Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics

Status:	Draft Final Report for Panel Review
Release Date:	May 10, 2024
Panel Meeting Date:	June 3 – 4, 2024

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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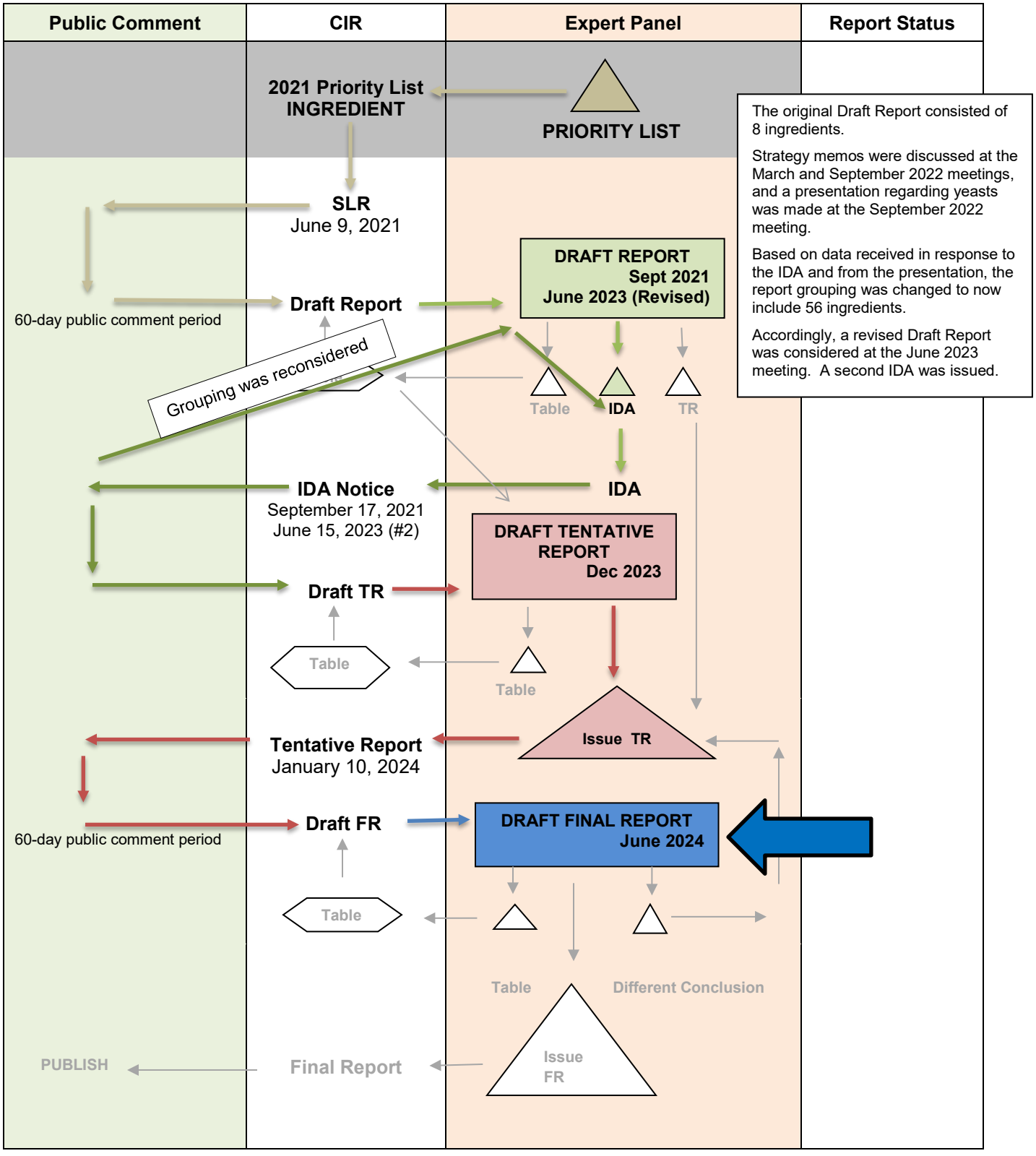
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Yeast-derived ingredients

MEETING June 2024



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, MS, Senior Scientific Analyst/Writer, CIR
Date: May 10, 2024
Subject: Draft Final Report on Yeast-Derived Ingredients

Enclosed is the Draft Final Report on the Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics (*report_Yeast_062024*). At the December 2023 meeting, the Panel issued a Tentative Report on these 56 yeast-derived ingredients with the conclusion that 11 yeast-derived ingredients and 22 generically-named yeast-derived ingredients, when derived from species of yeast included in the report with both dermal sensitization and food use status, are safe in cosmetics. The Panel determined that the data were insufficient to make a determination for the remaining 23 ingredients. Since the issuing of the Tentative Report, the following information has been received:

- *data1_Yeast_062024*
  - Anonymous. 2024. Summary information *Candida oleophila* (includes a summary of an HRIPT).
    - EFSA statement – *Candida oleophila* has been added as a synonym to *Yarrowia lipolytica*; therefore the QPS status that is currently present for *Yarrowia lipolytica*, is extended to *Candida oleophila*
    - Summary of an HRIPT on a Yeast Extract derived from *Candida oleophila* (final test concentration of 0.285%; n = 100)
- *data2_Yeast_062024*
  - Anonymous. 2024. Composition and Use Information Pichia Heedii Extract and Yeast Extract made from Pi*chia naganishii;* Summary of Food Use of *Pichia* spp.
    - composition information on Pichia Heedii Extract
    - composition information on a Yeast Extract derived from *Pichia naganishii*
    - reported use concentration of Pichia Heedii Extract in skin care products at up to 0.096%
    - reported use concentration of Yeast Extract derived from *Pichia naganishii* in skin care products at up to 0.105%
    - summary information/bibliography of *Pichia* spp. used in foods (it should be noted that the majority of the species (excluding *Pichia naganishii)* provided in the summary and noted in the references in the bibliography are not species that are reported to be used in cosmetics)
- *data3_Yeast_062024*
  - Eurofins. 2016. Confirmation in human of the skin compatibility and absence of allergenic potential of one cosmetic product after repeated application under patch.
    - HRIPT of a trade name mixture containing 10% Pichia Ferment Lysate Filtrate (n = 55; further test article details not provided)
- *data4_Yeast_062024*
  - ICCR-Roßdorf GmbH. 2023. *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay
    - Ames assay on pure Pichia Ferment Lysate Filtrate
- *data5_Yeast_062024*
  - ICCR-Roßdorf GmbH. 2023. In vitro eye irritation: human cornea model test – OECD 492
    - In vitro ocular irritation assay on pure Pichia Ferment Lysate Filtrate
- *data6_Yeast_062024*
  - Xylome. 2024. Information on Lipomyces Oil Extract and Lipomyces Lipid Bodies
    - manufacturing information on Lipomyces Oil Extract

    composition data on Lipomyces Lipid Bodies and Lipomyces Oil Extract
    - information regarding the potential use of Lipomyces Lipid Bodies as a loading agent for hydrophobic drugs and active ingredients

In addition to the information above, it should be noted that according to the wINCI *Dictionary*, Yeast Ferment Extract is derived from *Saccharomyces cerevisiae*; therefore, this ingredient may be considered safe as a non-generic yeast-derived ingredient. According to all of the information received, and the current method the Panel has employed to determine the safety of the ingredients in this report, the following ingredients may be considered for addition to the list of safe ingredients as they now have both QPS or GRAS status/food use/systemic toxicity data and sensitization data:

Yeast-derived ingredients:
Yarrowia Lipolytica Extract
Yarrowia Lipolytica Ferment Lysate
Yarrowia Lipolytica Oil
Yeast Ferment Extract

Generic yeast-derived ingredients:
Yeast Extract derived from *Candida oleophila*
Yeast Extract derived from *Pichia naganishii*

The data profile (*dataprofile_Yeast_062024*) included herein is composed of three tables (new data since the last iteration of the report have been marked in the data profile with a bolded, red **X**). 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.

Other items 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_062024*), a search strategy (*search_Yeast_062024)*, flow chart (*flow_Yeast_062024*), report history (*history_Yeast_062024*), comments on the Tentative Report from Council (*PCPCcomments_Yeast_062024*), and responses to these comments (*response-PCPCcomments_Yeast_062024*). In addition, the presentation given to the Panel at the September 2022 meeting can be found using the following link: https://www.cir-safety.org/sites/default/files/presentation_Yeast_062024.pdf

The Panel should carefully consider the Abstract, Discussion, and Conclusion presented in this report. If these are satisfactory, the Panel should issue a Final Report.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: February 1, 2024

SUBJECT: Tentative Report: Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics (release date January 19, 2024)

The Personal Care Products Council respectfully submits the following comments on the tentative report, Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics.

Key Issues

As Lipomyces Oil and Yarrowia Lipolytica Oil are mixtures of triglycerides, perhaps additional information on composition would be sufficient to consider these ingredients safe. For example, the following article suggests that the composition of Lipomyces Oil (using *Lipomyces starkeyi*) is similar to vegetable oil.

> Zhang L, Lim EY, Loh K-C, et al. 2021. Two-stage fermentation of *Lipomyces starkeyi* for production of microbial lipids and biodiesel. Microorganisms 9 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8399642/pdf/microorganisms-09-01724.pdf

Yarrowia Lipolytica Oil is GRAS (see Table 8) and the distribution of fatty acids found in this species is provided in Table 4. The following review may provide additional information about the composition and use of this oil.

> Zinjarde SS. 2014. Food-related applications of *Yarrowia lipolytica*. Food Chemistry 152: 1-10. https://doi.org/10.1016/j.foodchem.2013.11.117.

Although it has a generic INCI name, Yeast Ferment Extract is defined as "the extract of the product obtained by the fermentation of *Saccharomyces cerevisiae*." Because it is made from *Saccharomyces cerevisiae* it should be moved with the ingredients considered safe.

It would be helpful if the Discussion included a table showing the specific additional data needed for each insufficient data ingredient.

Additional Considerations

Yeast Strain Identification and Biosafety – As the term "baker's yeast" is used for the first time in this section, please identify it as *Saccharomyces cerevisiae*.

Method of Manufacture – As this section is about cosmetic ingredients, please revise: "in the finished cosmetic product" to "in the cosmetic ingredient".

Composition and Impurities, *Lipomyces starkeyi* – Please indicate that the oil from this species is edible and similar in composition to palm oil (as stated in reference 15).

Composition and Impurities, *Saccharomyces cerevisiae* – It is misleading to state that baker's yeast "must contain" low levels of heavy metals. 21 CFR184.1983 states: "The ingredient meets the following specifications on a dry weight basis" followed by the levels. The CIR report should also state that baker's yeast must meet the following specifications (rather than it "must contain").

Acute – Rather than "parenteral" please be more specific and state the route that was used ("subcutaneous").

Case Reports – In the first paragraph, it should also be noted that the ingredients under review do not contain live organisms.

Table 3 – As Yeast Ferment Extract is defined as being made from *Saccharomyces cerevisiae*, this INCI name should be moved to the row with Saccharomyces Cerevisiae Extract.

Table 7 – The title of this table needs to be corrected to "Yeast-derived [ingredients] not reported to be use[d] according to 2023 frequency of use and 2021/2023 concentration of use data" (add the word "ingredients" and add "d" to use).

Table 8 – As it is defined as being made from *Saccharomyces cerevisiae,* Yeast Ferment Extract should be added to the row with Hydrolyzed Saccharomyces Cell Wall and Saccharomyces Cerevisiae Extract.

Table 10 – The studies in reference 42 (oral, parenteral) were done in rats so it should say "strain" was not specified (it currently states that "species" was not specified).

Table 11 – In the Results column of reference 44, "ration" needs to be corrected to "ratio"

Table 12 – Please be consistent in using the abbreviations for the positive controls, e.g., the first row uses 9-AA, the second row uses 9-aminoacridine. In the abbreviations list at the bottom of the table, "9-aminoadridine" needs to be corrected to "9-aminoacridine".

Table 15 – The text indicates that Galactomyces Ferment Filtrate was tested "neat". Therefore, there was no vehicle (rather than NR it should say none), and in the Concentration/Dose column it should say "neat" rather than "concentration not stated".

Yeast-Derived Ingredients - June 2024 – Priya Cherian	
Comment Submitter: PCPC **Date of Submission: February 1, 2024**	
Comment	**Response/Action**
As Lipomyces Oil and Yarrowia Lipolytica Oil are mixtures of triglycerides, perhaps additional information on composition would be sufficient to consider these ingredients safe. For example, the following article suggests that the composition of Lipomyces Oil (using *Lipomyces starkeyi*) is similar to vegetable oil. Zhang L, Lim EY, Loh K-C, et al. 2021. Two-stage fermentation of *Lipomyces starkeyi* for production of microbial lipids and biodiesel. Microorganisms 9 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8399642/pdf/microorganisms-09-01724.pdf	Study added to report.
Yarrowia Lipolytica Oil is GRAS (see Table 8) and the distribution of fatty acids found in this species is provided in Table 4. The following review may provide additional information about the composition and use of this oil. Zinjarde SS. 2014. Food-related applications of *Yarrowia lipolytica*. Food Chemistry 152: 1-10. https://doi.org/10.1016/j.foodchem.2013.11.117.	Study added to report.
Although it has a generic INCI name, Yeast Ferment Extract is defined as "the extract of the product obtained by the fermentation of *Saccharomyces cerevisiae*." Because it is made from *Saccharomyces cerevisiae* it should be moved with the ingredients considered safe.	Addressed.
It would be helpful if the Discussion included a table showing the specific additional data needed for each insufficient data ingredient.	Table added to report.
Yeast Strain Identification and Biosafety – As the term "baker's yeast" is used for the first time in this section, please identify it as *Saccharomyces cerevisiae*.	Addressed
Method of Manufacture – As this section is about cosmetic ingredients, please revise: "in the finished cosmetic product" to "in the cosmetic ingredient".	Addressed.
Composition and Impurities, *Lipomyces starkeyi* – Please indicate that the oil from this species is edible and similar in composition to palm oil (as stated in reference 15).	Addressed.
Composition and Impurities, *Saccharomyces cerevisiae* – It is misleading to state that baker's yeast "must contain" low levels of heavy metals. 21 CFR184.1983 states: "The ingredient meets the following specifications on a dry weight basis" followed by the levels. The CIR report should also state that baker's yeast must meet the following specifications (rather than it "must contain").	Addressed
Acute – Rather than "parenteral" please be more specific and state the route that was used ("subcutaneous").	Addressed
Case Reports – In the first paragraph, it should also be noted that the ingredients under review do not contain live organisms.	Addressed
Table 3 – As Yeast Ferment Extract is defined as being made from *Saccharomyces cerevisiae*, this INCI name should be moved to the row with Saccharomyces Cerevisiae Extract.	It has been kept in the same spot in the table; however, the order, family, genus, and associated species names have been updated
Table 7 – The title of this table needs to be corrected to "Yeast-derived [ingredients] not reported to be use[d] according to 2023 frequency of use and 2021/2023 concentration of use data" (add the word "ingredients" and add "d" to use).	Addressed
Table 8 – As it is defined as being made from Saccharomyces cerevisiae, Yeast Ferment Extract should be added to the row with Hydrolyzed Saccharomyces Cell Wall and Saccharomyces Cerevisiae Extract.	Addressed
Table 10 – The studies in reference 42 (oral, parenteral) were done in rats so it should say "strain" was not specified (it currently states that "species" was not specified).	Addressed
Table 11 – In the Results column of reference 44, "ration" needs to be corrected to "ratio"	Addressed
Table 12 – Please be consistent in using the abbreviations for the positive controls, e.g., the first row uses 9-AA, the second row uses 9-aminoacridine. In	Addressed

the abbreviations list at the bottom of the table, “9-aminoadridine” needs to be corrected to “9-aminoacridine”.	
Table 15 – The text indicates that Galactomyces Ferment Filtrate was tested “neat”. Therefore, there was no vehicle (rather than NR it should say none), and in the Concentration/Dose column it should say “neat” rather than “concentration not stated”.	Addressed

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 and issues Tentative Report with safe as used conclusion for 11 yeast-derived ingredients and 22 generic named yeast-derived ingredients, when derived from species of yeast included in the report with both dermal sensitization and food use status, are safe in cosmetics; insufficient for remaining 23 ingredients
- Tentative Report posted

February 2024

- Comments received from Council on Tentative Report
- Data received - *Candida oleophila* – HRIPT and EFSA statement that Candida oleophila has QPS status and is synonymous to *Yarrowia lipolytica*
- Data received – composition information on Pichia Heedii Extract and Yeast Extract derived from *Pichia naganishii*; reported use concentrations for these ingredients from supplier; bibliography provided stating use of *Pichia* spp in foods

March 2024

- Data received - Pichia Ferment Lysate Filtrate – HRIPT, Ames assay, and in vitro ocular irritation assay

- Data received – manufacturing information on Lipomyces Oil Extract, composition data on Lipomyces Lipid Bodies and Lipomyces Oil Extract, and information regarding use of Lipomyces Lipid Bodies as loading agent for hydrophobic substances

June 2024

- Panel reviews Draft Final Report

Table 1. Data profile on ingredients with reported species (33 total ingredients) - June 2024 - 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		
*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		X	X																							
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 (33 total ingredients) - June 2024 - 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													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														X									X	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	
	Saccharomyces Ferment Extract																										

Table 1. Data profile on ingredients with reported species (33 total ingredients) - June 2024 - 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		
Schizosaccharomyces pombe					X	X																					
	Schizosaccharoymces Pombe Extract			X																							
Yarrowia lipolytica			X		X	X																					
	Yarrowia Lipolytica Extract (synonymous to Yeast Extract derived from *Candida oleophila*)																								X		
	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																		X								
Pichia Extract																													
Saccharomyces																													
Saccharomyces Extract																													
Saccharomyces Ferment	X						X				X				X														
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	X			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		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 – June 2024 – 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.

Full Panel – September 14, 2021

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
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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.

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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.

Full Panel – June 13, 2023

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.

DECEMBER 2023 MEETING – DRAFT TENTATIVE REPORT

Belsito Team – December 4, 2023

DR. BELSITO: Okay, yeast. Another beast. So, the easiest way that I had was -- and thank you Priya for putting this table together that tells us whether it's food use, and that other category that I can't remember. The QPS status and then whether we have sensitization on it.

And then just going down that area, I think there are a number of this specific (audio skip) we can say are okay. But before we go through that list of specific yeasts, I guess the problem that I have are these -- on Table 2 these generics that are like yeasts.

What is yeast? Which yeast is yeast? And that has the highest use. We don't know what it is. I mean it's defined as yeast and we know that we have all these species, so I don't know how to deal with that. You know, yeast extract.

So, I think one of the biggest questions -- because the material that has the largest use, we don't know really what it is, right? So, I mean, I don't know if we're going to solve -- because medic industry's problem unless the INCI dictionary gives us a definition of what the heck yeast is in terms of species.

DR. RETTIE: So, Don, doesn't Table 3 help out a little bit there? It lists 12 yeast species known to be used in the preparation of yeast extract. So, we get a little information.

DR. BELSITO: Yeah.

DR. RETTIE: So much data.

DR. BELSITO: Oh, gosh. Hold on, because for some reason my computer is not letting me save my prostaglandin changes.

DR. HELDRETH: Trying to process all that data. Historically, in a situation like this the Panel has either put insufficient for an ingredient like this that's very vague or has come to a conclusion where we limited what can be considered the ingredient. We did it before when we had some different oligomers, and one ingredient name could mean three different oligomers, and the Panel came to a conclusion of safety when only this one oligomer of the three was used. And so, I mean that's a potential pathway the Panel could go here.

I would say if there's a species like saccharomyces cerevisiae that the Panel feels comfortable with, they could say if all the data suggested it's safe, they could say safe as used when the species is used for yeast extract. Something to that effect. Those are the historical options the Panel has followed.

DR. BELSITO: Well, that'd be nice because then we could go through all these species that we felt were safe and then basically say that yeast extract/yeast ferment produced from these species would be considered safe as used as well.

DR. HELDRETH: That would be in line with previous conclusions by the Panel.

DR. BELSITO: Okay. So then if we go down the list from Table 1, we have candida saitoana which has a food use and has human irritation/sensitization. So that would be okay. And then the question is, is the hydrolyzed version of that okay as well for which we have reported use but do not have the data? Would we expect hydrolysis to change, in any way, the systemic toxicity?

DR. HELDRETH: Audrey, did you have a comment or something to add? You're on mute. There you go.

DR. BELSITO: We're not hearing you. No. No. At the top of your screen. Sometimes my microphone mutes me out. Can you check to make sure your mic is open up at the top on your -- yeah. They're you go.

MS. POKRZYWA: Yes? Okay. Okay. I'm sorry for the technical problem. The thing about the (inaudible) of yeast. I convinced PCPC (inaudible) of yeast as defined by the (inaudible). And so, each name is generic. As a class of (inaudible) is very defined by (inaudible) information.

About the hydrolyzed (inaudible), as I share some information, if (inaudible) the yeast are food data and sensitization (inaudible) the hydrolyzed extract should be (inaudible) as safe because it is a clarification of steps of the yeast (inaudible) yeast.

DR. BELSITO: So, what you're saying is the hydrolysis, if anything, would produce a safer material?

MS. POKRZYWA: Yes, but only if the yeast, the starting (inaudible) yeast (inaudible) and in this case (inaudible) safe or not is the case.

DR. BELSITO: Right. Curt, Paul, Allan, you're okay with that?

DR. RETTIE: Yeah, I think so.

DR. BELSITO: Curt and Paul? In the absence of hearing anything I'll presume that you're okay with it.

DR. SNYDER: Yeah, I'm okay. Sorry, stepped away there for a minute.

DR. BELSITO: Candida saitoana is okay. Then here under the galactomyces we have three different species and they're all lumped together and with an X for food and then we have animal and human data for dermal sensitization. Priya, do you know why all three of those species were lumped together as food? Does that essentially mean they all have food use?

MS. CHERIAN: I don't know if all of them have food uses but it's all of those species can be used in that ingredient. That's why all three of those are listed there.

DR. BELSITO: I see.

MS. CHERIAN: Also, I don't know if it's helpful, but I have a table that I had for myself and it has all the ingredients with food and sensitization, the ones that don't have either and the ones that have either/or. So, if you need to see that I have it.

DR. BELSITO: Oh, that would be great because I'm just working off of Table 1 and I just checked the ones that I think are safe and then the ones I had questions on.

MS. CHERIAN: I'll share my screen now.

DR. BELSITO: So, Allan, this is what we did -- Priya did for us with algae.

MS. CHERIAN: Yeah. Okay, is it big enough?

DR. BELSITO: It could be bigger for my eyes, but.

MS. CHERIAN: Oops.

DR. BELSITO: That's better.

MS. CHERIAN: So, this table on this side would be the ingredients that we have everything for, food and sensitization. I included the hydrolyzing ingredients too. And then the generic ingredients aren't part of this since we don't know the specific species, but we do know some species that yeast extract -- which species are used with yeast extract. Not all of them, we have some of them. So, this isn't an actual INCI ingredient, but I just listed it here as yeast extract derived from candida magnoliae since we know that's a species that could be used there.

So, it's not 18 safe ingredients, it's technically 11 plus maybe 12 if you're counting yeast extract as safe.

DR. BELSITO: Yeah, I think we decided to go with defining the yeast extracts that we would consider to be safe. So, making that definition so that would be 12.

MS. CHERIAN: Right.

DR. BELSITO: I'm Just looking --

DR. RETTIE: Can I ask (audio skip) with algae? I wasn't involved in algae. Did you require clean data for sensitization as well as food use?

DR. BELSITO: Yes, that's how we did that. Yes.

DR. RETTIE: You decided you needed both?

DR. BELSITO: Yes.

DR. RETTIE: Okay, thanks.

DR. BELSITO: Okay, well that simplifies matters. So then, I don't know who's reporting on this tomorrow but if you could send along that slide because it would clearly show which ones were safe and then what our needs were which would either be like a chronic oral toxicity if it doesn't have food use, right? Would be what the data need would be or 28-day dermal, I guess.

MS. CHERIAN: Sure. This specific list is in the memo, the one with both food and sensitization. The other three lists are not in the memo. But I can send this to you for a more comprehensive view.

DR. BELSITO: Right. So, for --

DR. HELDRETH: Yeah, I actually forwarded it. You should all have it in your email.

MS. CHERIAN: Okay.

DR. BELSITO: So, for those that don't have food use, what would our needs be, 28-day dermal and if evidence of absorption of systemic toxicity data may be needed. What are we asking for in those cases?

DR. SNYDER: That would be our standard, yeah, 28-day dermal if absorbed and additional endpoints may be needed. That's fair.

DR. BELSITO: Okay.

DR. SNYDER: Yep.

DR. BELSITO: And then for the ones without sensitization, obviously sensitization.

DR. SNYDER: Yeah. You're reporting on this, Don, so I think that table made it just a lot easier for you, right then and there.

DR. BELSITO: Yeah. So, I did not see that table so where is it? You're sending it out?

DR. HELDRETH: I just sent that email to this team that has all of Priya's tables.

DR. BELSITO: Okay. I shut off my emails otherwise I'm getting bombarded constantly during these meetings. Okay. Great. Thank you. Well, that made yeast a short report. Any comments on the document in terms of -- okay.

DR. SNYDER: That table has all of wave two data, Priya? All the Wave 2 data's in there with all that sensitization data?

MS. CHERIAN: Yes.

DR. SNYDER: Okay, thank you.

DR. BELSITO: My comments of food, no dermal. Okay. And we're okay with the fact that galactomyces is used up to 90.7 percent. Doesn't really bother me but we're okay with that?

DR. HELDRETH: I agree.

DR. BELSITO: Okay. And then I just, in terms of discussion development, obviously the usual impurities and that the food use mitigated our need for DART studies (audio skip) and then the inhalation boilerplate, right?

And in the draft discussion that you started, Priya, you just in volatile compounds you mentioned benzaldehyde. One that bothered me was hexane is apparently used as well and I would just add that one and then put et cetera after it.

And then just one other point I think that would be helpful for future quickness of looking, because when you're doing in vitro sensitization data basically the rule is two out of three. So, you have the DPRA and you have the KeratinoSens and then you have dendritic cell activation, which could be h-CLAT, U-SENS or IL-8 Luc.

It may be nice under in vitro to put three columns, DPRA, Kerat- -- you know, AOP1, which would be DRPA, AOP2, which would be KeratinoSens, AOP3 which could be either of the three dendritic cell activations and put whether they were done. Because if only one was done then it's not helpful. You can't make a decision off the in vitro, you need at least two that are negative. I just think it would be cleaner.

MS. CHERIAN: Do you want that distinction in the data profile too? Do you think that would be helpful or no?

DR. BELSITO: Yeah, I think it would be helpful in the data profile too for quickly going down it. And then on the sensitization table, this is PDF page 115. For the saccharomyces ferment lysate filtrate, did it say what species of saccharomyces or just it's saccharomyces?

MS. CHERIAN: Just saccharomyces.

DR. BELSITO: Yeah. This was so -- okay. And --

MS. CHERIAN: And for those I can't really say if it's used in/safe in foods, either, because I don't have it.

DR. BELSITO: Right, yeah. No, I think, you know, what Bart said is we're just going to define the yeast that are safe, and the same thing will be true with the saccharomyces. But I guess that was just data anyway. It doesn't really matter here. Okay, those were my only comments on yeast. Curt, Paul, Allan, do you have other comments on the document?

DR. RETTIE: Nope.

DR. BELSITO: And the Wave 2 data has been included you said, Priya, correct? It will be included --

MS. CHERIAN: In the --

DR. BELSITO: Yeah.

MS. CHERIAN: Yeah.

DR. BELSITO: In your table. Okay. Great. Thank you, another two-hour report. Okay. So, are we going to something simple now?

Cohen Team – December 4, 2023

DR. COHEN: I'd like to move on to yeast. Okay, at the June 2023 meeting the panel reviewed the revised draft report on 56 yeast-derived ingredients and issued a second IDA for this ingredient group. The first was issued in September 2021. In this IDA in order to determine safety, the panel requested confirmatory dermal sensitization data and data on food use such as GRAS on the yeast species used to derive these ingredients.

In lieu of food use -- so GRAS -- 28-day dermal tox data may be considered. At the June meeting, we requested information about QPS as designated by the E.U. in order to determine if this parameter may be used to clear systemic tox of food use for these ingredients. Table 3 is very much appreciated and gives us food use, QPS status, and sensitization data.

Of note, we removed three yeast derived ingredients which are hydrolyzed yeast protein, yeast beta-glucan, and yeast polysaccharides. These are distinct molecules, and we wouldn't include them here. On PDF 131 we had a good explanation of QPS and there's a link to the QPS reference on PDF page 127 and reference 131 which I found very useful.

I guess the question for the group was I see we have highlighted, I think it's 12 items where we have tox clearance either by GRAS or by QPS or food use and sensitization, but some are just in vitro and were we agreeing to that? I don't recall us doing that for -- what did we do this for, the coral or -- I'm trying to remember.

DR. BERGFELD: Bone algae.

DR. COHEN: Algae, yes. Algae. Were we using in vitro for the algae? I thought we needed --

DR. BERGFELD: It was GRAS.

DR. COHEN: GRAS plus sensitization like HRIPT.

DR. SLAGA: Yeah.

DR. COHEN: And the other thing is I thought for saccharomyces, while it's listed in Table -- well the Table 1, the derm sensitization was in vitro, I think we had human data on them. You know, in Table 13 we have saccharomyces ferment, lysate filtrate. We have a number of HRIPTs in people, so I felt that was okay. But, I guess, the question for the group is the phaffia really cleared? Maybe it's not on Table 3 anyway.

DR. BERGFELD: It's not there. I didn't see it.

DR. COHEN: One, two, three, four -- it's funny it comes as highlighted in Table 1. Is everyone seeing what I'm seeing which is in Table 1 it comes across as highlighted.

