Safety Assessment of Brown Algae-Derived Ingredients as Used in Cosmetics

Status:	Draft Tentative Report for Panel Review
Release Date:	November 9, 2018
Panel Meeting Date:	December 3-4, 2018

The 2018 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Lillian C. Becker, former Scientific Analyst/Writer and Priya Cherian, Scientific Analyst/Writer.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Priya Cherian, Scientific Writer/Analyst
Date: November 9, 2018
Subject: Draft Tentative Report of the Safety Assessment on Brown Algae-Derived Ingredients

Enclosed is the Draft Tentative Report of the Safety Assessment of Brown Algae-Derived Ingredients as Used in Cosmetics. (It is identified as *broalg122018rep* in the pdf document).

At the September 2018 meeting, the Panel issued an Insufficient Data Announcement for these 82 ingredients. The Panel's data needs were:

- Composition and organic constituent data for each of these Brown Algae-derived cosmetic ingredients
- 28-Day dermal toxicity data for those ingredients that are not GRAS
- Sensitization data at relevant use concentrations for all ingredients (e.g., Macrocystis Pyrifera (Kelp) Extract at 36.4%)
- Genotoxicity data for those ingredients that are not GRAS

Since the September Panel meeting, CIR has received the following data, which have been incorporated into the report and have been designated by highlighting (*broalg122018data1* through *broalg122018data11*).

- Manufacturing information, composition data, and an in vivo skin irritation study of several trade name mixtures containing Pelvetia Canaliculata Extract and Laminaria Digitata Extract (*broalg122018data1*)
- An in vivo skin sensitization study of a trade name mixture containing Undaria Pinnatifida Extract in caprylic/capric triglyceride (*broalg122018data2*)
- An in vitro skin irritation study on a trade name mixture containing Undaria Pinnatifida Extract in caprylic/capric triglyceride (*broalg122018data3*)
- Manufacturing information, composition data, a 24 hour patch test, a sensitization study, and an in vitro skin irritation study on several trade name mixtures containing Undaria Pinnatifida Extract (*broalg122018data4*)
- Information received from UNITIS regarding several brown algae ingredients (*broalg122018data5*)
- A dermal irritation study, an in chemico skin sensitization study, an in vitro skin sensitization study, a bacterial reverse mutation study , and an ocular irritation study of a trade name mixture containing Undaria Pinnatifida Cell Culture Extract (0.5-2%) (*broalg122018data6*)
- A dermal irritation test, ocular irritation test, and in vitro sensitization study using a test substance containing 1.3% Sargassum Filipendula Extract (*broalg122018data7*)
- A reverse mutation study, a dermal irritation study, a sensitization study, and an ocular irritation study regarding a trade name mixture containing approximately 4% Macrocystis Pyrifera (Kelp) Extract (*broalg122018data8*)
- A dermal irritation test, ocular irritation test, a genotoxicity study, and composition data of a trade name mixture consisting of Cystoseira Amentacea/Caespitosa/Brachycarpa Extracts (48%) and water (52%) (*broalg122018data9*)
- A dermal irritation test, ocular irritation test, and composition data of a trade name mixture consisting of Himanthalia Elongata Extract (20%), Undaria Pinnatifida Extract (37%) and water (43%) (*broalg122018data10*)
- A genotoxicity test, dermal irritation test, dermal sensitization test, ocular irritation test, and composition data of 48% Halidrys Siliquosa Extract in 52% water. (*broalg122018data11*)

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The data that were presented to the Panel in Wave 2 and Wave 3 for the September meeting have also been incorporated into the report. (These data are not highlighted.)

Comments provided by the Council prior to the September meeting on the draft report have been addressed (*broalg122018pcpc)*. In addition, the flow chart (*broalg122018flow*), updated data profile (*broalg122018prof*), VCRP data (*broalg122018FDA*), minutes (*broalg122018min*), history (*broalg122018hist*), and search strategy (*broalg122018strat*), have been included in this packet.

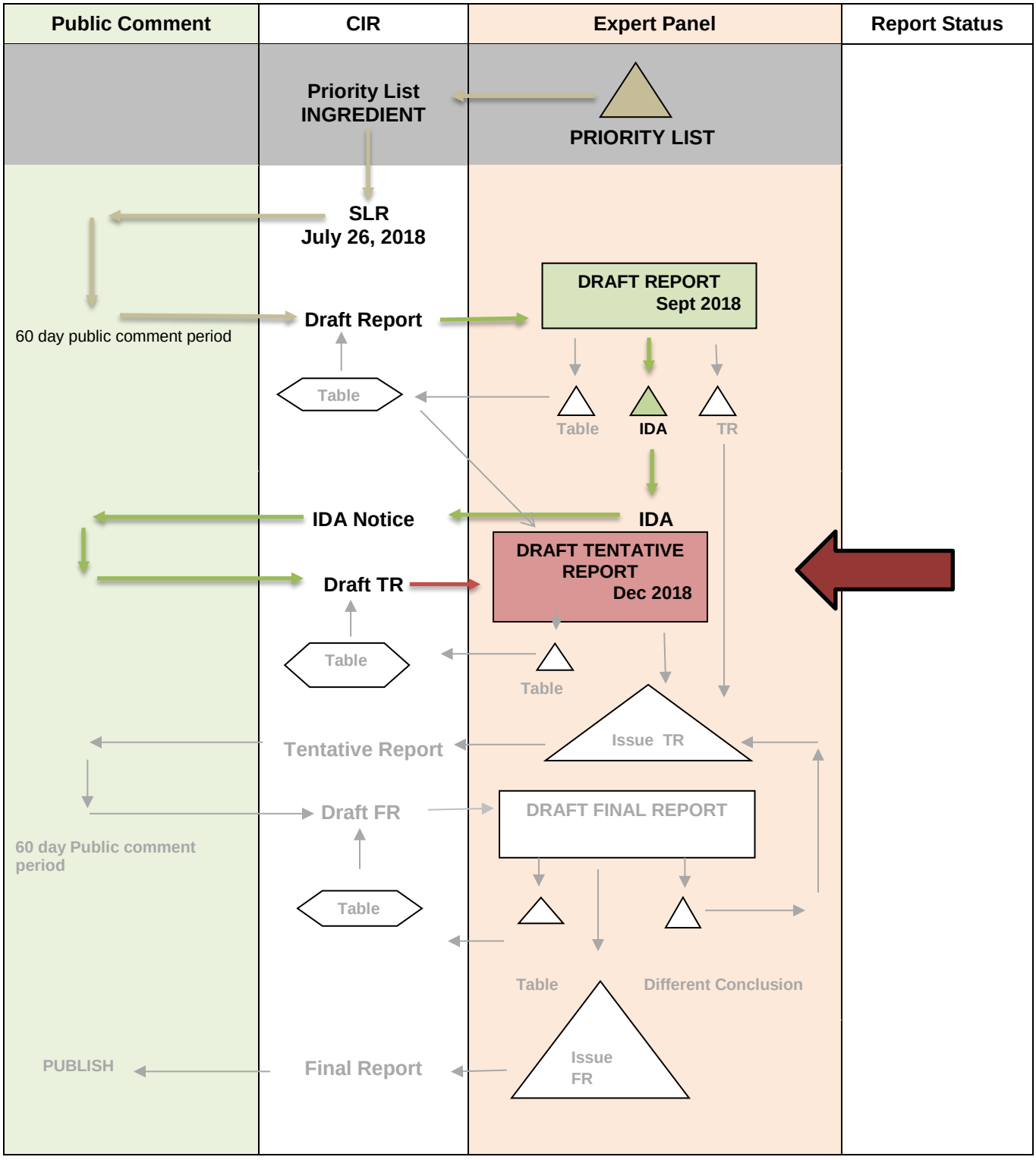
A draft Discussion has been incorporated into the report, based on the proceedings and comments from the September meeting. The Discussion draft addresses irritation/sensitization data, impurities concerns, estrogenic effects, and the outstanding data needs. Please determine if these concerns are addressed properly, and identify any other issues that need to be discussed.

The Panel should carefully consider the data presented in this report, and issue a Tentative Report with a safe, safe with qualifications, insufficient data, or split conclusion.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Brown Algae-derived ingredients

MEETING December 2018



History of Brown Algae

August 2018: SLR announced for public comment

September 2018: draft report reviewed by Panel; the Panel issued an IDA; the Panel requested the following data:

- Composition and organic constituent data for each of these Brown Algae-derived cosmetic ingredients
- 28-Day dermal toxicity data for those ingredients that are not GRAS
- Sensitization data at relevant use concentrations for all ingredients (e.g., Macrocystis Pyrifera (Kelp) Extract at 36.4%)
- Genotoxicity data for those ingredients that are not GRAS

Following the September 2018 meeting, information regarding manufacturing, composition, genotoxicity, sensitization, skin irritation, and ocular irritation regarding several brown algae ingredients were received.

December 2018: the Panel reviews the draft tentative report

Brown Algae Data Profile for ***December*** **2018**. Writer – Priya Cherian																				
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
1. Agarum Cribrosum Extract	x																				
2. Alaria Esculenta Extract	x x												x			x					
3. Ascophyllum Nodosum							x														
4. Ascophyllum Nodosum Extract	x			x						x		x			x				x		
5. Ascophyllum Nodosum Powder	x	x																			
6. Cladosiphon Novae-Caledoniae Extract																					
7. Cladosiphon Okamuranus Extract	x																				
8. Cystoseira Amentacea/Caespitosa/ Branchycarpa Extract											x		x						x		
9. Cystoseira Baccata Extract													x			x					
10. Cystoseira Balearica Extract																					
11. Cystoseira Caespitosa Extract																					
12. Cystoseira Compressa Extract				x															x		
13. Cystoseira Compressa Powder																					
14. Cystoseira Tamariscifolia Extract													x								
15. Dictyopteris Membranacea Extract (Retired)																					
16. Dictyopteris Polypodioides Extract	x																				
17. Dictyota Coriacea Extract																					
18. Durvillea Antarctica Extract	x																				
19. Ecklonia Cava Extract				x			x												x		
20. Ecklonia Cava Water							x														
21. Ecklonia Kurome Extract																					
22. Ecklonia Kurome Powder																					
23. Ecklonia/Laminaria Extract																					
24. Ecklonia Maxima Extract																					
25. Ecklonia Maxima Powder																					
26. Ecklonia Radiata Extract	x																				
27. Eisenia Arborea Extract																					
28. Fucus Serratus Extract	x																				
29. Fucus Spiralis Extract														x		x					
30. Fucus Vesiculosus	x																				
31. Fucus Vesiculosus Extract	x x			x			x							x		x			x		
32. Fucus Vesiculosus Powder	x																				
33. Halidrys Siliquosa Extract											x		x			x			x		
34. Halopteris Scoparia Extract																					
35. Himanthalia Elongata Extract	x										x		x								
36. Himanthalia Elongata Powder													x								
37. Hizikia Fusiforme Extract																				x	
38. Hizikia Fusiformis Water																					

Brown Algae Data Profile for ***December*** **2018**. Writer – Priya Cherian																					
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
39. Hizikia Fusiformis Callus Culture Extract																					
40. Hydrolyzed Ecklonia Cava Extract																					
41. Hydrolyzed Fucus Vesiculosus Extract																					
42. Hydrolyzed Fucus Vesiculosus Protein																					
43. Kappaphycus Alvarezii Extract																					
44. Laminaria Angustata Extract (Retired)																					
45. Laminaria Cloustoni Extract	x																				
46. Laminaria Diabolica Extract																					
47. Laminaria Digitata Extract	x											x	x x	x		x					
48. Laminaria Digitata Powder	x																				
49. Laminaria Hyperborea Extract	x												x			x					
50. Laminaria Japonica Extract	x						x				x		x	x							
51. Laminaria Japonica Powder							x														
52. Laminaria Longissima Extract																					
53. Laminaria Ochotensis Extract (Retired)																					
54. Laminaria Ochroleuca Extract	x												x								
55. Laminaria Saccharina Extract													x			x			x		
56. Lessonia Nigrescens Extract	x																				
57. Lessonia Nigrescens Powder																					
58. Macrocystis Pyrifera (Kelp)	x										x					x			x		
59. Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract																					
60. Macrocystis Pyrifera (Kelp) Extract	x										x			x							
61. Macrocystis Pyrifera (Kelp) Juice																					
62. Macrocystis Pyrifera (Kelp) Protein	x																				
63. Nereocystis Luetkeana Extract											x			x							
64. Pelvetia Canaliculata Extract	x												x			x					
65. Pelvetia Siliquosa Extract																					
66. Phyllacantha Fibrosa Extract																					
67. Saccharina Angustata Extract																				x	
68. Saccharina Japonica Extract																					
69. Saccharina Longicruris Extract																					

Brown Algae Data Profile for ***December*** **2018**. Writer – Priya Cherian																					
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
70. Sargassum Filipendula Extract	x										x			x		x	x				
71. Sargassum Fulvellum Extract				x																	
72. Sargassum Fusiforme Extract																					
73. Sargassum Glaucescens Extract													x								
74. Sargassum Horneri Extract																					
75. Sargassum Muticum Extract	x																				
76. Sargassum Pallidum Extract																				x	
77. Sargassum Siliquastrum Extract																					
78. Sargassum Thunbergii Extract																					
79. Sargassum Vulgare Extract	x																				
80. Sphacelaria Scoparia Extract	x												x			x					
81. Undaria Peterseniana Extract																					
82. Undaria Pinnatifida Extract	x						x						x		x	x					
83. Undaria Pinnatifida Cell Culture Extract											x			x			x		x		
84. Undaria Pinnatifida Leaf/Stem Extract																				x	
85. Undaria Pinnatifida Powder	x						x													x	
86. Undaria Pinnatifida Root Powder																					

