24, 2019
- The US Centers for Disease Control (CDC) urgent threats list

Toxicological data – Food uses

- Bibliographical toxicological data has been found on the large majority of yeasts of each family belonging to Saccharomycetes class (complete data available on request)

NB : Data about Biocontrol were not taken into account since fruits and vegetables are supposed to be cleaned before consumption

Family	Food use – Bibliographical data
Ascoideaceae	Listed in the publication : Diganta Narzary, Nitesh Boro et al. (2021), Community structure and metabolic potentials of the traditional rice beer starter 'emao'
Debaryomycetaceae	Most of strains are : - Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use"** - Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), **International Dairy Federation bulletin 514/2022** - Listed in publications about fruits fermentation for liquor (Camu-Camu, Agave) - 1 strain notified for **QPS status** : Candida oleophila - Listed in 1 patent : Method for producing beverages by acid removal (EP2866594A1)

Toxicological data – Food uses

Family	Food use – Bibliographical data
Dipodascaceae	Most of strains are : - Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use“** - Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), **International Dairy Federation bulletin 514/2022** - 1 strain with **QPS status :** Yarrowia lipolytica
Endomycetaceae	Listed in the Patent US3296090A - Fermentation process for producing 1-tryptophane (one of the essential amino acids necessary for nutrition), 1984

Toxicological data – Food uses

Family	Food use – Bibliographical data
Metschnikowiaceae	Most of strains are : - Listed in publications about fermentations of beers and wines - Listed in publication: Hiroyuki Sasaharaa, Ken Izumori, (2005), **"Production of L-talitol from L-psicose by Metschnikowia koreensis LA1 isolated from soy sauce mash", Journal of Bioscience and Bioengineering** - Listed in the patent: EP 1 065 276 A1, (1999) **Methods for producing D-arabitol, D-xylulose and xylitol using the yeast Metschnikowia** - Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use“** - Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), **International Dairy Federation bulletin 514/2022** - 1 strain with **GRAS status:** Metschnikowia pulcherrima

Toxicological data – Food uses

Family	Food use – Bibliographical data
Phaffomycetaceae	Strains are : - Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use“** - Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), **International Dairy Federation bulletin 514/2022** - 1 strain with **QPS status :** Wickerhamomyces anomalus - 1 strain notified for **QPS status** : Komagataella pastoris
Pichiaceae	Most of strains are : - Listed in publications about wine and distilled Agave beverages - Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), **International Dairy Federation bulletin 514/2022**

Toxicological data – Food uses

Family	Food use – Bibliographical data
Saccharomycetaceae	Strains are : - Listed in the publication : François Bourdichon, Serge Casaregola et al. (2011) "**Food fermentations: Microorganisms with technological beneficial use“** - Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), **International Dairy Federation bulletin 514/2022** - 3 strains with **QPS status :** Kluyveromyces marxianus, Kluyveromyces lactis, Zygosaccharomyces rouxii - 1 strain notified for **QPS status** : Eremothecium ashbyii - 1 strain with **GRAS status :** Saccharomyces Cerevisiae
Saccharomycetales incertae sedis	- Listed in the publication : Roxane Detry, Noa Simon-Delso, (2020), "**Specialisation of Yeast Genera in Different Phases of Bee Bread Maturation**", Microorganisms