MS. CHERIAN: So, that's because those are ingredients that have a species reported. In table 2 those are all generic yeast ingredients so I don't have a species, so therefore you can't really say for sure that we have food use and sensitization on the ingredient as whole because it could be any species.

DR. COHEN: Thank you, Priya. So, is it table 3 that was where we're clear?

MS. CHERIAN: Table 1 and table 3.

DR. SLAGA: Yeah.

MS. CHERIAN: And the memo lists all of the ones that have both food use and sensitization.

DR. COHEN: So, I'm just shooting back and forth here. Is the phaffia rhodozyma on a cleared list now? No. Phaffia. Got to go back. I was trying to print these.

MS. CHERIAN: Yes. Yes, it is. It is.

DR. COHEN: And is that based solely on the in vitro sensitization?

MS. CHERIAN: Yes. If it had in vitro, animal, or human it was marked as having sensitization data.

DR. COHEN: Oh, I know tomorrow we're going to have a discussion about that but --

DR. TILTON: I think I noted this before, but I was okay with the in vitro sensitization data.

DR. COHEN: This was just EpiDerm. It was just for phaffia it was just one EpiDerm assay, right? This was for irritation. Let me just go to sensitization.

MS. FIUME: PDF page 115. It has two studies.

DR. COHEN: Okay. And ARE (inaudible). KerotinoSens. Okay. Okay, I mean, we can just discuss it tomorrow. What's the rest of the group feel?

DR. BERGFELD: It's going safe.

DR. COHEN: I --

DR. BERGFELD: Safe.

DR. COHEN: Yeah, yeah. Well, just for those, right?

DR. BERGFELD: Yeah.

DR. ROSS: So, for me, I looked at this and we got 18 ingredients of both food use or QPS status and sensitization. So, I came out of it thinking we can clear those 18.

MS. FIUME: Right.

DR. ROSS: Can I ask a question? Well, yeast extract itself has the most uses so what about yeast extract? In the memo, our beautiful little table 3 which was so informative, thank you very much, it showed 12 species but all but two pichia species have GRAS or QPS status and these two have some sensitization tests showing a lack of sensitization as used. And that's the pichia heedii and pichia naganishii. But the pichia heedii and the apichia naganishii don't have acute tox data but there's only one use for yeast extract; it could result in incidental ingestion. So, I thought we should clear yeast extract as well.

And that would leave around about another 26 or so that don't have GRAS, QPS, and sensitization data. I don't know what we do with those.

DR. BERGFELD: Insufficient.

DR. ROSS: Yeah. I'm fine with insufficient and clear as many as we can and --

DR. SLAGA: Yeah. The rest insufficient.

DR. ROSS: Yeah.

DR. COHEN: Right. For just yeast, yeast extract, yeast ferment extract why am I thinking that this was saccharomyces but --

DR. BERGFELD: I think that was the main yeast. Saccharomyces.

DR. ROSS: That's a different -- I think the nitrolic list, isn't that a different product?

MS. CHERIAN: They're all derived from the -- the yeasts are derived from the class saccharomyces. I don't know if they're derived from the genus saccharomyces.

DR. ROSS: Yeah.

DR. COHEN: Ah.

DR. ROSS: I was trying not to open this up but I'm going to have to open it.

DR. COHEN: So how are we clearing yeast when we don't know what it is, again? Just remind me.

DR. ROSS: Is Table 3 in the memo and --

DR. COHEN: Table 3.

DR. ROSS: Priya can tell you what the (audio skip) is. Species in yeast extract.

DR. COHEN: The yeast species listed in this table are only known species of yeast used in the production of yeast extract. But in table 2, yeast, yeast extract, yeast ferment extract --

MS. CHERIAN: They're all there. The only ingredient -- only generic ingredient -- that I know the species of is yeast extract because we've got information from manufacturers and whatever species came in in wave two for saccharomyces, I think cell wall or something.

DR. COHEN: Yes.

MS. CHERIAN: So those were the only generic ingredients I know the species of. I don't know the species of yeast or any of the other ones.

DR. COHEN: We don't know.

MS. CHERIAN: No. And even with yeast extract I don't know if that's comprehensive. I don't know if that includes all of the species, I just know that those have been reported. So, if you make a conclusion on yeast extract you might have to say it's safe when formulated using the species that I listed for just yeast extract.

DR. COHEN: Yeah. I think we have to because it's so generic a term, right?

DR. BERGFELD: There's a definition somewhere.

DR. COHEN: Is that a question thing or is that a final conclusion thing?

MS. FIUME: I believe in the past an issue like that would've gone into the discussion.

DR. COHEN: Even though in the conclusion we're going to spell out every one of the ones that we're clearing, right?

MS. CHERIAN: Right.

MS. FIUME: Correct.

MS. CHERIAN: But when it comes to ingredient number two, I wouldn't include yeast extract derived from candida magnoliae as a number. It's not 18 ingredients, it's 12 if that makes sense, because one, two, three, four, five, six, seven that are safe on that list in the memo are these extracts derived from a certain species. But that's not an INC name. But I'd still list it in the safe list in the report.

DR. COHEN: Okay.

MS. FIUME: So, you're saying yeast extract would be listed?

MS. CHERIAN: Yes, yes.

MS. FIUME: Yes.

MS. CHERIAN: Yes.

DR. COHEN: Yeast extract.

MS. CHERIAN: But it would probably also be listed under insufficient because it's not comprehensively safe.

DR. COHEN: Right. That's where I'm all uncomfortable here with this.

DR. ROSS: Now I'm comfortable.

MS. CHERIAN: I'm sorry.

DR. COHEN: Because with yeast extract, right, which is a generic term, Table 3 is the ones we know about.

MS. CHERIAN: Right.

DR. ROSS: Yeah.

DR. COHEN: We don't know about the ones we don't know about.

MS. CHERIAN: Right.

DR. COHEN: Right, right? So, how do we in our conclusion clear that without specifically saying yeast extracts derived from Table 3 are the only ones that are cleared? Yes.

MS. FIUME: So, procedurally the way you could do it, is you would address that in the discussion and then the conclusion defaults to as described in the report. So, they would have been described in the report. So, you would've expressed in the discussion, they would be spelled out in the body of the report and that's generally when something is unclear like that how it's handled and covered.

DR. COHEN: I like that a lot. It's simple and I can understand it.

MS. FIUME: Unlike my last explanation, so I'm glad that made it easy.

DR. COHEN: Which is (audio skip) for me lately on this. All right.

DR. ROSS: So, what's the conclusion, Dr. Cohen?

DR. COHEN: Well, the conclusion is those will be considered safe as used as described in the report. Right? And the report will describe which yeast extracts we're referring to.

DR. BERGFELD: Are you going to have a list of insufficient?

DR. COHEN: This is Don's tomorrow, but I think -- I hate to be so cheeky about it, but I would say the insufficients are all the others. Is that --

DR. BERGFELD: And for what reason?

DR. COHEN: Well, we either don't have sensitization data or we don't have GRAS or QPS data.

DR. BERGFELD: Okay.

DR. COHEN: One or the other. Right? And it would be you know who you are.

DR. BERGFELD: I like it.

DR. COHEN: Right? And what we could do is refer to -- we could refer to table one.

MS. CHERIAN: I had a really comprehensive list I made for myself yesterday that has a list of which ingredient has sensitization, which is missing what basically. And I sent it to the other team so I could get Bart to send this to you too if you want. Bart can share it here.

DR. COHEN: I mean, sure.

MS. CHERIAN: Okay.

DR. COHEN: Okay.

MS. FIUME: I was going to say for simple purposes now it would be all the other ingredients not listed in the memo?

MS. CHERIAN: Right.

MS. FIUME: Okay.

DR. GRIFFIN: Before we jump to the next ingredient, I just have one quick question and my apologies if I'm misunderstanding this. My understanding was that the respiratory exposure boilerplate had been changed but I think at PDF page 88 this is the old boilerplate still.

DR. COHEN: Well, let's see. PDF --

DR. BERGFELD: Is that in the discussion?

DR. GRIFFIN: Yes. The draft discussion. The last paragraph of the draft discussion.

DR. BERGFELD: Susan?

DR. TILTON: I'm not muted. So, are you saying that this is a prior boilerplate?

DR. COHEN: We have the aer- --

DR. BERGFELD: Not up to date. It's not up to date.

DR. GRIFFIN: I don't think it's up to date. However, the panel noted that in aerosol products the majority of the droplets/particles -- that whole section.

DR. COHEN: I think that's the problem with the old -- that's the problem with the boilerplate.

DR. TILTON: Well, we don't have a different one at this point. But you're not talking about that here. You're saying that the one that we're currently using, that's not what this is?

DR. GRIFFIN: Well, is that what we are currently using because -- and is that reflective of the most current up to date knowledge because my understanding is it is not.

DR. COHEN: Well, are you referring to the inhalation discussion that we've had?

DR. GRIFFIN: Yes.

DR. COHEN: Well, I think the boilerplate needs to be redone, right, and that's the issue. But I think in that discussion we are referring people to the inhalation document, right? The inhalation doc.

DR. BERGFELD: It says there there's this website for it.

DR. GRIFFIN: That's right, yeah.

DR. BERGFELD: But it pulls out one of the yeast ferments as having a face powder use and possibly inhalation exposure so the question could this be shortened and say we cannot determine safety? We have no inhalation data.

DR. COHEN: Hmm. Are you talking about a conclusion now that says no- --

DR. BERGFELD: No, no, no. It's in the discussion. It's in the discussion.

DR. COHEN: What are you suggesting?

DR. BERGFELD: Well, Courtney was suggesting it's out of date, the paragraph regarding inhalation under discussion. The question is should it be changed now, or should it just go away and a whole position or should we go forward with it? Are we going forward safe with a number of ingredients, insufficient for many more. But maybe it's a discussion to be had at the panel.

DR. COHEN: Well, look, the issue is we have in the past not cleared things when incidentally inhaled.

DR. ROSS: Yeah. That's correct. And I --

DR. COHEN: So.

DR. ROSS: You could do that here and --

DR. BERGFELD: This is potentially inhaled, yeah? Mm-hmm. Face powder.

DR. ROSS: You don't have the particle size data.

DR. BERGFELD: Doesn't look like it.

DR. COHEN: Boy, that's going to --

DR. TILTON: And also, as long as we have the link to the living document.

DR. COHEN: That's true, but it is -- we do use the inhalation boilerplate when we know things are going to be inhaled and we have some inhalational tox. Right? We don't have any of that here.

DR. BERGFELD: No. No, we don't have particle size. And we don't have the delivery system either. I suspect it's probably not in the applicator, but --

DR. COHEN: Well, we have the airbrush boilerplate, but it really does speak to changing the conclusion a bit.

DR. BERGFELD: It could.

DR. ROSS: I mean, you're getting to a point here where everything we need a particle size distribution for us to clear it. I mean, that's the direction you're going.

DR. BERGFELD: Right.

DR. COHEN: Or pulmonary tox, right?

DR. BERGFELD: Yeah.

DR. COHEN: Right. So, all right. I'll --

DR. BERGFELD: There is a pulmonary tox topic.

DR. COHEN: That was a hat-trick there.

DR. GRIFFIN: Sorry.

DR. COHEN: No, no. Because this is all voluntary. This data's based on VCRP, right? It's not based on where we actually know where this is being used. Once MoCRA goes into effect we might find this stuff in lots of aerosolized products. And since our only data requirement is GRAS or dermal sensitization and not asking for anything else we probably can't conclude anything much more than that. Okay. Okay. I look forward to tomorrow on that one.

DR. BERGFELD: There is some pulmonary tox I was trying to see what -- it's on 82.

DR. ROSS: Yeah, I just -- the Australian patients. Yeah, causes of all patients.

DR. COHEN: That's for geotrichum, right?

DR. ROSS: Yeah.

DR. BERGFELD: Yeah.

DR. COHEN: The problem is the inhalational one is for different -- that's for --

DR. BERGFELD: No, it's for a different yeast.

DR. COHEN: Galatomyces.

DR. BERGFELD: Yeah. This is from (inaudible) in house. Hmm. I think we have to change it. We don't have any information. It's insufficient.

DR. COHEN: Wilma, we're changing the conclusion.

DR. BERGFELD: Yeah, I think we have to change it.

DR. COHEN: Yeah. Okay. I think that'll be an interesting conversation that I don't think too long. Okay. Okay.

Full Panel – December 5, 2023

DR. BELSITO: So while I'm bringing this up, I'll as if Bart could just post the table that Priya provided us with yesterday, it'll make it a lot easier.

At the June 2023 meeting we reviewed the revised Draft Report on 56 yeast-derived ingredients and issued a second insufficient data for this ingredient group. In this IDA, in order to determine the safety of these ingredients the Panel requested confirmatory dermal sensitization data, data on food use generally recognized as safe status on yeast species used to derive

these ingredients for those where it was absent, and in lieu of food use GRAS studies 28-day dermal tox could be considered. We requested information on Qualified Presumption of Safety (QPS) status as designated by the EU, in order to determine whether that would clear systemic toxicity food use needs for these yeasts.

We got lots of new information including some information in Wave 2 on Hydrolyzed Saccharomyces Cell Wall. And, what you can see here in this table that Bart's brought up is, you can see the ingredients that have both food use and sensitization, which we would say are all safe as used.

And then we have ingredients that are highlighted in yellow with food use but no sensitization. Those would require sensitization data at the concentration of use.

We have in the sort of yellow-amber the Pichia Heedii Extract where we have sensitization but no food use. That would require a 28-day dermal, and, if evidence of absorption, other systemic endpoints would be needed.

And then we have in sort of the orange color there where we have no food or sensitization. For three materials we would need both a 28-day dermal and sensitization at concentration of use.

If you can scroll down a little bit more, Bart, to that ingredient that cannot be stated to have food use or dermal sensitization. The way we handled that was, first, we did receive information on Hydrolyzed Saccharomyces Cell Wall in Wave 2. And, we have information on Saccharomyces Cerevisiae so we thought that the Hydrolyzed Saccharomyces Cell Wall derived from the Cerevisiae is safe as used.

We also went on to say that the Hydrolyzed Yeast, Hydrolyzed Yeast Extract, were safe as used if they were derived from yeast ingredients that we found to be safe as used. And that the Saccharomyces group at the bottom, again, if they were derived from Saccharomyces Cerevisiae which we've determined to be safe as used, then they are also safe as used. If they're derived from other Saccharomyces for which we don't have data, it would be insufficient for those specific data needs as mentioned above. So a very split conclusion.

DR. BERGFELD: So your conclusion includes a number of them that will be safe.

DR. BELSITO: Right.

DR. BERGFELD: And then many that are insufficient, and the reason for it under different categories.

DR. BELSITO: Right.

DR. BERGFELD: Okay.

DR. BELSITO: So all the highlighted in blue, and in terms of the ingredients below those that are safe as highlighted in blue, so like a Yeast Extract that came from Candida Saitoana or Saccharomyces Cerevisiae would be fine, but if it came from any of the other yeast that we need data on, it would not be.

DR. BERGFELD: And that would also go in an explanation in the Discussion?

DR. BELSITO: Yes, the Discussion would be quite long.

DR. BERGFELD: Yeah. Dr. Cohen, do you have a comment or a second?

DR. COHEN: I have a comment. Number one, that was tremendously presented and even clearer than we had laid it out. And I do like the way you handled the yeast extracts by referring back to those that have been cleared.

Don, one issue that came up in our discussion is if we clear these, they're being cleared based on skin sensitization and their implicit safety is food or the like, we don't -- and some of these are in powders. And the question is do we split conclusions and indicate that we don't have information or they're not cleared for incidental inhalation.

DR. BELSITO: I think that we sort of deal with that in the report. I mean, we do discuss the IgE-mediated effects.

DR. COHEN: Yeah. I mean, you're talking about, you know, the immediate type hypersensitivity but we don't have real pulmonary data for inhalation of yeast powders.

DR. BELSITO: I'm not sure what you mean. I mean, so clearly our usually respiratory boilerplate. There's some reason you don't think it's sufficient. And I can only assume that's because you're concerned about Type 1 allergic reactions. Is that correct?

DR. COHEN: Listen, that's what precipitated the conversation, but in the absence of having any specific pulmonary tox data I don't know what I don't know about them.

DR. BELSITO: Well, we know that per our respiratory boilerplate they're not going to get to the alveoli, so they should not really cause any significant lung issues. Again, the only issue I could see would be if you're concerned about Type 1 hypersensitivity, which I thought we addressed in the report.

DR. COHEN: Well, but there are fungi that cause hypersensitivity reactions that are not Type 1. Right?

DR. BELSITO: Living fungi.

DR. COHEN: Yeah.

DR. BELSITO: These aren't living.

DR. COHEN: Well, we just don't have the data. I'd like to hear from some of the other people about it, but I think the respiratory boilerplate is something we're going to talk about in the Inhalation Resource discussion.

DR. BELSITO: And I think by the time you extract the yeast, or you make a cell wall, you're not dealing with living organisms anymore.

DR. COHEN: No, but --

DR. BELSITO: What other kind of reactions --

DR. COHEN: Hyper -- well, I'm not sure if some of the other pulmonary hypersensitivity disorders require that the fungus to be alive or not. I just don't know. Any comments from your team, or, Susan, do you want to comment?

DR. TILTON: I mean, from a toxicological perspective, I think the boilerplate is sufficient in this case.

DR. COHEN: Okay.

DR. TILTON: Some of your concerns sound a little bit more on the clinical side. You said in terms of reactivity, but I wouldn't have concerns about a significant amount of absorption through inhalation route of exposure.

DR. COHEN: Okay. Then I'm going to second Don's motion.

DR. BERGFELD: All right.

DR. HELDRETH: There are a couple hands up, from Priya and then from Audrey.

DR. BERGFELD: Okay, Priya first.

MS. CHERIAN: I just wanted to say that in the Wave 2 data there are a couple species that were listed under Hydrolyzed Saccharomyces Cell Wall. And there are two uses for Saccharomyces Pastorianus and Saccharomyces Cerevisiae, and the data we received was an ingredient derived from Pastorianus. So that can also be a species that's considered safe. Even though that's not an INCI name, it's a generic ingredient that's associated with a species that we know about.

DR. BELSITO: Okay.

DR. BERGFELD: Audrey, did you have a comment?

MS. POKRZYWA: I just wanted to confirm information said by Dr. Belsito. In these two case of the positive inhalation with cases with yeast were alive. But as we already stated, during also manufacturing processes of cosmetic ingredients, yeast cannot be alive. There are dead. And I think toxicity by inhalation is very, very low. And in the draft reports, in the Uses section, it's well mentioned that no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type. Therefore, the absence of information about inhalation use is already taken into account in this report. So, I think it's already discussed.

DR. BERGFELD: Thank you. I have a question of you, Don, and your table which I really appreciated as well. Did you put the QPS in there as well, the presumed to be safe, as part of the food?

DR. BELSITO: Yeah.

DR. BERGFELD: So that's a combo of GRAS plus?

DR. BELSITO: But don't thank me, thank Priya. This is all her work.

DR. BERGFELD: Priya, wonderful, actually wonderful, thank you. All right, if there are no more comments than we can call the question. Dr. Belsito, would you just repeat it for clarity, please, in general.

DR. BELSITO: All the materials in the blue are safe as used. The ingredients below that cannot be stated as food use or dermal sensitization, when they're derived from the yeast that we've approved, are also safe as used. Then if you go to the green box where we have food use but no sensitization, we need sensitization data. Where we have sensitization and no food use, we need 28-day dermal and if absorbed then other systemic endpoints may be needed. And where we have no food and no sensitization, again, we need a 28-day dermal and we need sensitization at concentration of use.

DR. BERGFELD: So we have safe as used for a list in blue; insufficient for green, yellow and orange, but for different reasons those will be listed in categories. Okay.

DR. BELSITO: Right.

DR. BERGFELD: All right, and, David, did you second that?

DR. COHEN: I did.

DR. BERGFELD: Okay, I'm going to call the question then. All those in favor of this conclusion -- excuse me, I'm going to do it the other way -- opposed? Abstaining? Unanimously approve, thank you. The next one is again, Dr. Cohen, Octoxynols.

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-aminoacridine
ADME	absorption, distribution, metabolism, and excretion
AF-2	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide
ALT	alanine aminotransferase
AOP	adverse outcome pathway
ARE	antioxidant response element
BAL	bronchoalveolar lavage
BSL	biosafety level
B16F10	melanocytes
Caco-2	human colon epithelial cells from a male with colorectal adenocarcinoma
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
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DLD1	human colorectal adenocarcinoma cell line
DMSO	dimethyl sulfoxide
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-nitroguanidine
EP-2	natural yeast extract isolated by ethanol precipitation
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GPMT	guinea pig maximization test
GRAS	generally recognized as safe
GST	glutathione S-transferase
HaCaT	human keratinocytes
HCC70	non-metastatic breast cancer cell line
HCT116	human colorectal carcinoma cell line
h-CLAT	human cell line activation test
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
KE	key event
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 breast cancer line with estrogen, progesterone, and glucocorticoid receptors
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 concentration
t_{50}	duration of exposure resulting in a 50% decrease in MTT conversion
THP-1	human monocytic cell line
US	United States
U-SENS™	U937 cell line activation test
UVA	ultraviolet A
VCRP	Voluntary Cosmetic Registration Program
ZR-75-1	mammary gland epithelial cell line from a female with ductal carcinoma

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, such as heavy metals and pesticide residues, according to limits set by the US Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA). The Panel reviewed the available data to determine the safety of these ingredients and concluded that 11 yeast-derived ingredients and 22 generically-named yeast-derived ingredients, when derived from species of yeast included in the report with both dermal sensitization and food use status, are safe in cosmetics in the present practices of use and concentration described in this safety assessment. The Panel also concluded that the available data are insufficient to make a determination of safety for the remaining 23 ingredients under the intended conditions of use in cosmetic formulations.

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 (*Saccharomyces cerevisiae*)) 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 dubliniensis*, *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 ingredient.

Hydrolyzed Saccharomyces Cell Wall

According to a manufacturer, Hydrolyzed Saccharomyces Cell Wall is prepared via the enzyme treatment, acid treatment, and neutralization of *Saccharomyces pastorianus*.[13] Supernatants are removed to produce the final 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).[14] 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 Oil Extract

In order to produce Lipomyces Oil Extract, multiple genes that are naturally present in *Lipomyces starkeyi* are over-expressed and manipulated to create a hyper-lipogenic strain.[15,16] This strain produces a mixture of triglycerides (*Lipomyces* oil extract). Sugar is converted to the oil extract via the yeast at a high rate, filling the cells to > 90% of the yeast volume. Upon fermentation and harvest of the hyper-lipogenic yeast strain, the *Lipomyces* oil extract inside of heat-treated cells is released by homogenization and purified. Homogenized cells are extracted, resulting in the pure oil.

Lipomyces starkeyi

Lipomyces starkeyi oil is prepared by first culturing the yeast, followed by cell crushing, filtration, organic solvent extraction, and oil purification.[17] 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).[18] 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.[19] 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.[20] 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.[21] 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.[22]

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).[23] The specific fatty acids observed can be found in Table 4.

Hydrolyzed Saccharomyces Cell Wall

According to a manufacturer, Hydrolyzed Saccharomyces Cell Wall may be derived from the yeast species *Saccharomyces bayanus*, *Saccharomyces cerevisiae*, or *Saccharomyces pastorianus*.[13] This ingredient should not contain more than 2 µg/g lead, 1.5 µg/g arsenic, and 5.6% nitrogen.

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.[24] 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.[25] 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.[14] 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.[26]

Lipomyces Lipid Bodies

Full genomic sequencing and polymerase chain reaction tests were performed on a cream containing 100% Lipomyces Lipid Bodies.[27] This cream contained no foreign genes or antibiotic resistance traits. This single-ingredient cream is composed of large, isolated yeast lipid bodies in water.[15] These lipid bodies are approximately 10 µm in size. Approximately 87.5% of the lipid body mass is composed of the mixture of triglycerides that is the same Lipomyces Oil Extract.[15] The remaining 12.5% consists of the shell wall that contains hydrophilic lipids, specifically diacylglycerides, trace levels of proteins, and yeast beta-glucans.

Lipomyces Oil Extract

Lipomyces Oil Extract is reported to have a lipid profile similar to that of refined, bleached, and deodorized palm oil.[15,16] However, Lipomyces Oil Extract is less than 50% saturated and is not bleached, omitting chlorinated hydrocarbons, colored contaminants, sterols, and trans-fats. The lipid profile of Lipomyces Oil Extract was evaluated via gas chromatography and was determined to consist of 40% palmitic acid, 39% oleic acid, 6% lineolic acid, 6% stearic acid, 5% palmitoleic acid, and 4% other acid residues.

Lipomyces starkeyi

The main components of *Lipomyces starkeyi* are triglycerides.[17] Yeast oil derived from this species is rich in palmitic and oleic acid residues and is an edible oil similar to palm oil. Lipid samples of *Lipomyces starkeyi* processed via different fermentation methods were determined to have compositions similar to the main components of vegetable oil.[28] The fatty acid composition of these samples consisted of oleic acid (46.6 – 48.12%), palmitic acid (33.6 – 38.43%), stearic acid (4.59 – 5.97%), palmitoleic acid (3.01 – 3.96%), and linoleic acid (1.12 – 2.93%).

Pichia Heedii Extract

Pichia Heedii Extract consists of 20% monosaccharides of glucose and mannose, 44% oligosaccharides and polysaccharides of glucose and mannose, and 10% oligopeptides.[29] The extract also contains 26% mineral ash (chloride, sodium, potassium, and phosphorous).

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, torulene 0.058 ± 0.002 mg/g, and beta-carotene 0.024 ± 0.001 mg/g.[30] 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 status conditions, the ingredient must meet the following specifications: 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.[31] 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.[32] 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.[33] 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.[34] 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.[14] 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.[35]

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.[19]

Schizosaccharomyces pombe

The fatty acid profile of a *Schizosaccharomyces pombe* extract was evaluated via gas chromatography.[36] 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).[37]

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).[20] 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).[23] 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.[38] 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 oleophila, 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% minerals (as determined by pyrolysis), and 7 - 60% proteins.[22] The sum of heavy metals in these extracts were reported to be < 20 ppm. Yeast Extract derived from *Pichia naganishii* is reported to consist of 12% oligosaccharides and polysaccharides of glucose and mannose, 29% minerals (as determined by pyrolysis), and 59% oligopeptides.[29]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from US 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 included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database in 2023 (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council; maximum use concentrations). The data were provided by cosmetic product categories, based at that time 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).[39] 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).[40] 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.

According to a supplier, Pichia Heedii Extract is reported to be used in skin care products at 0.032 - 0.096%.[29] The same supplier reported that Yeast Extract derived from *Pichia naganishii* is used in skin care products at 0.0105 – 0.105%.

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.[41]

Non-Cosmetic

Yeasts are commonly used worldwide in the food and beverage industry, mainly in baking and alcohol production as a fermentative agent.[42] 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*.[22] 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*).[43] 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).[4,35,44-46] 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 subcutaneous study performed in rats.[46] 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).[47]

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).[35] 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).[48] 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.[49] 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).[45] 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 Ferment Lysate Filtrate (in dimethyl sulfoxide (DMSO); 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).[4,43,45,49-53] 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.[54-57] 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 guilliermondii*, *Candida humicola*, *Candida norvegica*, *Candida utilis*, *Cryptococcus albidus*, *Geotrichum candidum, Pitryosporum pachydermatis, Pitryosporum ovale, Rhodotorula minuta, Rhodotorula rubra, Saccharomyces cerevisiae, Torulopsis glabrata*, and *Trichosporon cutaneum*) was evaluated.[58] 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.[47] 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*).[18] 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.[59] 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.[60] 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.[61] 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.[62] 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.[63] 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% Galactomyces 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.[64] 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.[65] 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%).[66,67] 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 addition, Table 14 provides an overview of the available sensitization data per ingredient, along with an indication as to whether the studies assess key events (KE) in the adverse outcome pathway (AOP) for skin sensitization.[68] Notations are also provided if guinea pig maximization tests, Buehler tests, or human repeated insult patch tests (HRIPTs) were performed.