Brown Algae

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	Web
1. Agarum Cribrosum Extract	-	-	X	X	X	X	X	X					N	N	N	n	n	√
2. Alaria Esculenta Extract	-	-	X	X	X	X	X	X										√
3. Ascophyllum Nodosum	-	-	√	X	√	X	X	X										√
4. Ascophyllum Nodosum Extract	84775-78-0	√	√	X	√	X	X	C										√
5. Ascophyllum Nodosum Powder	-	-	√	X	X	X	X	X										√
6. Asterionellopsis Glacialis Extract	-	-	X	X	X	X	X	X										X
7. Cladosiphon Novae-Caledoniae Extract	-	-	X	X	X	X	X	X										X
8. Cladosiphon Okamuranus Extract	-	-	X	X	X	X	X	X										
9. Cystoseira Amentacea/ Caespitosa/ Branchycarpa Extract	-	-	X	X	X	X	X	X										
10. Cystoseira Baccata Extract	-	-	X	X	X	X	X	X										
11. Cystoseira Balearica Extract	-	-	X	X	X	X	X	X										
12. Cystoseira Caespitosa Extract	-	-	X	X	X	X	X	X										
13. Cystoseira Compressa Extract	-	-	X	√	X	X	X	X										
14. Cystoseira Compressa Powder	-	-	X	X	X	X	X	X										
15. Cystoseira Tamariscifolia Extract	-	-	X	X	X	X	X	X										
16. Dictyopteris Membranacea Extract (Retired)	-	-	X	√	X	X	X	X										
17. Dictyopteris Polypodioides Extract	-	-	X	X	X	X	X	X										
18. Dictyota Coriacea Extract	-	-	X	X	X	X	X	X										
19. Durvillea Antarctica Extract	-	-	X	X	X	X	X	X										
20. Ecklonia Cava Extract	-	-	X	√	X	X	X	X										

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	Web
21. Ecklonia Cava Water	-	-	X	X	X	X	X	X										
22. Ecklonia Kurome Extract	-	-	X	X	X	X	X	X										
23. Ecklonia Kurome Powder	-	-	X	X	X	X	X	X										
24. Ecklonia/Laminaria Extract	-	-	X	X	X	X	X	X										
25. Ecklonia Maxima Extract	-	-	X	X	X	X	X	X										
26. Ecklonia Maxima Powder	-	-	X	X	X	X	X	X										
27. Ecklonia Radiata Extract	-	-	X	X	X	X	X	X										
28. Eisenia Arborea Extract	-	-	X	X	X	X	X	X										
29. Fucus Serratus Extract	94167-02-9	-	√	√	X	X	X	X										
30. Fucus Spiralis Extract	-	-	√	X	X	X	X	X										
31. Fucus Vesiculosus	-	-	X	√	√	X	X	X										
32. Fucus Vesiculosus Extract	283-633-7	√	X	X	X	X	X	X										
33. Fucus Vesiculosus Powder	-	√	X	√	X	X	X	X										
34. Halidrys Siliquosa Extract	-	-	X	X	X	X	X	X										
35. Halopteris Scoparia Extract	-	-	X	X	X	X	X	X										
36. Himanthalia Elongata Extract	-	-	X	X	√	X	X	X										
37. Himanthalia Elongata Powder	-	-	X	X	X	X	X	X										
38. Hizikia Fusiforme Extract	-	-	X	X	√	X	X	X										
39. Hizikia Fusiformis Water	-	-	X	X	X	X	X	X										
40. Hizikia Fusiformis Callus Culture Extract	-	-	X	X	X	X	X	X										
41. Hydrolyzed Ecklonia Cava Extract	-	-	X	X	X	X	X	X										
42. Hydrolyzed Fucus Vesiculosus Extract	84696-13-9	-	√	X	X	X	X	X										
43. Hydrolyzed Fucus Vesiculosus Protein	-	-	X	X	X	X	X	X										
44. Kappaphycus Alvarezii Extract	1220882-73-4 (generic)	-	X	X	X	X	X	X										

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	Web
45. Laminaria Angustata Extract (Retired)	-	√	X	X	X	X	X	X										
46. Laminaria Cloustoni Extract	90046-11-0 92128-82-0	-	X	X	X	X	X	X										
47. Laminaria Diabolica Extract	-	-	X	X	X	X	X	X										
48. Laminaria Digitata Extract	90046-12-1 92128-82-0	√	√	X	√	√	X	X										
49. Laminaria Digitata Powder	-	√	X	X	X	√	X	X										
50. Laminaria Hyperborea Extract	90046-13-2 92128-82-0	√	√	X	√	√	X	X										
51. Laminaria Japonica Extract	92128-82-0	√	√	√	√	√	X	X										
52. Laminaria Japonica Powder	-	-	X	X	X	X	X	X										
53. Laminaria Longissima Extract	-	-	X	X	X	X	X	X										
54. Laminaria Ochotensis Extract (Retired)	-	√	X	X	X	√	X	X										
55. Laminaria Ochroleuca Extract	92128-82-0	-	√	X	X	X	X	X										
56. Laminaria Saccharina Extract	90046-14-3 92128-82-0	√	√	X	X	√	X	X										
57. Lessonia Nigrescens Extract	-	-	X	X	X	X	X	X										
58. Lessonia Nigrescens Powder	-	-	X	X	X	X	X	X										
59. Macrocystis Pyrifera (Kelp)	-	√	√	X	X	X	X	X										
60. Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/ Stipe Juice Extract	-	-	X	X	X	X	X	X										
61. Macrocystis Pyrifera (Kelp) Extract	347174-92-9	√	√	X	X	√	X	X										
62. Macrocystis Pyrifera (Kelp) Juice	-	-	X	X	X	X	X	X										
63. Macrocystis Pyrifera (Kelp) Protein	-	-	X	X	X	X	X	X										
64. Nereocystis Luetkeana Extract	-	√	X	X	X	√	X	X										
65. Pelvetia Canaliculata Extract	223751-75-5	-	√	X	X	X	X	X										
66. Pelvetia Siliquosa Extract	-	-	X	X	X	X	X	X										

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	Web
67. Phyllacantha Fibrosa Extract	-	-	X	X	X	X	X	X										
68. Rissoella Verruculosa Extract	-	-	X	X	X	X	X	X										
69. Saccharina Angustata Extract	-	-	X	X	X	X	X	X										
70. Saccharina Japonica Extract	-	-	X	X	X	X	X	X										
71. Saccharina Longicruris Extract	-	-	X	X	X	X	X	X										
72. Sargassum Filipendula Extract	-	-	X	X	X	X	X	X										
73. Sargassum Fulvellum Extract	-	-	X	X	X	X	X	X										
74. Sargassum Fusiforme Extract	-	-	X	X	X	X	X	X										
75. Sargassum Glaucescens Extract	-	-	X	X	X	X	X	X										
76. Sargassum Horneri Extract	-	-	X	X	X	X	X	X										
77. Sargassum Muticum Extract	-	-	X	X	√	X	X	X										
78. Sargassum Pallidum Extract	-	-	X	X	X	X	X	X										
79. Sargassum Siliquastrum Extract	-	-	X	X	√	X	X	X										
80. Sargassum Thunbergii Extract	-	-	X	X	X	X	X	X										
81. Sargassum Vulgare Extract	-	-	X	X	X	X	X	X										
82. Sahel Scenedesmus Extract	-	-	X	X	X	X	X	X										
83. Sphacelaria Scoparia Extract	-	-	X	X	X	X	X	X										
84. Undaria Peterseniana Extract	-	-	X	X	X	X	X	X										
85. Undaria Pinnatifida Extract	-	-	X	√	X	X	X	X										
86. Undaria Pinnatifida Cell Culture Extract	-	-	X	X	X	X	X	X										
87. Undaria Pinnatifida Leaf/Stem Extract	-	-	X	X	X	X	X	X										
88. Undaria Pinnatifida Powder	-	√	X	X	X	√	X	X										
89. Undaria Pinnatifida Root Powder	-	√	X	X	X	√	X	X	N	N	N							

Botanical and/or Fragrance Websites (if applicable)

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	IFRA	RIFM
1. Agarum Cribrosum Extract	-						
2. Alaria Esculenta Extract	-						
3. Ascophyllum Nodosum	-						
4. Ascophyllum Nodosum Extract	-						
5. Ascophyllum Nodosum Powder	84775-78-0						
6. Asterionellopsis Glacialis Extract	-						
7. Cladosiphon Novae-Caledoniae Extract	-						
8. Cladosiphon Okamuranus Extract	-						
9. Cystoseira Amentacea/Caespitosa/ Branchycarpa Extract	-						
10. Cystoseira Baccata Extract	-						
11. Cystoseira Balearica Extract	-						
12. Cystoseira Caespitosa Extract	-						
13. Cystoseira Compressa Extract	-						
14. Cystoseira Compressa Powder	-						
15. Cystoseira Tamariscifolia Extract	-						
16. Dictyopteris Membranacea Extract (Retired)	-						
17. Dictyopteris Polypodioides Extract	-						
18. Dictyota Coriacea Extract	-						
19. Durvillea Antarctica Extract	-						
20. Ecklonia Cava Extract	-						

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	IFRA	RIFM
21. Ecklonia Cava Water	-						
22. Ecklonia Kurome Extract	-						
23. Ecklonia Kurome Powder	-						
24. Ecklonia/Laminaria Extract	-						
25. Ecklonia Maxima Extract	-						
26. Ecklonia Maxima Powder	-						
27. Ecklonia Radiata Extract	-						
28. Eisenia Arborea Extract	-						
29. Fucus Serratus Extract	94167-02-9						
30. Fucus Spiralis Extract	-						
31. Fucus Vesiculosus	-						
32. Fucus Vesiculosus Extract	-						
33. Fucus Vesiculosus Powder	-						
34. Halidrys Siliquosa Extract	-						
35. Halopteris Scoparia Extract	-						
36. Himanthalia Elongata Extract	-						
37. Himanthalia Elongata Powder	-	X	X	X	X	X	X
38. Hizikia Fusiforme Extract	-						
39. Hizikia Fusiformis Water	-						
40. Hizikia Fusiformis Callus Culture Extract	-						
41. Hydrolyzed Ecklonia Cava Extract	-						
42. Hydrolyzed Fucus Vesiculosus Extract	84696-13-9						
43. Hydrolyzed Fucus Vesiculosus Protein	-						

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	IFRA	RIFM
44. Kappaphycus Alvarezii Extract	1220882-72-4 (generic)						
45. Laminaria Angustata Extract (Retired)	-						
46. Laminaria Cloustoni Extract	90046-11-0 92128-82-0						
47. Laminaria Diabolica Extract	-						
48. Laminaria Digitata Extract	90046-12-1 92128-82-0						
49. Laminaria Digitata Powder	-						
50. Laminaria Hyperborea Extract	90046-13-2 92128-82-0						
51. Laminaria Japonica Extract	92128-82-0						
52. Laminaria Japonica Powder	-						
53. Laminaria Longissima Extract	-						
54. Laminaria Ochotensis Extract (Retired)	-						
55. Laminaria Ochroleuca Extract	92128-82-0						
56. Laminaria Saccharina Extract	90046-14-3 92128-82-0						
57. Lessonia Nigrescens Extract	-						
58. Lessonia Nigrescens Powder	-						
59. Macrocystis Pyrifera (Kelp)	-						
60. Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract	-						
61. Macrocystis Pyrifera (Kelp) Extract	347174-92-9						
62. Macrocystis Pyrifera (Kelp) Juice	-						
63. Macrocystis Pyrifera (Kelp) Protein	-						

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	IFRA	RIFM
64. Nereocystis Luetkeana Extract	-						
65. Pelvetia Canaliculata Extract	223751-75-5						
66. Pelvetia Siliquosa Extract	-						
67. Phyllacantha Fibrosa Extract	-						
68. Rissoella Verruculosa Extract	-						
69. Saccharina Angustata Extract	-						
70. Saccharina Japonica Extract	-						
71. Saccharina Longicruris Extract	-						
72. Sargassum Filipendula Extract	-						
73. Sargassum Fulvellum Extract	-						
74. Sargassum Fusiforme Extract	-						
75. Sargassum Glaucescens Extract	-						
76. Sargassum Horneri Extract	-						
77. Sargassum Muticum Extract	-						
78. Sargassum Pallidum Extract	-						
79. Sargassum Siliquastrum Extract	-						
80. Sargassum Thunbergii Extract	-						
81. Sargassum Vulgare Extract	-						
82. Sahel Scenedesmus Extract	-						
83. Sphacelaria Scoparia Extract	-						
84. Undaria Peterseniana Extract	-						

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	IFRA	RIFM
85. Undaria Pinnatifida Extract	-						
86. Undaria Pinnatifida Cell Culture Extract	-						
87. Undaria Pinnatifida Leaf/Stem Extract	-						
88. Undaria Pinnatifida Powder	-						
89. Undaria Pinnatifida Root Powder	-						

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

SciFinder

INCI names and CAS No.