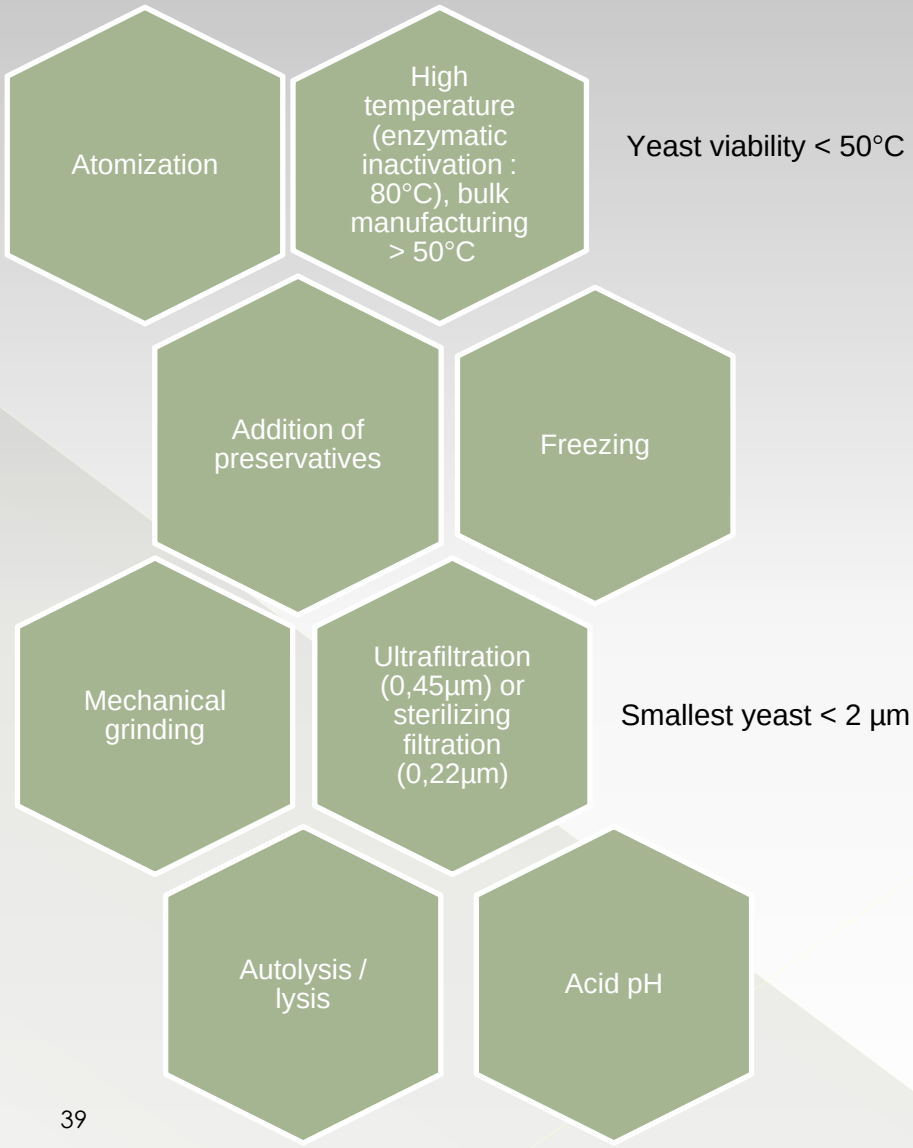
Toxicological data – Food uses

Family	Food use – Bibliographical data
Saccharomycodaceae	Listed in the Publication : Nuno Bourbon-Melo, Margarida Palma, (2021), "**Use of Hanseniaspora guilliermondii and Hanseniaspora opuntiae to enhance the aromatic profile of beer in mixed-culture fermentation with Saccharomyces cerevisiae"**, Food Microbiology
Saccharomycopsidaceae	Listed in the Publication : Zai-Bin Xie, Kai-Zheng Zhang (2021), "**Saccharomycopsis fibuligera in liquor production: A review**", European Food Research and Technology
Trichomonascaceae	Listed in the Publication : Pradnya Chavan, Sarika Mane, Girish Kulkarn et al. (2009), "**Natural yeast flora of different varieties of grapes used for wine making in India**", Food Microbiology

Cosmetic ingredient manufacturing process

Manufacturing processes used to obtain cosmetic ingredients and products are incompatible with the viability of yeast.

Thus, no yeast can be alive in a cosmetic product



Conclusion

- Since the dawn of time, more and more yeasts have been **used in food**, especially in fermentation
- Identification and analytical characterization are key-information to guarantee the **quality, stability and safety** of the yeasts used
- As demonstrated before, from **taxonomy** and **manufacturing process** points of view, all yeast-related INCI names can be grouped into one generic INCI name YEAST
- Thanks to robust and well mastered manufacturing processes of cosmetic ingredients, the quality of yeasts is perfectly **reproductible and stable**

Conclusion

- Due to the existence of food use for the majority of strains from Saccharomycetes class, all **can be grouped together** in the "Yeast" INCI name and can be considered safe for use as a cosmetic ingredient

- Processes used to manufacture cosmetic products guarantee the **absence of live organisms**

- Even if the safety of the yeast is demonstrated, we strongly recommend a pre-market **safety evaluation** of the cosmetic ingredient, consistent with CIR reviews of other ingredients with a history of safe use in the diet, additional data concerning the potential for local effects, e.g., dermal irritation and sensitization, are needed

Thank you for attending
Any questions?