In vitro dermal irritation assays yielded negative results (majority of studies performed were EpiDerm™ assays).[2,13,69-75] Tests were performed using a trade name mixture containing 8-10% Hydrolyzed Saccharomyces Cell Wall, 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.[43] In dermal patch tests in humans, the following were tested and found to be non-irritating: a *Galactomyces* ferment filtrate (tested neat; multiple patch test); a trade name mixture containing 8-10% Hydrolyzed Saccharomyces Cell Wall (tested neat; single patch test); Metschnikowia Agaves Extract, Pichia Anomala Extract, Pichia Heedii Extract, Pichia Minuta Extract, and Yeast Extract derived from *Candida magnoliae, Candida saitoana*, *Metschnikowia pulcherrima*, and *Metschnikowia reukaufii* (all tested at 15% aq.; single patch tests); a cosmetic formulation containing 1% *Saccharomyces cerevisiae* extract (tested neat; single patch test).[13,22,76,77] In a 4-wk use study, no irritation was observed when subjects applied a cream containing 100% Lipomyces Lipid Bodies to the hands and face.[27]

No sensitization potential was observed in several in chemico/in vitro sensitization assays. Direct peptide reactivity assays (DPRA; assesses KE1 in the AOP) were performed using Phaffia Rhodozyma Extract (100 mM in acetonitrile) and trade name mixture containing 24% Saccharomyces Ferment Lysate Filtrate (100 mM acetonitrile).[78,79] KeratinoSens™ assays (assess KE2 in the AOP) were performed using a trade name mixture containing 8 - 10% Hydrolyzed Saccharomyces Cell Wall, 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* (majority of test substances tested at up to 2000 µM).[13,22,43,79-81] Human cell line activation tests (h-CLAT; assesses KE3 in the AOP) were performed using Hydrolyzed Yeast (up to 5000 µg/ml) and Yeast Extract (derived from *Pichia naganishii* (concentration tested not stated)).[43,80] A U937 cell line activation test (U-SENS™; also assesses KE3 in the AOP) was performed using Pichia Minuta Extract (concentration tested not stated).[43]

Local lymph node assays (LLNA; assess KE4 in the AOP) were performed in mice for *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 procedure, the test substance was considered to be non-sensitizing. In guinea pig studies, no sensitization was observed in a guinea pig maximization test (GPMT) of *Galactomyces* ferment filtrate (tested neat),[82] and no sensitization was observed in a Buehler assay performed using a mixture

containing 90% yeast (*Saccharomyces cerevisiae*) cell wall in 10% HSCAS (tested at 49.5% in water and carboxymethylcellulose).[4]

Human studies were performed for several of the yeast-derived ingredients. HRIPTs of a skincare product containing 1.485% Galactomyces Ferment Filtrate (tested neat; n = 104), a facial treatment essence containing 92.675% Galactomyces Ferment Filtrate (tested neat; (n = 100)), a trade name mixture containing 0.4% Hydrolyzed Yeast (tested at 0.01%; final test concentration of Hydrolyzed Yeast: 0.00004%; n = 51), a trade name mixture containing 8-10% Hydrolyzed Saccharomyces Cell Wall (tested neat; n = 50), a trade name mixture containing 10% Pichia Ferment Lysate Filtrate (tested neat; n = 55), a cream containing 0.0135% Saccharomyces Ferment Lysate Filtrate (n = 52), a trade name mixture containing 2% Saccharomyces Ferment Lysate Filtrate (tested neat; n = 105), a cream containing 0.028% Saccharomyces Lysate Extract (tested neat; n = 50), and a trade name mixture containing 25% Saccharomyces Lysate Extract (tested at 10% in water; final test concentration of Saccharomyces Lysate Extract: 2.5%; n = 50), a lotion containing 0.0045% Yeast Extract (n = 52), Yeast Extract derived from *Candida oleophila* (final test concentration of 0.285%; n = 100), 15% aq. Metschnikowia Agaves Extract (n = 112), Pichia Anomala Extract (n = 100 and n = 104), Pichia Heedii Extract (n = 106), Pichia Minuta Extract (n = 107), a Yeast Extract derived from *Candida saitoana* (n = 112), and a Yeast Extract derived from *Metschnikowia reukaufii* (n = 104) were negative for sensitization.[13,22,83-92]

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%).[93,94] Similarly, no phototoxicity or photosensitization was observed in assays performed on animals using *Galactomyces* ferment filtrate (tested neat; n = 3 rabbits in phototoxicity assay, n = 10 guinea pigs/group in photosensitization assay).[95,96]

OCULAR IRRITATION STUDIES

Details on the ocular irritation studies summarized below can be found in Table 15.

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 8-10% Hydrolyzed Saccharomyces Cell Wall, a trade name mixture containing 49% Phaffia Rhodozyma Extract, Pichia Ferment Lysate Filtrate (100%), 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*.[2,43,69-75,97,98]

No irritation was observed in an ocular irritation assay performed in rabbits using *Galactomyces* ferment filtrate (tested neat).[99] 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.[4,43] 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.[100-123] These reports, however, were found in immunocompromised or post-surgical patients; therefore, their relevancy to cosmetic safety is unlikely. In addition, yeast-related infections are associated with live yeast strains, but no live yeasts are expected to be present in finished cosmetic products.

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.[46] 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.[124] 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.[125] 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, *Penicillium 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.[126] 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.[127] 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.

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 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 subcutaneous study)) was considered to be non-toxic in acute inhalation and acute subcutaneous 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 Ferment Lysate Filtrate, 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 pseudotropicalis* (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 8-10% Hydrolyzed Saccharomyces Cell Wall (tested neat), 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 neat), a trade name mixture containing 8-10% Hydrolyzed Saccharomyces Cell Wall (tested neat), 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. DPRAs (assess KE1 in the AOP) were performed using Phaffia Rhodozyma Extract (100 mM in acetonitrile) and trade name mixture containing 24% Saccharomyces Ferment Lysate Filtrate (100 mM acetonitrile). KeratinoSens™ assays (assess KE2 in the AOP) were performed using a trade name mixture containing 8-10% Hydrolyzed Saccharomyces Cell Wall, 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* (majority of test substances tested at up to 2000 µM). h-CLATs (assess KE3 in the AOP) were performed using Hydrolyzed Yeast (up to 5000 µg/ml) and Yeast Extract (derived from *Pichia naganishii* (concentration tested not stated)). A U-SENS™ (also assesses KE3 in the AOP) was performed using Pichia Minuta Extract (concentration tested not stated).

Several LLNAs (assess KE4 in the AOP) 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 procedure, the test substance was considered to be non-sensitizing. No sensitization was observed in a GPMT of *Galactomyces* ferment filtrate (tested neat) or in a Buehler 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).

Human studies were performed for several of the yeast-derived ingredients, all with negative results. HRIPTs of a skincare product containing 1.485% Galactomyces Ferment Filtrate (tested neat; n = 104), a facial treatment essence containing 92.675% Galactomyces Ferment Filtrate (tested neat; (n = 100)), a trade name mixture containing 0.4% Hydrolyzed Yeast (tested at 0.01%; final test concentration of Hydrolyzed Yeast: 0.00004%; n = 51), a trade name mixture containing 8-10% Hydrolyzed Saccharomyces Cell Wall (tested neat; n = 50), a trade name mixture containing 10% Pichia Ferment Lysate Filtrate (tested neat; n = 55), a cream containing 0.0135% Saccharomyces Ferment Lysate Filtrate (n = 52), a trade name mixture containing 2% Saccharomyces Ferment Lysate Filtrate (tested neat; n = 105), a cream containing 0.028% Saccharomyces Lysate Extract (tested neat; n = 50), and a trade name mixture containing 25% Saccharomyces Lysate Extract (tested at 10% in water; final test concentration of Saccharomyces Lysate Extract: 2.5%; n = 50), a lotion containing 0.0045% Yeast Extract (n = 52), Yeast Extract derived from Candida *oleophila* (final test concentration of 0.285%; n = 100), 15% aq. Metschnikowia Agaves Extract (n = 112), Pichia Anomala Extract (n = 100 and n = 104), Pichia Heedii Extract (n = 106), Pichia Minuta Extract (n = 107), a Yeast Extract derived from *Candida saitoana* (n = 112), and a Yeast Extract derived from *Metschnikowia reukaufii* (n = 104) were negative for sensitization.

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 (nested neat).

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 8-10% Hydrolyzed Saccharomyces Cell Wall, a trade name mixture containing 49% Phaffia Rhodozyma Extract, Pichia Ferment Lysate Filtrate, 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 (tested neat). Minimal irritation was observed in an ocular irritation assay performed in rabbits using a mixture containing 90% *Saccharomyces cerevisiae* cell wall in 10% 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 tests 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.

DISCUSSION

The 56 ingredients in this report are derived from various species of yeast; the majority of the yeasts are from the Saccharomycetes class. The Panel reviewed these yeast-derived ingredients and determined that the data are sufficient to conclude that 11 yeast-derived ingredients and 22 generically-named yeast-derived ingredients, when derived from species of yeast included in the report with both dermal sensitization and food use status, are safe in cosmetics in the present practices of use and concentration; the Panel also concluded that data were insufficient to determine the safety of the remaining 23 ingredients. Data profiles for these ingredients were considered sufficient when food use data (via published literature, GRAS status, and/or QPS status) and sensitization data were available for the ingredient itself, or for the species of yeast used to derive the ingredient. (The need for systemic toxicity data was mitigated for those ingredients that are used in foods, have a GRAS status, or QPS status because exposure via ingestion would be expected to be far greater than exposure via cosmetics.) Some of the yeast-derived ingredients reviewed herein are generic, and it is unknown which species, or how many species, are used to manufacture the ingredients (e.g., Yeast Extract). These generic ingredients were considered to be safe by the Panel if formulated using a species of yeast included in this report that had both food use and dermal sensitization data. These species include *Candida magnoliae*, *Candida saitoana*, *Metschnikowia agaves*, *Metschnikowia reukaufii*, *Pichia anomala*, *Pichia minuta*, *Phaffia rhodozyma*, *Saccharomyces cerevisiae*, and *Saccharomyces pastorianus.* Ingredients lacking

some or all of the data components described herein were considered to have insufficient safety data, and depending on which data are lacking, systemic toxicity data (via 28-d dermal toxicity assay), sensitization data, or both, are required (food use/GRAS status/QPS status may be used in lieu of systemic toxicity data). It should be noted that if 28-d dermal toxicity data are provided and these data indicate absorption of the ingredient, other toxicity endpoints would be required to determine safety (e.g., developmental and reproductive toxicity). A comprehensive listing the data needs of each ingredient is provided in Table 16.

The Panel noted that elevated levels of heavy metals and pesticide residues may be present in these yeast-derived ingredients and stressed that the cosmetics industry should continue to use the necessary procedures to minimize impurities in cosmetic formulations according to limits set by the FDA and EPA. In addition, the Panel noted that volatile compounds (e.g., benzaldehyde, hexane) 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.

The Panel also noted incidences of IgE-mediated hypersensitivity following inhalation exposure to certain yeast species (e.g., *Saccharomyces cerevisiae*). 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 was 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 that could result with the use of some of these ingredients in cosmetics (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.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Therefore, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 11 of the 56 yeast-derived ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Hydrolyzed Candida Saitoana Extract
Galactomyces Ferment Filtrate
Hydrolyzed Metschnikowia Agaves Extract*
Metschnikowia Agaves Extract*
Hydrolyzed Metschnikowia Reukaufii Extract*
Metschnikowia Reukaufii Lysate Extract*
Phaffia Rhodozyma Extract*
Phaffia Rhodozyma Ferment Extract*
Pichia Anomala Extract
Pichia Minuta Extract*
Saccharomyces Cerevisiae Extract

In addition, the Panel concluded that the following 22 generic-named yeast-derived ingredients (ingredients in which the species of yeast used in manufacturing was not provided in the *Dictionary*), when derived from species of yeast included in the report, are safe in cosmetics in the present practices of use and concentration described in this safety assessment:

Hydrolyzed Saccharomyces Cell Wall*
Hydrolyzed Saccharomyces Extract*
Hydrolyzed Saccharomyces Lysate Extract*
Hydrolyzed Yeast
Hydrolyzed Yeast Extract
Lactic Yeasts*
Pichia Extract*
Saccharomyces*
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*
Yeast
Yeast Extract
Yeast Ferment Extract

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

The Panel also concluded that the available data are insufficient to make a determination of safety for the remaining 23 ingredients under the intended conditions of use in cosmetic formulations.

Hydrolyzed Candida Bombicola Extract**
Hydrolyzed Kluyveromyces Extract**
Hydrolyzed Metschnikowia Shanxiensis**
Hydrolyzed Torulaspora Delbrueckii Extract**
Kluyveromyces Extract
Lipomyces Lipid Bodies**
Lipomyces Oil**
Lipomyces Oil Extract**
Metschnikowia Henanensis Extract**
Metschnikowia Viticola Extract**
Pichia Caribbica Ferment**
Pichia Ferment Extract Filtrate**
Pichia Ferment Lysate Filtrate
Pichia Heedii Extract**
Pichia Pastoris Ferment 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**

*** There are currently no uses reported for these ingredients.*

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	21
Color	clear-pale yellow	21
Odor	characteristic	21
Water Solubility	soluble	21
Specific Gravity (@ 25ºC)	1.05 – 1.15	21
Refraction Index (RIU (@ 25°C))	1.3920 – 1.5000	21

*derived from *Saccharomyces cerevisiae*

Table 3. Taxonomy of yeast-derived ingredients[1,128]

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*	-	-
	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	*Saccharomyces bayanus*	*Saccharomyces abulensis*
	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	*Saccharomyces cerevisiae*	*Mycoderma cerevisiae* *Candida robusta* *Saccharomyces capensis* *Saccharomyces italicus* *Saccharomyces oviformis* *Saccharomyces uvarum var. melibiosus*
	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	*Saccharomyces pastorianus*	*Saccharomyces carlsbergensis* *Saccharomyces monacensis*
Hydrolyzed Saccharomyces Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-
Hydrolyzed Saccharomyces Lysate Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	-	-

Table 3. Taxonomy of yeast-derived ingredients[1,128]

INCI Ingredient	Class	Order	Family	Genus	Associated Genus and Species/Synonyms	Synonyms**
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	-	-	-	-	-
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*	-

Table 3. Taxonomy of yeast-derived ingredients[1,128]

INCI Ingredient	Class	Order	Family	Genus	Associated Genus and Species/Synonyms	Synonyms**
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*
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*

Table 3. Taxonomy of yeast-derived ingredients[1,128]

INCI Ingredient	Class	Order	Family	Genus	Associated Genus and Species/Synonyms	Synonyms**
Torulaspora Delbrueckii Ferment	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Torulaspora*	*Torulapora delbrueckii*	*Saccharomyces delbrueckii* *Saccharomyces fermentati* *Saccharomyces rosei* *Candida colliculosa*
Yarrowia Lipolytica Extract	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Yarrowia*	*Yarrowia lipolytica*	*Endomycopsis lipolytica* *Mycotorula lipolytica* *Candida lipolytica* *Candida oleophila*
Yarrowia Lipolytica Ferment Lysate	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Yarrowia*	*Yarrowia lipolytica*	*Endomycopsis lipolytica* *Mycotorula lipolytica* *Candida lipolytica* *Candida oleophila*
Yarrowia Lipolytica Oil	Saccharomycetes	Saccharomycetales	*Dipodascaceae*	*Yarrowia*	*Yarrowia lipolytica*	*Endomycopsis lipolytica* *Mycotorula lipolytica* *Candida lipolytica* *Candida oleophila*
Yeast	Saccharomycetes	-	-	-	-	-
Yeast Extract***	Saccharomycetes	-	-	-	-	-
	Saccharomycetes	Saccharomycetales	*NR*	*Starmerella*	*Candida magnoliae*	*Starmerella magnoliae* *Torulopsis magnoliae*
	Saccharomycetes	Saccharomycetales	*Debaryomycetaceae*	*Kurtzmaniella*	*Candida oleophila*	*Yarrowia lipolytica*
	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*

Table 3. Taxonomy of yeast-derived ingredients[1,128]

INCI Ingredient	Class	Order	Family	Genus	Associated Genus and Species/Synonyms	Synonyms**
Yeast Ferment Extract	Saccharomycetes	Saccharomycetales	*Saccharomycetaceae*	*Saccharomyces*	*Saccharomyces cerevisiae*	*Mycoderma cerevisiae* *Candida robusta* *Saccharomyces capensis* *Saccharomyces italicus* *Saccharomyces oviformis* *Saccharomyces uvarum var. melibiosus*

*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[21,22] 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)[23]

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*[129]

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)[39] and concentration (2021/2023)[40,130,131] 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)[39] and concentration (2021/2023)[40,130,131] 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)[39] and concentration (2021/2023)[40,130,131] 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)[39] and concentration (2021/2023)[40,130,131] 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)[39] and concentration (2021/2023)[40,130,131] 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)[39] and concentration (2021/2023)[40,130,131] 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)[39] and concentration (2021/2023)[40,130,131] 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)[39] and concentration (2021/2023)[40,130,131] 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)[39] and concentration (2021/2023)[40,130,131] 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 ingredients not reported to be used 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	132,133
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	134
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	135,136
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	48,137-140
Hydrolyzed Metschnikowia Agaves Extract Metschnikowia Agaves Extract	*Metschnikowia agaves* can be found in blue agave used to make tequila	-	141
Hydrolyzed Metschnikowia Reukaufii Extract Metschnikowia Reukaufii Lysate Extract Yeast Extract derived from *Metschnikowia reukaufii*	*Metschnikowia reukaufii* is used in beer fermentation	-	142
Hydrolyzed Saccharomyces Cell Wall Saccharomyces Cerevisiae Extract Yeast Ferment Extract	*Saccharomyces bayanus* is used in wine and beer-making *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 *Saccharomyces pastorianus* is used in the production of lager beer	Inactivated yeast (*Saccharomyces cerevisiae*) cells are used in animal feed and over-the-counter nutritional supplements	42,140,143
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	-	144-146
Lipomyces Lipid Bodies (when derived from *Lipomyces starkeyi*)	*Lipomyces starkeyi* is GRAS in probiotics	Cream consisting of 100% Lipomyces Lipid Bodies is being researched for use as a loading agent for hydrophobic drugs and active ingredients; a new class of antifungal in *Lipomyces* oil cream is being developed for topical burn treatment	16

Table 8. Food use/presence and non-cosmetic uses of yeast species

Associated Ingredients	Food Use/Presence	Other Non-Cosmetic Uses	Reference
Lipomyces Oil Lipomyces Oil Extract	*Lipomyces starkeyi* is GRAS in probiotics	-	140,147
Metschnikowia Viticola Extract	*Metschnikowia viticola may be present in wine* *Metschnikowia viticola has been isolated from grapes grown in Hungary*	-	148,149
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	140,150-153
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	154-156
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* (cattle) -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	-	157
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	158-162
Pichia Heedii Extract	-	*Pichia heedii* may be used to assimilate D-xylose	163
Pichia Minuta Extract	*Pichia minuta* may be found in wine	*Pichia minuta* has been isolated from olive tree cultures	164,165
Phaffia Rhodozyma Extract Phaffia Rhodozyma Ferment Extract	*Phaffia rhodozyma* – QPS status	Astaxanthin-rich *Phaffia rhodozyma* may be used in feed for salmon and trout	140,166
Schizosaccharomyces Pombe Extract	*Schizosaccharomyces pombe* is used in cachaça (alcoholic beverage made from fermented sugarcane juice) and kombucha *Schizosaccharomyces pombe* – QPS status	-	140,144
Yarrowia Lipolytica Extract Yarrowia Lipolytica Ferment Lysate Yarrowia Lipolytica Oil Yeast Extract when derived from *Candida oleophila* (synonymous to *Yarrowia lipolytica*)	*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	*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 *Yarrowia lipolytica* may be used for commercial production of food grade citric acid [21 CFR 173.165]	46,136,140,167-171

Table 8. Food use/presence and non-cosmetic uses of yeast species

Associated Ingredients	Food Use/Presence	Other Non-Cosmetic Uses	Reference
	omega-3 fatty acid, docosahexaenoic acid [21 CFR 184.1472] *Yarrowia lipolytica* – QPS status *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	*Yarrowia lipolytica* is used in the manufacture of foods and may produce biosurfactants/emulsifers	
Yeast Extract (when derived from *Candida magnoliae*)	*Candida magnoliae* has been isolated from lime honey and honeycomb	-	[172-174]
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	[175-180]
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	[181,182]
Yeast Extract (when derived from *Pichia naganishii*)	*Pichia naganishii* may be present in the fermented liquid "ersho" used in Ethiopian foods *Pichia naganishii* may be found in injera sourdough	-	[183]

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	[22]
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	[22]
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	[22]
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	[22]
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	[22]
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	[22]
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	[22]

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	[43]
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	[43]
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	44
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.	35
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.	45
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	46
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 (strain, 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	46
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	47
Yeast Extract (when derived from *Candida oleophila*)	*Candida oleophila* strain O**	NR	rats (strain, 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	46

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 ($p < 0.05$).	[35]
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 ratio 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.	[48]
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	[49]
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	[45]
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	[45]

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	52
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-AA, 2-NF, 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	49
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	50
Pichia Ferment Lysate Filtrate	Pichia Ferment Lysate Filtrate	DMSO	experiment 1: 3, 10, 33, 100, 333, 1000, 2500, and 5000 µg/plate experiment 2: 33, 100, 333, 1000, 2500, and 5000 µg/plate	*S. typhimurium* strains TA1537, TA98, TA1535, and TA100; *E. coli* WP2 *uvrA*	Ames assay; OECD TG 471; performed with and without metabolic activation; concurrent untreated and solvent controls used; positive controls: sodium azide, 4-nitro-o-phenylene-diamine, 2-AA	Non-mutagenic; controls gave expected results	53
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	43
Pichia Minuta Extract	Pichia Minuta Extract	NR	NR	TK6 lymphoblastoid human cells	micronucleus assay	Non-mutagenic	43
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	45
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	45
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	51
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	46

Table 12. Genotoxicity studies*

Ingredient	Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
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	[46]
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	[43]
Yeast Extract (when derived from *Pichia naganishii*)	Yeast Extract (derived from *Pichia naganishii*)	NR	NR	L5178Y TK+/- mouse lymphoma cells	micronucleus assay	Non-mutagenic	[43]
				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	[49]
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-aminoacridine; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide; DMSO = dimethyl sulfoxide; 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						
Hydrolyzed Saccharomyces Cell Wall (derived from *Saccharomyces pastorianus*)	trade name mixture containing Hydrolyzed Saccharomyces Cell Wall (8-10%), phenoxyethanthol (0.5%), lactic acid (0.16 – 0.22%), alcohol (4%), fragrance (< 0.1%), and water (residual)	100%; 25 µl	reconstructed human epidermis model	LabCyte EPI-MODEL24 SIT; OECD TG 439; negative control of water; positive control of sodium dodecyl sulfate; 15 min application time	non-irritating	13
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	70
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	71
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	74
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	69
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	72
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	75
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	73
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

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
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	[46]
Human						
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate****	100%	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.	[76]
Hydrolyzed Saccharomyces Cell Wall (derived from *Saccharomyces pastorianus*)	trade name mixture containing Hydrolyzed Saccharomyces Cell Wall (8-10%), phenoxyethanol (0.5%), lactic acid (0.16 – 0.22%), alcohol (4%), fragrance (< 0.1%), and water (residual)	100%	20 subjects	24-h patch test; occlusive conditions; sites evaluated 60 min and 24 h after patch removal	non-irritating	[13]
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	[27]
Metschnikowia Agaves Extract	Metschnikowia Agaves Extract	15% in water	11 subjects	patch test; no other details provided	non-irritating	[22]
Pichia Anomala Extract	Pichia Anomala Extract	15% in water	10 subjects	patch test; no other details provided	non-irritating	[22]
Pichia Anomala Extract	Pichia Anomala Extract	15% in water	10 subjects	patch test; no other details provided	non-irritating	[22]
Pichia Heedii Extract	Pichia Heedii Extract	15% in water	10 subjects	patch test; no other details provided	non-irritating	[22]
Pichia Minuta Extract	Pichia Minuta Extract	15% in water	11 subjects	patch test; no other details provided	non-irritating	[22]
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	[77]
Yeast Extract	Yeast Extract derived from *Candida magnoliae*	15% in water	10 subjects	patch test; no other details provided	non-irritating	[22]
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	[22]
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	[22]
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	[22]

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
SENSITIZATION						
In Chemico/In Vitro						
Hydrolyzed Saccharomyces Cell Wall (derived from *Saccharomyces pastorianus*)	trade name mixture containing Hydrolyzed Saccharomyces Cell Wall (8-10%), phenoxyethanol (0.5%), lactic acid (0.16 – 0.22%), alcohol (4%), fragrance (< 0.1%), and water (residual)	up to 400 µg	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	[13]
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	[80]
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	h-CLAT; OECD TG 442E	no sensitization potential	[80]
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	[78]
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	[81]
Pichia Minuta Extract	Pichia Minuta Extract	NR	KeratinoSens™ cell line	ARE-Nrf2 Luciferase Test; OECD TG 442D	no sensitization potential	[43]
Pichia Minuta Extract	Pichia Minuta Extract	NR	U937 cell line	U-SENS™; OECD TG 442E	no sensitization potential	[43]
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	[79]
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	[79]
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	[43]
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	[22]
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	[43]
Yeast Extract (when derived from *Pichia naganishii*)	Yeast Extract (derived from *Pichia naganishii*)	NR	THP-1 cell line	h-CLAT; OECD TG 442E	no sensitization potential	[43]

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	[82]
Hydrolyzed Saccharomyces Cell Wall (*derived from Saccharomyces cerevisiae*)	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)	Buehler test; 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						
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	84
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	89
Hydrolyzed Saccharomyces Cell Wall (derived from *Saccharomyces pastorianus*)	trade name mixture containing Hydrolyzed Saccharomyces Cell Wall (8-10%), phenoxyethanol (0.5%), lactic acid (0.16 – 0.22%), alcohol (4%), fragrance (< 0.1%), and water (residual)	100%	50 subjects	HRIPT; level of occlusion and patch size not stated; 9 induction patches; challenge patch applied 2 wk after last induction patch	non-irritating and non-sensitizing	13
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 conditions (patch size: 4 cm^2); 9 induction patches; challenge patch applied 2 wk after last induction patch	non-irritating and non-sensitizing	85
Metschnikowia Agaves Extract	Metschnikowia Agaves Extract	15% in water	112 subjects	HRIPT; no other details provided	non-sensitizing	22
Pichia Anomala Extract	Pichia Anomala Extract	15% in water	104 subjects	HRIPT; no other details provided	non-sensitizing	22
Pichia Anomala Extract	Pichia Anomala Extract	15% in water	100 subjects	HRIPT; no other details provided	non-irritating; non-sensitizing	22
Pichia Ferment Lysate Filtrate	trade name mixture containing 10% Pichia Ferment Lysate Filtrate	100%	55 subjects	HRIPT; occlusive conditions (patch size: 50 mm^2); 9 induction patches over 3 wk challenge applied 2 wk after last induction	non-irritating; non-sensitizing	92
Pichia Heedii Extract	Pichia Heedii Extract	15% in water	106 subjects	HRIPT; no other details provided	non-irritating; non-sensitizing	22
Pichia Minuta Extract	Pichia Minuta Extract	15% in water	107 subjects	HRIPT; no other details provided	non-sensitizing	22
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	86
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	88
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	83

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
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	90
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	87
Yeast Extract	1.90% Yeast Extract derived from *Candida oleophila* (final test concentration of extract: 0.285%)	15% in water	100 subjects	HIRPT; 6-wk study; no other details provided	non-sensitizing	91
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	22
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	22
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	93
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	94
Animal						
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate****	100%	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	96

Table 13. Dermal irritation and sensitization studies*

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
PHOTOSENSITIZATION						
Animal						
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate****	100%	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 (1.2×10^8 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	[95]

ARE = antioxidant response element; dpm = disintegrations per minute; DPRA = direct peptide reactivity assay; h-CLAT = human cell line activation test; 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; U-SENS = U937 cell line activation test; 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. Sensitization data profile per ingredient, identifying those assessing AOP key events, animal studies, and human studies

Ingredient	Key Event 1	Key Event 2	Key Event 3	Key Event 4	GPMT/Buehler	HRIPT
Galactomyces Ferment Filtrate					GPMT	HRIPT
Hydrolyzed Saccharomyces Cell Wall (derived from *Saccharomyces cerevisiae*)					Buehler	
Hydrolyzed Saccharomyces Cell Wall (derived from *Saccharomyces pastorianus*)		KeratinoSens™				HRIPT
Hydrolyzed Yeast		KeratinoSens™	h-CLAT			HRIPT
Metschnikowia Agaves Extract						HRIPT
Phaffia Rhodozyma Extract	DPRA	KeratinoSens™				
Pichia Anomala Extract						HRIPT
Pichia Heedii Extract						HRIPT
Pichia Minuta Extract		KeratinoSens™	U-SENS			HRIPT
Saccharomyces Cerevisiae Extract				LLNA		
Saccharomyces Ferment Lysate Filtrate	DPRA	KeratinoSens™				HRIPT
Saccharomyces Lysate Extract						HRIPT
Yeast Extract						HRIPT
Yeast Extract (derived from *Candida magnoliae*)		KeratinoSens™				
Yeast Extract (derived from *Candida saitoana*)						HRIPT
Yeast Extract (derived from *Metschnikowia reukaufii*)		KeratinoSens™				HRIPT
Yeast Extract (derived from *Pichia naganishii*)		KeratinoSens™	h-CLAT			

DPRA = direct peptide reactivity assay; GPMT = guinea pig maximization test; h-CLAT = human cell line activation test; HRIPT = human repeated insult patch test; U-SENS = U937 cell line activation test

Table 15. 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	97
Hydrolyzed Saccharomyces Cell Wall (derived from *Saccharomyces pastorianus*)	trade name mixture containing Hydrolyzed Saccharomyces Cell Wall (8-10%), phenoxyethanol (0.5%), lactic acid (0.16 – 0.22%), alcohol (4%), fragrance (< 0.1%), and water (residual)	NR	100%; 50 µl	human corneal epithelial cells	LabCyte CORNEA-MODEL24 EIT; OECD TG 492; phosphate-buffered saline used as negative control; ethanol used as positive control; 1 min exposure period	non-irritating; controls gave expected results	13
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	70

Table 15. Ocular irritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Pichia Ferment Lysate Filtrate	Pichia Ferment Lysate Filtrate	NR	100%; 50 µl	corneal epithelial model (EpiOcular™)	EpiOcular™ assay; OECD TG 492; 30 min incubation; MTT assay performed; phosphate-buffered saline used as negative control; methyl acetate used as positive control	non-irritating; controls gave expected results	98
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	43
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	71
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	74
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.	69
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
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	72
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	73
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	75

Table 15. Ocular irritation studies

Ingredient	Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
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	[43]
				ANIMAL			
Galactomyces Ferment Filtrate	*Galactomyces* ferment filtrate***	none	0.1 ml (neat)	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	[99]
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	[46]

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*)

Table 16. Safety designation of yeast-derived ingredients

Yeast-derived ingredients considered safe	
Hydrolyzed Candida Saitoana Extract	Phaffia Rhodozyma Extract
Galactomyces Ferment Filtrate	Phaffia Rhodozyma Ferment Extract
Hydrolyzed Metschnikowia Agaves Extract	Pichia Anomala Extract
Metschnikowia Agaves Extract	Pichia Minuta Extract
Hydrolyzed Metschnikowia Reukaufii Extract	Saccharomyces Cerevisiae Extract
Metschnikowia Reukaufii Lysate Extract	
Generic yeast-derived ingredients considered safe*	
Hydrolyzed Saccharomyces Cell Wall	Saccharomyces Ferment Extract Lysate Filtrate
Hydrolyzed Saccharomyces Extract	Saccharomyces Ferment Filtrate
Hydrolyzed Saccharomyces Lysate Extract	Saccharomyces Ferment Lysate Extract
Hydrolyzed Yeast	Saccharomyces Ferment Lysate Filtrate
Hydrolyzed Yeast Extract	Saccharomyces Lysate
Lactic Yeasts	Saccharomyces Lysate Extract
Pichia Extract	Saccharomyces Lysate Extract Filtrate
Saccharomyces	Saccharomyces Lysate Filtrate
Saccharomyces Extract	Yeast
Saccharomyces Ferment	Yeast Extract
Saccharomyces Ferment Extract	Yeast Ferment Extract
Yeast-derived ingredients considered insufficient due to lack of systemic toxicity/food use/GRAS/QPS data	
Pichia Ferment Lysate Filtrate	
Pichia Heedii Extract	
Yeast-derived ingredients considered insufficient due to lack of sensitization data	
Hydrolyzed Candida Bombicola Extract	Pichia Pastoris Ferment Filtrate
Hydrolyzed Kluyveromyces Extract	Hydrolyzed Torulaspora Delbrueckii Extract
Kluyveromyces Extract	Torulaspora Delbrueckii Extract
Lipomyces Lipid Bodies	Torulaspora Delbrueckii Ferment
Lipomyces Oil	Schizosaccharomyces Pombe Extract
Lipomyces Oil Extract	Yarrowia Lipolytica Extract
Metschnikowia Viticola Extract	Yarrowia Lipolytica Ferment Lysate
Pichia Caribbica Ferment	Yarrowia Lipolytica Oil
Pichia Ferment Extract Filtrate	
Yeast-derived ingredients considered insufficient due to lack of both systemic toxicity/food use/GRAS/QPS data and sensitization data	
Metschnikowia Henanensis Extract	
Hydrolyzed Metschnikowia Shanxiensis	
Schizosaccaromyces Ferment Extract Filtrate	
Schizosaccharomyces Ferment Filtrate	

*these generic ingredients are considered safe as used when derived from the following species of yeast: *Candida magnoliae*, *Candida saitoana*, *Metschnikowia agaves*, *Metschnikowia reukaufii*, *Pichia anomala*, *Pichia minuta*, *Phaffia rhodozyma*, *Saccharomyces cerevisiae*, and *Saccharomyces pastorianus*

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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: February 2, 2024

SUBJECT: Yeast Extract derived from *Candida oleophila*

Anonymous. 2024. Summary information *Candida oleophila* (includes a summary of an HRIPT).