Ascophyllum Nodosum – 33 substance hits; 0 useful
Ascophyllum Nodosum Extract – 1 substance hits; 0 useful
Ascophyllum Nodosum Powder – 1 substance hit; 0 useful
Fucus Serratus Extract – 1 substance hit; 0 useful
Fucus Spiralis Extract – 1 substance hit; 0 useful
Hydrolyzed Fucus Vesiculosus Extract – 1 substance hit; 0 useful
Kappaphycus Alvarezii Extract – 1 substance hit; 0 useful
Laminaria Cloustoni Extract – 2 substance hits; 0 useful
Laminaria Digitata Extract – 2 substance hits; 0 useful
Laminaria Hyperborea Extract – 2 substance hits; 0 useful
Laminaria Japonica Extract – 1 substance hit; 0 useful
Laminaria Saccharina Extract – 2 substance hits; 0 useful
Laminaria Ochroleuca Extract – 1 substance hit; 0 useful
Macrocystis Pyrifera – 79 substance hits; 0 useful
Macrocystis Pyrifera (Kelp) Extract – 1 substance hit; 0 useful
Pelvetia Canaliculata Extract – 1 substance hit; 0 useful
Saccharina Angustata Extract – 1 substance hit; 0 useful

PubMed

((((((((((((((Agarum Cribrosum Extract) OR Alaria Esculenta Extract) OR Ascophyllum Nodosum) OR Ascophyllum Nodosum Extract) OR Ascophyllum Nodosum Powder) OR Asterionellopsis Glacialis Extract) OR Cystoseira Tamariscifolia Extract) OR Cladosiphon Novae-Caledoniae Extract) OR Cladosiphon Okamuranus Extract) OR Cystoseira Amentacea/Caespitosa/ Branchycarpa Extract) OR Cystoseira Baccata Extract) OR Cystoseira Balearica Extract) OR Cystoseira Caespitosa Extract) OR Cystoseira Compressa Extract) OR Cystoseira Compressa Powder) OR 84775-78-0 AND (tox[sb]) = 55 hits, 5 possibly useful.

((((((((((((((Cystoseira Tamariscifolia Extract) OR Dictyopteris Membranacea Extract) OR Dictyopteris Polypodioides Extract) OR Dictyota Coriacea Extract) OR Durvillea Antarctica Extract) OR Ecklonia Cava Extract) OR Ecklonia Cava Water) OR Ecklonia Kurome Extract) OR Ecklonia Kurome Powder) OR Ecklonia/Laminaria Extract) OR Ecklonia Maxima Extract) OR Ecklonia Maxima Powder) OR Ecklonia Radiata Extract) OR Eisenia Arborea Extract) OR Fucus Serratus Extract) OR **94167-02-9** AND (tox[sb]) = 41 hits, 4 possibly useful.

((((((((((((((Fucus Spiralis Extract) OR Fucus Vesiculosus) OR Fucus Vesiculosus Extract) OR Fucus Vesiculosus Powder) OR Halidrys Siliquosa Extract) OR Halopteris Scoparia Extract) OR Himanthalia Elongata Extract) OR Himanthalia Elongata Powder) OR Hizikia Fusiforme Extract) OR Hizikia Fusiformis Water) OR Hizikia Fusiformis Callus Culture Extract) OR Hydrolyzed Ecklonia Cava Extract) OR Hydrolyzed Fucus Vesiculosus Extract) OR 84696-13-9) OR Hydrolyzed Fucus Vesiculosus Protein) OR Kappaphycus Alvarezii Extract OR 1220882-73-4) AND (tox[sb]) = 231 hits, 4 possibly useful.

((((((((((((((Laminaria Angustata Extract) OR Laminaria Cloustoni Extract) OR 90046-11-0) OR 92128-82-0) OR Laminaria Diabolica Extract) OR Laminaria Digitata Extract) OR Laminaria Digitata Powder) OR 90046-12-1) OR 92128-82-0) OR Laminaria Hyperborea Extract) OR 90046-13-2) OR 92128-82-0) OR Laminaria Japonica Extract) OR 92128-82-0) OR Laminaria Japonica Powder) OR Laminaria Longissima Extract) OR Laminaria Ochotensis Extract) AND (tox[sb]) = 31 hits, 1 possibly useful.

((((((((((((((Laminaria Ochroleuca Extract) OR Laminaria Saccharina Extract) OR Lessonia Nigrescens Extract) OR Lessonia Nigrescens Powder) OR Macrocystis Pyrifera) OR kelp) OR Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract) OR Macrocystis Pyrifera (Kelp) Extract) OR Macrocystis Pyrifera (Kelp) Juice) OR Macrocystis Pyrifera (Kelp) Protein) OR Nereocystis Luetkeana Extract) OR **92128-82-0**) OR **90046-14-3**) OR **92128-82-0**) OR **347174-92-9**) OR **223751-75-5** AND (tox[sb]) = 1 hit, not useful

((((((((((((((Pelvetia Canaliculata Extract) OR **223751-75-5**) OR Pelvetia Siliquosa Extract) OR Phyllacantha Fibrosa Extract) OR Rissoella Verruculosa Extract) OR Saccharina Angustata Extract) OR Saccharina Japonica Extract) OR Saccharina Longicruris Extract) OR Sargassum Filipendula Extract) OR Sargassum Fulvellum Extract) OR Sargassum Fusiforme Extract) OR Sargassum Glaucescens Extract) OR Sargassum Horneri Extract) OR Sargassum Muticum Extract) OR Sargassum Pallidum Extract) OR Sargassum Siliquastrum Extract AND (tox[sb]) 40 hits, 5 possibly useful

((((((((((Sargassum Thunbergii Extract) OR Sargassum Vulgare Extract) OR Sahel Scenedesmus Extract) OR Sphacelaria Scoparia Extract) OR Undaria Peterseniana Extract) OR Undaria Pinnatifida Extract) OR Undaria Pinnatifida Cell Culture Extract) OR Undaria Pinnatifida Leaf/Stem Extract) OR Undaria Pinnatifida Powder) OR Undaria Pinnatifida Root Powder) AND (tox[sb]) = 21 hits, 3 possibly useful

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon https://java.epa.gov/oppt_chemical_search/
https://java.epa.gov/oppt_chemical_search/
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc

Botanical Websites, if applicable
Dr. Duke's https://phytochem.nal.usda.gov/phytochem/search
Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
Sigma Aldrich plant profiler http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html

Fragrance Websites, if applicable
IFRA (International Fragrance Association) – http://www.ifraorg.org/
the Research Institute for Fragrance Materials (RIFM) should be contacted

Brown Algae Minutes

September 2018 Meeting

Day 1 – Group 1

DR. MARKS: This is a first review of these 83 ingredients. They're complex if I interpreted Dr. Lowe's presentation, which was excellent from two years ago, I guess. On page 156, they are a functional group of plants and protozoa and unique organisms. They don't fit real nicely into one little bundle.

I think we're going to have to clarify whether all 83 in this report are seaweed kelp brown algae, or whether there's a unique brown algae in this, or there's protozoa. As with all of these botanicals -- and this even more, I think, complex chemistry and composition -- they vary in their composition levels depending on the species.

We have 83 here, no small number. How they were grown, where they're harvested, what sea they were grown in, how they were manufactured. They've been contaminated with heavy metals, specifically arsenic. We eat them, so at least some of them are grass.

And so that leads to my first question. Tom and Rons, do you like all 83? Are there ones we should eliminate, and if we do I'd like to know why. But presumably, the only reason you eliminate it is, it isn't a brown algae. I assume these are all brown algae.

DR. HELDRETH: Yes. Our understanding from the presentation that Dr. Lowe gave was we arranged those that he determined brown algae. Then we also sent a message to the INCI nomenclature committee, who has a biologist who's an expert in the taxonomy of these types of things to give us an analysis. And to our best understanding, all of the ingredients in this report are along the lines of a kelp or seaweed-type of brown algae.

DR. MARKS: I think that's really important to capture. And I will, in the introduction, indicate -- or somewhere that these are all brown algae, and they're all seaweed, basically. Okay. Tom, Ron and Ron, go back to the question, is there any reason to eliminate any one of these?

DR. HILL: I have a question. Do we have any sort of a phylogenetic tree that fits these algae? I mean, because otherwise we're looking at -- is there a group of deciduous trees, or something, which may be closely related or not very closely related. I'm not sure how much read across one can do from one species to the next, perhaps none. But if I know that two are closely related, that's a start.

DR. HELDRETH: It was Dr. Lowe's assertion that these were related.

DR. HILL: Well, I know they're all brown algae; but again, I mean, that's like saying all

flowering plants. That's about as close as that gets me, I believe.

DR. HELDRETH: From Dr. Lowe's explanation, it wasn't really just based on whether or not they were the color brown.

DR. HILL: Yeah. I know that.

DR. HELDRETH: It was a classification of a specific kelp-type of algae that excluded things like, you know, little bacteria or other things that get classified in the very vague name algae. And he suggested not only based on the similarities within the brown group, but how they're often used in things like food and stuff that these ingredients were similar enough to be group.

DR. HILL: Okay. But I still wonder if it's possible to get a phylogenetic tree.

DR. EISENMANN: She did. If you look at Table 4.

DR. HILL: Okay. That's effective, what she's got?

DR. EISENMANN: It does break it down into subclass or family. There's actually three -- or four. And this is from a website, algae database. You can tell that some are more related than others.

DR. HILL: Right. I'm a visual learner guy. Like, you know, when you get a phylogenetic tree it's very pictorially useful. But, I mean, that's a lot to ask. If there's not one commercially available, which there probably isn't, but maybe there is, that would be helpful.

DR. MARKS: I guess when -- let's see, it was Dr. Row, correct?

DR. SHANK: Lowe.

DR. MARKS: Lowe. His presentation, he says we employ four main criteria, pigmentation, obviously, that's the brown. Storage products. I assume we're going to get that from composition. And we're going to read across -- hopefully we'll get multiple compositions so that we can read across. On flagella, I don't think we're worried about that in this case.

I'm looking on page 165 is where he talks about algal divisions, Ron Hill. And then if you go right before that on page 163, I liked how that figure was labeled. Hypothetical. That doesn't help much, does it? And they have those nice little arrows going into different divisions and whatever.

DR. HILL: I have a colleague that works on algae symbionts in the context of natural products. And basically, he sends them off now to get genetic profiling at Aberdeen so that we have a better idea than just that. I do remember this slide because it's very colorful.

DR. MARKS: I think we've settled on all the ingredients are okay, unless we hear differently.

DR. SHANK: Okay to be, inserted better.

DR. MARKS: Included. I'm sorry. Included in this report.

DR. SLAGA: Yes.

DR. MARKS: Yes. Exactly. I'm sorry. I didn't get into what are the needs yet. Yeah, I have some needs too also. Okay. They are glass, but how many of these specific species are grass? Or is that just kelp that's grass?

DR. SLAGA: A lot of them are kelp.

DR. MARKS: Yeah. But if you use the word kelp, is that inclusive of all? I don't know. Ron Shank, Ron is now getting down to the meat of this.

DR. SHANK: Well, the grass ones are Laminaria and Undaria. And from the page where all these ingredients are numbered, the Laminaria are number 44 to 55. And the Undaria are numbers 81 to 86. And these are grass food additives. I'd say all we need is skin sensitization. There's also grass for Hizikia, numbers 37 to 39, but they're not currently used. If they were used, we'd have to have skin sensitization data.

Then in Wave 2 we did get some skin sensitization on some, but not many. For all the rest, I would say we need a 28 dermal toxicity study. And a skin sensitization study for all the extracts, at least. Assuming that the extracts contain the components in the other preparations, which is a huge assumption I think. I don't know if that helps, but that's where I come from.

DR. MARKS: I think it's a really good start, because it gives us some framework. You're really focusing initially on the grass --

DR. SHANK: The grass ingredients.

DR. MARKS: -- grass brown algae. And that -- again, I'll summarize this in a minute, make sure I have the right numbers. But I like that. I can tell you with the skin sensitization, you were mentioning that, Ron. I took the ones with the highest uses. Like the fucus vesiculosus extract, 6 percent.

Wave 3 had a mixture and an HRIPT, but I didn't see that the percentage of that brown algae in the mixture was mentioned. Was it? Did I overlook that? Because then I couldn't -- if I knew it was 6 percent, I'd say fine, that looks okay. The same you'd talked about the Laminaria that all we really need is the skin sensitization. In Wave 3 we got another HRIPT. That's of the extract, but it didn't tell me what the percentage was.

Is that correct?

MS. CHERIAN: That's the percentage that wasn't mentioned in there.

DR. MARKS: No. Neither one of them. I would say I'd want --

DR. EISENMANN: All the ones from BiotechMarine did give concentration in the extract. They gave it kind of as a range, the dry extract is, and they tested it neat. Most of that material was either in glycol water or caprylic/capric triglyceride. There was a few propylene glycol extract.

Their information did give -- I mean, occasionally I had to go back and ask them for it, and that's written on each thing by hand. Then there was another table from a different company, and they were reluctant to give concentration. They just said it was in the range of .5 to 10 percent for all of them on that one table, which was less than desirable.

But from all the information from BiotechMarine, they did -- and it's not in all of the summaries that are for Wave 2, but it's all --

DR. MARKS: We'll need to go back and get that.

DR. EISENMANN: Right.

DR. MARKS: Because if that's the case, and if it's up to 10 percent -- the one was 6 percent for the fucus extract. The Laminaria digitata extract, 5 percent. If they were tested up to 10 percent, then that becomes a nonissue. Because the HRIPT's were normal.

DR. EISENMANN: I don't know if they ever have that high of concentration of an extract, that company don't. I'd have to look back. But they all say how much -- they don't give exact, but they give a reasonable sized range. Not like the other summary that came in, that is for all of the extract was .5 to 10 percent. Not helpful.