Summary Information – *Candida oleophila*

Synonym

The December 2023 update of the "list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA" (at: Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 19: Suitability of taxonomic units notified to EFSA until September 2023 (wiley.com)) states that: "*Candida oleophila* has been added as a synonym of *Yarrowia lipolytica*".

HRIPT

An ingredient containing 1.90% Yeast Extract made from *Candida oleophila,* diluted at 15% in water (tested concentration of the extract 0.285%) was tested in 100 subjects (6-week study).

Results: Non-sensitizing

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: February 15, 2024

SUBJECT: *Pichia* species

Anonymous. 2024. Composition and Use Information Pichia Heedii Extract and Yeast Extract made from Pichia naganishii; Summary of Food Use of *Pichia* spp.

February 2024

Composition and Use Information (provided by a cosmetic ingredient supplier)
Pichia Heedii Extract and Yeast Extract made from *Pichia naganishii*

Typical composition of Pichia Heedii Extract:

- Monosaccharides of glucose and mannose: 20%
- Oligosaccharides and polysaccharides of glucose and mannose: 44%
- Mineral ash: 26% (Chloride, Sodium, Potassium, Phosphorus)
- Peptides: 10% oligopeptides

The common use concentrations in skin care finished products are: 0.032% to 0.096%

Typical composition of Yeast Extract derived from *Pichia naganishii*:

- Oligosaccharides and polysaccharides of glucose and mannose: 12%
- Mineral ash: 29% (Chloride, Potassium, Phosphorus, Sodium, Sulfur)
- Peptides: 59% oligopeptides

The common use concentrations in skin care finished products are: 0.0105% to 0.105%

Summary of Food Use of *Pichia* spp (Bibliography attached)

Many species of *Pichia* spp. have been found in various foods and drinks that we consume (GRAS Notice GRN 938, Pichia Kluyveri DSM 33235; Hammes et al. 2005): bakery sourdoughs (Michel et al. 2023; Nuobariene, Arneborg and Hansen 2014, 2014; Boyaci- Gunduz and Erten 2020; Nuobariene, Arneborg, and Hansen 2014), cheese (Pereira-Dias et al. 2000; Banjara, Suhr, and Hallen-Adams 2015), olive brine (Marquina et al. 1992), and wine (Drumonde -Neves et al. 2017; Carbonetto et al. 2018; Jolly, Augustyn and Pretorius 2017).

Yeasts of the *Pichia* genus have also been found in traditional dishes or drinks (Steinkraus 1996):

• Seafood, rice and meat mixtures made in acid fermentation in the Philippines (p262-264) ;

• In a palm wine fermented in Nigeria (p381-382) ;

• In a drink called Mexican Pulque made from agave juice (p389-393).

The *Pichia naganishii* species was identified in a fermented liquid, “ersho”, used in the composition of an Ethiopian specialty called “enjera”, itself made from a cereal, teff (Eragrostis tef) (Steinkraus 1996; Ashenafi 1994; Mengesha, Tebeje et Tilahun 2022, 2022, 2022; Neela et Fanta 2020; Tesfaw, Oner et Assefa 2021; Charlotte Urien 2015).

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Study / Product References:

Coordinator Centre: **EH 16-1111/0/16.0505**

Investigator Centre: **ER 16/081-17/16-0363**

CONFIRMATION IN HUMAN OF THE SKIN COMPATIBILITY AND ABSENCE OF ALLERGENIC POTENTIAL OF ONE COSMETIC PRODUCT AFTER REPEATED APPLICATION UNDER PATCH

Human Repeated Insult Patch Test (HRIPT)

SPONSOR: INFINITEC ACTIVOS S.L.
Baldiri i Reixac 15-21
08028 Barcelona - SPAIN

For: Mrs. Marisabel MOURELLE

TEST PRODUCT: PH4 Ferment Collagen Explosion REF: FE0001

Study report
version 1

Bucharest, 27th October 2016

25 pages in this report including 12 in Appendices

CONFIRMATION IN HUMAN OF THE SKIN COMPATIBILITY AND ABSENCE OF ALLERGENIC POTENTIAL OF ONE COSMETIC PRODUCT AFTER REPEATED APPLICATION UNDER PATCH

Repeated Insult Patch Test (HRIPT)

TABLE OF CONTENTS

I. AIM AND PRINCIPLE OF THE STUDY

This study intended to confirm the skin compatibility and the absence of allergenic potential of several cosmetic products including the product **PH4 Ferment Collagen Explosion REF: FE0001**, after repeated application to the skin under exaggerated experimental conditions.

The product was applied under patch for a defined time. The applications were repeated 9 times over a period of 3 consecutive weeks, period necessary for the possible induction of an allergy.

After a minimal 2-week rest period, with no treatment, a single application of each product under patch, to the induction site and to a virgin site and for a defined time, enabled to reveal a possible induced allergy.

II. RELEVANCE OF THE STUDY

Ethics

The study aiming at confirming the skin compatibility of the test product and the foreseeable risk incurred by the subjects who took part in the study being minor, there was a suitability between the aim of the study, its possible risks and the potential troubles related to the modalities planned in the protocol.

All the subjects were included in the study the same day, taking into account their number.

The applications were performed at the Investigating Centre by the dermatologist helped by the technician in charge of the study.

A clinical examination by the dermatologist helped by the technician in charge of the study was performed after each passage at the Investigating Centre.

In case of important reactivity to one of the products, the applications could be interrupted in the subjects concerned.

Methodological approach

The skin compatibility of the product was confirmed by the dermatologist who has an appropriate experience.

The experimental conditions adopted created a certain occlusion and favoured the penetration of the ingredients through the skin. If some of them had an allergenic potential, this one could be more easily proved by this kind of approach.

The methodology used was an adaptation from that described by **Marzulli and Maibach** (Human Repeated Insult Patch Test for delayed contact hypersensitivity: HRIPT)

- Marzulli F.N., Maibach H.I., Contact allergy: predictive testing in man, Contact Dermatitis, 1976, 2, pp. 1-17.

The patch material and the conditions of use of the product were adapted to the type of test product in accordance with the corresponding procedure.

The experimental area chosen (back) enabled to test easily the product. The sites of application of the different products were chosen at random to get rid of the variability of the skin reactivity according to the site.

A control site (without test product) served as control to avoid the possible intercurrent effects not directly related to the test product.

Panel

Referring to the experience acquired in the field of contact allergy to cosmetic products and to the accurate knowledge of the ingredients incorporated into the test products, the number of subjects, defined in the protocol, was acceptable to confirm, in first approach, the absence of allergenic potential of the test products.

Results

The results were mainly expressed as descriptive data and did not require a statistical treatment.

If the test product had a good skin compatibility, under these experimental conditions, by extrapolation it should be safe for human health when applied under normal conditions of use.

III. TYPE OF THE STUDY

This mono-centric study was performed in open.

The subject was used as own control.

It was performed according to the general conditions of EUROFINS EVIC ROMANIA, established for the performance of Human test project.

The test project was submitted to the previous agreement of the internal committee of EUROFINS EVIC ROMANIA (opinion n° CI-187/16 of September 05th 2016).

IV. INVESTIGATOR AND COORDINATOR CENTRE

IV.1.Investigator Centre

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08013, BARCELONA (Spain)

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V. DATES OF PERFORMANCE OF THE STUDY

Beginning on: September 05th, 2016

End on: October 14th, 2016

VI. TEST PRODUCTS

VI.1. Total number of products simultaneously tested in the study

Fifteen (15) products were tested in this study.

This number complied with the corresponding procedure which defines the maximal number of test products according to the chosen experimental area and patch material.

This report concerns only the product **PH4 Ferment Collagen Explosion REF: FE0001.**

One control patch, corresponding to the type of patch material used, containing an ad hoc quantity of distilled water, was applied at the same time.

VI.2. Identification of the test product

Denomination	PH4 Ferment Collagen Explosion
Reference	FE0001
Batch number	Y1608261
Eurofins EVIC Product Testing Spain reference	16.0505
EUROFINS EVIC ROMANIA reference	16-0363
Galenic form and organoleptic characteristics	Colourless liquid
Number and type of samples	2 plastic jars + 1 plastic flask
Content of the samples	150g + 10g

VI.3. Information concerning the test product

The document relating to the test product supplied with the samples was the Sponsor's letter of agreement particularly concerning the conformity of the formula to the regulations in force and its safety.

VII. SUBJECTS

VII.1. Number

The number of subjects whose data had to be exploitable at the end of the study was 50, with a lower acceptable limit of 50, in accordance with the corresponding procedure.

In order to compensate for the possible withdrawals during the study and to obtain this quota of subjects at the end of the study, about 12% of extra people were recruited.

- The subjects whose data were exploitable at the end of the study:

- To check the skin compatibility of the test products, corresponded to all the subjects included as long as they were submitted at least to one post application examination at the defined time or else,

- To check the absence of allergenic potential of the test products (in absence of allergic reaction during the induction phase), corresponded to all the subjects included as long as they were submitted to the challenge.

56 subjects were included in the study.

1 subject discontinued (ref. 6d) for personal reasons independent of the study and no exclusion was decided by the investigator.

The compatibility of the test product was therefore assessed in 55 subjects.

The confirmation of the absence of allergenic potential of the test product was assessed in 55 subjects.

VII.2. Specific inclusion criteria

The specific inclusion criteria, defined in the protocol, were the following ones:

- Age: 18-70 years old
- Sex: female / male
- Phototype (Fitzpatrick): II to IV
- With all types of skin.

All the subjects corresponded to these specific inclusion criteria. Their typological characteristics are defined in **Appendices 1/1 to 1/3.**

VII.3. Specific non inclusion criteria

The specific non inclusion criteria were the following ones:

- cutaneous marks on the experimental area which could interfere with the assessment of skin reactions (pigmentation troubles, scar elements, over-developed pilosity, ephelides and naevi in too great quantity, sunburn…..),
- eczematoid reaction still visible, scar or pigmentary sequelae of previous tests on the experimental area,
- allergy to colophony, to nickel,
- personal history of adverse reactions to the same type of product as the one tested,
- atopy,
- reactivity to adhesive plaster,
- participation in more than 5 tests under exaggerated use conditions (under patch) within 12 months before the study, including 3 hypoallergenicity tests at the most,
- intensive sun exposure within the month before the study,
- forecast of intensive sun or UVA exposure (UV lamps) during the test period,
- forecast of bath (bathtub, sea or swimming-pool), sauna or hammam sessions during the test period,
- intensive or regular practice of one or several sports whose temporary interruption created difficulties,
- treatment with Vitamin A acid or its derivatives within 3 months before the beginning of the study,
- treatment with topical corticoids on the experimental area within 8 days before the study,
- treatment with PUVA or UVB within 1 month before the study,
- forecast of vaccination during the test period or last vaccination within 3 weeks before the study.

All the subjects corresponded to these specific non inclusion criteria.

VIII. METHODOLOGY

VIII.1. Experimental area and sites of application of the test products

The chosen experimental area was the back.

The site of application of the products was chosen by the dermatologist or the technician in charge of the study. Skin appearance was taken into account and the areas of friction with clothes were avoided.

The product was applied by the dermatologist or the technician in charge of the study, to one of the sites localized by a clockwise distribution, altering of one rank from a subject to another.

VIII.2. Experimental conditions of application of the test product

The experimental conditions defined in the protocol were the following ones:

Patch material	Experimental conditions of use	Quantity applied
Finn Chamber standard®	As it is provided by the sponsor	20 µl

Occlusive patch

-Finn Chamber standard®: aluminium cupula in which the product was put down (20 µl or approximately 20 mg), kept in position by a hypoallergenic adhesive: Scanpor® (inner diameter: 8 mm, surface: 50 mm²).

The quantities of product had to be measured with a micropipette (with a single use tip).

All the experimental conditions of application at the Investigating Centre, defined in the protocol, were respected.

VIII.3. Chronology of the study

The applications of the test product, the removal of the patches and the controls were performed by the dermatologist or the technician in charge of the study.

- **Induction phase:** 3 consecutive weeks.

  * application of the product to a perfectly delimited site, under patch on D1, D3, D5, D8, D10, D12, D15, D17, D19.

  * patch removal
    - after 48 h of contact on D3, D5, D10, D12, D17, D19.
    - after 72 h of contact on D8, D15, D22.

  * controls: skin examination and questioning (paragraph VIII.6) before patching on D1 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D3, D5, D8, D10, D12, D15, D17, D19, D22.

- **Rest period:** 2 consecutive weeks at least (4 weeks at the most).

  * no application of product.

- **Challenge:** 1 week.

  * application of the product to a perfectly delimited virgin site and to the site defined for the induction phase, under patch on D36.

  * patch removal after 48 h of contact on D38.

  * controls: skin examination and questioning (paragraph VIII.6) before patching on D36 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D38, D39, D40 (48, 72, 96 h after application).

All the experimental conditions of application were respected except the following ones:

- application of the product at the end of induction period (D22) with patch removal in D24 by the test subject at home for the first 37 subjects (ref 1d to 37d), the subjects were instructed that in case of reactions or discomfort sensations they had to come at the investigating centre for clinical examination. No subject reported reactions or discomfort sensations.
- rest period of 12 days instead of 14 days for the first 37 subjects (ref 1d to 37d).

These deviations occurred due to the fact that the product arrived later to the investigating centre.
The investigator judged these deviations with no major incidence on the interpretation of the results.

VIII.4. Constraints of the study

The constraints imposed on the subjects were the following ones:

- no application of other products (than the tested ones) to the experimental area,
- no wearing of too tight or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patches,
- no bath (bathtub or swimming-pool or sea), no hammam or sauna sessions during the study,
- if shower, protection of the experimental area or no violent projection of water and no application of soap to the experimental area to avoid patch removal or appearance of intercurrent phenomena and very gentle wiping if necessary,
- no excessive sweating and no intensive sport liable to cause unsticking of the patches,
- no intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal,
- neither anti-allergic, anti-inflammatory (systemic or topical corticotherapy…) treatment nor treatment with patent medicines containing vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen),
- no vaccination during the study,
- at least 14 passages at the Investigating Centre (15 if a pre-inclusion visit was necessary).

VIII.5. Control of the observance of the modalities of the protocol

The investigator checked the respect of the **constraints**.

The subjects were questioned at the end of the study. The Investigator assessed the importance of the possible deviations in comparison with the experimental conditions required at the beginning of the study.

The synthesis of the answers obtained is enclosed in **Appendices 2/1 and 2/2.**

All the deviations from the protocol were analysed and the investigator assessed their effect on the validity of the results.

All the constraints of the study, defined in the protocol, were respected by the subjects.

VIII.6. Confirmation of the compatibility (absence of irritant effect) and absence of allergenic potential

VIII.6.1. Frequency of the examinations

The skin examination and joint questioning had to be performed by the dermatologist helped by the technician in charge of the study.

The examination had to be performed, visually under standard "daylight", according to the frequency mentioned on paragraph VIII.3.

All the examinations were performed in accordance with the conditions defined in the protocol.

VIII.6.2. Expression of the results of the skin examination and questioning

The expression of the results of the skin examination and questioning was that defined for this type of study in accordance with the corresponding procedure.

In case of reactivity:

- **The main visible signs were noted, i.e.:**

Erythema, Œdema, Vesicle, Bulla, Papule, Scab, Dryness, Coloration, Soap effect.

The intensity of the **erythema and oedema** was assessed according to an ordinal scale: very slight, slight, moderate, severe.

The appearance of the **erythema** was specified: diffuse, punctuated, peripheral (around the application site).

The importance of the number of **vesicles and papules** was assessed according to an ordinal scale: 1 to 2 vesicles or papules, more than 2 vesicles or papules.

Bulla, scab, dryness, coloration and soap effect were described.

The importance of the **dryness and coloration** was assessed according to an ordinal scale: very slight, slight, moderate, severe.

- **the main sensations of discomfort were described, i.e. :**

Heating, Stinging, Pruritus (itching).

The results were expressed:

- **in percentage of reactive subjects** : for this calculation only the visible signs of reactivity were taken into account : erythema, oedema, vesicle, bulla, papule, scab.
- **in a descriptive manner** for the other visible signs or for the sensations of discomfort : when the frequency of appearance of these signs justified it, the percentage of reactive subjects was possibly calculated.

VIII.6.3. Interpretation of the results of the skin examination and questioning

All the subjects included in the study were taken into account to confirm the skin compatibility of the test product as long as they were submitted at least to one post application examination at the defined time or else.

All the subjects included in the study were taken into account to confirm the absence of allergenic potential of the test product (in absence of allergic reaction during the induction phase) as long as they were submitted to the challenge.

The interpretation of the results of the skin examination and questioning was that defined for this type of study in accordance with the corresponding procedure.

The possible reactions observed during the induction phase were either **irritation reactions** or **revelation of an allergy previously contracted or revelation of an allergy precociously induced** by the test product.

The possible reactions observed during the challenge on the "virgin" site were compared to those observed on the "induction" site at the same times. They were either **irritation reactions** or **revelation of an allergy induced during the induction phase** by the test product.

The natures, intensity, time of appearance, time of disappearance, location (induction site and/or virgin site) of the skin reaction were taken into account for the interpretation of the results.

To appreciate the skin compatibility and possible irritation reactions, the interpretation of the results, performed by the dermatologist was absolute (referring to **the experience of the investigator centre** in this field and especially to the **data acquired** on products of same cosmetic category tested under similar conditions). The test product could therefore have a **very good, good, moderate or bad skin compatibility**.

To appreciate the allergenic potential, the interpretation of the results was partly based on the allergenicity evaluation scale established by the **ICDRG** (International Contact Dermatitis Research Group) and took into account the visible reactions (clinical signs) and the possible reactions appeared on the control site:

ICDRG	NT	non tested
	?+	doubtful reaction, only slight erythema
	+	positive reaction (with no vesicle): erythema, infiltration, sometimes some papules
	++	strong positive reaction: presence of erythema, papules, vesicles
	+++	violent positive reaction: with presence of bullae
	-	negative reaction
	IR	irritation reaction

IX. RESULTS / DISCUSSION

The individual data of the skin examination and questioning of the subjects are enclosed in **Appendices 3/1 to 3/3 and 4/1 to 4/3.**

In brief:

Induction phase	
Type of reactivity on the induction site	**Number and percentage of reactive subjects**
None	0 / 0%

Challenge	
Type of reactivity on the induction site and virgin site	**Number and percentage of reactive subjects**
None	0 / 0%

Discussion:

During the induction phase, no irritation reaction was observed

During the challenge, no allergic reaction was observed.

X. CONCLUSION

Under the experimental conditions adopted the repeated applications of the product **PH4 Ferment Collagen Explosion REF: FE0001**, under occlusive patch, in a panel of 55 subjects with **all types of skin**, induced no reaction of irritation and the product **has a very good skin compatibility**.

Moreover, **no allergic reaction** was detected. Thus, the product may be considered as "hypoallergenic", under this specific context.

Signatures and dates

Investigator: Dr. Rozalia OLSAVSZKY (dermatologist) 03/11/2016

I the undersigned, Rozalia OLSAVSZKY declare that the overall conduct of the study was carried out under my responsibility and in spirit with the principles of Good Clinical Practices for cosmetics (International recommendations ICH E 6, step 4, of 1/5/1996).

Quality Assurance Personnel: Alina BOBOC 03/11/2016

I the undersigned, Alina BOBOC, declare that:
- this type of study was audited according to the procedure of the investigator centre on 21/09/2016,
- the final report was examined on 27/10/2016
- the results reported accurately and completely reflect the raw data of the study.

Head manager of the investigator centre: Alina NANU 03/11/2016

I the undersigned, Alina NANU, declare to have designated Rozalia OLSAVSZKY as investigator and ensured that she approved the study protocol with full knowledge of the facts.

APPENDICES

Appendix 1/1

TYPOLOGICAL CHARACTERISTICS OF THE SUBJECTS

Subject ref.	Age (years)	Sex F=female M=male	Phototype*
1d	68	M	IV
2d	60	F	III
3d	68	M	II
4d	64	F	III
5d	66	F	III
6d	55	M	III
7d	67	M	III
8d	64	F	III
9d	61	F	III
10d	64	M	III
11d	58	F	III
12d	48	F	III
13d	63	F	II
14d	58	F	IV
15d	61	F	II
16d	64	F	III
17d	69	M	III
18d	61	F	III
19d	62	F	II
20d	64	F	III

Appendix 1/2

TYPOLOGICAL CHARACTERISTICS OF THE SUBJECTS

Subject ref.	Age (years)	Sex F=female M=male	Phototype*
21d	65	F	III
22d	52	F	III
23d	67	M	III
24d	63	F	IV
25d	61	M	III
26d	64	M	III
27d	65	F	III
28d	69	F	IV
29d	62	F	III
30d	39	F	IV
31d	60	F	III
32d	61	F	III
33d	63	F	II
34d	62	F	III
35d	55	F	IV
36d	63	F	III
37d	48	M	IV

Appendix 1/3

TYPOLOGICAL CHARACTERISTICS OF THE SUBJECTS

Subject ref.	Age (years)	Sex F=female M=male	Phototype*
38d	62	F	II
39d	63	F	III
40d	64	F	III
41d	41	F	III
42d	65	F	III
43d	55	F	IV
44d	65	F	II
45d	65	F	II
46d	57	F	III
47d	59	M	III
48d	67	M	III
49d	28	F	III
50d	36	F	III
51d	43	M	III
52d	50	F	III
53d	59	M	III
54d	36	F	II
55d	62	F	II
56d	20	M	II

Legends:

	Withdrawal

*__phototype according to Fitzpatrick__, established on the principle of a first 30 to 40-minute sun exposure after the winter or a period without exposure of an equivalent duration:

Type I	Always burns easily, never tans
Type II	Always burns easily, tans minimally
Type III	Burns moderately, tans gradually
Type IV	Burns slightly, always tans easily
Type V	Burns rarely, tans intensely
Type VI	Never burns, strongly pigmented

Appendix 2/1

CONTROL OF THE OBSERVANCE
Constraints

Constraints **(55 exploitable results)**	**Number** of subjects who respected the constraints	**Percentage** of subjects who respected the constraints
No application of other products than the tested ones to the experimental area Deviation: none	55	100%
No wearing of too tight or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patch Deviation: none	55	100%
No bath (bathtub, swimming pool or sea), no hammam or sauna sessions during the study Deviation: none	55	100%
If shower, protection of the experimental area or no violent projection of water and no application of soap to the experimental area to avoid patch removal or appearance of intercurrent phenomena and very gentle wiping if necessary Deviation: none	55	100%
No excessive sweating and no intensive sport liable to cause unsticking of the patch Deviation: none	55	100%

Appendix 2/2

CONTROL OF THE OBSERVANCE
Constraints

Constraints **(55 exploitable results)**	**Number** of subjects who respected the constraints	**Percentage** of subjects who respected the constraints
No intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal Deviation: none	55	100%
Neither anti-allergic, anti-inflammatory (systemic or topical corticotherapy…) treatment nor treatment with patent medicines containing Vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen) – no medical treatment which could interfere with the study Deviation: none	55	100%
No vaccination during the study Deviation: none	55	100%
At least 13 passages at the Investigating Centre (14 if a pre-inclusion visit was necessary) Deviation: none	55	100%

Appendix 3/1

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Subject ref.	Reactivity									
	D3[1]	D5	D8	D10	D12	D15	D17	D19	D22[2]	D24[3]
1d	/	/	/	/	/	/	/	/	/	/
2d	/	/	/	/	/	/	/	/	/	/
3d	/	/	/	/	/	/	/	/	/	/
4d	/	/	/	/	/	/	/	/	/	/
5d	/	/	/	/	/	/	/	/	/	/
6d	/	Withdrawal								
7d	/	/	/	/	/	/	/	/	/	/
8d	/	/	/	/	/	/	/	/	/	/
9d	/	/	/	/	/	/	/	/	/	/
10d	/	/	/	/	/	/	/	/	/	/
11d	/	/	/	/	/	/	/	/	/	/
12d	/	/	/	/	/	/	/	/	/	/
13d	/	/	/	/	/	/	/	/	/	/
14d	/	/	/	/	/	/	/	/	/	/
15d	/	/	/	/	/	/	/	/	/	/
16d	/	/	/	/	/	/	/	/	/	/
17d	/	/	/	/	/	/	/	/	/	/
18d	/	/	/	/	/	/	/	/	/	/
19d	/	/	/	/	/	/	/	/	/	/
20d	/	/	/	/	/	/	/	/	/	/

[1] Before 1st product application

[2] Before last product application

[3] Based on the fact the subject didn't report reactions or discomfort sensations

Appendix 3/2

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Subject ref.	Reactivity									
	D3[1]	D5	D8	D10	D12	D15	D17	D19	D22[2]	D24[3]
21d	/	/	/	/	/	/	/	/	/	/
22d	/	/	/	/	/	/	/	/	/	/
23d	/	/	/	/	/	/	/	/	/	/
24d	/	/	/	/	/	/	/	/	/	/
25d	/	/	/	/	/	/	/	/	/	/
26d	/	/	/	/	/	/	/	/	/	/
27d	/	/	/	/	/	/	/	/	/	/
28d	/	/	/	/	/	/	/	/	/	/
29d	/	/	/	/	/	/	/	/	/	/
30d	/	/	/	/	/	/	/	/	/	/
31d	/	/	/	/	/	/	/	/	/	/
32d	/	/	/	/	/	/	/	/	/	/
33d	/	/	/	/	/	/	/	/	/	/
34d	/	/	/	/	/	/	/	/	/	/
35d	/	/	/	/	/	/	/	/	/	/
36d	/	/	/	/	/	/	/	/	/	/
37d	/	/	/	/	/	/	/	/	/	/

[1] Before 1st product application

[2] Before last product application

[3] Based on the fact the subject didn't report reactions or discomfort sensations

Appendix 3/3

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Subject ref.	Reactivity								
	D3	D5	D8	D10	D12	D15	D17	D19	D22
38d	/	/	/	/	/	/	/	/	/
39d	/	/	/	/	/	/	/	/	/
40d	/	/	/	/	/	/	/	/	/
41d	/	/	/	/	/	/	/	/	/
42d	/	/	/	/	/	/	/	/	/
43d	/	/	/	/	/	/	/	/	/
44d	/	/	/	/	/	/	/	/	/
45d	/	/	/	/	/	/	/	/	/
46d	/	/	/	/	/	/	/	/	/
47d	/	/	/	/	/	/	/	/	/
48d	/	/	/	/	/	/	/	/	/
49d	/	/	/	/	/	/	/	/	/
50d	/	/	/	/	/	/	/	/	/
51d	/	/	/	/	/	/	/	/	/
52d	/	/	/	/	/	/	/	/	/
53d	/	/	/	/	/	/	/	/	/
54d	/	/	/	/	/	/	/	/	/
55d	/	/	/	/	/	/	/	/	/
56d	/	/	/	/	/	/	/	/	/

Legends:

/	nothing to report
E	Erythema
Œ	Œdema
Ve	Vesicle
D	Dryness
S	Soap effect

NA	no applied
Bu	Bulla
Pa	Papule
Sc	Scab
C	Coloration
Pr	Pruritus
Hea	Heating
Sti	Stinging
Vesicules or papules	1 (1 or 2) ; 2 (>2)

0.5	Very slight intensity
1	Slight intensity
2	Moderate intensity
3	Severe intensity
d	diffuse
p	punctuated
peri	peripheral

Appendix 4/1

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Subject ref.	Reactivity						According to the ICDRG criteria
	Induction site			Virgin site			
	D36	D38	D40	D36	D38	D40	
1d	/	/	/	/	/	/	-
2d	/	/	/	/	/	/	-
3d	/	/	/	/	/	/	-
4d	/	/	/	/	/	/	-
5d	/	/	/	/	/	/	-
6d	Withdrawal						
7d	/	/	/	/	/	/	-
8d	/	/	/	/	/	/	-
9d	/	/	/	/	/	/	-
10d	/	/	/	/	/	/	-
11d	/	/	/	/	/	/	-
12d	/	/	/	/	/	/	-
13d	/	/	/	/	/	/	-
14d	/	/	/	/	/	/	-
15d	/	/	/	/	/	/	-
16d	/	/	/	/	/	/	-
17d	/	/	/	/	/	/	-
18d	/	/	/	/	/	/	-
19d	/	/	/	/	/	/	-
20d	/	/	/	/	/	/	-

Appendix 4/2

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Subject ref.	Reactivity						According to the ICDRG criteria
	Induction site			Virgin site			
	D36	D38	D40	D36	D38	D40	
21d	/	/	/	/	/	/	-
22d	/	/	/	/	/	/	-
23d	/	/	/	/	/	/	-
24d	/	/	/	/	/	/	-
25d	/	/	/	/	/	/	-
26d	/	/	/	/	/	/	-
27d	/	/	/	/	/	/	-
28d	/	/	/	/	/	/	-
29d	/	/	/	/	/	/	-
30d	/	/	/	/	/	/	-
31d	/	/	/	/	/	/	-
32d	/	/	/	/	/	/	-
33d	/	/	/	/	/	/	-
34d	/	/	/	/	/	/	-
35d	/	/	/	/	/	/	-
36d	/	/	/	/	/	/	-
37d	/	/	/	/	/	/	-
38d	/	/	/	/	/	/	-
39d	/	/	/	/	/	/	-
40d	/	/	/	/	/	/	-

Appendix 4/3

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Subject ref.	Reactivity						According to the ICDRG criteria
	Induction site			Virgin site			
	D36	D38	D40	D36	D38	D40	
41d	/	/	/	/	/	/	-
42d	/	/	/	/	/	/	-
43d	/	/	/	/	/	/	-
44d	/	/	/	/	/	/	-
45d	/	/	/	/	/	/	-
46d	/	/	/	/	/	/	-
47d	/	/	/	/	/	/	-
48d	/	/	/	/	/	/	-
49d	/	/	/	/	/	/	-
50d	/	/	/	/	/	/	-
51d	/	/	/	/	/	/	-
52d	/	/	/	/	/	/	-
53d	/	/	/	/	/	/	-
54d	/	/	/	/	/	/	-
55d	/	/	/	/	/	/	-
56d	/	/	/	/	/	/	-

Legends:

/	nothing to report
E	Erythema
Œ	Œdema
Ve	Vesicle
D	Dryness
S	Soap effect

NA	no applied
Bu	Bulla
Pa	Papule
Sc	Scab
C	Coloration
Pr	Pruritus
Hea	Heating
Sti	Stinging
Vesicules or papules	1 (1 or 2) ; 2 (>2)

0.5	Very slight intensity
1	Slight intensity
2	Moderate intensity
3	Severe intensity
d	diffuse
p	punctuated
peri	peripheral

ICDRG	NT	non tested
	?+	doubtful reaction, only slight erythema
	+	positive reaction (with no vesicle): erythema, infiltration, sometimes some papules
	++	strong positive reaction: presence of erythema, papules, vesicles
	+++	violent positive reaction: with presence of bullae
	-	negative reaction
	IR	irritation reaction

Report

C4004-210510: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay

ICCR Study Number:	4068911
Sponsor Name:	INFINITEC ACTIVOS S.L.
ESIMS Code:	2023-00073-EGM
Version ID:	Final
Issue Date:	19 October 2023
Study Director:	Dr. Steffi Chang
Testing Facility:	ICCR-Roßdorf GmbH In den Leppsteinswiesen 19 64380 Rossdorf Germany

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COMPLIANCE WITH GOOD LABORATORY PRACTICE

C4004-210510: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

- "Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) in its currently valid version
- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17
- EC Commission Directive 2004/10/EC

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

S.C

Dr. Steffi Chang
Study Director
ICCR-Roßdorf GmbH

19 October 2023

Date

QUALITY ASSURANCE STATEMENT

C4004-210510: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay

Study based activities at the Test Facility ICCR-Roßdorf GmbH were audited and inspected. The details of these audits and inspections are given below.

Type of Inspection	Date(s) of Inspection	Date Reporting to Study Director, Test Facility Management
Study plan verification 1st Audit	11 Jul 2023	11 Jul 2023
Process-based Test item preparation, test system preparation and application	19 Jul 2023	19 Jul 2023
Report audit	29 Sep 2023	29 Sep 2023

General facilities and activities where this study was conducted were inspected on an annual basis and results are reported to the relevant responsible person and Management.

The statement is to confirm, that this report reflects the raw data.

Quality Assurance

S. Ebert

19 October 2023

Date

Sabine Ebert

Quality Assurance Auditor
ICCR-Roßdorf GmbH

1 SUMMARY

This study was performed to investigate the potential of C4004-210510 to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and the *Escherichia coli* strain WP2 *uvrA*.
The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.
The test item was tested at the following concentrations:

Pre-Experiment/Experiment I:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

No precipitation of the test item occurred up to the highest investigated dose.

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in all strains with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with C4004-210510 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies, which were clearly within the historical laboratory control data range. Thus, the sensitivity of the test system was demonstrated.

Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.
Therefore, C4004-210510 is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

2 INTRODUCTION AND PURPOSE

The study was performed to assess the potential of the test item to induce gene mutations by means of two independent *Salmonella typhimurium* and *Escherichia coli* reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since a negative result was obtained in this experiment, experiment II was performed as a pre-incubation assay.

The most widely used assays for detecting gene mutations are those using bacteria. They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency at which an agent abolishes or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to grow in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The *Salmonella typhimurium* histidine (his) and the *Escherichia coli* tryptophan (trp) reversion system measures $his^- \rightarrow his^+$ and $trp^- \rightarrow trp^+$ reversions, respectively. The *Salmonella typhimurium* and *Escherichia coli* strains are constructed to differentiate between base pair (TA 1535, TA 100, and WP2 *uvrA*) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect at least six dose levels with adequately spaced concentrations were tested. The maximum dose level was 5000 µg/plate.

To validate the test, reference mutagens were tested in parallel to the test item.

2.1 Study Details

Sponsor	**INFINITEC ACTIVOS S.L.** Can Parellada 22, Nave 2-3 08170 Montornés del Vallés, Barcelona Spain
Study Monitor	Dr. Andrea Marburger Evonik Operations GmbH Nutrition & Care Rodenbacher Chaussee 4 63457 Hanau-Wolfgang Germany

2.2 Study Schedule

Study initiation date	12 July 2023
Experimental start date	21 July 2023
Experimental completion date	07 August 2023

2.3 Regulatory Testing Guidelines

This study was designed to be compatible with the procedures indicated by the following internationally accepted guidelines and recommendations:

- Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471: Bacterial Reverse Mutation Test, corrected June 26, 2020
- Commission Regulation (EC) No. 440/2008 B13/14, dated May 30, 2008

3 MATERIALS AND METHODS

3.1 Test Item and Supporting Information

Information as provided by the Sponsor.

Identification:	C4004-210510
Alternative name:	Pichia Ferment Lysate Filtrate
Batch:	210510
Purity:	Not applicable
Appearance:	Colorless*, liquid
Recertification Date:	05/2025
Storage Conditions:	Freezer
Stability in Solvent:	Not indicated by the Sponsor
Purpose of Use:	Cosmetic product

* Determined by ICCR-Roßdorf staff

No correction for purity was made.

3.2 Special Conditions

Maximum concentration:	50 mg/mL
Solvent:	DMSO

3.3 Study Controls

3.3.1 Negative Controls

Concurrent untreated and solvent controls were performed.

3.3.2 Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Purity:	≥ 99 %
Dissolved in:	deionised water
Concentration:	10 µg/plate
Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Purity:	≥ 98%
Dissolved in:	DMSO (purity > 99%)
Concentration:	10 µg/plate in strain TA 98, 50 µg/plate in strain TA 1537

Strain:	WP2 *uvrA*
Name:	methyl methane sulfonate, MMS
Purity:	98.7%
Dissolved in:	deionised water
Concentration:	2.0 µL/plate

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 *uvrA*
Name:	2-aminoanthracene, 2-AA
Purity:	≥ 96%
Dissolved in:	DMSO (purity > 99%)
Concentration:	2.5 µg/plate (10.0 µg/plate in WP2 *uvrA*)

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range is sufficient evidence of biological stability.

3.4 Test Item Preparation

On the day of the experiment, the test item C4004-210510 was dissolved in DMSO (purity > 99%). The solvent was chosen because of its solubility properties and its relative nontoxicity to the bacteria (Maron et al.; 1981).

All formulations were prepared freshly before treatment and used within two hours of preparation. The formulation was assumed to be stable for this period unless specified otherwise by the Sponsor.

3.5 Test System

3.5.1 Characterisation of the *Salmonella typhimurium* Strains and *Escherichia coli* Strain

The histidine dependent strains are derived from *Salmonella typhimurium* strain LT2 through mutations in the histidine locus. Additionally due to the "deep rough" (rfa-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "uvrB-minus". In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker.

The strain *Escherichia coli* WP2 and its derivatives carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (Trp^+) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the *uvrA* derivative is deficient in the DNA repair process (excision repair damage). Such a repair-deficient strain may be more readily mutated by agents.

When summarized, the mutations of the *S. typhimurium* strains and the *E. coli* strain, used in this study, can be described as follows:

Strains	Genotype	Type of mutations indicated
Salmonella typhimurium		
TA 1537	*his* C 3076; *rfa⁻; uvrB⁻*	frame shift mutations
TA 98	*his* D 3052; *rfa⁻; uvrB⁻*; R-factor	" "
TA 1535	*his* G 46; *rfa⁻; uvrB⁻*	base-pair substitutions
TA 100	*his* G 46; *rfa⁻; uvrB⁻*; R-factor	" "
Escherichia coli		
WP2 *uvrA*	*trp⁻; uvrA⁻*	base-pair substitutions and others

Regular checking of the properties of the *Salmonella typhimurium* and *Escherichia coli* strains regarding the membrane permeability, ampicillin resistance; UV sensitivity, and amino acid requirement as well as normal spontaneous mutation rates is performed in ICCR-Roßdorf GmbH according to Ames *et al.* (1977) and Maron and Ames (1983). Thus, it is ensured that the experimental conditions set down by Ames are fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, and WP2 *uvrA* were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

3.5.2 Storage

The strain cultures are stored as stock cultures in ampoules with nutrient broth plus 5% DMSO in liquid nitrogen.

3.5.3 Precultures

The thawed bacterial suspension was transferred into 250 mL Erlenmeyer flasks containing 50 mL nutrient medium. A solution of 50 µL ampicillin (25 µg/mL) was added to the strains TA 98 and TA 100. This nutrient medium contains per litre:

8 g Nutrient Broth
5 g NaCl

The bacterial cultures were incubated in a shaking water bath for 4 hours at 37°C. The optical density of the bacteria was determined by absorption measurement and the obtained values indicated that the bacteria were harvested at the late exponential or early stationary phase (10^8-10^9 cells/mL).

3.5.4 Selective Agar

Plates with selective agar (without histidine/tryptophan) were used.

3.5.5 Overlay Agar

The overlay agar contains per litre:

for *Salmonella typhimurium*:			**for *Escherichia coli*:**		
7.0	g	Agar Agar	7.0	g	Agar Agar
6.0	g	NaCl	6.0	g	NaCl
10.5	mg	L-Histidine×HCl×H_2O	10.2	mg	Tryptophan
12.2	mg	Biotin			

Sterilisations were performed at 121°C in an autoclave.

3.6 Mammalian Microsomal Fraction S9 Homogenate

Due to the limited capacity for metabolic activation of potential mutagens in *in vitro* methods an exogenous metabolic activation system is necessary.

Phenobarbital/β-naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 was prepared and stored according to the currently valid version of the SOP for rat liver S9 preparation. Each batch of S9 was routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

The protein concentration of the S9 preparation was 31.6 mg/mL (Lot. No.: 031122K) in both experiments.

3.6.1 S9 Mix

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution, to result in a final concentration of approx. 10% (v/v) in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM	$MgCl_2$
33 mM	KCl
5 mM	glucose-6-phosphate
4 mM	NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment, the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames *et al.* (1977).

3.6.2 S9 Mix Substitution Buffer

The S9 mix substitution buffer contains per litre:

700 mL 100 mM sodium-ortho-phosphate-buffer pH 7.4
300 mL KCl solution 0.15 M

During the experiment, the S9 mix substitution buffer was stored in an ice bath.

3.7 Experimental Design and Study Conduct

3.7.1 Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described for the experiment I below (plate incorporation test).

Toxicity of the test item results in a reduction in the number of spontaneous revertants (below a factor of 0.5) or a clearing of the bacterial background lawn.

The pre-experiment is reported as main experiment I since the acceptance criteria are met (cf. 3.8.2).

3.7.2 Dose Selection

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as experiment I. Since no toxic effects were observed 5000 µg/plate were chosen as maximal concentration. The concentration range included two logarithmic decades.

The following concentrations were tested in experiment II:

33; 100; 333; 1000; 2500; and 5000 µg/plate

3.7.3 Experimental Performance

For each strain and dose level, including the controls, three plates were used.

Experiment I (Plate Incorporation)

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL	Test solution at each dose level (solvent or reference mutagen solution (positive control)),
500 µL	S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL	Bacteria suspension (cf. 3.5.3 Precultures),
2000 µL	Overlay agar

Experiment II (Pre-Incubation)

The following materials were mixed in a test tube and incubated at 37°C ± 1.5°C for 60 minutes.

100 µL	Test solution at each dose level (solvent or reference mutagen solution (positive control)),
500 µL	S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL	Bacteria suspension (cf. 3.5.3 Precultures),

After pre-incubation 2.0 mL overlay agar (45°C) was added to each tube.

The mixture was poured on minimal agar plates. After solidification the plates were incubated upside down for at least 48 hours at 37°C ± 1.5°C in the dark.

In parallel to each test a sterile control of the test item was performed and documented in the raw data. Therefore, 100 µL of the stock solution, 500 µL S9 mix / S9 mix substitution buffer were mixed with 2.0 mL overlay agar and poured on minimal agar plates.

3.8 Data Evaluation

3.8.1 Data Recording

The colonies were counted using a validated computer system (cf. 3.9, Major computerized systems), which was connected to a PC with printer to print out the individual values, the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results).

3.8.2 Acceptability of the Assay

The *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control;
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data;
- the positive control substances should produce an increase above the threshold of twofold (strains TA 98, TA 100, and WP2 *uvrA*) or threefold (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control;
- a minimum of five analysable dose levels should be present with at least three dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.

The current historical control data are presented in Annex 1.

3.8.3 Evaluation of Results

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants of twofold or above (strains TA 98, TA 100, and WP2 *uvrA*) or threefold or above (strains TA 1535 and TA 1537) the spontaneous mutation rate of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is reached or exceeded at more than one concentration.

An increase of revertant colonies equal or above the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

3.8.4 Biometry

According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

3.9 Major Computerized Systems

Petri Viewer Sorcerer Colony Counter 3.0 (Instem, Suffolk IP33 3TA, UK) with the software program Ames Study Manager (v1.24) and Ames Archive Manager (v1.01).

4 DEVIATIONS FROM STUDY PLAN

There were no deviations from study plan.

5 ARCHIVING

Records and documentation relating to this study will be maintained in the archives of ICCR-Roßdorf GmbH for a period of 4 years from the date on which the Study Director signs the final report. This will include but may not be limited to the Study Plan, any amendments, electronic and paper raw data, and Report.

At termination of the aforementioned period, the records and documentation will be transferred to the GLP compliant archive Rhenus Archiv Services GmbH, Frankfurt am Main for further archiving up to a total archiving period of 15 years.

A sample of the test item will not be archived.

ICCR-Roßdorf GmbH will retain in its archive a copy of the study plan and final report, and any amendments indefinitely.

6 RESULTS AND DISCUSSION

The test item C4004-210510 was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and the *Escherichia coli* strain WP2 *uvrA*.
The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls were tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

No precipitation of the test item occurred up to the highest investigated dose.

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with C4004-210510 at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies, which were clearly within the historical laboratory control data range. Thus, the sensitivity of the test system was demonstrated.

7 CONCLUSION

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

8 REFERENCES

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Mutation Res. 88, 343-350

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Mutation Res. 113, 173-215

TABLES

Table 1 Summary of Experiment I

Study Name: 4068911 Study Code: ICCR 4068911
Experiment: 4068911 VV Plate Date Plated: 21.07.2023
Assay Conditions: Date Counted: 27.07.2023

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	DMSO		14 ± 1	10 ± 3	22 ± 1	140 ± 6	61 ± 11
	Untreated		13 ± 1	12 ± 2	23 ± 8	136 ± 14	59 ± 6
	C4004-210510	3 µg	14 ± 2	11 ± 3	18 ± 1	129 ± 7	64 ± 11
		10 µg	14 ± 2	9 ± 4	26 ± 5	146 ± 13	56 ± 9
		33 µg	10 ± 3	11 ± 2	29 ± 8	135 ± 13	55 ± 6
		100 µg	14 ± 3	7 ± 1	33 ± 9	136 ± 10	57 ± 4
		333 µg	13 ± 6	12 ± 3	27 ± 7	136 ± 8	50 ± 7
		1000 µg	16 ± 2	8 ± 2	28 ± 5	139 ± 12	58 ± 12
		2500 µg	9 ± 2	9 ± 3	21 ± 4	129 ± 8	59 ± 8
		5000 µg	17 ± 2	9 ± 3	26 ± 6	136 ± 13	59 ± 6
	NaN3	10 µg	1090 ± 89			1632 ± 137	
	4-NOPD	10 µg			478 ± 27		
	4-NOPD	50 µg		96 ± 6			
	MMS	2.0 µL					1232 ± 81
With Activation	DMSO		12 ± 6	20 ± 4	37 ± 1	149 ± 1	69 ± 5
	Untreated		16 ± 6	13 ± 3	37 ± 9	142 ± 7	60 ± 13
	C4004-210510	3 µg	11 ± 3	12 ± 3	35 ± 5	132 ± 22	58 ± 5
		10 µg	14 ± 1	13 ± 2	35 ± 10	130 ± 2	57 ± 6
		33 µg	9 ± 4	17 ± 5	32 ± 6	143 ± 8	63 ± 6
		100 µg	15 ± 5	11 ± 3	38 ± 5	130 ± 12	56 ± 6
		333 µg	13 ± 3	13 ± 4	30 ± 9	140 ± 14	63 ± 12
		1000 µg	12 ± 5	14 ± 1	39 ± 2	130 ± 18	61 ± 11
		2500 µg	8 ± 0	13 ± 3	36 ± 7	141 ± 2	77 ± 7
		5000 µg	7 ± 1	11 ± 2	37 ± 8	134 ± 6	67 ± 10
	2-AA	2.5 µg	225 ± 21	547 ± 13	2157 ± 527	3350 ± 172	
	2-AA	10.0 µg					290 ± 15

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Table 2 Summary of Experiment II

Study Name: 4068911 Study Code: ICCR 4068911
Experiment: 4068911 HV2 Pre Date Plated: 02.08.2023
Assay Conditions: Date Counted: 07.08.2023

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	DMSO		15 ± 3	10 ± 3	28 ± 4	122 ± 18	60 ± 9
	Untreated		15 ± 6	15 ± 2	37 ± 6	147 ± 8	55 ± 11
	C4004-210510	33 µg	19 ± 3	16 ± 6	35 ± 9	137 ± 6	50 ± 12
		100 µg	15 ± 5	15 ± 2	32 ± 9	131 ± 11	64 ± 4
		333 µg	15 ± 2	13 ± 5	31 ± 8	140 ± 5	57 ± 7
		1000 µg	13 ± 4	14 ± 6	34 ± 2	128 ± 9	55 ± 12
		2500 µg	13 ± 8	13 ± 6	38 ± 4	130 ± 8	51 ± 2
		5000 µg	16 ± 3	10 ± 2	35 ± 5	136 ± 12	64 ± 5
	NaN3	10 µg	1495 ± 38			2014 ± 196	
	4-NOPD	10 µg			493 ± 70		
	4-NOPD	50 µg		119 ± 11			
	MMS	2.0 µL					837 ± 62
With Activation	DMSO		15 ± 4	13 ± 5	38 ± 9	138 ± 19	62 ± 8
	Untreated		11 ± 6	18 ± 6	53 ± 7	123 ± 7	53 ± 7
	C4004-210510	33 µg	16 ± 3	11 ± 6	46 ± 11	134 ± 12	66 ± 4
		100 µg	16 ± 7	15 ± 3	49 ± 3	136 ± 13	73 ± 8
		333 µg	14 ± 1	13 ± 5	50 ± 6	144 ± 23	68 ± 14
		1000 µg	16 ± 5	11 ± 3	42 ± 7	126 ± 20	57 ± 2
		2500 µg	15 ± 6	19 ± 5	42 ± 2	133 ± 9	73 ± 4
		5000 µg	14 ± 0	12 ± 2	48 ± 3	129 ± 8	54 ± 7
	2-AA	2.5 µg	218 ± 7	475 ± 49	2850 ± 246	4220 ± 127	
	2-AA	10.0 µg					292 ± 19

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Table 3 Individual Results of Experiment I

Study Name: 4068911
Experiment: 4068911 VV Plate
Assay Conditions:

Study Code: ICCR 4068911
Date Plated: 21.07.2023
Date Counted: 27.07.2023

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**C4004-210510**	3 µg	*13.7*	*1.5*	*1.0*	15, 14, 12
		10 µg	*14.3*	*2.3*	*1.0*	13, 13, 17
		33 µg	*10.3*	*2.5*	*0.7*	13, 8, 10
		100 µg	*14.3*	*2.5*	*1.0*	17, 14, 12
		333 µg	*13.3*	*5.5*	*1.0*	13, 19, 8
		1000 µg	*16.3*	*2.1*	*1.2*	17, 14, 18
		2500 µg	*9.3*	*2.3*	*0.7*	12, 8, 8
		5000 µg	*16.7*	*2.3*	*1.2*	14, 18, 18
	DMSO		*14.0*	*1.0*		14, 13, 15
	Untreated Control		*13.0*	*1.0*		14, 12, 13
TA 1537	**C4004-210510**	3 µg	*11.0*	*3.5*	*1.1*	9, 9, 15
		10 µg	*9.3*	*4.2*	*0.9*	14, 6, 8
		33 µg	*10.7*	*2.3*	*1.1*	12, 12, 8
		100 µg	*7.3*	*1.2*	*0.7*	6, 8, 8
		333 µg	*11.7*	*2.5*	*1.2*	12, 14, 9
		1000 µg	*8.0*	*2.0*	*0.8*	8, 6, 10
		2500 µg	*9.0*	*3.0*	*0.9*	6, 12, 9
		5000 µg	*9.0*	*3.0*	*0.9*	12, 6, 9
	DMSO		*10.0*	*2.6*		8, 13, 9
	Untreated Control		*12.0*	*1.7*		13, 10, 13
TA 98	**C4004-210510**	3 µg	*18.0*	*1.0*	*0.8*	18, 17, 19
		10 µg	*25.7*	*4.7*	*1.2*	22, 31, 24
		33 µg	*28.7*	*8.0*	*1.3*	28, 21, 37
		100 µg	*33.0*	*8.9*	*1.5*	43, 26, 30
		333 µg	*27.0*	*7.0*	*1.2*	22, 35, 24
		1000 µg	*27.7*	*4.9*	*1.3*	22, 30, 31
		2500 µg	*21.3*	*4.0*	*1.0*	19, 19, 26
		5000 µg	*25.7*	*6.1*	*1.2*	27, 31, 19
	DMSO		*22.0*	*1.0*		23, 21, 22
	Untreated Control		*22.7*	*7.5*		23, 15, 30
TA 100	**C4004-210510**	3 µg	*129.3*	*6.7*	*0.9*	122, 135, 131
		10 µg	*146.0*	*13.2*	*1.0*	131, 151, 156
		33 µg	*135.0*	*13.5*	*1.0*	146, 139, 120
		100 µg	*135.7*	*9.7*	*1.0*	144, 138, 125
		333 µg	*136.0*	*7.5*	*1.0*	128, 143, 137
		1000 µg	*139.3*	*11.5*	*1.0*	128, 139, 151
		2500 µg	*128.7*	*8.4*	*0.9*	134, 119, 133
		5000 µg	*135.7*	*12.7*	*1.0*	121, 144, 142
	DMSO		*140.0*	*5.6*		135, 146, 139
	Untreated Control		*136.0*	*14.4*		120, 148, 140

Study Name: 4068911
Experiment: 4068911 VV Plate
Assay Conditions:

Study Code: ICCR 4068911
Date Plated: 21.07.2023
Date Counted: 27.07.2023

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	**C4004-210510**	3 µg	*63.7*	*11.0*	*1.0*	75, 63, 53
		10 µg	*55.7*	*9.1*	*0.9*	57, 46, 64
		33 µg	*55.3*	*5.5*	*0.9*	49, 59, 58
		100 µg	*57.0*	*4.0*	*0.9*	61, 53, 57
		333 µg	*50.3*	*7.1*	*0.8*	44, 49, 58
		1000 µg	*58.3*	*11.6*	*1.0*	64, 45, 66
		2500 µg	*59.0*	*8.2*	*1.0*	66, 50, 61
		5000 µg	*59.3*	*5.5*	*1.0*	63, 53, 62
	DMSO		*61.0*	*10.8*		73, 58, 52
	Untreated Control		*59.3*	*5.9*		66, 55, 57
TA 1535	**NaN3**	10 µg	*1090.0*	*88.6*	*77.9*	1148, 1134, 988
TA 1537	**4-NOPD**	50 µg	*96.3*	*6.4*	*9.6*	100, 89, 100
TA 98	**4-NOPD**	10 µg	*478.0*	*27.4*	*21.7*	457, 509, 468
TA 100	**NaN3**	10 µg	*1631.7*	*137.0*	*11.7*	1729, 1691, 1475
WP2 uvrA	**MMS**	2.0 µL	*1231.7*	*81.0*	*20.2*	1190, 1325, 1180

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Study Name: 4068911
Experiment: 4068911 VV Plate
Assay Conditions:

Study Code: ICCR 4068911
Date Plated: 21.07.2023
Date Counted: 27.07.2023

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**C4004-210510**	3 µg	*11.0*	*2.6*	*0.9*	10, 9, 14
		10 µg	*14.0*	*1.0*	*1.2*	15, 13, 14
		33 µg	*9.0*	*3.6*	*0.8*	6, 13, 8
		100 µg	*15.3*	*4.7*	*1.3*	19, 10, 17
		333 µg	*13.0*	*2.6*	*1.1*	10, 14, 15
		1000 µg	*12.0*	*5.2*	*1.0*	9, 9, 18
		2500 µg	*8.0*	*0.0*	*0.7*	8, 8, 8
		5000 µg	*6.7*	*1.2*	*0.6*	6, 8, 6
	DMSO		*11.7*	*5.5*		18, 9, 8
	Untreated Control		*16.0*	*5.6*		17, 10, 21
TA 1537	**C4004-210510**	3 µg	*11.7*	*2.5*	*0.6*	14, 12, 9
		10 µg	*13.0*	*1.7*	*0.7*	15, 12, 12
		33 µg	*17.3*	*4.9*	*0.9*	23, 14, 15
		100 µg	*11.3*	*3.1*	*0.6*	14, 8, 12
		333 µg	*13.3*	*3.5*	*0.7*	17, 13, 10
		1000 µg	*14.3*	*0.6*	*0.7*	14, 14, 15
		2500 µg	*13.0*	*2.6*	*0.7*	14, 10, 15
		5000 µg	*10.7*	*2.1*	*0.5*	10, 13, 9
	DMSO		*19.7*	*4.2*		21, 15, 23
	Untreated Control		*12.7*	*2.5*		13, 15, 10
TA 98	**C4004-210510**	3 µg	*35.0*	*5.3*	*1.0*	41, 33, 31
		10 µg	*34.7*	*9.7*	*0.9*	24, 37, 43
		33 µg	*32.3*	*5.9*	*0.9*	28, 39, 30
		100 µg	*38.3*	*5.0*	*1.0*	33, 39, 43
		333 µg	*30.3*	*9.3*	*0.8*	41, 26, 24
		1000 µg	*39.0*	*2.0*	*1.1*	37, 41, 39
		2500 µg	*36.3*	*6.5*	*1.0*	43, 30, 36
		5000 µg	*37.3*	*7.8*	*1.0*	31, 35, 46
	DMSO		*36.7*	*0.6*		36, 37, 37
	Untreated Control		*37.0*	*8.9*		27, 44, 40
TA 100	**C4004-210510**	3 µg	*131.7*	*22.0*	*0.9*	109, 153, 133
		10 µg	*129.7*	*1.5*	*0.9*	128, 130, 131
		33 µg	*143.3*	*8.0*	*1.0*	151, 135, 144
		100 µg	*130.0*	*12.5*	*0.9*	120, 126, 144
		333 µg	*140.3*	*13.9*	*0.9*	144, 152, 125
		1000 µg	*130.0*	*18.0*	*0.9*	130, 148, 112
		2500 µg	*140.7*	*2.1*	*0.9*	140, 139, 143
		5000 µg	*134.0*	*5.6*	*0.9*	129, 133, 140
	DMSO		*148.7*	*0.6*		149, 148, 149
	Untreated Control		*142.0*	*7.2*		134, 148, 144