DR. MARKS: We still need to be sure of what the percentage of the extract was in these HRIPTs. We can't say it's safe if I don't know the percentage. It gets back to, Ron, your question. The other was Laminaria again and other grass group. Digitata powder, that was up to 40 percent in a leave-on. We need to have sensitization on that.

And then I was picking just the ones with a high either use or concentration. Macrocystis pyrifera kelp extract is used up to 36 percent, so I want to see sensitization on that. Yeah, I had an IDA. I figured we'd get to an insufficient data announcement. Now the question is which ones.

That's the initial sensitization but, Ron, I'm going to go back on what you said because I gave specific species. But you were more general in terms of groups that we needed, which is good.

DR. SHANK: To start off, yes.

DR. MARKS: And then you had the 28-day tox also on the others. Then really, what is it, Undaria, is that what it is?

DR. SHANK: Undaria.

DR. MARKS: Undaria species. Again, you felt just the sensitization data on them. And I would think if we used the same reasoning we've done in the past, we really wanted -- most of these are extracts anyway. But if we get the extract, presumably it would be a concentrated form of the contents or ingredients. Ron Hill, yeah?

DR. HILL: Well, just with the caveat that it may depend on what -- the trouble is when you get a percentage added to a formulation and it's so much percent of the extract, is that a .1 percent extract of what you're adding at 5 percent?

DR. MARKS: That was the problem I had with these. I couldn't decide, on Wave 3, how much of the actual brown algae percentage was in that testing, because it was X percentage of a mixture.

DR. HILL: And so, lacking that information, I don't know how you --

DR. MARKS: That's one of the data we'll request. Okay. I like the way Ron divided things up, Ron Shank. Shall we start with that in terms of that's the way we would start with this large group of ingredients? And then we'll see how the approach from the other team is. And then as time goes on, we'll even be more focused.

DR. BERGFELD: Can I ask Ron a question? Ron Shank? When you have grass ingredients and people ingest all these, at what concentrations, are they 100 percent?

DR. SHANK: Usually they don't -- you know, grass usually don't give a concentration.

DR. BERGFELD: Is it the whole though? Whatever it is, the whole algae, they're just eating that?

DR. SHANK: Oh no, that's defined. But how much is used in individual products usually isn't stated as far as I remember.

DR. KATZ: They usually don't state it; although there may be some exceptions, but they usually don't. And I think it's important, as I mentioned before, when you're talking about grass, please make sure that you say grass as related to food additives, so that it's clear that it's not grass as related to a cosmetic ingredient.

DR. BERGFELD: Do you think it would be worthwhile exploring what the grass food additives have actually done? And in any way they might have talked about mixtures or full, just consumption of the actual algae. I mean, with all these vegetarians and funny eaters, I mean, they may be ingesting 100 percent of a product, of food stuff.

DR. SHANK: Very good question. In the literature search, were there FDA files that listed the grass ingredients and what data were supplied to show it was grass?

MS. CHERIAN: I'm not sure. I didn't do that part, but I can go back and check.

DR. SHANK: Okay. Because my experience with it is a lot of it is just a number of scientists, and researchers, responded to FDA and said, this has been used widespread for a long time and it's generally recognized as safe. But there isn't a huge database to confirm the safety. That's my recollection.

DR. BERGFELD: Jim, one of the audience wants to.

DR. MARKS: Oh, I'm sorry. Thank you. Come on right up to one of the microphones so we capture it. Thank you. Thanks, Wilma. I was trying to capture Ron's divisions.

DR. ZIMMERMAN: Merle Zimmerman, American Herbal Products Association. A bunch of these brown algae that are identified are in wide used as food ingredients. I know I eat at least two of the species in this list with my lunch at the sushi bar on Monday. That might also be a relevant piece of information for purposes of exposure and safety.

DR. SHANK: Yes.

DR. ZIMMERMAN: I can do some searches. If you'd like me to bring some stuff back, let me know.

DR. BERGFELD: That really would be great. Because as I'm listening to all of this, and the need for sensitization, if you could establish sort of the amount that's ingested in historical review, we might be able to come up with not such a great need for sensitization. Because we know about nickel. If you're sensitized to nickel, if you eat it, you break out, if nickel is incorporated in any of the food stuff.

DR. MARKS: I would still want to see their local lymph node assay, just to get an idea of is it a sensitizer or not. Then either getting pig max or more importantly an HRIPT. I wouldn't assume just because we eat it and we don't break out in a rash, that if we put it on topically, we would be okay. I'd like to see the skin sensitivity. As far as the 28-day tox, if you can tell Dr. Shank what you're eating of those other ones today, and if

you come back tomorrow, we you know it's probably grass. That's, of course, a joke. Ron, thank you for laughing.

Let me see if I have this right, Ron. I want to be sure. And if not, either I'll -- I was thinking about asking you to do your division, but I figured that would be it.

I'm going to second a motion tomorrow. I suspect it's going to be an insufficient data announcement. And with our discussions we took the Ron Shank approach. If you want, I can leave that out. That the grass ingredients, and they were number 37 to 29, that's the Hizikia species, the 44 to 55, the Laminaria species, and the 81 to 86, the Undaria species, we need sensitization data. For the rest of the ingredients we need 28-day tox and sensitization.

DR. SHANK: Those numbers that I used come from the table that begins on page 12 and list all 86. And each one is numbered.

DR. MARKS: Is this one that is from the -- let me see here. The table I'm using is this one here that gives you what tests have been done.

DR. SHANK: Are they numbered?

DR. MARKS: And it's numbered 1 through --

DR. SHANK: Eighty-six.

DR. MARKS: Is it 86? I said 83, I thought.

DR. EISENMANN: There's a few that have been taken off of that table because they weren't actually brown.

DR. MARKS: Okay, that's why.

DR. EISENMANN: Because I think the actual number is 82.

DR. MARKS: Oh, now it's 82.

DR. SHANK: Okay.

DR. EISENMANN: I keep trying to find the 83rd[d] ingredient and I haven't found it. If you find me an 83rd ingredient, I'll put it in.

DR. MARKS: Can we have an auctioneer here as far as how many ingredients?

DR. KATZ: Do you know which three or four should be removed?

DR. MARKS: Well, that can be clarified in the next rendition, I think.

DR. SHANK: The table on 12 goes to 89, one through 89.

DR. HILL: There's two tables and they both go to 89.

DR. MARKS: Oh yeah, there's Wave 3 again. Do you have the Wave 3 table where -- in multicolor?

DR. SHANK: No. This is in the original document.

DR. MARKS: Okay. I think I had that one here. Does that corresponds? It's the Hizikia, 37 to 39. There's Hizikia extract, water and callus culture extract. Are those the three that -- I think I heard you right, 37 to 39, Ron?

DR. SHANK: Yes. That's what I said. Actually, it looks like -- well, unfortunately it's which table you use.

DR. MARKS: Okay.

DR. SHANK: It's the Hizikias. And in the very first table we got, that would be 38 to 40. But in the other table it's 37 to 39.

DR. MARKS: Well, maybe what I should do is just put -- rather than the numbers, put --

DR. SHANK: The actual names.

DR. MARKS: Yeah. I have the names in parentheses.

DR. SHANK: Okay.

DR. MARKS: I thought this was the original one. This isn't? Again, I think we got three different tables. Because the last one was the one that had the multicolor original submission, Wave 2 and Wave 3, in red and blue. Maybe I'll use that one. Let me see what number Hizikia is there. Thirty-seven, 38, 39. It's again 37 to 39. Okay. And then the next ones are the Laminaria group. And I'll say approximately 44 through 55.

DR. SHANK: Yes.

DR. MARKS: Okay. And then the last group of the grass kelp is 81 to 86, the Undaria species. And I assume these are all species, right? I'm correct in saying species?

DR. EISENMANN: Mm-hmm.

DR. MARKS: Okay. And we need the sensitization and actually, specifically, I mentioned some other ones where we need -- because of either the frequent use or the high concentration -- individual I put down there. And then for the rest of the ingredients, we need a 28-day tox since they're not grass. And then we also need sensitization for them.

That makes it actually pretty straight forward at this point. We'll see how complex the Belsito team makes it. But does that sound good? This is actually going a little more -- thank you, Ron, for suggesting that way of approaching it.

DR. SHANK: Okay.

DR. MARKS: Any other comments? Tom?

DR. SLAGA: No.

DR. MARKS: Ron Hill? We're obviously going to see it again, particularly if it goes out as an insufficient data announcement, which is hard for me to imagine it won't. Priya, any questions?

MS. CHERIAN: No.

DR. MARKS: Any others from industry? Okay.

DR. SLAGA: It's a very nice summary.

DR. MARKS: Oh, yeah.

DR. SLAGA: That helped a lot.

DR. MARKS: Okay. Thanks, Priya. Let's go ahead and with that we will move on to hydrogen peroxide, one of our favorite disinfectants.

Day 1 – Group 2

DR. BELSITO: Oh my God.

DR. LIEBLER: Kelp.

DR. BELSITO: Wave 2. Now here -- we're getting Wave 4.

DR. LIEBLER: Wave 4 is just the greatest hits of Waves 2 and 3.

MS. CHERIAN: It's just a summarization of the sensitization and dermal to make it easier.

DR. BELSITO: Okay. Well, we definitely need to limit arsenic. We need limits on heavy metals. What about these extractions? Methanol, hexane, chloroform?

DR. LIEBLER: You know, so I thought we actually had a lot of information about the different prep methods, and they seem to me to fall into a couple of categories. Maybe two or three categories to get these ground-up powders, to get these alcohol extractions or these aqueous extracts.

And I wonder if it might not be possible to prepare a kind of a map diagram that just shows the

major ways in which brown algae is converted to cosmetic products. Maybe not so much with a high level of detail in the map, but under method of manufacture it could be right there. I would imagine maybe sort of an inverted pitchfork trident thing, you know, with three pathways. Because then you'd have a table with lots of information for the individual ingredients.

MS. CHERIAN: Okay.

DR. BELSITO: Okay. Now, we know a lot about the impurities, we know a lot about the method of manufacture, we know zilch about composition.

DR. LIEBLER: Yeah. I had a more specific question about composition, which was do we -- because of Wave 2, we now have data on the actual cosmetic ingredients, not just on some representative algae from the literature.

DR. BELSITO: Right.

DR. LIEBLER: So, that's good. And I had a question about constituents of concern with respect to sensitization for example. And we don't have data on those for any representative, at least -- I might have missed it in the blizzard of Wave 2 or Wave 3.

DR. BELSITO: Well first of all, the two biggies are Laminaria digitata and macrocystis. Those are the ones that are most frequently used, right?

MS. CHERIAN: Yes.

DR. BELSITO: And we have an HRIPT on 46 humans for laminaria, but we have no data for macrocystis.

MS. CHERIAN: We have some data for that ingredient --

DR. BELSITO: We have no sensitization data.

MS. CHERIAN: -- either in Wave 2 or 3.

DR. BELSITO: I didn't see it.

MS. CHERIAN: Okay. Let's see.

DR. BELSITO: And all of the times that these were irritant, it was always with propylene glycol. And I thought propylene glycol was the irritant there. I was okay with the irritation, but we have no sensitization data for macrocystis. And we have just an HRIPT on 46 individuals for laminaria.

And we also have no tox data for either one of them. And at most, we have 28-day tox data. And

that raises the whole issue of iodine concentration and thyroid effects.

DR. LIEBLER: You're talking about macrocystis?

DR. BELSITO: Yeah. Now the thyroid issues with ingestion of these kelps were extremely high amounts, but we don't have absorption data. And then we don't really have good genotox data. And then we have some endocrine effects. We don't have photo, we don't have composition, we don't have 28-day dermal absorption. We don't have sensitization on macrocystis, we don't have photo. The genotox, there's some report of endocrine affects.

DR. LIEBLER: Yeah. I'm trying to get some idea of how widespread food consumption is with the ones that we're using. Macrocystis, laminaria digitata, laminaria saccharina approved as food additive or direct food addition, food for human consumption as a source of iodine or as a dietary supplement. I don't know to what extent that factors into our need for dermal tox or additional tox data.

My hunch with these is that we may be treating these more the way we treat other kinds of botanicals, where our major concerns is going to be sensitization and constituents of concern. Maybe that's not accurate, but that how I first approached these.

DR. KLAASSEN: Well, they are considered food additives, especially for animals to quite a high extent, without apparent toxicity, which gives me some support.

DR. LIEBLER: In the acute oral toxicity study, it's Table 21, PDF Page 55, we have a relatively small selection of brown algae compounds that have been tested -- or brown algae that have been tested. For our report, the fucus vesiculosus, there are three different studies in Swiss mice.

But if you look at all the brown algae that have been tested there's, let's see one, two, three, four, five, six, seven, eight studies, all of which have oral LD50s in the thousands. These are sort of the profile of nontoxic substances.

As far as dermal absorption, you know, it's basically a botanical. So, it's got sort of a wide variety of chemical substances, many of which are not absorbed at all.

DR. BELSITO: But we don't even know what they are.

DR. LIEBLER: That's a concern I have is the chemical composition of these. But I would say, particularly with respect to constituents of concern relative to sensitization. And of course, I didn't realize that these tended to accumulate arsenic so much.

DR. BELSITO: Right.

DR. LIEBLER: I found that interesting and surprising. Think of all the kelp in the world. This could actually be a major reservoir of arsenic other than the earth's crust.