Study Name: 4068911 Study Code: ICCR 4068911
Experiment: 4068911 VV Plate Date Plated: 21.07.2023
Assay Conditions: Date Counted: 27.07.2023

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	**C4004-210510**	3 µg	*58.3*	*4.6*	*0.8*	61, 53, 61
		10 µg	*57.0*	*6.2*	*0.8*	62, 50, 59
		33 µg	*63.3*	*5.5*	*0.9*	67, 57, 66
		100 µg	*55.7*	*5.5*	*0.8*	52, 62, 53
		333 µg	*63.3*	*12.1*	*0.9*	54, 77, 59
		1000 µg	*60.7*	*10.7*	*0.9*	54, 73, 55
		2500 µg	*77.0*	*6.6*	*1.1*	76, 71, 84
		5000 µg	*67.0*	*10.0*	*1.0*	57, 67, 77
	DMSO		*69.0*	*5.2*		63, 72, 72
	Untreated Control		*60.3*	*13.2*		72, 46, 63
TA 1535	**2-AA**	2.5 µg	*225.3*	*20.5*	*19.3*	225, 246, 205
TA 1537	**2-AA**	2.5 µg	*546.7*	*13.4*	*27.8*	541, 562, 537
TA 98	**2-AA**	2.5 µg	*2157.0*	*526.7*	*58.8*	1964, 1754, 2753
TA 100	**2-AA**	2.5 µg	*3350.0*	*172.0*	*22.5*	3280, 3546, 3224
WP2 uvrA	**2-AA**	10.0 µg	*290.0*	*15.1*	*4.2*	285, 278, 307

Key to Positive Controls

2-AA 2-aminoanthracene

Table 4 Individual Results of Experiment II

Study Name: 4068911
Experiment: 4068911 HV2 Pre
Assay Conditions:

Study Code: ICCR 4068911
Date Plated: 02.08.2023
Date Counted: 07.08.2023

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**C4004-210510**	33 µg	*19.3*	*2.5*	*1.3*	17, 22, 19
		100 µg	*14.7*	*4.5*	*1.0*	15, 10, 19
		333 µg	*15.3*	*1.5*	*1.0*	15, 14, 17
		1000 µg	*13.3*	*4.0*	*0.9*	9, 17, 14
		2500 µg	*13.3*	*7.5*	*0.9*	9, 22, 9
		5000 µg	*16.0*	*2.6*	*1.0*	18, 17, 13
	DMSO		*15.3*	*2.5*		13, 18, 15
	Untreated Control		*14.7*	*6.0*		9, 14, 21
TA 1537	**C4004-210510**	33 µg	*15.7*	*5.5*	*1.6*	22, 12, 13
		100 µg	*15.3*	*1.5*	*1.5*	15, 14, 17
		333 µg	*13.3*	*4.7*	*1.3*	17, 15, 8
		1000 µg	*14.0*	*6.2*	*1.4*	12, 9, 21
		2500 µg	*12.7*	*5.5*	*1.3*	19, 10, 9
		5000 µg	*10.3*	*1.5*	*1.0*	9, 12, 10
	DMSO		*10.0*	*2.6*		13, 9, 8
	Untreated Control		*15.0*	*2.0*		15, 13, 17
TA 98	**C4004-210510**	33 µg	*35.3*	*9.0*	*1.3*	44, 36, 26
		100 µg	*32.0*	*9.0*	*1.2*	32, 23, 41
		333 µg	*30.7*	*8.0*	*1.1*	23, 39, 30
		1000 µg	*34.3*	*2.1*	*1.2*	36, 32, 35
		2500 µg	*37.7*	*4.0*	*1.4*	40, 33, 40
		5000 µg	*34.7*	*4.5*	*1.3*	30, 35, 39
	DMSO		*27.7*	*3.5*		31, 28, 24
	Untreated Control		*37.0*	*6.2*		35, 44, 32
TA 100	**C4004-210510**	33 µg	*136.7*	*6.4*	*1.1*	133, 133, 144
		100 µg	*131.3*	*11.0*	*1.1*	124, 126, 144
		333 µg	*139.7*	*4.9*	*1.1*	142, 134, 143
		1000 µg	*128.3*	*8.5*	*1.1*	125, 122, 138
		2500 µg	*130.0*	*7.8*	*1.1*	135, 121, 134
		5000 µg	*136.0*	*11.5*	*1.1*	147, 124, 137
	DMSO		*122.0*	*17.6*		102, 135, 129
	Untreated Control		*147.3*	*8.1*		138, 151, 153
WP2 uvrA	**C4004-210510**	33 µg	*49.7*	*11.7*	*0.8*	45, 41, 63
		100 µg	*63.7*	*3.8*	*1.1*	62, 61, 68
		333 µg	*56.7*	*6.8*	*0.9*	49, 59, 62
		1000 µg	*54.7*	*11.6*	*0.9*	44, 67, 53
		2500 µg	*51.3*	*2.3*	*0.9*	50, 50, 54
		5000 µg	*64.3*	*4.6*	*1.1*	67, 59, 67
	DMSO		*59.7*	*9.0*		70, 54, 55
	Untreated Control		*55.0*	*10.8*		46, 52, 67

Study Name: 4068911
Experiment: 4068911 HV2 Pre
Assay Conditions:

Study Code: ICCR 4068911
Date Plated: 02.08.2023
Date Counted: 07.08.2023

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**NaN3**	10 µg	*1495.3*	*37.9*	*97.5*	1539, 1471, 1476
TA 1537	**4-NOPD**	50 µg	*119.3*	*11.0*	*11.9*	130, 108, 120
TA 98	**4-NOPD**	10 µg	*492.7*	*70.0*	*17.8*	537, 529, 412
TA 100	**NaN3**	10 µg	*2013.7*	*195.7*	*16.5*	1789, 2105, 2147
WP2 uvrA	**MMS**	2.0 µL	*837.3*	*61.8*	*14.0*	873, 766, 873

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Study Name: 4068911
Experiment: 4068911 HV2 Pre
Assay Conditions:

Study Code: ICCR 4068911
Date Plated: 02.08.2023
Date Counted: 07.08.2023

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**C4004-210510**	33 µg	*16.0*	*3.5*	*1.1*	18, 18, 12
		100 µg	*16.3*	*7.0*	*1.1*	23, 9, 17
		333 µg	*14.0*	*1.0*	*0.9*	13, 14, 15
		1000 µg	*15.7*	*4.7*	*1.0*	14, 21, 12
		2500 µg	*14.7*	*5.7*	*1.0*	13, 10, 21
		5000 µg	*14.0*	*0.0*	*0.9*	14, 14, 14
	DMSO		*15.0*	*4.4*		18, 10, 17
	Untreated Control		*11.3*	*6.1*		18, 6, 10
TA 1537	**C4004-210510**	33 µg	*11.0*	*5.6*	*0.8*	17, 10, 6
		100 µg	*15.0*	*3.5*	*1.1*	13, 19, 13
		333 µg	*13.3*	*4.5*	*1.0*	18, 9, 13
		1000 µg	*11.3*	*3.2*	*0.9*	10, 15, 9
		2500 µg	*19.3*	*4.7*	*1.4*	21, 23, 14
		5000 µg	*11.7*	*1.5*	*0.9*	13, 10, 12
	DMSO		*13.3*	*5.1*		12, 9, 19
	Untreated Control		*17.7*	*6.0*		12, 17, 24
TA 98	**C4004-210510**	33 µg	*46.3*	*10.7*	*1.2*	58, 37, 44
		100 µg	*49.3*	*3.1*	*1.3*	50, 46, 52
		333 µg	*50.3*	*5.7*	*1.3*	52, 44, 55
		1000 µg	*42.3*	*6.7*	*1.1*	35, 48, 44
		2500 µg	*42.3*	*2.1*	*1.1*	44, 43, 40
		5000 µg	*47.7*	*2.5*	*1.3*	50, 48, 45
	DMSO		*37.7*	*8.7*		40, 28, 45
	Untreated Control		*53.0*	*7.2*		59, 55, 45
TA 100	**C4004-210510**	33 µg	*134.0*	*12.2*	*1.0*	148, 128, 126
		100 µg	*136.3*	*12.7*	*1.0*	151, 130, 128
		333 µg	*144.0*	*22.6*	*1.0*	138, 125, 169
		1000 µg	*125.7*	*20.2*	*0.9*	113, 115, 149
		2500 µg	*133.3*	*8.5*	*1.0*	125, 142, 133
		5000 µg	*128.7*	*8.3*	*0.9*	126, 138, 122
	DMSO		*138.0*	*19.1*		160, 128, 126
	Untreated Control		*122.7*	*6.7*		117, 121, 130
WP2 uvrA	**C4004-210510**	33 µg	*66.3*	*3.5*	*1.1*	66, 63, 70
		100 µg	*72.7*	*8.1*	*1.2*	82, 68, 68
		333 µg	*67.7*	*13.5*	*1.1*	54, 81, 68
		1000 µg	*56.7*	*1.5*	*0.9*	57, 58, 55
		2500 µg	*72.7*	*3.8*	*1.2*	70, 71, 77
		5000 µg	*54.3*	*6.5*	*0.9*	48, 61, 54
	DMSO		*62.3*	*8.1*		71, 61, 55
	Untreated Control		*52.7*	*6.5*		53, 46, 59

Study Name: 4068911
Experiment: 4068911 HV2 Pre
Assay Conditions:

Study Code: ICCR 4068911
Date Plated: 02.08.2023
Date Counted: 07.08.2023

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**2-AA**	2.5 µg	*217.7*	*6.5*	*14.5*	224, 211, 218
TA 1537	**2-AA**	2.5 µg	*475.0*	*49.3*	*35.6*	527, 469, 429
TA 98	**2-AA**	2.5 µg	*2850.0*	*246.0*	*75.7*	2648, 2778, 3124
TA 100	**2-AA**	2.5 µg	*4220.0*	*127.0*	*30.6*	4103, 4202, 4355
WP2 uvrA	**2-AA**	10.0 µg	*292.3*	*18.6*	*4.7*	305, 271, 301

Key to Positive Controls

2-AA 2-aminoanthracene

ANNEXES

Annex 1 Historical Data

These data represent the laboratory´s historical control data from February 2022 until February 2023 representing approx. 310 experiments (WP2 uvrA the historical data are based on approx. 200 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	12	2.4	6	20	12	2.3	7	21
	Untreated control	12	2.7	7	23	13	2.4	7	20
	Positive control[1]	1198	208.4	401	1969	320	75.3	144	1070
TA 1537	Solvent control	11	2.1	7	18	13	2.7	7	26
	Untreated control	11	2.2	7	19	13	3.1	6	25
	Positive control[2]	125	21.9	59	191	307	95.7	135	552
TA 98	Solvent control	29	4.3	18	43	40	7.3	21	62
	Untreated control	30	4.5	19	46	43	6.9	18	65
	Positive control[3]	671	142.7	233	1099	3141	1095.8	407	7734
TA 100	Solvent control	120	21.8	73	210	117	22.6	76	204
	Untreated control	126	22.8	74	215	121	27.0	71	210
	Positive control[4]	1812	341.2	572	3414	3307	868.5	594	5263
WP2 uvrA	Solvent control	46	6.7	30	64	56	7.4	36	72
	Untreated control	48	6.9	30	65	57	8.5	33	74
	Positive control[5]	798	158.4	291	1295	337	144.5	120	1129

Mean = mean value of revertants/plate
SD = standard deviation
Min = minimal value
Max = maximal value

[1] Without S9 mix: 10 µg/plate NaN_3, with S9 mix: 2.5 µg/plate 2-aminoanthracene

[2] Without S9 mix: 50 µg/plate 4-Nitro-o-phenylene-diamine, with S9 mix: 2.5 µg/plate 2-aminoanthracene

[3] Without S9 mix: 10 µg/plate 4-Nitro-o-phenylene-diamine, with S9 mix: 2.5 µg/plate 2-aminoanthracene

[4] Without S9 mix: 10 µg/plate NaN_3, with S9 mix: 2.5 µg/plate 2-aminoanthracene

[5] Without S9 mix: 2 µL/plate Methyl methane sulfonate, with S9 mix: 10 µg/plate 2-aminoanthracene

Annex 2 S9 Certificate

ICCR ROßDORF

CERTIFICATE

ICCR-Roßdorf S9 Preparation Lot No. 031122K

Date of preparation: 03 Nov 2022
Recertification date: 25 Apr 2023

Protein assay: 31.6 mg protein / ml S9

Sterility: 0 colonies / ml S9 on glucose-minimal-agar

Salmonella typhimurium assay (AMES-test)

Treatment	µl S9 / plate	number of revertants in TA 98
negative	0	29
control	100	32
10 µg/plate	0	57
2-Aminoanthracene	100	1900
10 µg/plate	0	24
Benzo(a)pyrene	100	106

The S9 was obtained from the livers of male Wistar rats which received triple treatments of 80 mg / kg body weight Phenobarbital and ß-Naphthoflavone orally on consecutive days. The livers were prepared 24 hours after the last treatment.

S. Ebert Sabine Ebert — 04 May 2023
Quality Assurance Auditor, ICCR-Roßdorf GmbH — Date

S. Chang — 11. MAI 2023
Dr. Steffi Chang, Study Director, ICCR-Roßdorf GmbH — Date

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Geschäftsführer: Dr. Markus Schulz

SOP Origin TS-SOP S9_23

Annex 3 Copy of the General Study Plan (471.Ames.Evonik.1)

(13 pages)

General Study Plan: 471.Ames.Evonik.1

Reverse Mutation Assay "Ames Test" using *Salmonella typhimurium* and *Escherichia coli*

Testing Facility

ICCR-Roßdorf GmbH
In den Leppsteinswiesen 19
64380 Rossdorf
Germany

Table of Contents

1. Introduction and Purpose

The experiment will be performed to assess the potential of the test item to induce gene mutations in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay. The experiment will be performed as a plate incorporation assay. If a negative or equivocal result is obtained in this experiment, a second experiment will be performed as pre-incubation assay. In case of a clear positive response, a second experiment is not required. If necessary, additional experiments will be performed to establish the biological relevance of a result.

The most widely used assays for detecting gene mutations are those using bacteria (Hollstein et al.; 1979). They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and induce mutations.

Reverse mutation assays determine the frequency at which an agent abolishes or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to grow in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The *Salmonella typhimurium* histidine (his) and the *Escherichia coli* tryptophan (trp) reversion system measures $his^- \rightarrow his^+$ and $trp^- \rightarrow trp^+$ reversions, respectively. The *Salmonella typhimurium* and *Escherichia coli* strains are constructed to differentiate between base pair (TA 1535, TA 100, and WP2 *uvrA*) and frame shift (TA 1537, TA 98) mutations.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect at least 5 concentrations with adequately spaced intervals are tested. The maximum concentration will be 5000 µg/plate, unless limited by toxicity of the test item.

To validate the test, reference mutagens will be tested in parallel to the test item.

2. Regulatory Information

2.1 Good Laboratory Practice

The study will be conducted in compliance with principles of Good Laboratory Practice Standards as set forth in:

- "Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1)
- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17
- EC Commission Directive 2004/10/EC

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and

FDA), and Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

2.2 Regulatory Testing Guidelines

The study will be performed in compliance with the following regulations or guidelines:

- "Ninth Addendum to OECD Guidelines for Testing of Chemicals", Section 4, No. 471: "Bacterial Reverse Mutation Test", corrected June 26, 2020
- Commission Regulation (EC) No. 440/2008 B13/14, dated May 30, 2008

3. Test Item and Supporting Information

3.1 Test Item

The test item will be supplied by or on behalf of the Sponsor, including the following information if known; identification, description, batch, purity, expiry / retest date, storage conditions and safety precautions to be taken.

3.2 Controls

3.2.1 Negative Controls

Concurrent untreated and solvent controls will be performed.

3.2.2 Positive Control Substances

Without metabolic activation

Strains:	TA1535, TA100
Name:	sodium azide, NaN_3
Purity:	≥ 99%
Dissolved in:	deionised water
Concentration:	10 µg/plate

Strains:	TA1537, TA98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD (Maron & Ames)
Purity:	≥ 98%
Dissolved in:	dimethyl sulfoxide, DMSO (purity > 99%)
Concentration:	10 µg/plate in strain TA 98, 50 µg/plate in strain TA 1537

Strains:	WP2 *uvrA* (Green & Muriel)
Name:	methyl methane sulfonate, MMS
Purity:	≥ 97%
Dissolved in:	deionised water
Concentration:	2.0 µL/plate

With metabolic activation

Strains:	TA1535, TA1537, TA98, TA100, WP2 *uvrA*
Name:	2-aminoanthracene, 2-AA
Purity:	≥ 96%
Dissolved in:	DMSO (purity > 99%)
Concentration:	2.5 µg/plate (10.0 µg/plate in WP2 *uvrA*)

Batch numbers of positive control substances will be given in the study report.

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range will be sufficient evidence of biological stability and activity.

4. Test Item Preparation

Working solutions of the test item will be formulated in solvent. Solvents are chosen from water, DMSO, acetone, ethanol, dimethyl formamide (DMF), tetrahydrofuran (THF), or others where appropriate (as recommended by Maron *et al.*, 1981) on the basis of their ability to formulate the test item and their relative non-toxicity to the bacteria. In the event of the test item being non-soluble, then a doseable suspension will be used. Five or more concentrations of the test item will be plated, the highest usually being at the toxic or maximum recommended concentration limit. All formulations will be prepared freshly before treatment and used within two hours of preparation. The formulation will be assumed to be stable for this period unless specified otherwise by the Sponsor.

5. Test System and Supporting Information

5.1 Characterisation of the Salmonella typhimurium Strains and Escherichia coli Strains

The histidine dependent strains are derived from *Salmonella typhimurium* strain LT2 through mutations in the histidine locus. Additionally due to the "deep rough" (rfa^-) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named *uvrB*$^-$. In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker.

Strain *Escherichia coli* WP2 and its derivatives carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (Trp^+) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the *uvrA* derivative is deficient in the DNA repair process (excisable repair damage). Such a repair-deficient strain may be more readily mutated by agents.

When summarized, the mutations of the *Salmonella typhimurium* strains and the *Escherichia coli* strain used in this study can be described as follows:

Strains	Genotype	Type of mutations indicated
Salmonella typhimurium		
TA 1537	*his* C 3076; *rfa*$^-$; *uvrB*$^-$	frame shift mutations
TA 98	*his* D 3052; *rfa*$^-$; *uvrB*$^-$; R-factor	" "
TA 1535	*his* G 46; *rfa*$^-$; uvrB$^-$	base-pair substitutions
TA 100	*his* G 46; *rfa*$^-$; *uvrB*$^-$; R-factor	" "
Escherichia coli		
WP2 *uvrA*	*trp*$^-$; *uvrA*$^-$	base-pair substitutions and others

Regular checking of the properties of the *Salmonella typhimurium* and *Escherichia coli* strains regarding the membrane permeability, ampicillin resistance, UV sensitivity, and amino acid requirement as well as normal spontaneous mutation rates is performed in ICCR-Roßdorf GmbH according to Ames *et al.* (1977) and Maron and Ames (1983). Thus, it is ensured that the experimental conditions set down by Ames are fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, and WP2 *uvrA* were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

5.2 Storage

The strain cultures are stored as stock cultures in ampoules with nutrient broth plus 5% DMSO in liquid nitrogen.

5.3 Precultures

The thawed bacterial suspension will be transferred into 250 mL Erlenmeyer flasks containing 50 mL nutrient medium. A solution of 50 µL ampicillin (25 µg/mL) will be added to the strains TA 98 and TA 100. This nutrient medium contains per litre:

8 g Nutrient Broth
5 g NaCl

The bacterial culture will be incubated in a shaking water bath for up to 8 hours at 37° C. The optical density of the bacteria will be determined by absorption measurement and the obtained values indicated that the bacteria will be harvested at the late exponential or early stationary phase (10^8-10^9 cells/mL).

5.4 Selective Agar

Plates with selective agar (without histidine/tryptophan) will be used.

5.5 Overlay Agar

The overlay agar contains per litre:

for *Salmonella typhimurium*:			**for *Escherichia coli:***		
7.0	g	Agar Agar	7.0	g	Agar Agar
6.0	g	NaCl	6.0	g	NaCl
10.5	mg	L-Histidine×HCl×H_2O	10.2	mg	Tryptophan
12.2	mg	Biotin			

Sterilisations will be performed at 121°C in an autoclave.

5.6 Mammalian Microsomal Fraction S9 Mix

Due to the limited capacity for metabolic activation of potential mutagens in *in vitro* methods an exogenous metabolic activation system is necessary.

Phenobarbital/β-naphthoflavone induced rat liver S9 will be used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the ICCR-Roßdorf GmbH SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

5.6.1 S9 Mix

An appropriate quantity of S9 supernatant is thawed and mixed with S9 cofactor solution, to result in a final concentration of approx. 10% (v/v) in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM	$MgCl_2$
33 mM	KCl
5 mM	glucose-6-phosphate
4 mM	NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
During the experiment, the S9 mix is stored in an ice bath. The S9 mix preparation is performed according to Ames *et al*. (1977).

5.6.2 S9 Mix Substitution Buffer

The S9 mix substitution buffer contains per litre:

700	mL	100 mM sodium ortho-phosphate buffer pH 7.4
300	mL	KCl solution 0.15 M

During the experiment, the S9 mix substitution buffer will be stored in an ice bath.

6. Experimental Design and Study Conduct

6.1 Pre-Experiment for Toxicity (Plate Incorporation)

To evaluate the toxicity of the test item a pre-study will be performed with all strains used. Eight concentrations will be tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment will be the same as described below for the experiment I (cf. 6.3, experiment I, plate incorporation test).
Toxicity of the test item results in a reduction in the number of spontaneous revertants (below a factor of 0.5) or a clearing of the bacterial background lawn.

The pre-experiment will be reported as main experiment I if the acceptance criteria (7.2) are met.

6.2 Concentration Selection

According to the results of this pre-experiment the concentrations to be applied in the main experiments will be chosen.
The maximum concentration is 5000 µg/plate, unless limited by toxicity of the test item. The concentration range covers at least two logarithmic decades. In this study at least five adequately spaced concentrations will be tested. In case of a negative or equivocal result a second experiment will be performed.

6.3 Experimental Performance

For each strain and dose level, including the controls, three plates will be used.
The following materials will be mixed in a test tube and poured onto the selective agar plates:

Experiment I (Plate Incorporation)

100 µL*	Test solution at each concentration level (solvent or reference mutagen solution (positive control)),
500 µL	S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL	Bacteria suspension (cf. test system, pre-culture of the strains),
2000 µL	Overlay agar

Experiment II (Pre-Incubation)

If the pre-incubation method (Experiment II) will be performed 100 µL* test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer** and 100 µL bacteria suspension will be mixed in a test tube and incubated at 37°C for 60 minutes. After pre-incubation 2.0 mL overlay agar (approx. 45°C) will be added to each tube. The mixture will be poured on selective agar plates.

After solidification the plates will be incubated upside down for 48-72 hours at 37°C in the dark (de Serres and Shelby, 1979). Bacterial colonies on the plates are then counted, or the plates stored at 4°C until counted.

In parallel to each test a sterile control of the test item will be performed and is documented in the raw data. Therefore, 100 µL* of the stock solution, 500 µL S9 mix / S9 mix substitution buffer will be mixed with 2.0 mL overlay agar and poured on minimal agar plates.

* Since some solvents (i.e. ethanol, acetone, THF) are toxic to the bacteria a lower amount of test solution and solvent will be applied if one of these solvents will be used.

** Substitution buffer: 7 parts of 100 mM sodium-ortho-phosphate-buffer pH 7.4 with 3 parts of KCl solution 0.15 M

7. Data Evaluation

7.1 Data Recording

The colonies are counted using the Petri Viewer with the software program Ames Study Manager. The evaluation unit is connected to a PC with printer to print out the individual values, the means from the plates for each concentration together with standard deviations and induction factors as compared to the spontaneous reversion rates. The print outs are kept with the raw data. If precipitation of the test item precludes automatic counting the revertant colonies are counted manually.

7.2 Acceptability of the Assay

The *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control;
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data;
- the positive control substances should produce an increase above the threshold of twofold (strains TA 98, TA 100, and WP2 *uvrA*) or threefold (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control;
- a minimum of five analysable dose levels should be present with at least three dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.

The current historical control data will be given in the SSS.

7.3 Evaluation of Results

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants of twofold or above (strains TA 98, TA 100, WP2 *uvrA*) or of threefold or above (strains TA 1535 and TA 1537) the spontaneous mutation rate of the corresponding solvent control is observed (Hollstein *et al.*, 1979).

A concentration dependent increase is considered biologically relevant if the threshold is reached or exceeded at more than one concentration.

An increase of revertant colonies equal or above the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A concentration dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second

experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

8. Major Computerized Systems

Petri Viewer Sorcerer Colony Counter 3.0 (Instem, Suffolk IP33, 3TA, UK) with the software program Ames Study Manager and Ames Archive Manager (in their currently valid versions).

9. Quality Assurance

The following will be inspected or audited in relation to this study.

Study Plan	Study Plan and any amendments.
Process based inspections	Procedures will be inspected on representative studies, not necessarily on this study
Report Audit	The draft report and study data will be audited

QA findings will be reported to the Study Director and Test Facility Management promptly on completion of each action.

10. Study Plan Amendment and Deviation

Any intended change to the study plan (General Study Plan and Study Specific Supplement) will result in an amendment to the study plan approved by the Study Director and also signed by Test Facility Management and the Sponsor. Amendments will be distributed to all recipients of the study plan.

Deviations (unplanned changes) from the study plan (General Study Plan and Study Specific Supplement) will be documented and acknowledged by the Study Director.

Any revision of the General Study Plan will be agreed with the Sponsor.

11. Reporting

Draft Report	A GLP compliant report will be prepared. Following experimental completion a draft report will be provided to the Sponsor for their review. The Draft report will be provided electronically.
Final Report	After receipt and review of the Sponsor's comments, appropriate changes will be made and revisions provided to the Sponsor. Once authorized by the Sponsor, the audited, signed final report will be issued. Any additions or corrections to an authorized final report will be documented as a formal addendum/amendment to the final report. The Final report will be provided electronically in PDF format. One original of the final report will be issued. The Sponsor will receive a PDF file of the final report only.

In the absence of ongoing communications and after notification in writing to the Sponsor, ICCR-Roßdorf GmbH reserves the right to finalize, sign and issue the Final report from this study six months after the issue of the draft. Any subsequent requests for modifications, corrections or additions to the Final Report will be the subject of a formal report amendment (or new study, as appropriate) and will be subject to additional cost.

12. Archiving

Records and documentation relating to this study will be maintained in the archives of ICCR-Roßdorf GmbH for a period of 4 years from the date on which the Study Director signs the final report. This will include electronic and paper raw data, and report that support the reconstruction of the study.

At termination of the aforementioned period, the records and documentation will be transferred to the GLP compliant archive Rhenus Archiv Services GmbH, Frankfurt am Main, for further archiving up to a total archiving period of 15 years.

A sample of the test item will not be archived.

ICCR-Roßdorf GmbH will retain in its archive a copy of the study plan and final report, and any amendments indefinitely.

13. References

Ames, B.N., J. McCann, and E. Yamasaki (1977)
Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test
In: B.J. Kilbey *et al*. (Eds.) *Handbook of Mutagenicity Test Procedures* Elsevier, Amsterdam, 1-17

De Serres, F.J. and M.D. Shelby (1979)
Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay
Mutation Res. 64, 159-165

Hollstein, M., J. McCann, F.A. Angelosanto, and W.W. Nichols (1979)
Short-term tests for carcinogens and mutagens
Mutation Res. 65, 133-226

Green, M.H.L. and W.J. Muriel (1976)
Mutagen testing using Trp^+ reversion in Escherichia coli
Mutation Res. 38, 3- 32

Maron, D.M., J. Katzenellenbogen, and B.N. Ames (1981)
Compatibility of organic solvents with the Salmonella/Microsome Test
Mutation Res. 88, 343-350

Maron, D.M., and B.N. Ames (1983)
Revised methods for the Salmonella mutagenicity test
Mutation Res. 113, 173-215

General Study Plan: GSP471.Ames.Evonik.1

14. Signatures

Author	27 January 2023 Date

Dr. Steffi Chang
ICCR-Roßdorf GmbH

Approved by Test Facility Management	27 January 2023 Date

Dr. Markus Schulz
ICCR-Roßdorf GmbH

Reviewed by Quality Assurance M. Hahn	27 January 2023 Date

Annex 4 Copy of the Study Specific Supplement

(5 pages)

Study Specific Supplement

To General Study Plan: 471.Ames.Evonik.1

C4004-210510:

Reverse mutation assay "Ames Test" using *Salmonella typhimurium* and *Escherichia coli*

ICCR Study Number:	4068911
Sponsor Name:	INFINITEC ACTIVOS S.L.
ESIMS Code:	2023-00073-EGM
Version ID:	Final
Issue Date:	12 July 2023
Study Director:	Dr. Steffi Chang
Testing Facility:	ICCR-Roßdorf GmbH In den Leppsteinswiesen 19 64380 Rossdorf Germany

Table of Content

1. Proposed Schedule

Experimental Starting Date:	01 August 2023
Experimental Completion Date:	30 September 2023
Draft Report Date:	31 October 2023

2. Test Item

Information as provided by the Sponsor.