DR. KLAASSEN: I think that arsenic form is not so toxic. It says in here some place that they're arsenic sugars. And I know at least fish, also, concentrate arsenic and puts it in a form that's not toxic like the inorganic form is. But I'm not entirely positive about this. But yeah, that's kind of interesting.

DR. LIEBLER: Paul have comments?

DR. BELSITO: Brown algae. "Extracts to 36 percent. Powders to 40. Juices no concentration. Water no concentration. Many uses with no concentration data provided. Plant-like, seaweed, protozoa, unique kingdoms -- very diverse group, too diverse?? Impurities; phytosterols, alginic acid, heavy metals, especially, arsenic, and phthalates. No data on composition. Tox data limited, but no level of toxicity. This one is touch with such a diverse number of sources and ingredients; don't know where to begin other than composition and impurity data base on some sort of plausible grouping." And that was my problem. We're just sort of assuming these all have the same composition.

DR. LIEBLER: Well, yeah. I mean, I suppose implicitly we're assuming that they have similar enough composition to be grouped together. If we did play the mental exercise of deciding to break these up, how would we break them up?

DR. BELSITO: I don't know.

DR. LIEBLER: With what would seem to be anything other than arbitrary.

DR. BELSITO: But wouldn't it be nice if we had composition on a couple different -- like at least the two that are primarily used for laminaria and the macrocystis?

DR. LIEBLER: Right. No, I agree. That's one of the notes I had, is that we need data on composition for the representative of the major groups. Particularly, I thought constituents of concern. Maybe you're not as concerned about sensitization with these, Don?

DR. BELSITO: I don't know. I mean, that's was one of my needs. I raised to you was an HRIPT of 46 sufficient for the laminaria, but we have nothing on macrocystis, which is the other one that has a high concentration of use.

DR. LIEBLER: I think we definitely need that. And I think of these as botanical. And with

botanicals, we almost always are looking for constituents of concern. Flavonoids, terpenoids, things like that. And at least if we have representative data for the different classes, along with safety data on sensitization, then we can draw a conclusion.

We don't have genotox on major -- we have genotox on a couple of fucus vesiculosus?

DR. BELSITO: Mm-hmm.

DR. LIEBLER: But we don't have it on any of the laminaria, do we?

DR. BELSITO: Nope.

DR. LIEBLER: Or the macrocystis?

DR. BELSITO: Nope.

DR. LIEBLER: I think we need that.

DR. HELDRETH: Is there one for laminaria saccharina extract? At least according to Priya's table, it looks like there's genotox for Number 55.

DR. LIEBLER: I might have buzzed by it.

DR. BELSITO: The genotox is not on laminaria though.

DR. LIEBLER: We have laminaria digitata, prep method concentration not specified, AMES assay with and without metabolic activation. There's a reference, I didn't look at it. Is that what you're referring to, Bart?

DR. HELDRETH: In Priya's cheat sheet table here, number 55 in the table says laminaria saccharina extract.

DR. LIEBLER: Oh, sorry.

MS. CHERIAN: Oh, it's in Wave 2.

DR. HELDRETH: So, data came in Wave 2.

DR. LIEBLER: I think the other problem in reviewing this report is the data are spread out over so many reports, that I just was missing stuff.

MS. CHERIAN: And I think fucus vesiculosus was the highest number of uses and concentration. But the concentration might have gone down.

DR. LIEBLER: Okay, so the cheat sheet's only for the skin endpoints, right?

DR. KLAASSEN: Right.

DR. BELSITO: Mm-hmm.

DR. LIEBLER: Yup.

DR. HELDRETH: No. It has repro, geno.

DR. KLAASSEN: Oh, he's talking about the one she handed out 30 minutes ago.

MS. CHERIAN: That's the data profile.

DR. HELDRETH: Yeah, the data profile.

MS. CHERIAN: Yeah. So, it's not on there. The genotox data is not on there, it's in Wave 2. That's only skin sensitization and irritation.

DR. LIEBLER: Alright. I think -- it's hard to tell what we have at this point.

MS. CHERIAN: Yes. Yeah.

DR. BELSITO: But the genotox data is on laminaria saccharina and not digitata?

DR. HELDRETH: True.

DR. LIEBLER: And where are you getting that, Don?

DR. BELSITO: Wave 2.

DR. HELDRETH: So, on Page 6 of Wave 2, it says for laminaria saccharina extract, the genotox says, tradename mixture containing this ingredient in seawater and methylpropanediol AMES test, salmonella strains. It lists five of those with and without metabolic activation in dose 50 to 5000 micrograms per plate, non-mutagenic.

DR. LIEBLER: Okay. But I think we need to have representative genotox for the major classes. And it looks like we've got it for laminaria.

DR. BELSITO: But does that take care of laminaria digitata?

DR. LIEBLER: In addition to the Wave 2, there is what was in the report, Table 23, which said laminaria digitata -- this is PDF 60 in the original report. And it's an AMES assay with and without metabolic activation. But it doesn't specify concentrations.

DR. BELSITO: Right.

DR. LIEBLER: It's probably not a great study. So, it's thin and nonexistent for macrocystis.

DR. BELSITO: Right.

DR. LIEBLER: But we've got two fucus vesiculosus in the report, Table 23, with

concentrations. One is a common assay, which isn't the best; it's not very sensitive. And the other is the chromosome aberration OECD GL 487. So, we really need more on fucus vesiculosus unless that's in Wave 2.

MS. CHERIAN: There's no genotox.

DR. LIEBLER: None?

MS. CHERIAN: For that ingredient, no.

DR. LIEBLER: Okay.

DR. BELSITO: Macrocystis.

DR. LIEBLER: Fucus I was talking about. And then macrocystis. So, we're lacking genotox for both of those. We don't have any AMES for fucus.

MS. CHERIAN: No.

DR. LIEBLER: I mean, relatively to the number of ingredients is really spotty.

DR. BELSITO: Okay. So insufficient, is that fair to start with?

DR. LIEBLER: Yes. Right.

DR. BELSITO: Okay. And do we have enough on the residual impurities? Or do we just simply say restrict arsenic, heavy metals and extraction solvents?

DR. LIEBLER: I think actually we've got a lot of data on the residual metal impurities, or arsenic and metals. And we obviously should treat that in a discussion and say restrict. I'm more concerned about the lack of data on the organic constituents of concern.

DR. BELSITO: What do you mean, the extractants?

DR. LIEBLER: No.

DR. BELSITO: The solvents?

DR. LIEBLER: Like terpenoids and flavonoids. Not the impurities, the constituents of concern that could contribute to sensitization.

DR. BELSITO: Okay.

DR. LIEBLER: All the data we have so far are non-sensitizing?

MS. CHERIAN: Yes.

DR. KLAASSEN: It looks pretty clean.

DR. BELSITO: We don't have a lot of sensitization data.

DR. LIEBLER: I mean, how comfortable are you with the sensitization?

DR. BELSITO: I don't know what's in them.

DR. LIEBLER: Well, okay. If you were concerned about sensitization with these, then that increases the need for data on the constituents of concern that are associated with sensitization.

DR. BELSITO: Right.

DR. LIEBLER: If you had a very thorough list of studies that were to show non-sensitizing in humans, at use concentrations, then I wouldn't be so concerned about having data on terpenoids and flavonoids and so forth.

DR. BELSITO: So, we need composition on laminaria and macrocystis?

DR. LIEBLER: Yes.

DR. BELSITO: We need a 28-day dermal? Or are you happy with a grass status?

DR. LIEBLER: I think the grass status helps. We've got Table 22, Oral repeated dose. We hardly have any studies in which there's evidence of toxicity, either in acute or repeat dose.

DR. BELSITO: (Inaudible) dose with the extract for iodine.

DR. LIEBLER: Yeah.

DR. BELSITO: Thyroid affects.

DR. LIEBLER: Right. I mean, because it's such a big group, we don't have a comprehensive data set for toxicity with all of them. But for what we do, it's a pretty consistent message; these aren't really toxic.

DR. BELSITO: So, you don't need a 28-day dermal?

DR. LIEBLER: I don't think we need the 28-day dermal. If you take that information, plus the widespread use of these as dietary supplements or food additives.

DR. BELSITO: Okay, so we're not worried about dermal absorption because we have all of this grass status, dietary supplement, et cetera.

DR. LIEBLER: Right.

DR. BELSITO: Okay. And then we need composition on laminaria, macrocystis, sensitization and irritation and concentration of use for macrocystis. And we're okay with the 46 for laminaria?

DR. LIEBLER: If you're okay with it, I'm okay with it.

DR. BELSITO: Well, I guess we'll see what the composition looks like. Photo absorption?

DR. LIEBLER: Photo absorption?

DR. BELSITO: Yeah.

DR. LIEBLER: Oh, I'm sure they all absorb. I mean, they're complexed, you know, botanicals. They all absorb.

DR. BELSITO: So, then we need photosensitization/photo-irritation?

DR. LIEBLER: I don't think that necessarily follows. Do we have any photosensitization on any of them?

DR. BELSITO: Nope.

DR. LIEBLER: I mean, complexed organic mixtures all absorb, but not all of the absorbing materials -- I mean, most of the absorbing materials are not photo allergens or photosensitizers.

DR. BELSITO: Right. But some of them are.

DR. LIEBLER: I mean, with pure compounds, absorption tells you something.

DR. BELSITO: Right.

DR. LIEBLER: With mixtures, absorption doesn't tell you anything. So, the kind of logic use in RIFM where if it has absorption above or below the benchmark, clears it, that doesn't apply in mixtures like this.

DR. BELSITO: Right. So how do we deal with that?

DR. LIEBLER: If we had --

DR. BELSITO: Composition.

DR. LIEBLER: -- composition. Again, constituents of concern, including known photosensitizers. Flavonoid, terpenoid sensitizers. That's why I kept coming back to that point. If those are low, or minimal, or at least documented and the measured amounts are present in ingredients that have been tested, at least for sensitization, then I think we're okay.

For photo, that's really hard to predict for mixtures. For pure compounds, sure. But for mixtures, it's really hard to predict. And then I don't know that we're going to get very far by saying we want photosensitization on everything. I mean, we can ask for photosensitization on representative ingredients from the major groups.

DR. BELSITO: So, photosensitization, phototoxicity for laminaria and macrocystis, or concentration of use?

DR. LIEBLER: Yeah. And if we don't get that and they respond with data on constituents, particularly organic constituents that might be associated with photosensitization, then we can take that into consideration.

DR. BELSITO: What about genotox?

DR. LIEBLER: Based on what I've seen so far, I think the data are thin. We'd like more genotox data. Particularly for --

DR. BELSITO: For laminaria.

DR. LIEBLER: On the laminaria.

DR. BELSITO: On macrocystis.

DR. LIEBLER: Macrocystis, right. Yeah.

DR. BELSITO: Anything else? Developmental repro? No?

DR. LIEBLER: I really doubt it. I mean, I don't think we're going to need it.

DR. BELSITO: Are we clear on the genotox, on the idea that they're used as foods?

DR. LIEBLER: What do we have on carcinogenesis?

DR. BELSITO: Nothing.

DR. LIEBLER: Nothing.

DR. KLAASSEN: Well, you know, this is our first time around. I think we should ask for genotoxicity.

DR. LIEBLER: Yeah.

DR. BELSITO: Okay. For again, laminaria and macrocystis?

DR. KLAASSEN: Right.

DR. LIEBLER: I agree with you, Curt.

DR. KLAASSEN: And regarding phototoxicity, that's -- you know, these chlorophyll-type compounds and chlorophyll degradation products are photosensitizers. So therefore, to request those there is some reason.

DR. LIEBLER: I think we agreed on that. I think we agreed we're going to ask for that.

DR. KLAASSEN: But all I'm saying is it's not just grabbing out of nothing. There's a kind of a reason for it.

DR. BELSITO: The list I have so far is we would like some information on the composition of laminaria and macrocystis. Sensitization and irritation and concentration of use for macrocystis. Phototoxicity, photosensitization at concentration of use for macrocystis and laminaria. And some genotox on laminaria and macrocystis. That it?

DR. LIEBLER: Yes.

DR. BELSITO: Anything else?

DR. KLAASSEN: That should be good enough.

DR. BELSITO: Any other comments on brown algae? Okay.

DR. LIEBLER: I think this will be easier to deal with next time when we can have it all in one document.

DR. KLAASSEN: Yeah.

DR. BELSITO: Oh, well, then we still get Wave 7 and 8. Okay.

Day 2

DR. BELSITO: Well, this is huge and I'm not going to read all of them, but the two major ones laminaria digitate and macrocystis. And we thought we could use those as our sort of, for lack of a better word, read across to brown algae.

We thought that there was a lot of data about impurities, but we don't know what these are made of. We don't know composition. So, we're asking for the composition on laminaria and macrocystis to see how similar different types of brown algae were.

We do have sensitization and irritation on the laminaria, but not the macrocystis, and we're asking for that. And we're asking for genotoxicity on the laminaria and the macrocystis extract, as insufficient data.

DR. BERGFELD: So, that's a motion?

DR. BELSITO: That's a motion.

DR. BERGFELD: Dr. Marks?

DR. MARKS: We second the insufficient data announcement. We, or I might say Ron Shank, had a different approach which was appealing. Ron divided these -- what is it 83 ingredients -- into two groups, the grass group and the non-grass group. And the grass group was, depending on which list -- I think we got three

different tables -- but the hizikia species, 37 to 39, at least, in the table I use, and the laminaria species 44 to 55 numbered, and then the undaria species, 81 to 86. We wanted the sensitization data on those. For the rest of the ingredients, which were not grass ingredients, we wanted a 28-day tox and sensitization. We like, obviously, the composition. I didn't feel as comfortable with the sensitization data on several of the ingredients you mentioned.

In Wave 3 we did get HRIPT, which was good for the fucus vesiculosus, and the laminaria digitata extract at 5 percent. But, in both of those, I wasn't able to determine what the concentrations of those ingredients were in that mixture. They just said the mixture was tested and the HRIPT was okay; but I didn't know what percentage of that mixture was the actual algae ingredient.

For the laminaria digitata powder, that's being used at 40 percent, and I saw no evidence as far as sensitization confirming its safety. And the macrocystis extract, that's used at 36.4 percent and there was no data on sensitization.

So, I think we can roll together what both teams need. Our team found it appealing the way Ron Shank approached it. So, a lot of the toxicity data, such as the 28-day tox wouldn't be necessary for the grass ingredients of this large group.

DR. BERGFELD: Do you want to comment, Dan?

DR. LIEBLER: It actually disturbs me to realize I had the same idea as Ron Shank. But I'm curious as to whether or not we can actually get a good inventory of the ingredients, in our report, that are associated with grass for food enhancers and flavor adjuvants and so forth. So, can we get a good listing of that, do we know?

DR. MARKS: Well, that could certainly be in the insufficient data announcement.

DR. SHANK: So, we know which are which. Because I like the idea -- I mean, I recognize as well, all kidding aside, that many of these are widely consumed. And this could be very similar to some of our other botanical ingredients where, like apple or orange or something, that, you know, they're widely consumed and we mostly focused on the skin endpoints.

So, I agree with that. And I just want to make sure that we can -- I'd like to know to the extent to which we could get a good inventory, what is grass? What could be considered that way?

DR. BERGFELD: Linda, could you respond at all?

MS. KATZ: No, not really, because I don't do grass, it's not within my jurisdiction. I'm

presuming that you can always make a FOIA request and the FDA can provide that information; but it's not something from my group. One of the other groups, the Office of Food Additive Safety is the one who handles that determination.

DR. BERGFELD: Thank you. Any other comments?

DR. MARKS: I think, as again as this moves forwards we need to really, in the introduction and discussion, really emphasis how complex this is. And the algae that their definition, at least by Dr. Lowe's presentation, is that they're functional groups and that they're mixtures. I asked yesterday whether these are all seaweeds, and it appears they are all seaweeds. They aren't protozoa, or they aren't some unique kingdom. So, I think that's important.

And then, obviously, we not only have the complexity of botanicals in terms of their chemistry composition -- which we're going to ask for the composition. But these composition levels varied depending on species, varied on growing, harvesting, method of manufacture. And then, these particularly is concerned about contamination by heavy metals and arsenic, and that all needs to be captured in the discussion.

DR. BERGFELD: I want to make one comment since there's so much data here; that I personally, as a Chair, would like the data profile updated with all the Wave information that came through.

DR. MARKS: Oh sure, we'll see that in the next rendition.

DR. BERGFELD: Okay.

DR. SLAGA: That'll be very helpful.

DR. BELSITO: Priya did a very good job of putting all of those sensitization and irritation data together for us, so good work.

MS. CHERIAN: Thank you.

DR. MARKS: Yes, and method of manufacture and impurities. Each Wave came with another two pages of tables, or three or four.

DR. LIEBLER: I had suggested a map. No, just a schematic, because it seemed like there were some recurrent themes with a lot of little individual differences of the types of preparations that are made; sort of a powder versus an alcoholic extract versus an aqueous extract, et cetera. And maybe like an upside down trident that might have examples of some of the families and how they're -- just to orient the reader into how these things are turned from kelp, you know, algae to products that are more tangible. So, that's what I suggested to Priya.

DR. MARKS: And we certainly divided the botanical as to safe and insufficient, depending on whether the final product is an extract or a powder or a juice or whatever; so, that is important.

DR. BERGFELD: All right. I think that we've had enough discussion, then, to call to question. All those in favor of the conclusion of insufficient data announcement? Thank you. Unanimous. Well, that was quick. Thank you, Don, and thank you Jim. As I thought it would be longer. Let's move on to the next ingredient, the Acrylates Copolymers, Dr. Marks.

DR. HELDRETH: Before we move on, could we just get a reiteration of the needs, so that Priya has everything she needs for the announcement.

DR. BERGFELD: Okay.

DR. BELSITO: Why don't you go ahead Jim because you added some in. I'm fine adding as many insufficiencies as we need at this point.

DR. MARKS: The first thing would be which one of these algae really are grass designated? And then the second, we basically need sensitization for everything. But from the hizikia, the laminaria and the undaria, we felt we had enough to move forward since these are grass ingredients that all we need was sensitization. The rest of the ingredients we want the 28-day tox, along with sensitization -- and genotox.

And composition. Don, you had brought out a couple of lead ingredients for composition. As far as I'm concerned, let's get as much composition as we can get for as many different species of algae. But, Don, you were specific in naming species.

DR. BELSITO: Well, most of them are very low concentration of use with the exception of the laminaria digitata, which is at 50 percent. I'm sorry I made a mistake; I had written down that sensitization was at 50 percent propylene glycol, it's 5. So, I guess we need sensitization for that as well.

And the macrocystis, which is at 33, I think, .4 percent in a leave-on. So, I just sort of saw those as the two lead products. And if we can get composition on them, and seeing that the compositions of these algae are pretty similar, we could use data from the read-across for those two that are used in very high amounts, to help clear a lot of other information.

DR. MARKS: So, I think as much composition as we can get.

DR. BELSITO: But we have a lot of sensitization and irritation, as you can see, from the table that Priya provided for us yesterday.

DR. MARKS: Yeah. Unfortunately, they weren't in the ones that are most commonly used.

DR. BELSITO: Right.

DR. MARKS: And there were some big numbers of use. And that's why you picked those out, Don, I concur.

DR. BERGFELD: Are you okay with the needs assessment and what is needed?

MS. CHERIAN: Yes.

DR. BERGFELD: All right, we'll move on then. We're going on then, again, to the Copolymers, Dr. Marks?

Safety Assessment of Brown Algae-Derived Ingredients as Used in Cosmetics

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The 2018 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Lillian C. Becker, former Scientific Analyst/Writer and Priya Cherian, Scientific Analyst/Writer.

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ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of 82 brown algae-derived ingredients, which are frequently reported to function in cosmetics as skin-conditioning agents. The Panel reviewed the available data to determine the safety of these ingredients. Impurities, particularly arsenic, may be present in these ingredients. Industry should continue to use good manufacturing practices to limit these possible impurities. The Panel concluded that… [to be determined].

INTRODUCTION

This is a safety assessment of 82 brown algae-derived ingredients as used in cosmetics (Table 1). The ingredients in this review are extracts, powders, juices, or waters derived from one or multiple species of brown algae. These ingredients are a highly complex group, with intricate chemistry and compositions that vary depending on species type, harvesting, and method of manufacture. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), these brown algae-derived ingredients are most commonly used as skin conditioning agents (Table 2).[1] These ingredients are also reported to be used as absorbents, antioxidants, binders, hair conditioning agents, oxidizing agents, pH adjusters, and viscosity increasing agents.

There are several major groups of algae (as described in "Algae Identification" section). However, this safety assessment focuses only on brown algae. The names of the ingredients in this report are written in accordance with the INCI naming conventions, i.e., capitalized without italics or abbreviations. When referring to the algae from which these ingredients are derived, the standard taxonomic practice of using *italics* is followed (e.g., *Agarum cribrosum*). The term "kelp" is commonly used when referring to brown algae. Kelp are large brown algae that belong to the order Laminariales.[2]

Several brown algae constituents, such as phytosterols,[3] phytosteryl ingredients,[3] and alginic acid[4] were found to be safe as used by the Panel. The full reports on these ingredients can be accessed on the CIR website (https://www.cir-safety.org/ingredients); therefore, information regarding these ingredients will not be included in this report.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

The European Chemical Agency (ECHA)[5,6] website provides summaries of data generated by industry, and is cited throughout the report as appropriate. Also referenced in this safety assessment are summary data found in other reports, including those published by the European Medicines Agency (EMA),[7,8] the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA),[9] and Food Standards Australia New Zealand (FSANZ).[10,11]

CHEMISTRY

Definitions

The ingredients in this safety assessment are derived from various species of brown algae. "Algae" is not a taxonomic group, but a functional group of convenience.[12] Not all algae should be considered to be plant-like (seaweed; macroalgae). While some algae are seaweed, some are protozoa, and some are unique and belong in other kingdoms. However, these aquatic and oxygenic organisms are all part of the eclectic group called "algae."

Algae Identification

There are several major groups of algae, and they are commonly referred to as brown algae (*Phaeophyceae),* green algae (*Chlorophyta*), diatoms (*Bacillariophyceae*), chrysophytes (*Chrysophyta*), blue-green algae (*Cyanophyta*), red algae (*Rhodophyta*), dinoflagellates (*Pyrrhophyta*), and euglenoids (*Euglenophyta*). A description of these major algal groups can be seen in Table 3. The different types of algae are differentiated by storage products, pigmentation, and cell wall composition.[12] A list of the brown algae-derived ingredients based on their subclass, order, family and genus is presented in Table 4.

Brown algae are mostly comprised of large, leathery seaweeds and are classified in about 265 genera with more than 1500 species.[12,13] The actual color varies depending on the proportion of brown pigment (fucoxanthin) to green pigment (chlorophyll). This algal group contains alginic acid and fucoidan in its complex cell walls. General characteristics and the geographic distribution of the specific species of brown algae in this report are presented in Table 5

As with plant-derived ingredients, the constituent composition of these seaweed ingredients can vary widely depending on growing conditions, age of the organisms, local environmental aspects, harvesting conditions, methods of extraction, and many other variables. For example, the concentration of the most abundant carotenoid pigment in brown algae, fucoxanthin, varies remarkably depending on the age of the alga, and the protein content in brown algae varies considerably depending on the season in which it is harvested.[14,15]

Physical and Chemical Properties

Physical and chemical properties of Ascophyllum Nodosum Extract, Ascophyllum Nodosum Powder, and Ecklonia Cava Extract are presented in Table 6. Using the sieve method, 93.5% of the particle sizes of Ascophyllum Nodosum Extract, as a fully dried extract, were less than 0.250 mm and greater than 0.045 mm.[6]

Harvesting

Originally, the only source of brown algae was in the wild; but since the mid-twentieth century, demand has exceeded the supply that could be harvested from wild sources, and methods for cultivation have been developed.[16] Consequently, today, commercial brown seaweed comes mainly from farming rather than wild sources. *Laminaria japonica* and *Undaria pinnatifida* are among the most cultivated species of brown algae.[17] Several species, such as *Laminaria japonica*, are grown on suspended ropes in the ocean.[16] Repeated harvesting of *Macrocystis pyrifera* over a 3-month period did not significantly impact tissue chemical properties (i.e. alginate yield; viscosity and strength; nutritional quality, such as protein, carbohydrate, lipid, crude fiber, ash, and energy content; and tissue carbon/nitrogen ratios).[18]

Method of Manufacture

Numerous methods of manufacture are provided in Table 7. Several of these methods have a target constituent or composition (e.g., high in fucoidan). The characterization of the final extract is provided in the table. A general overview of a method of manufacture for the relevant brown algae-derived ingredients can be seen in Figure 1.

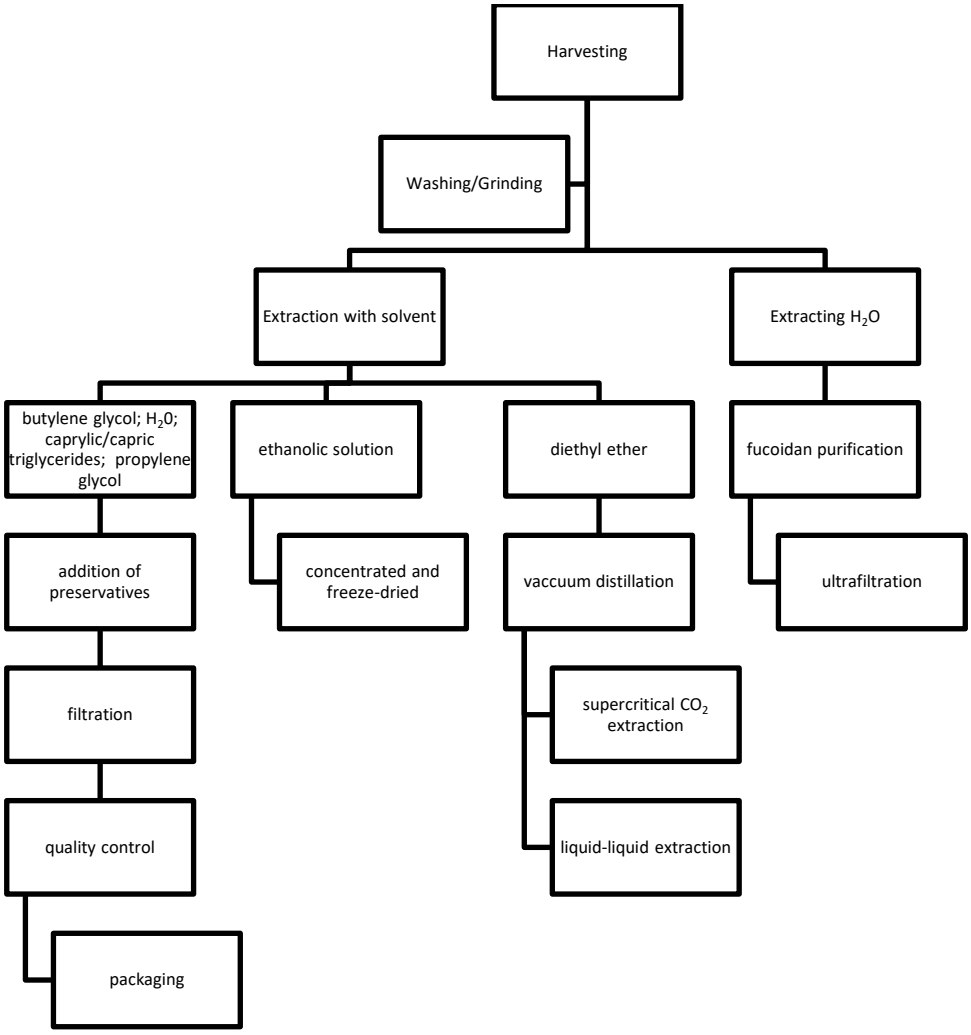

Figure 1. Overview of methods of manufacture for brown algae-derived ingredients. [9,19-28,28-52, CIR STAFF]