Identification:	C4004-210510
Alternative name:	Pichia Ferment Lysate Filtrate
Batch:	210510
Purity:	Not applicable
Appearance:	Colorless*, liquid
Recertification Date:	05/2025
Storage Conditions:	Freezer
Purpose of Use:	Cosmetic product
Safety Precautions:	Routine hygienic procedures will be sufficient to ensure personnel health and safety.

* Determined by ICCR-Roßdorf staff

Correction for purity will not be made.

3. Special Conditions

Maximum concentration:	50 mg/mL
Solvent:	DMSO (purity > 99%)

4. Contact Details

Sponsor	**INFINITEC ACTIVOS S.L.** Can Parellada 22, Nave 2-3 08170 Montornés del Vallés, Barcelona Spain
Study Monitor	Dr. Andrea Marburger Evonik Operations GmbH Nutrition & Care Rodenbacher Chaussee 4 63457 Hanau-Wolfgang Germany Tel.:+49 6181 59 3419 Email: andrea.marburger@evonik.com
Study Director	Dr. Steffi Chang Tel: +49 6154-807211 Email: steffi.chang@iccr-rossdorf.de

5. Signatures

C4004-210510: Reverse mutation assay "Ames Test" using Salmonella typhimurium and Escherichia coli

12 July 2023

Study Director
Dr. Steffi Chang
ICCR-Roßdorf GmbH

Date

12 July 2023

Test Facility Management
Dr. Markus Schulz
ICCR-Roßdorf GmbH

Date

12 July 2023

Sponsor Representative
Dr. Andrea Marburger
Evonik Operations GmbH

Date

Note: These signatures also acknowledge the procedures described in the corresponding General Study Plan.

Annex 5 GLP Certificate

HESSEN

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

ICCR-Roßdorf GmbH
Institute for Competent Contract Research
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**2** Toxicity studies
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**3** Mutagenicity studies

28.06. bis 30.06.2022
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP- Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Brandt

Dr. Astrid Brandt, Referentin, Wiesbaden, den **01.November 2022**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, KLIMASCHUTZ, LANDWIRTSCHAFT UND VERBRAUCHERSCHUTZ

Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80, D 65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)

Report

C4004 - 210510:
In vitro Eye Irritation:
Human Cornea Model Test – OECD 492

ICCR Study Number:	4068912
ESIMS Code:	2023-00079-EGT
Sponsor Name:	Infinitec Activos S.L.
Version ID:	Final
Issue Date:	10 October 2023
Study Director:	M.Sc. Anja Dehmelt
Test Facility:	ICCR-Roßdorf GmbH In den Leppsteinswiesen 19 64380 Rossdorf Germany

TABLE OF CONTENTS

LIST OF ANNEXES

DEFINITIONS AND ABBREVIATIONS

ATLA	Alternative to Laboratory Animals
CC	Colorant Control
DMEM	Dulbecco's Minimum Essential Medium
DPBS	Dulbecco's Phosphate Buffered Saline
EC	European Commission
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Community
EU	European Union
GHS	Globally Harmonised System
GLP	Good Laboratory Practice
KC	Killed Control
MTT	3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazoliumbromide
NADH	Reduced Nicotinamide adenine dinucleotide
NSKC	Non-specific Killed Control
OD	Optical Density
OECD	Organisation for Economic Co-Operation and Development
p.p.	Percentage Points
QA	Quality Assurance
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RH	Relative Humidity
TG	Test Guideline
UN	United Nations

COMPLIANCE WITH GOOD LABORATORY PRACTICE

C4004 - 210510: *In vitro* Eye Irritation: Human Cornea Model Test – OECD 492

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

- "Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) in its currently valid version
- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17
- EC Commission Directive 2004/10/EC

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

A. Dehmelt

M.Sc. Anja Dehmelt
Study Director
ICCR-Roßdorf GmbH

10 October 2023

Date

QUALITY ASSURANCE STATEMENT

C4004 - 210510: *In vitro* Eye Irritation Test: Human Cornea Model Test – OECD 492

Study based activities at the Test Facility ICCR-Roßdorf GmbH, Rossdorf, were audited and inspected. The details of these audits and inspections are given below.

Type of Inspection	Date(s) of Inspection	Date Reporting to Study Director, Test Facility Management
Study plan verification	11 Jul 2023	11 Jul 2023
Process based Assessment of response	27 Jul 2023	27 Jul 2023
Report audit	07 Sep 2023	07 Sep 2023

General facilities and activities where this study was conducted were inspected on an annual basis and results are reported to the relevant responsible person and Management.

The statement is to confirm, that this report reflects the raw data.

Quality Assurance

L. Tsiklauri

Quality Assurance Auditor
ICCR-Roßdorf GmbH

10 Oct 2023

Date

1 SUMMARY

This *in vitro* study was performed to assess the eye irritation potential of C4004 - 210510 by means of the Human Cornea Model Test.

The test item did not prove to be a MTT reducer in the MTT interference pre-experiment. Also, its intrinsic color was not intensive and the OD of the test item in deionised water or isopropanol at 570 nm after blank correction was < 0.08. Therefore, additional tests with freeze-killed tissues or viable tissues (without MTT addition) did not have to be performed.

50 µL of the test item, the negative control (deionised water) or the positive control (methyl acetate) were applied to duplicate EpiOcular^{TM} tissues for 30 minutes, respectively.

The mean OD of the tissue replicates treated with the negative control was in the range of >0.8 and < 2.8, thus assuring the quality of the tissues.

Treatment with the positive control resulted in a decrease of viability below 50% compared to the negative control value in the relative absorbance, thus assuring the validity of the test system.

The difference of relative viability between the two relating tissues was < 20 p.p. in the same run (for test item, positive and negative control tissues).

After treatment with the test item C4004 - 210510 the mean relative cell viability value increased to 102.32% compared to the mean value of the negative control. This value is above the threshold for irritancy of ≤ 60%. Therefore, the test item is considered **not** to be an eye irritant.

In conclusion, it can be stated that in this study and under the experimental conditions reported, C4004 - 210510 is considered not to be an eye irritant according to UN GHS.

2 INTRODUCTION AND PURPOSE

Eye irritation is generally defined as "the production of reversible changes in the eye". The potential for chemical induced eye irritation is an important consideration in establishing procedures for the safe handling, packing and transport of chemicals. It was usually determined in the *in vivo* Draize rabbit eye irritation test as described in OECD guideline 405. In a pre-validation study performed by Avon Products Inc. and MatTek Corporation, the *in vitro* eye test using the human cornea model EpiOcular™ and measurement of cell viability by dehydrogenase conversion of MTT into a blue formazan salt have turned out as a sufficiently promising predictor for eye irritancy potential.

A limitation of the Test Guideline OECD 492 is that it does not allow discrimination between eye irritation/reversible effects on the eye (Category 2) and serious eye damage/irreversible effects on the eye (Category 1), nor between eye irritants (optional Category 2A) and mild eye irritants (optional Category 2B), as defined by UN GHS. For these purposes further testing with other suitable test methods is required.

The EpiOcular™ Eye Irritation Test (EIT) was validated by the European Union Reference laboratory for Alternatives to Animal Testing (EURL ECVAM) and cosmetics Europe between 2008 and 2013.

The test consists of a topical exposure of the neat test item to a human reconstructed cornea model followed by a cell viability test. Cell viability is measured by dehydrogenase conversion of MTT [(3-4,5-dimethyl thiazol 2-yl) 2,5-diphenyl-tetrazoliumbromide], present in cell mitochondria, into a blue formazan salt that is quantitatively measured after extraction from tissues. The relevant reduction of cell viability in comparison of untreated negative controls is used to predict eye irritation potential.
The technical proficiency of the test system according to OECD Guideline 492 guideline recommended proficiency substances was demonstrated. The respective proficiency certificate given by MatTek is annexed to this report.

2.1 Study Details

Sponsor:
INFINITEC ACTIVOS S.L.
Can Parellada 22, Nave 2-3
08170 Montornnés del Vallés, Barcelona
Spain

Study Monitor:
Dr. Andrea Marburger
Evonik Operations GmbH
Rodenbacher Chaussee 4
63457 Hanau-Wolfgang
Germany

2.2 Study Schedule

Study initiation date	13 July 2023
Experimental start date:	20 July 2023
Experimental completion date:	18 August 2023

2.3 Regulatory Testing Guidelines

The study was performed in compliance with the following regulations or guidelines

- OECD Guideline for Testing of Chemicals 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage (18 June 2019).

- MatTek Corporation Protocol: EpiOcular^{TM} Eye Irritation Test (OCL-200-EIT) For the prediction of acute ocular irritation of chemicals: Identification of chemicals not requiring classification and labeling for eye irritation or serious eye damage; Version 02/02/2021

3 TEST ITEM AND SUPPORTING INFORMATION

3.1 Test Item

The test item was supplied by or on behalf of the Sponsor including the following information:

Identification:	C4004 - 210510
Alternative Name:	Pichia Ferment Lysate Filtrate
Batch:	210510
Purity:	Not applicable
Appearance:	Colorless*, Turbid liquid
Expiry Date:	May 2025
Storage Conditions:	In the freezer
Stability in Solvent:	In water/aqueous solvents: In the refrigerator: 1 day (shake before use) In the freezer: 1 week (shake before use)
Purpose of Use:	Cosmetic product

*detected by ICCR-Roßdorf GmbH laboratory staff

3.2 Study Controls

Concurrent controls were used for several ICCR-Roßdorf GmbH studies performed simultaneously. Each 50 µL were applied on duplicate tissues for 30 minutes.

3.2.1 Negative control

- DPBS (ICCR-Roßdorf GmbH)

3.2.2 Positive control

- Methyl acetate (purity: ≥ 98%) (MatTek)

3.3 Test Item Application

The test item was tested neat.

4 TEST SYSTEM AND SUPPORTING INFORMATION

4.1 Reconstructed Human EpiOcular (OCL) model purchased from MatTek

The EpiOcular tissue construct is a non-keratinized epithelium prepared from normal human keratinocytes. It models the cornea epithelium with progressively stratified, but not cornified cells. These cells are not transformed or transfected with genes to induce an extended life span in culture. The "tissue" is prepared in inserts (MILLICELL®, 10 mm ∅) with a porous membrane through which the nutrients pass to the cells. A cell suspension is seeded into the insert in specialized medium. After an initial period of submerged culture, the medium is removed from the top of the tissue so that the epithelial surface is in direct contact with the air (surface 0.6 cm^2). This allows the test item to be directly applied to the epithelial surface in a fashion similar to how the corneal epithelium would be exposed *in vivo*.

4.2 Standard Culture Conditions

Each incubation of the tissues was performed under 37 ± 1.5°C and 5 ± 0.5% CO_2 in DMEM Medium.

4.3 EpiOcular™ Kit

Standard Assay Kit and MTT-100 Kit were purchased from MatTek Corporation (82105 Bratislava, Slovakia, Lot No.: 38542). The quality certificate of the test kits demonstrating its robustness is annexed to the report.

4.3.1 Standard Assay Kit Components

Sealed 24-well plate	Contains 12/24 inserts with EpiOcular™ tissues on agarose
Serum-free assay Medium	DMEM-Medium
Positive control	Methyl Acetate (CAS#79-20-9)
12-well plate	Holding plate
24-well plates	For MTT viability assay
6-well plates	For storing inserts, or for topically applying test agents
Ca++Mg++-Free DPBS	Dulbecco´s Phosphate Buffered Saline

4.3.2 MTT-100 Assay Kit Components

1 vial, 2 mL	MTT concentrate	
1 vial, 8 mL	MTT diluent (supplemented DMEM)	For diluting MTT concentrate prior to use in the MTT assay
60 mL	Extractant solution (isopropanol)	For extraction of formazan crystals

4.4 MTT Solution

On the day of the experiment a MTT solution of 1 mg/mL in DMEM was prepared.

5 EXPERIMENTAL DESIGN AND STUDY CONDUCT

5.1 Pre-Experiment

Test items which might absorb light in the same range as formazan dye (naturally or after treatment) and test items which might be able to directly reduce the vital dye MTT (to MTT formazan) may interfere with the tissue viability measurements and need the use of additional controls for corrections. Therefore, two pre-experiments were performed to determine the color interference and the MTT interference as described below.

1) **The Color Interference – Test**

   Therefore, 50 µL of the test item was added to 2 mL of deionised water and mixed. 2 mL of deionised water was used as control (blank). Both were incubated for 3 hours under standard conditions.

   In parallel, 50 µL of the test item was added to 2 mL of isopropanol and mixed. A control (2 mL of isopropanol, blank) was run concurrently. Both were incubated for 3 hours at room temperature.

   After incubation the presence of the staining was evaluated by OD measurement (see section 5.5 Measurement).

2) **The MTT Interference – Test**

   To test if a test item directly reduces MTT, 1 mL of a MTT solution (1 mg/mL) including 50 µL of the test item was incubated for 3 hours under standard conditions. 50 µL deionised water in 1 mL MTT solution was used as negative control.

   After incubation the change of color was determined by the unaided eye.

5.2 Option for the Main Experiment

Since the OD of the test item in deionised water or isopropanol at 570 nm after blank correction was < 0.08 in the first and did not interfere with MTT in the second pre-experiment, no additional tissues were necessary.

5.3 Main Experiment

5.3.1 Pre-warming of EpiOcular™ Tissues

The plastic bag containing the 24-well plate with epidermal tissues was opened under sterile conditions. Under an airflow using forceps, the gauze was removed and the inserts were taken out. Any remaining agarose that adheres to the outer sides of the inserts was removed by gentle blotting on the sterile filter paper or gauze and prior to the exposure of the test item and of the controls the EpiOcular™ tissues was inspected for quality:

It was taken care, that

- air bubbles between agarose and insert were not > 30% of the total surface,
- liquid on top of the insert was removed with sterile cotton tips,
- if again moisture was observed on top of the inserts after the pre-incubation or in case of visible defects the respective skin models were discarded.

EpiOcular™ tissues were equilibrated at room temperature for 15 minutes. The inserts with the tissues were transferred into 6-well-plates containing 1.0 mL assay medium and incubated for 60 minutes under standard conditions. Afterwards, the medium was changed and a further pre-incubation for 16 - 24 hours at standard incubation conditions follows.

5.3.2 Treatment

After pre-warming of the EpiOcular™ tissues was completed, and prior to application of the test item respectively the controls, all tissues were pre-wetted with 20 µL $Ca^{2+}Mg^{2+}$free-DPBS and incubated for 30 minutes.

According to OECD guideline 492 a minimum of 83.3 µL/cm^2 ≙ 50 µL for the liquid test item were used for treatment.

Concurrent negative, positive control and the test item were applied in duplicate tissues at a volume of 50 µL atop the tissue surface and incubated for 30 min. Afterwards all tissues were rinsed several times with PBS and incubated for 12 min in 5 mL assay medium in a 12-well plate at room temperature (post exposure immersion). At the end of this incubation the tissues were transferred into a 6-well plate with 1 mL assay medium and incubated for a post-treatment incubation for 120 min at standard conditions.

5.4 MTT-Assay

The tissues were extracted from both, the top and the bottom of the tissues.

For the MTT-Assay, tissues were incubated for 180 minutes in 300 µl MTT solution. Each tissue was extracted with isopropanol within 4-72 hours at 2-8°C without shaking. To mix the extract, the plates were placed on an orbital plate shaker and shaken for 3.5 hours at room temperature. Then, the extracts were mixed and two 200 µL aliquots were transferred to a 96-well plate for OD measurement. 200 µL of isopropanol were added to the wells designated as blanks for 96-well plate.

5.5 Measurement

The optical density (OD_{570nm}) was determined spectrophotometrically in duplicates by a microplate reader (Versamax® Molecular Devices). The absorbance values were determined using the software SoftMax Pro Enterprise (version 4.7.1).

5.6 Data Recording

The data generated were recorded in the raw data file. The results are presented in tabular form, including experimental groups with the test item and the controls.

6 Data Evaluation

6.1 General Calculations

1) The mean OD value of the two wells for each tissue and the blank control (OD_{Blk}) was calculated (Mean [OD_{570}] (well 1 and well 2).

2) The mean OD_{Blk} was subtracted from each mean OD value of the two wells. (Mean [OD_{570}] blank corr. (well 1 and well 2)). These values were used for all further calculations below.

3) The mean OD of the two relating tissues for each test group (negative control (NC), positive control (PC)) and the test item (TI) was calculated with the blank corrected mean OD (Mean [OD_{570}] of T1 and T2)

4) The percent viability of each test group relative to the negative control (= 100%) was calculated:

$$Viability\ (\%) = 100\ \times \frac{mean\ OD_{TI/PC/NC}}{mean\ OD_{NC}}$$

5) The relative OD of each tissue per test group was calculated. 100 divided by the mean $OD_{NC\ T1\ and\ T2}$ x mean OD of each tissue.

6) The difference of the viability values between duplicate tissues was calculated: The relative OD of T2 was subtracted from T1.

6.2 Acceptability of the Assay

The results are acceptable if:

1) The mean negative control OD is > 0.8 and < 2.8.

2) The mean relative viability of the positive control is below 50% of the mean negative control viability.

3) The difference of viability between the duplicate tissues of each test group is < 20 percentage points (p.p.) in the same run.

The historical control data of the positive and negative controls are presented in Annex 1. The results of the positive and negative controls of the test method demonstrate reproducibility over time.

6.3 Interpretation of Results – Prediction model

If the test item-treated tissue viability is > 60% relative to the negative control treated tissue viability, the test item is identified as not requiring classification and labelling according to UN GHS (No Category).

If the test item-treated tissue viability is ≤ 60% relative to negative control treated tissue viability, no prediction can be made for this test item.

A single test composed of at least two tissue replicates should be sufficient for a test chemical, when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 60±5%, a second test should be considered, as well as a third one in case of discordant results between the first two tests.

7 DEVIATIONS FROM STUDY PLAN

There were no deviations from study plan.

8 ARCHIVING

Records and documentation relating to this study will be maintained in the archives of ICCR-Roßdorf GmbH for a period of 4 years from the date on which the Study Director signs the final report. This will include but may not be limited to the Study Plan, any Amendments, raw data and Report of this study.

A sample of the test item will not be archived.

At termination of the aforementioned period, the records and documentation will be transferred to the GLP compliant archive Rhenus Archiv Services GmbH, Frankfurt am Main, for further archiving up to a total archiving period of 15 years.

ICCR-Roßdorf GmbH will retain in its archive a copy of the study plan, final report and any amendments indefinitely.

9 RESULTS AND DISCUSSION

9.1 Pre-Experiment

9.1.1 Assessment of Color Interference

Treatment Group	OD 570 nm Well 1	OD 570 nm Well 2	Mean OD of 2 Wells	Mean OD of 2 Wells blank corrected	Evaluation Mean OD_{570} (blank corrected) > 0.08
Blank Aqua Deion.	0.036	0.037	0.037		
Test Item + Aqua Deion.	0.038	0.039	0.038	0.002	no
Blank Isopropanol	0.036	0.038	0.037		
Test Item+ Isopropanol	0.077	0.076	0.077	0.040	no

The mean OD of the test item in deionised water or isopropanol was < 0.08 and therefore, an additional test with viable tissues without MTT addition was not necessary in the main experiment.

9.1.2 Assessment of MTT Interference

Optical evaluation of the MTT-reducing capacity of the test item with MTT-reagent did not show color change. Therefore, an additional test with freeze-killed tissues was not necessary.

9.2 Main experiment

Results after treatment with C4004 - 210510 and the controls for 30 minutes:

Test Group	Tissue No.	Well 1 [OD_{570}]	Well 2 [OD_{570}]	Mean [OD_{570}] (Well 1 and well 2)	Mean [OD_{570}] blank corr. (Well 1 and well 2)	Mean [OD_{570}] of T1 and T2	Tissue viabil. [%]	Viabil. of T1 and T2 [%]	Diff. of viabil. between T1 and T2 [p.p.]
Blank		0.038	0.037	0.037					
Negative Control	1	2.025	1.951	1.988	1.950	1.953	100.0	99.9	0.25
	2	1.972	2.013	1.993	1.955			100.1	
Positive Control	1	0.837	0.824	0.831	0.793	0.780	39.96	40.6	1.32
	2	0.801	0.809	0.805	0.767			39.3	
Test Item	1	2.088	2.036	2.062	2.025	1.998	102.32	103.7	2.72
	2	2.008	2.009	2.009	1.972			101.0	

9.3 Discussion

This *in vitro* study was performed to assess the eye irritation potential of C4004 - 210510 by means of the Human Cornea Model Test according to OECD TG 492.

The test item did not prove to be a MTT reducer in the MTT interference pre-experiment. Also, its intrinsic color was not intensive and the OD of the test item in deionised water or isopropanol at 570 nm after blank correction was < 0.08. Therefore, additional tests with freeze-killed tissues or viable tissues (without MTT addition) did not have to be performed.

50 µL of the test item, the negative control (deionised water) or the positive control (methyl acetate) were applied to duplicate EpiOcular^{TM} tissues for 30 minutes, respectively.

The mean OD of the tissue replicates treated with the negative control was > 0.8 and < 2.8 (1.950 and 1.955), thus assuring the quality of the tissues.

Treatment with the positive control induced a decrease below 50% viability (39.96%) compared to the negative control value in the relative absorbance, thus assuring the validity of the test system.

The difference of relative viability between the two relating tissues was < 20 p.p. (values between 0.25 p.p. and 2.72 p.p.) in the same run (for test item, positive and negative control tissues).

Regarding the reproducibility of the data, the absorbance values of the negative and positive controls were within the historical range of absorbance.

After treatment with the test item C4004 - 210510 the mean relative viability value increased to 102.32% compared to the relative absorbance value of the negative control. This value is above the threshold for irritancy of ≤ 60%. Therefore, the test item is considered **not** need to be an eye irritant.

10 CONCLUSION

In conclusion, it can be stated that in this study and under the experimental conditions reported, C4004 - 210510 is considered not to be an eye irritant according to UN GHS.

11 REFERENCES

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Regulation (EC) No. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures (CLP)

ANNEXES

Annex 1 Historical Control Data

Positive Control; OD at 570 nm after exposition to Methyl acetate		**Negative Control OD at 570 nm Deionised water (MatTek)**	
Mean Viability	33.31%	**Mean Absorption***	1.95
Standard Deviation	11.29 p.p.	**Standard Deviation**	0.29
Range of Viabilities	6.63% - 48.38%	**Range of Absorbance***	1.27–2.49
Mean Absorption	0.66	* should be 0.8–2.8 (OECD 492) or 1.0–2.5 (MatTek)	
Standard Deviation	0.26		
Range of Absorbance	0.11 - 1.17		

Data of 54 sets of controls shared between 114 studies performed from May 2014 until October 2022. (p.p. – percentage points)

Annex 2 Test Kit Certificate

MATTEK
A BICO COMPANY

Certificate of Analysis

Product: EpiOcular™ Tissue

Lot Number: 38542

Part#: OCL-200, OCL-212, OCL-200-EIT, OCL-212-EIT

Description: Reconstructed ocular tissue containing normal human keratinocytes. *This product is for research use only. Not for use in animals, humans or diagnostic purposes.*

I. Cell source

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

Keratinocyte Strain: 4F1188

II. Analysis for potential biological contaminants

The cells used to produce EpiOcular™ tissue are screened for potential biological contaminants. Tests performed for each of the potential biological contaminant listed in the analysis that follows, where performed according to the test method given. The product resulted in "no detection" for the following potential biological contaminants determined by the stated test method:

Keratinocytes:

HIV-1 virus – Oligonucleotide-directed amplification	Not detected
HIV-2 virus – Oligonucleotide-directed amplification	Not detected
Hepatitis B virus – Oligonucleotide- directed amplification	Not detected
Hepatitis C virus – Oligonucleotide- directed amplification	Not detected
Bacteria, yeast, and other fungi – long term antibiotic, antimycotic free culture	Not detected

III. Analysis for tissue functionality

Test	Specification	Acceptance criteria	Result and QA Statement	
Tissue viability	MTT QC assay, 1 hour, n=3	OD (540-570 nm) [1.1-3.0]	1.805 ± 0.064	**Pass**
Barrier function	ET-50 assay, 100 µl 0.3% Triton X-100, 3 time-points, n=2, MTT assay	ET-50 [12.2-37.5 min]	22.55 min	**Pass**
Sterility	Long term antibiotic and antimycotic free culture	No contamination	Sterile	**Pass**

Tissue viability and the barrier function tests are within the acceptable ranges and indicate appropriate formation of the mucosal barrier and a viable basal cell layer.

Initials: IS
Date: 8/15/23

Nelson Rivas
Quality Assurance Department
Document Control Manager

August 15, 2023
Date

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

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QC-10-012-0011 Rev. B

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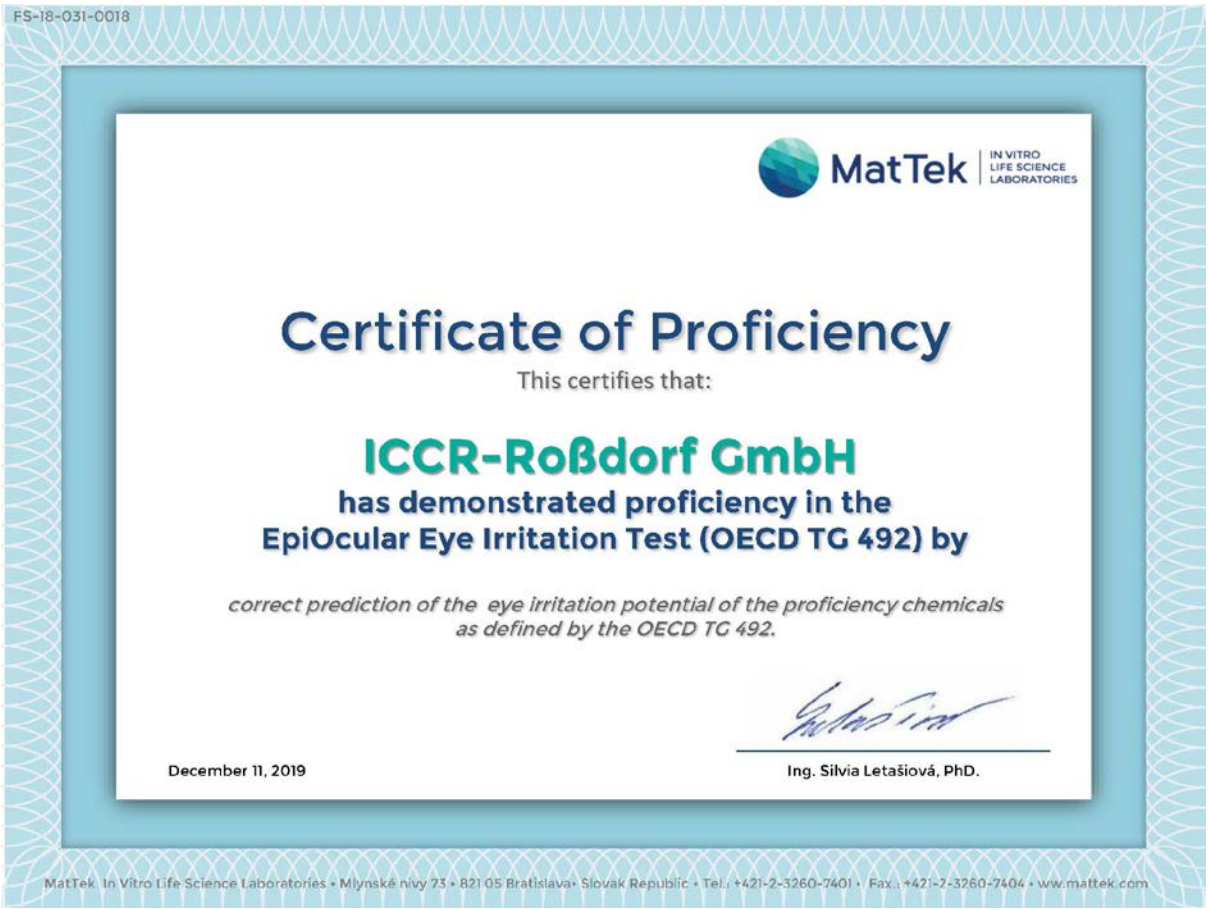
Annex 3 Certificate of Proficiency



Annex 4 GLP Certificate

HESSEN

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

ICCR-Roßdorf GmbH
Institute for Competent Contract Research
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**2** Toxicity studies
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**3** Mutagenicity studies

28.06. bis 30.06.2022
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP- Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Brandt

Dr. Astrid Brandt, Referentin, Wiesbaden, den **01.November 2022**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, KLIMASCHUTZ, LANDWIRTSCHAFT UND VERBRAUCHERSCHUTZ

Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80, D 65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)

Lipomyces Oil Extract (**YOIL®**, an RBD-Palm Oil Replacement, Made in Yeast) INCI Designated

Lipomyces Lipid Bodies (**YOIL-Cream®,** an aqueous moisturizer, no emulsifiers, 87% Oil) INCI Designated

From:
Xylome Corporation, Dr. Tom Kelleher, Ph.D. Univ. Research Park, 510 Charmany Drive, Labs 61-62, Madison WI 53719
To:
Dr. Bart Heldreth, CIR Executive Director, Cosmetic and Ingredient Review, 1620 L Street NW, Suite 1200, Washington D.C. 20036-4702, 202-331-0651 cirinfo@cir-safety.org

Dr. Heldreth and the Expert Panel:
The Xylome scientists were thrilled to see the pending expert panel report of Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics, which was released on January 10th, 2024. The contents are very informative, and we agree with how the expert panel handled the safety evaluations.

Xylome's team has worked hard to create a pure bioidentical replacement for white refined bleached and deodorized (RBD) palm oil. Because a true palm-oil bioidentical replacement such as **Lipomyces Oil Extract** (Product 1) and a very large **Lipomyces Lipid Body Product** (Product 2) have not previously existed, we have provided some bullet points that highlight the unique features and a peer-reviewed publication (reference attached) related to safety for these two sustainable cosmetic ingredients.