Arsenic is a constituent of concern in certain brown algae [see Constituents of Concern].[10,11,53,54] There are methods to remove the arsenic, including extraction with water, methanol, or water/methanol mixtures accompanied with sonication or mechanical agitation.[55] Extraction with microwave-assisted heating and accelerated solvent extraction systems are described in the literature.[55] Soaking the algae in water at room temperature followed by simmering in the water is shown to be

effective for removing inorganic arsenic.[56] Another variation entails repeated boiling in seawater, and replacing the water three times, after initial soaking.[53] Soaking the algae in a simmering 4% acetic acid or a 4% sodium hydrogen carbonate aqueous solution has also been shown to remove arsenic.[57]

Composition

Some constituents and constituent groups that are found in brown algae, in general, are presented in Table 8; included therein are alkaloids, laminarins, pheromones, phytohormones, terpenoids, amino acids, betaines, and characteristic pigments such as chlorophyll a and c, β-carotene, fucoxanthin, and several other xanthophylls.[58] Constituents found in *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Laminaria digitata* are listed in Table 9.

Sterols are also found in brown algae.[59,60] Sterols reported to be in *Cystoseira tamariscifolia*, *Fucus spiralis*, and *Sargassum vulgare* are provided in Table 10.

Methanol, hexane, and chloroform extracts from *Cystoseira compressa* were examined for flavonoid and phenolic content.[61] The flavonoid content of the methanol, hexane, and chloroform extract were 0.291 ± 0.02, 0.88 ± 0.07, and 0.804 ± 0.07 mg/g, respectively. The phenolic content of hexane (1.541 ± 0.09 mg/g) was considerably higher than the phenolic content of the methanol (0.161 ± 0.08 mg/g) and chloroform (0.45 ± 0.04 mg/g) extracts.

Constituents of ethanolic extracts of *Fucus spiralis* and *Sargassum vulgare* are presented in Table 11. The constituent with the highest concentration in both extracts is vaccenic acid (21,690 and 2848 ppm, respectively).[62]

Approximately 0.64 – 1.99 grams of polyphenols can be found in *Himanthalia elongata* extract.[63] In addition, phlorotannins can also be found in this extract (0.2 % dry weight). These include fucols, diphloroethol, and several fucophloroethols. Polyphenols are also found in *Undaria pinnatifida* extract in amounts of 0.08 – 0.60 g/ 100 g extract. Fucoidans extracted from the sporophylls of *Undaria pinnatifida* show a higher sulfate and L-fucose content than other fucoidans. The concentration of polyphenols in an aqeous extract of *Halidrys siliquosa* was reported to be 0.16 %.[64] The total protein and mineral content present in *Halidrys siliquosa* is approximately 9.6 and 11.19%, respectively.

The composition of a water/propylene glycol extract of *Laminaria japonica* is provided in Table 12.[51] The compositions of extracts of *Laminaria japonica*[52] that are produced via enzyme hydrolysis are presented in Table 13.

The specifications for an alcohol extract of *Ecklonia cava*, as a food/dietary supplement, include a combined phlorotannin content of 90.0 ± 5.0%; the content of dieckol, a specific phlorotannin, is 6.6% to 9.9% (Table 14).[9] The extract is to contain no insoluble substances, and it is reported to contain calcium (4800 ± 400 mg/kg), magnesium (1300 mg/kg), potassium (700 ± 200 mg/kg), and iodine (220 ± 40 mg/kg).

An *Undaria pinnatifida* extract rich in fucoidan (extraction method presented in Table 7) was characterized as having 27% uronic acid, 53% monosaccharides, and 7.4% sulfate.[65] Major monosaccharides included 54% fucose and 35% galactose. The minor monosaccharides were 3% rhamnose, 4% arabinose, and 1% xylose, glucose, and mannose.

A desalinated *Undaria pinnatifida* powder was reported to consist of 532 mg/g dietary fiber, mostly in the form of alginates, and 209 mg/g protein.[66] The composition profile is presented in Table 15.

Impurities/Constituents of Concern

Arsenic – Inorganic

Arsenic, usually in the form of arsenosugars, is a natural constituent of some brown algae, including *Ecklonia radiate*, *Laminaria japonica*, and *Sargassum fusiforme*.[10,11,52,54,67] The amount of arsenic is inconsistent due to varied uptake of inorganic arsenic by brown algae varieties and the influence of external factors (e.g., temperature, season, and pH). Compared to many other foods, algae contain greater inorganic arsenic levels as a proportion of the total arsenic (e.g., 60% to 73%). A trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water was reported to have ≤ 2 ppm arsenic.[68] The amounts of arsenic that have been measured in various brown algae are presented in Table 16. The different arsenic-containing moieties found in four brown algae species are presented in Table 17. A comparison of the amount of arsenic found in *Laminaria japonica* and a *Laminaria japonica* extract (equivalence to cosmetic ingredients not confirmed) is presented in Table 18.

Heavy Metals

Brown algae, in general, exhibit an affinity for heavy metals, which are believed to be absorbed from the water column.[58,69] Heavy metal concentrations in algae are strongly dependent on environmental parameters of the sampling sites (e.g., salinity, temperature, pH, light, nutrient concentrations, oxygen, etc.) and the structural differences among the algae. These seaweeds also absorb heavy metals from the sediment.[70,71] A trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water was reported to have ≤ 20 ppm heavy metals.[68] An overview of the amount of heavy metals found in brown algae species is provided inTable 19. Information regarding heavy metal impurities in trade name mixtures containing brown algae can be found in Table 20.

An edible, phlorotannin-rich, ethanol extract of *Ecklonia cava* has specifications issued by the European Commission.[9] According to the Commission, this extract must contain < 3 mg/kg lead, < 0.1 mg/kg mercury, < 3 mg/kg cadmium, < 25 mg/kg arsenic, and 150 - 650 mg/kg iodine.

Phthalates

Dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) were shown to occur naturally in *Laminaria japonica* at concentrations of 60 and 70%, respectively.[72] These phthalates were also present in *Undaria pinnatifida* (concentrations not given).

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetic industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to surveys conducted by the Personal Care Products Council (Council), of maximum reported use concentration by product category.

According to VCRP data received in 2018, Fucus Vesiculosus Extract is reported to be used in 287 formulations (201 in leave-on formulations, 75 in rinse-off formulations, and 11 diluted for the bath; Table 21).[73] Laminaria Digitata Extract is reported to be used in 235 formulations, Macrocystis Pyrifera (Kelp) Extract in 188 formulations, and Laminaria Saccharina Extract is used in 132 formulations. All other in-use ingredients are reported to be used in 77 formulations or fewer.

Ascophyllum Nodosum Extract was reported in the VCRP as Ascophyllum Nodosum (Seaweed) Extract and Fucus Vesiculosus Extract was reported as Fucus Vesiculosus (Bladderwrack) Extract. Laminaria Saccharina Extract is reported in the VCRP as Saccharina Latissima (Kelp) Extract; the accepted scientific name for *Laminaria saccharina* is *Saccharina latissima*. There were also entries in the VCRP for ingredients that may be related to the listed brown algae-derived ingredients: kelp (24 uses), kelp extract (15 uses), Laminaria extract (4 uses), Phaeophyceae (brown algae; 4 uses), and seaweed extract (82 uses). It is not known to what extent these erroneous names are connected to any of the ingredients in this report.

According to the VCRP data received, the ingredient name "Algae Extract" is still reported to be used in 932 cosmetic formulations, though the name was retired in 2015.[1,73] It is likely, therefore, that some of these uses of the retired name represent brown algae ingredients.

The results of the concentration of use surveys conducted by the Council in 2015 and 2016 indicate Laminaria Digitata Powder has the highest reported maximum concentration of use; it is used at up to 40% in face and neck formulations.[74,75] Macrocystis Pyrifera (Kelp) Extract is reported to be used at up to 36.4% in eye lotions. The other ingredients are reported to be used at 6% or less.

In some cases, reports of uses were received in the VCRP, but concentration of use data were not provided. For example, Ascophyllum Nodosum Powder is reported to be used in 4 cosmetic formulations, but no use concentration data were reported. In other cases, no uses were reported in the VCRP, but concentration of use data were reported in the industry survey; Fucus Vesiculosus had no reported uses in the VCRP, but a use concentration in shampoos, moisturizing formulations, and suntan formulations was provided in the industry survey. Therefore, it should be presumed there is at least one use in every category for which a concentration is reported. The ingredients not in use according to 2018 VCRP data and the 2015 and 2016 Council surveys are listed in Table 22.

Several of these ingredients are used in formulations that are used near the eye (e.g., Macrocystis Pyrifera (Kelp) Extract at up to 36.4% in eye lotion and Fucus Vesiculosus Extract in mascara at up to 5%), incidentally ingested (e.g., Macrocystis Pyrifera (Kelp) Extract in lipsticks at up to 0.079%), and in formulations that come in contact with mucous membranes (e.g., Fucus Vesiculosus Extract and Laminaria Digitata Extract at up to 5% in bubble baths and Laminaria Japonica Extract and Macrocystis Pyrifera (Kelp) Extract at up to 5% in bath oils, tablets and salts).

Additionally, some of the brown algae-derived ingredients are used in cosmetic sprays and could possibly be inhaled; for example, Macrocystis Pyrifera (Kelp) Extract is reported to be used at up to 0.79% in spray face and neck products. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles < 10 µm compared with pump sprays.[76,77] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[78,79] Laminaria Japonica Extract and Macrocystis Pyrifera (Kelp) Extract were reported to be used in face powders at concentrations up to 0.0035%. Conservative estimates of inhalation exposures to respirable particles during the use of loose-powder cosmetic products are 400- to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[80-82]

None of the brown algae-derived ingredients named in this report are restricted from use in any way under the rules governing cosmetic products in the European Union.[83]

Non-Cosmetic

Brown seaweeds are consumed around the world and come mostly, but not only, from the *Laminaria*, *Undaria*, and *Hizikia* species.[16] According to the US FDA, brown algae (i.e., several species of seaweeds that are harvested principally in coastal waters of the northern Atlantic and Pacific oceans) are direct food substances that are generally recognized as safe (GRAS) for human consumption for use as flavor enhancers and flavor adjuvants, when the maximum level in food does not exceed the current good manufacturing practice (GMP). [21CFR184.1120] "Kelp" (the dehydrated, ground product prepared from *Macrocystis pyrifera, Laminaria digitata, Laminaria saccharina*, and *Laminaria cloustoni*) is approved as a food additive for direct addition to food for human consumption as a source of iodine or as a dietary supplement. [21CFR172.365] An overview of the species of brown algae that are GRAS in the US can be seen in Table 23. In New Zealand, Japan and other Asian countries, dried sea kelp is a common food; the exact species of kelp used varies according to location.[16] The EFSA NDA Panel concluded that an alcohol extract of *Ecklonia cava* is safe for the use in food supplements at a maximum intake level of 163 mg/day for adolescents from 12 to 14 years of age, 230 mg/day for adolescents above 14 years of age, and 263 mg/day for adults.[9] In addition, a listing of brown algae species that are frequently ingested by humans is provided in Table 24.

In animal drugs, feeds, and related products, brown algae (kelp; *Laminaria* spp. and *Nereocystis* spp.) are GRAS as natural substances [21CFR582.30] and as solvent-free natural extractives [21CFR582.40] used in conjunction with spices and other natural seasonings and flavorings.