Both Food-Grade GMP products are made by the same yeast strain, in a precision (pure-culture) fermentation process. The fermentations are heat-killed at harvest, and all yeast are removed during the processing of the oil and the lipid bodies. Several publications cite the GRAS nature of *Lipomyces* and it is used in some probiotics. As already presented to the FDA, the modifications in our *Lipomyces* strain are endogenous (native) to the strain and it contains no foreign genes or antibiotic selection markers. The strain has an EPA MCAN Exemption for large-scale production.

After multiple reviews of the three leading medical databases, Xylome's M.D. Safety Advisor has found no reports of opportunistic infections caused by the *Lipomyces* genera during the 75-year history of this yeast. The fundamental safety reason is that *Lipomyces* fails to grow above 32°C. so it cannot be a systemic pathogen at human body temperatures, around 37°C. Also, *Lipomyces* does not have the phenotypes associated with pathogenic yeast.

Both of Xylome's cosmetic ingredients are in the customer validation phase of development with 1-30 Kilogram samples from our nominal metric ton level GMP facility, and we are working on a path-forward for globally impactful commercial scale-up production, as a true replacement for RBD palm oil. There are no other bioidentical replacements for solid white RBD palm oil currently from any yeast sources. Our motivation is to provide an alternative, which avoids tropical deforestation, which is driven by the growth of palm oil, as described by J. Zuckerman's book "*Planet Palm*" 2021.

Finally, we have provided our peer-reviewed publication, which complements the unpublished Xylome reference in the report. Title: Precision Fermentation of Bioidentical Palm Oil Alternatives. T.W. Jeffries, Ph.D., T.J. Kelleher, Ph.D., and D.Z. Mokry, Ph.D. Feb 27th, 2023, Cosmetics & Toiletries (Attached copy follows for convenience).

Please contact us, if you have any questions and we will respond promptly.

Best regards,
Tom Kelleher Ph.D., and the Scientists at Xylome [contact: tkelleher@xylome.com or 805-603-9736]

Lipomyces Oil Extract (**YOIL®**, an RBD-Palm Oil Replacement, Made in Yeast) INCI Designated

Lipomyces Lipid Bodies (**YOIL-Cream®**, an aqueous moisturizer, no emulsifiers, 87% Oil) INCI Designated

Xylome Comments and Reference for:

Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics

INGREDIENTS:

- ***Yoil®*** *(INCI:* ***Lipomyces Oil Extract****)*
- ***Yoil-Cream®*** *(formulated) (INCI:* ***Lipomyces Lipid Bodies*** *(and) Citric Acid (and) Citrate (and) Gluconolactone (and) Glycerol (and) Vitamin E Acetate (and) Sodium Benzoate)*

COMMENTS: LIPOMYCES OIL EXTRACT

- Lipomyces Oil created by Xylome is a white fat at room temperature and a clear liquid oil above 34°C. made in a GRAS yeast with no foreign genes and no antibiotic resistance markers. The yeast is heat-killed (to minus 6-logs) and eliminated from the oil during processing.
- Secondly, Xylome's Lipomyces Oil is essentially a pure neutral triglyceride with a lipid composition that is engineered specifically to be a direct bioidentical replacement for refined, bleached and deodorized (RBD) palm oil. The quality and purity level exceeds that of current commercial RBD palm oils and Lipomyces Oil Extract contains no chlorinated hydrocarbons, sterols or pigments found in RBD palm oil.
- Xylome's Lipomyces Oil is purified either with classical hexane extraction or without the use of solvents. Commercial economics may impact which processing is performed for which markets. However, we believe a solvent-free process can be scaled for the cosmetic industry.

COMMENTS: LIPOMYCES LIPID BODIES

- The Lipomyces Lipid Bodies created by Xylome are obtained from the same GRAS strain and fermentation process as the Lipomyces Oil Extract.
- The Lipomyces Lipid Bodies are much larger (~10 microns) than occur in nature, and without additives they form a white, aqueous-feeling moisturizer, containing 87% Lipomyces Oil (RBD palm oil replacement) without the need for any emulsifying agents.
- Lipomyces Lipid Bodies can be used for loading hydrophobic active ingredients and hydrophobic therapeutics for topical drug delivery.
- Unformulated Lipomyces Lipid Bodies (without antimicrobial stabilizers) can be repeatedly steam sterilized or repeatedly frozen without disrupting the lipid body structures.
- Finally, Lipomyces Lipid Bodies for room temperature stability use the INCI designation ingredients, which are based on the Environmental Working Group (EWG) recommendations.

A peer-reviewed publication follows, which summarizes the technology for both products, including the human exposure evaluation (n=579) with the Lipid Bodies.

Lipomyces Oil Extract (**YOIL®**, an RBD-Palm Oil Replacement, Made in Yeast) INCI Designated

Lipomyces Lipid Bodies (**YOIL-Cream®**, an aqueous moisturizer, no emulsifiers, 87% Oil) INCI Designated

REFERENCE:

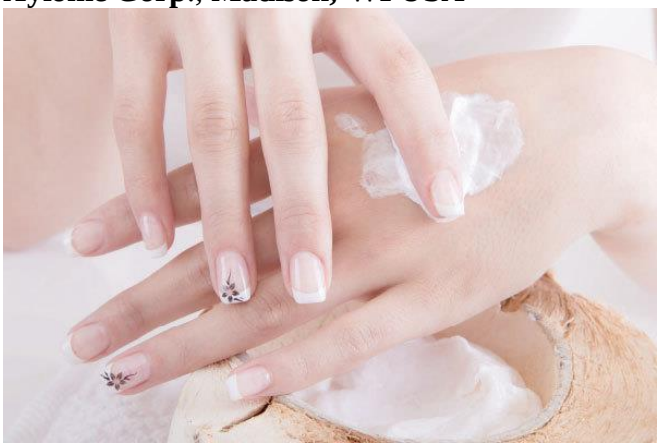
Title: Precision Fermentation of Bioidentical Palm Oil Alternatives. T.W. Jeffries, Ph.D., T.J. Kelleher, Ph.D., and D.Z. Mokry, Ph.D. Feb 27th, 2023, Cosmetics & Toiletries

FORMULATING | C&T PEER-REVIEWED

Precision Fermentation of Bioidentical Palm Oil Alternatives

IN SECTION: FORMULATING | C&T PEER-REVIEWED

T.W. Jeffries, Ph.D., T.J. Kelleher, Ph.D., and D.Z. Mokry, Ph.D.
Xylome Corp., Madison, WI USA



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KEY POINTS

- **Sustainable ingredients that act as drop-in replacements for refined, bleached and deodorized (RBD) palm oil are sought.**
- **Proposed here are a yeast oil extract and emulsifier-free oil-cream derived through precision *Lipomyces* fermentation, which are characterized and tested for loading capabilities as described here.**

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Tropical palm oil, particularly white, refined, bleached and deodorized (RBD) palm oil and its derivatives, can be found in almost 50% of consumer goods. In recent decades, the increase in palm oil consumption has

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Lipomyces Lipid Bodies (**YOIL-Cream®**, an aqueous moisturizer, no emulsifiers, 87% Oil) INCI Designated

triggered the rapid loss of tropical ecosystems and species extinction due to deforestation for new palm plantations. This situation is not sustainable. Indeed, for the past 180 years, palm oil sourcing has been responsible for many social and economic problems in tropical regions around the planet.[Display footnote number:1] Demands by environmental groups and aware consumers have created a market for sustainable replacements for RBD palm oil. The challenge has been to create an alternative that is bioidentical to white RBD palm oil – which dominates the consumer goods market thanks to the unique characteristics of being a solid white fat at room temperature and a liquid at body temperature.

Research focused on this challenge has recently achieved bioidentical substitutes for white RBD-palm oil: a *Lipomyces* oil extract[Display footnote number:a] and an oil-cream[Display footnote number:b] derived through precision fermentation of the yeast. These ingredients were characterized and tested for loading capabilities in preliminary evaluations described here.

Lipomyces Oil Extract Production

In brief, to produce the yeast oil, multiple genes naturally present in the lipogenic yeast *Lipomyces starkeyi*, which is generally regarded as safe (GRAS), are over-expressed. Manipulating the number of genes copied and their location creates a hyper-lipogenic strain, designated XYL403, which produces a neutral triglyceride; i.e., the *Lipomyces* oil extract. The yeast converts sugar into the oil extract at a high rate, filling the cells to > 90% of the yeast volume (see **Figure 1a**), which supports its commercial viability.

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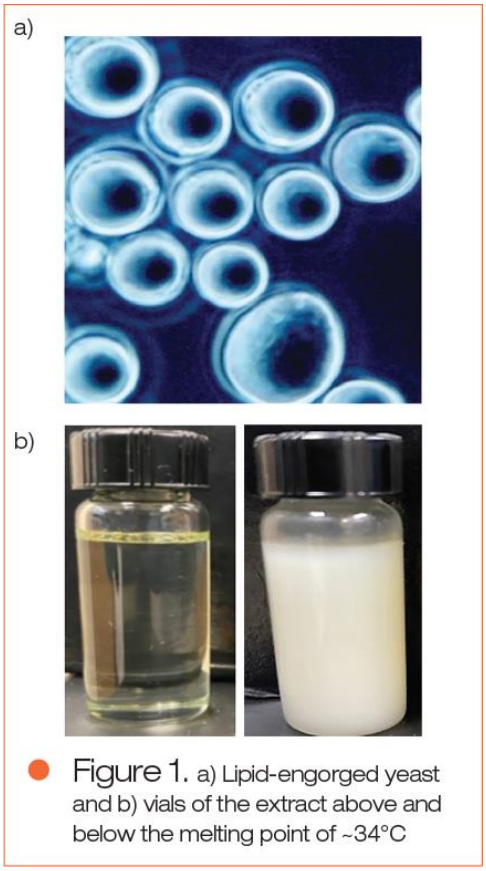

Figure 1. a) Lipid-engorged yeast and b) vials of the extract above and below the melting point of ~34°C

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Upon the fermentation and harvest of *Lipomyces* XYL403, the *Lipomyces* oil extract inside of heat-treated cells is released by homogenization and purified away from fermentation residues. The homogenized cells are extracted to make a pure oil.

Lipomyces Oil Extract Characterization

The resulting oil extract has a lipid profile that is approximately 90% identical to RBD palm oil. It is a white solid at room temperature and a nearly clear liquid oil at body temperature (see **Figure 1b**), which provides the same desirable texture and feel commonly associated with RBD-palm oil. **Figure 2** shows a visual comparison of the *Lipomyces* oil extract and commercial RBD palm oil.

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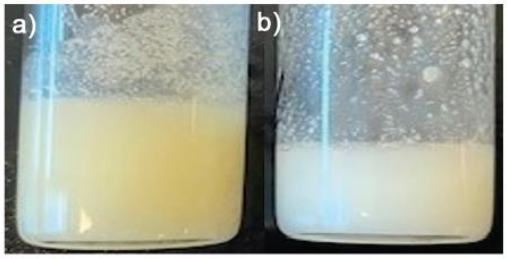

Figure 2. Comparison of a) commercial RBD palm to b) *Lipomyces* oil extract

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Furthermore, free fatty acids, pigments, sterols and bleaching-related chlorinated hydrocarbons can be present in RBD palm oil due to bleaching, elevated temperature refining and deodorization that is normally performed on crude red tropical palm oil to make it a white fat. The *Lipomyces* oil extract is less than 50% saturated and is not bleached – omitting chlorinated hydrocarbons or colored contaminants; it also is essentially trans-fat and sterol-free.

GC: The *Lipomyces* oil extract produced as described was analyzed by gas chromatograph (GC) using a standardized derivatization and injected into a reference standard-controlled GC to obtain the lipid profile. **Figure 3** compares the nearly identical profile of palm oil with *Lipomyces* oil extract. One minor lipid found at slightly elevated levels in *Lipomyces* oil extract was palmitoleic acid (C16:1), which is a common lipid found in human skin. At the low level of 3-7%, however, it has little impact on the desirable palm-oil-like physical properties, such as solid or liquid feel and melting point of the transition from a solid to liquid at 34°C.

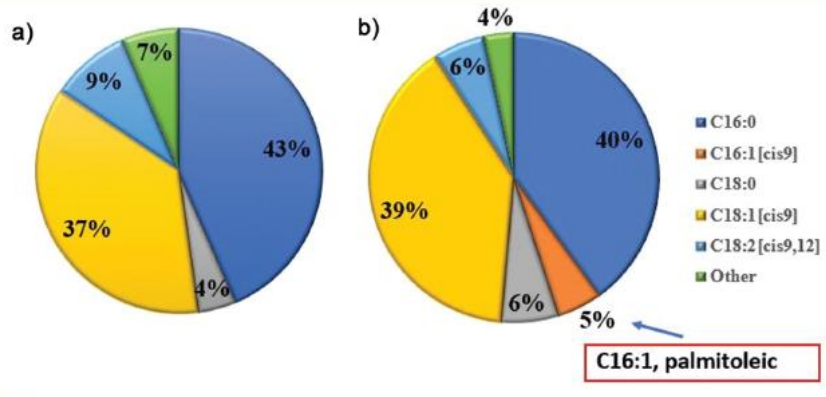

Figure 3. Gas chromatography profile of a) palm oil and b) *Lipomyces* oil extract

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Lipomyces Oil-cream Production

In addition to *Lipomyces* oil extract, during fermentation, *Lipomyces* XYL403 assembles purified lipid bodies in a novel aqueous-feeling, white composition typical of moisturizers. This oil-cream[Display footnote number:b], single-ingredient moisturizer forms without emulsifiers and is composed solely of large, isolated yeast lipid bodies in water. The lipid bodies are ~10 microns in size – approximately 100 × larger than commercial hydrophobic liposomes, which are 0.1 microns in size according to vendor literature and specifications. The structures contain internal *Lipomyces* oil extract and the outer "shell" of the lipid body structures (see **Figure 4**).

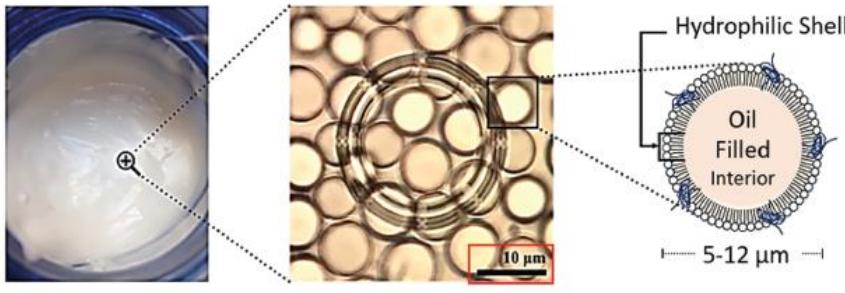

Figure 4. (From L) *Lipomyces* oil-cream, magnification and structural schematic

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These large lipid bodies are isolated by mild hydrolysis, then the culture medium, cell debris and other residuals are separated by flotation of the oil-filled lipid bodies – i.e, the denser aqueous bottom layer is removed. The lipid bodies are washed multiple times under mild acidic, alkaline and neutral pH conditions until an odorless, tasteless white oil-cream is produced.

Lipomyces Oil-cream Characterization

GC: The *Lipomyces* oil-cream also was characterized by GC and found to have an internal lipid composition comparable to RBD palm oil (see **Figure 5**).

GC-MS-MS: The large 10-micron lipid bodies were additionally subjected to lipidomic and proteomic studies, which confirmed the composition and ratio of internal *Lipomyces* oil extract is comparable to the ratios found in much smaller lipid bodies (~1-2 microns) of the parental strain of *Lipomyces* (see **Figure 6**).[Display footnote]

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Lipomyces Lipid Bodies (**YOIL-Cream®**, an aqueous moisturizer, no emulsifiers, 87% Oil) INCI Designated number:2 Approximately 87.5% of the lipid body mass is composed of the internal neutral triglyceride that is the same as *Lipomyces* oil extract. The remaining 12.5% of the lipid body is the shell wall that contains hydrophilic (ionic) lipids – specifically diacylglycerides (66% of the shell), phospholipids and trace levels of proteins and yeast beta-glucans.

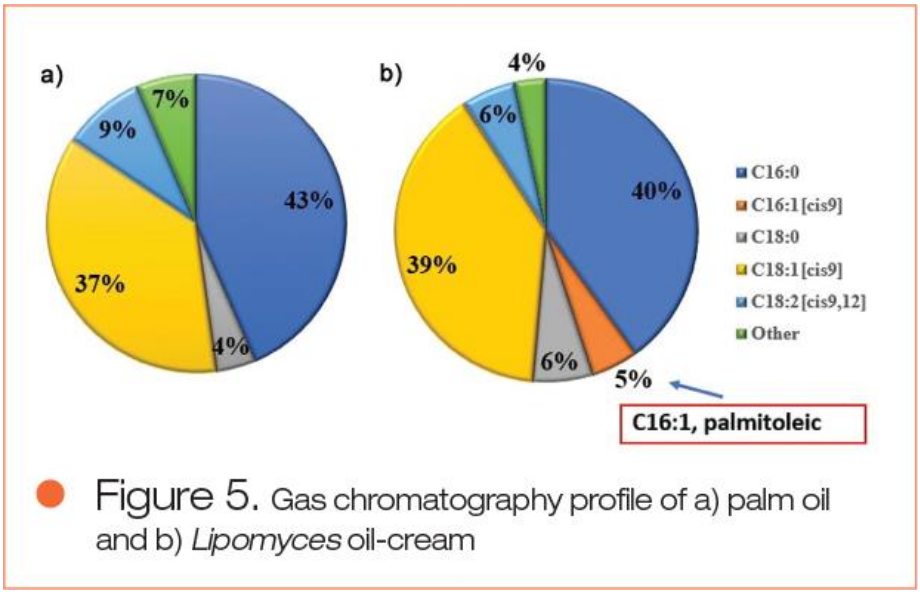

Figure 5. Gas chromatography profile of a) palm oil and b) *Lipomyces* oil-cream

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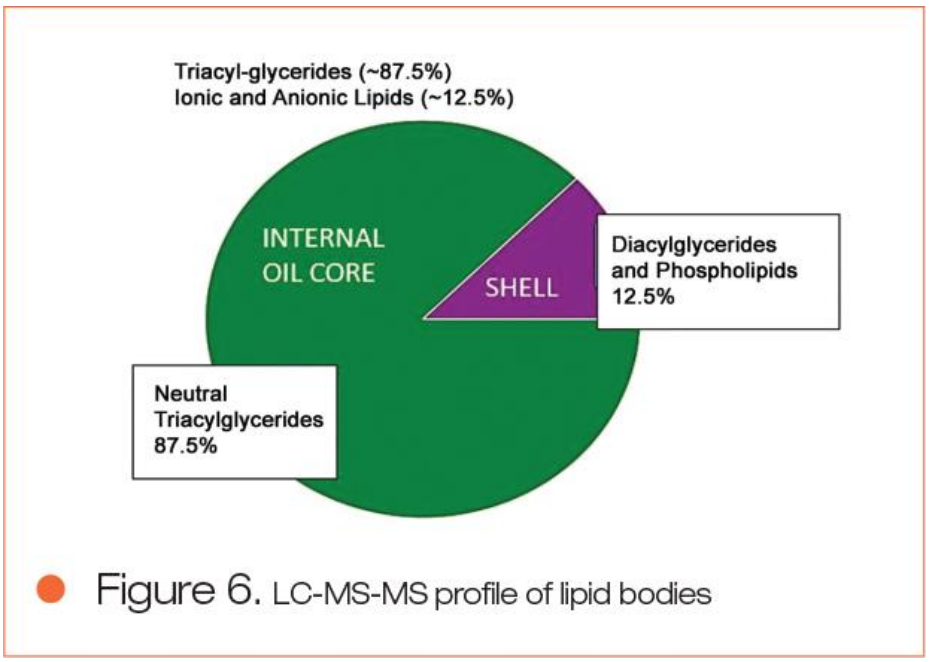

Figure 6. LC-MS-MS profile of lipid bodies

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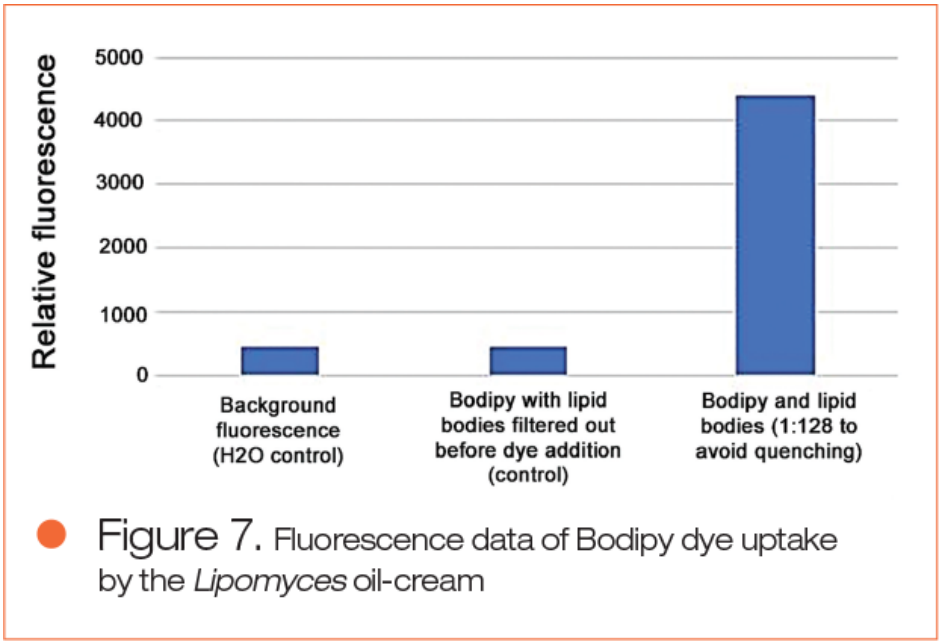

Figure 7. Fluorescence data of Bodipy dye uptake by the *Lipomyces* oil-cream

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The size of the lipid bodies combined with their hydrophobic oil-filled core present unique physical properties for cosmetic applications and topical drug delivery. Examples include their water miscibility (despite the oil content) and aqueous feel. The latter was confirmed in an internal study of women (n = 579) ages 28-65 who used it as a face and hand cream. The lipid bodies also are tolerant to multiple steam sterilizations, as demonstrated by another internal study exposing them to five cycles at 121ºC for 20 min each; no change in the lipid bodies was observed.

The oil-cream also has the potential to increase the delivery of hydrophobic drugs by an order of magnitude relative to liposomes and nanoparticles, based on the greater volumetric content of the oil core as volume to surface area. Furthermore, the barrier film created by the lipid body shells remaining on skin after product application is another area for product innovation.

Hydrophobic Ingredient Loading

As demonstrated, the structure of the *Lipomyces* oil-cream has a high volume to surface ratio between the internal oil and the lipid body shell. This hydrophobic core acts as a reservoir for compounds and is suitable for various forms of drug delivery, particularly when the drug or active is hydrophobic.

To visualize the uptake of compounds, surrogate hydrophobic dyes were used. Bodipy (fluorescent), Nile Red (color) and Sudan Black (color) were mixed with the *Lipomyces* oil-cream at 50 mg/mL in water after initial

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solubilization in DMSO. **Figure 7** shows fluorescence data of the uptake of the Bodipy dye by the oil-cream's lipid bodies; **Figure 8a** shows the uptake of Nile Red and **8b** shows the uptake of Sudan black in centrifuged samples.

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The loading of the Nile Red and Sudan Black appear to be completely bound to the low-density lipid bodies, based on the clarity of the lower water phase solutions; these were uniformly colored before the addition of the lipid bodies and centrifugation. To further confirm that the binding was due to loading into the lipid bodies, Sudan Black was examined microscopically under 1,000 × magnification. The movement of the Sudan Black into the lipid bodies is shown in **Figure 9.**

The results from the hydrophobic dyes support the expected mechanism of action for the loading of hydrophobic active ingredients and drugs. Currently, a program is under way for the loading of a new class of antifungal into *Lipomyces* oil-cream for topical burn treatment; additional loadings have included lidocaine, griseofulvin, benzocaine, colchicine, hydroxychloroquine and other hydrophobic drugs (not shown).

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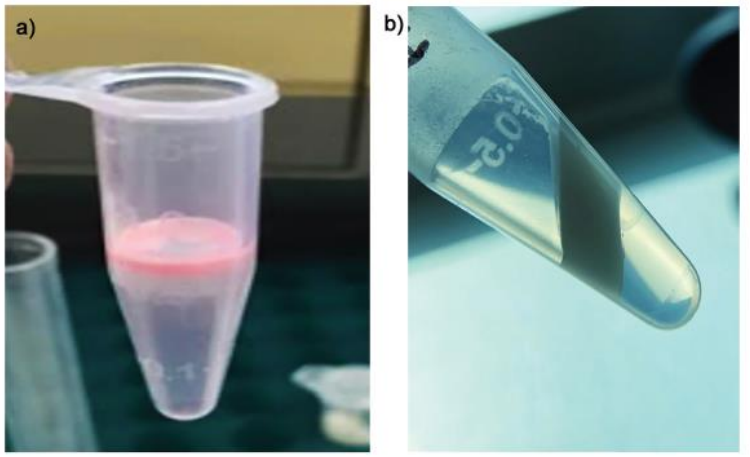

Figure 8. Centrifuged tubes showing uptake of a) Nile Red and b) Sudan Black by the *Lipomyces* oil-cream lipid bodies

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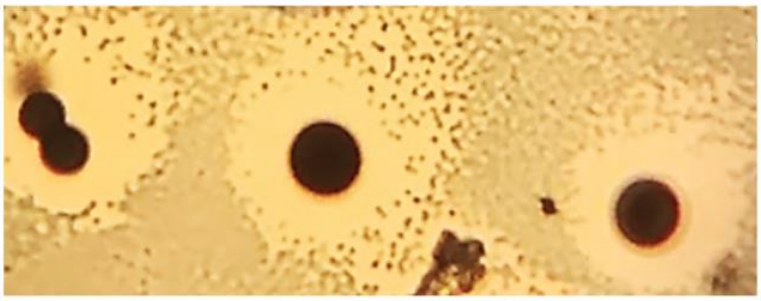

Figure 9. Sudan Black uptake to lipid bodies under microscope (1,000 x)

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Discussion

The precision fermentation of *Lipomyces* oil extract and oil-cream is a significant advancement in sustainability and more importantly, creates an alternative to tropical palm oil. The *Lipomyces* oil extract was found to be nearly identical to RBD palm oil, and could potentially be useful as a drop-in replacement in personal care and drug products. The similarity of the two was apparent in the lipid profile by GC and in physical properties such as melting point and texture (solid versus liquid at the physical state transition temperature of 34ºC). These properties have not yet been achieved with other microbial-derived palm oil alternatives, which are generally not solid white fats at room temperature.

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The *Lipomyces* oil-cream created in tandem with the oil extract is an emulsifier-free material whose 10-micron lipid bodies could provide advantages over palm oil emulsions, liposomes and nanoparticles. For example, the lipid bodies tolerated multiple steam sterilizations and a wide range of acidic and basic conditions, as demonstrated during drug-loading experiments measured by HPLC (not shown). Indeed, factors such as total hydrophobicity, ionic environment and pH can play a role when loading active ingredients that are less hydrophobic than the surrogate dyes reported here, making the oil-cream potentially useful for the development of active ingredient formulations.

The large volume of *Lipomyces* oil extract within the oil-cream lipid bodies also acts as a reservoir for hydrophobic compounds, which obviates the need for emulsifiers. The utility of the *Lipomyces* oil-cream as a delivery system for hydrophobic drugs is the topic of ongoing studies. Here, the fluorescence of lipid bound Bodipy dye suggests its complete quantitative uptake, compared with controls. Similar results were observed visually for the hydrophobic dyes Nile Red and Sudan Black. Confirmation of the lipid bodies as a passive reservoir for hydrophobic compounds was made by the microscopic examination of Sudan Black, whose particles were taken up by lipid bodies. This opens the door to a new frontier in skin care ingredients that are simpler and more sustainable.

Conclusion

White RBD palm oil represents nearly all (> 90%) of the palm oil used in North America, according to the Malaysian Palm Council in Washington, D.C. The introduction of a fermentation-derived alternative, *Lipomyces* oil extract, may provide a path forward to solve many social and ecological problems associated with current palm oil ingredients.[Display footnote number:1]

In addition, the emulsifier-free *Lipomyces* oil-cream co-created during fermentation holds potential for the delivery of hydrophobic active ingredients to skin; for example, to impart oil and moisture via an aqueous-feeling cream. Lastly, the ingredient could provide a barrier film composed of the lipid body shells, which offers another area for innovation for the delivery and retention of active ingredients.

Authors' note: The described ingredients hold potential for personal care products and drug delivery. Commercialization and equity partners will be required for full scale manufacturing once solid market validation is achieved with the current GMP pilot-scale processes.

[a] Yoil (INCI: Lipomyces Oil Extract) and

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[b] ***Yoil-Cream (patent pending) (INCI: Lipomyces Lipid Bodies (and) Citric Acid (and) Citrate (and) Gluconolactone (and) Glycerol (and) Vitamin E Acetate (and) Sodium Benzoate)***

Both Trade Names are registered trademarks of Xylome.

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1. Zuckerman, J. (2021). *Planet Palm*. The New York Press, NY. ISBN 978-1-62097-523-7, 335 p.

2. Uzuka, Y., Kanamori, T., Tanaka, K. and Kola T.T. (1975). Isolation and chemical composition of intracellular oil globules from the yeast *Lipomyces starkeyi. J Gen Appl Microbio*. 21(3) 157-168.

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