In the US, "kelp" is present in OTC medications for weight loss. [21CFR310.545] However, there are inadequate data to establish a general recognition of the safety and effectiveness of this ingredient for that specified use. Several other sources refer to the use of *Fucus vesiculosus* for weight loss.[84,85]

Pastes of seaweed, made by cold grinding or freeze crushing, are used in thalassotherapy, in which the pastes are applied to the body and then warmed under infrared radiation.[16] This treatment, in conjunction with seawater hydrotherapy, is said to provide relief for rheumatism and osteoporosis. In folk medicine, preparations of *Fucus vesiculosus* are used to treat hypothyroidism, iodine deficiency, arteriosclerosis, digestive disorders, menstrual abnormalities, cellulite, and sprains.[84,86] In herbal folk medicine, *Laminaria hyperborean* is used for thyroid regulation, and *Macrocystis Pyrifera* is used to treat thyroid conditions, anemia in pregnancy, and hypertension, weight loss, and as an immunity booster.[84]

Brown algae have been used as fertilizers and soil conditioners (*Ascophyllum*, *Sargassum*, *Ecklonia*, and *Fucus* species), animal feed for sheep, cattle, horses, pigs, and chickens (*Alaria esculenta*, and *Ascophyllum* and *Laminaria* species), feed and feed binder for fish and abalone (*Macrocystis pyrifera*), and biomass fuel (*Macrocystis pyrifera*), and they have been used for waste water/effluent treatment and removal of heavy metals (*Sargassum*, *Laminaria*, and *Ecklonia* species).[16,58] Brown algae are used as biomonitors for heavy metal pollution in estuarine and coastal waters worldwide, and to evaluate the quality of their surrounding environment.[69]

TOXICOKINETIC STUDIES

Obtaining data on the toxicokinetics of uncharacterized, complex mixtures would be impractical, as is the case with many botanical ingredients. No toxicokinetics studies were discovered in the published literature, and no unpublished data were submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

No acute dermal or inhalation toxicity studies were discovered in the published literature, and no unpublished data were submitted. Acute oral toxicity studies summarized below are presented in Table 25**.**

Oral

The LD_{50} was > 2000 mg/kg when Sprague-Dawley rats were dosed with Ascophyllum Nodosum Extract. No other details regarding this study were provided.[87] Cystoseira Compressa Extract was not toxic to mice when given a single dose of up to 2000 mg/kg by gavage. [88] The oral LD_{50}s of two Fucus Vesiculosus Extracts were 1000 and 500 mg/kg for male mice and between 1000 and 2000 mg/kg and < 750 mg/kg for female mice.[89] In rats (sex not stated), the oral LD_{50}s of two Fucus Vesiculosus Extracts were between 1000 and 2000 mg/kg for one extract and > 2000 mg/kg for the second extract.[89] There were no signs of toxicity when rats were given a single dose of up to 4000 mg/kg Laminaria Japonica Extract via oral gavage.[90] Sargassum Fulvellum Extract and Sargassum Thunbergii Extract were not toxic to mice that were given a single dose of 5000 mg in 10 mL Tween-80 via gavage.[50]

Short-Term, Subchronic, and Chronic Toxicity Studies

No repeated dose dermal or inhalation toxicity studies were discovered in the published literature, and no unpublished data were submitted. Short-term, subchronic, and chronic oral toxicity studies summarized below are presented in Table 26.

Oral

Ascophyllum Nodosum was not toxic when it was fed to pigs via a 10% oral diet for 23 days, or rats fed a 15% diet for 4 weeks.[45,88] Ecklonia Cava Extract was not toxic to rats dosed with up to 3000 mg/kg via oral gavage once daily in rats, and twice daily in dogs, for 13 weeks.[9,91] An enzyme extract of Ecklonia Cava Extract (starting at doses of 2000 mg/kg) administered by gavage for 2 weeks caused reduced ovary and brain weights in female rats.[91] Hepatic effects in rats were observed when animals were dosed with 2000 mg/kg/day via gavage of an alcohol Ecklonia Cava Extract for 4 weeks. When rats were dosed with the same extract in doses of 1500 mg/kg/day for 13 weeks, there were also decreases in body weight gain and organ weights (the hepatic effects resolved after 4 weeks recovery).[9]

Increased liver weights were apparent when two ethanol Fucus Vesiculosus Extracts (starting at doses of 200 mg/kg/day) were administered by gavage for 4 weeks in male rats.[89] While consuming high-fat diets, there were no adverse effects caused by alcohol Ecklonia Cava Extract when mice were given doses of up to 5 mg/day via gavage for 4 weeks.[92] An ethanol Laminaria Japonica Extract (up to 400 mg/kg) administered by gavage for 6 weeks caused decreased body weight gain, fat-pad weights, and serum and hepatic lipid levels in rats.[46] Vomiting was the only adverse effect when Ecklonia Cava Extract in capsules was orally administered (in increasing amounts up to 1000 mg/kg over 8 days) to dogs.[9] A *Ecklonia cava* powder (up to 0.15%; inference for Ecklonia Cava Extract and Ecklonia Cava Water) administered in feed for 28 days was not toxic to weanling pigs.[93] An *Undaria pinnatifida* extract (hydrolyzed in hydrochloric acid) administered orally for 28 days was not toxic to rats up to 1000 mg/kg/day, but alanine aminotransferase (ALT) and triglyceride levels in males and high-density lipoprotein (HDL) cholesterol in females increased at 2000 mg/kg/day.[65]

In rats, doses of 1200 to 4000 mg/kg Cladosiphon Okamuranus Extract given once a day for 3 months via gavage caused a dose-dependent increase in clotting time and decrease in alkaline phosphatase (ALP) that was not observed with lower doses.[47] There were no other adverse effects reported.

Laminaria Japonica Powder (up to 5%) was incorporated in the feed of mice from the age of 7 weeks until death. There were no dose-dependent effects on the lifespan of mice.[48] Undaria Pinnatifida Extract administered via drinking water (1.5 g in 1000 mL water) did not cause any toxic effects in rats when administered for 32 weeks.[94] Undaria Pinnatifida Extract (up to 5%) incorporated into feed of rats for 36 weeks did not cause any toxic effects.[48] The no observable adverse effect level (NOAEL) of a Laminaria Japonica Extract administered to rats by gavage for 6 months was 300 mg/kg/day.[90] In females, a decrease in aspartate aminotransferase (AST) was observed starting at 300 mg/kg/day and, at 2500 mg/kg/day, there was decreased serum glucose concentration. After a 1-month recovery period, these changes in glucose and AST returned to baseline.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

No DART studies were discovered in the published literature, and no unpublished data were submitted.

GENOTOXICITY STUDIES

The in vitro and in vivo genotoxicity studies summarized below are presented in Table 27.

In Vitro

Ascophyllum Nodosum Extract was not genotoxic in two Ames assays (up to 5000 µg/plate), a mammalian cell gene mutation test (up to 500 µg/mL), or in chromosomal aberration assays (up to 5 mg/mL); in a mammalian cell gene mutation test, Ascophyllum Nodosum Extract was genotoxic to Chinese hamster ovary (CHO) cells starting at 1500 µg/mL.[6,87] An Ames test was performed according to the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 471 on a trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water.[68] No mutagenic activity was reported. Ecklonia Cava Extract was not genotoxic in Ames assays (up to 5000 µg/plate) or chromosomal aberration assays (up to 350 µg/plate).[9,91] Halidrys Siliquosa Extract was non-mutagenic in an Ames assay, performed according to OECD TG 471, at up to 5 µL/plate.[64] Aqueous Fucus Vesiculosus Extract was not genotoxic in a chromosomal aberration assay (up to 1 mg/mL) or a comet assay (up to 1 mg/mL).[95] Laminaria Japonica Extract (up to 5000 µg/plate) was not mutagenic in an Ames assay or a chromosomal aberration assay.[52] A trade name mixture containing Laminaria Saccharina Extract in sea water and methylpropandiol was non-mutagenic in an Ames assay (up to 5000 µg/plate).[96] Macrocystis Pyrifera (Kelp) Extract was non-mutagenic in an Ames assay (1 mL test substance in 10 mL 0.9% sodium chloride; concentration of extract was approximately 4%).[97] Undaria Pinnatifida Extract was not genotoxic in Ames assays (up to 5000 µg/plate)[65,98,99] or chromosomal aberration assays (up to 5000 µg/mL).[98,99] A trade name mixture containing 24% Undaria Pinnatifida Cell Culture Extract was not mutagenic in a bacterial reverse mutation assay (up to 5000

µg/plate).[100] No genotoxicity was reported in a chemiluminescent 3D assay using water 52% and Cystoseira Amentacea/ Caespitosa/Brachycarpa Extract (48%) as the test substance at up to 10%.[101]

In Vivo

Ecklonia Cava Extract was not genotoxic in micronucleus assays up to 3000 mg/kg.[9,91] Laminaria Japonica Extract and Undaria Pinnatifida Extract were not genotoxic in micronucleus assays at up to 2000 mg/kg.[52,73,98,99]

CARCINOGENICITY STUDIES

No carcinogenicity studies were discovered in the published literature, and no unpublished data were submitted.

Tumor Promotion

Tumor promotion studies summarized below are presented in Table 28. The brown algae-derived ingredients that were tested were not tumor promoters; instead, decreases in the number, incidence, and size of tumors in rats and mice were observed.

Dermal

Mice were treated dermally with a single dose of 7,12-dimethylbenz[a]anthracene (DMBA; a carcinogen) followed by biweekly treatments for fifteen weeks with 12-*O*-tetradecanoylphorbol-13-acetate (TPA; a tumor promotor) or Undaria Pinnatifida Extract (1 mg).[102] The mice treated with Undaria Pinnatifida Extract had a delayed appearance of skin tumors (14 vs 8 weeks) and fewer tumors (average 0.2 vs 3.7) compared to the TPA-treated mice.

Oral

Rats injected with azoxymethane (AOM; a carcinogen) and then fed a diet containing Hizikia Fusiforme Extract (2% and 6%) had a reduced number of colorectal tumors (21 vs 58) compared to rats injected with AOM and fed a normal diet.[103] A *Saccharina angustata* powder (5%; inference for Saccharina Angustata Extract) in feed delayed the appearance and reduced the incidences of mammary tumors in rats orally administered DMBA.[104]

Rats administered *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG; a carcinogen) followed by Sargassum Pallidum Extract (0, 400, 600 and 800 mg/kg/day) in drinking water for 8 weeks had decreased inflammatory responses; serum IL-6, IL-1β, and TNF-α levels and concentration of serum and gastric mucosa malondialdehyde (MDA; an oxidant) were decreased in a dose-dependent manner.[105] In rats administered Undaria Pinnatifida Powder (0, 1.0% or 5.0% in feed) for 8 weeks after oral administration of DMBA, the mean combined weight of all mammary tumors of each rat in treatment groups was lower than that in the control group (approximately 7 vs 20 g).[106] Undaria Pinnatifida Extract (100% as drinking water) for 32 weeks reduced the incidence of mammary tumors (22% vs 100%) after female rats were orally administered DMBA.[94]

OTHER RELEVANT STUDIES

Endocrine Effects

In Vitro

Fucus vesiculosus extract

Human granulosa cells (obtained from 8 women) were treated with a water:ethanol (1:1) *Fucus vesiculosus* extract (25, 50, or 75 µmol/l) for 9 days.[107] Ethanol (50%) served as the vehicle control. At 50 and 75 µmol/l, the extract significantly reduced 17-β-estradiol levels in human granulosa cells and also competed with estradiol (E2) and progesterone for binding to their receptors.

Affinity of this extract for estrogen receptor-α (ERα), ERβ, and progesterone receptor (PR)-B was determined by radiometric competitive binding assays.[107] Dried extract (0.5, 5, or 50 µmol/l final concentration) was re-solubilized in dimethyl sulfoxide and combined with ERα or ERβ and 0.5 nmol/l estradiol. Non-specific binding was estimated in the presence of 1 µmol/l diethylstilbesterol. To test PR-B binding, the extract was incubated with PR-B and 1.4 nmol/l radiolabeled progesterone. Non-specific binding was estimated in the presence of 1 µmol/l progesterone. The extract competed for and bound to ERα (IC_{50} = 42.2 µmol/l), ERβ (IC_{50} = 31.8 µmol/l), and PR-B (IC_{50} = 31.8 µmol/l), with a slightly greater affinity for ERβ. The inhibition of progesterone production was less prominent, and there was no concentration-response relationship. In contrast, there was a concentration-dependent occupancy of the estrogen and progesterone receptors. Compounds found in *Fucus vesiculosus* could act as estradiol antagonists by decreasing the affinity of either ERα or ERβ for its ligand.

In competitive radio-ligand binding assays, aromatase activity was estimated by measuring the incorporation of tritium from androstenedione into water in the presence or absence of a *Fucus vesiculosus* extract (10, 50, or 100 µmol/L).[107] Aromatase activity following treatment of human luteinized granulosa cells (hLGCs) with this extract did not change.

A chemically activated luciferase reporter (CALUX®) assay was used to determine the effect of an aqueous *Fucus vesiculosus* extract on activation of the ER.[108] Aromatase enzymatic activity was measured to determine the potential effect of this extract on E2 biosynthesis. In co-treatments with E2, this extract (2%) reduced the activation of the luciferase reporter by up to 50%, exhibiting potent ER antagonistic effects. The effect of this extract (0 to 2%) on aromatase activity was measured using recombinant human CYP19 enzymatic hydrolysis of the fluorescent substrate, 7-methoxy-4-trifluoromethyl coumarin, in a 96-well plate. Ketoconazole was used as the positive marker of aromatase inhibition. This extract inhibited aromatase activity (IC_{50} 2.0%). ER-dependent and -independent cancer cell lines showed significantly decreased viability with increasing *Fucus vesiculosus* extract concentrations; altered morphological features suggested apoptosis and autophagy. The cell line-specific sensitivity suggests that *Fucus vesiculosus* extract was not toxic at up to 2%, but instead induces cell death through modulated pathways.

Animal

Fucus vesiculosus powder

Female Sprague-Dawley rats (n = 8), that had two confirmed normal estrous cycles, were administered a *Fucus vesiculosus* powder (0, 175, or 350 mg/kg/day) on an apple wedge daily for 4 weeks.[107] Vaginal smears were obtained and daily logs were maintained to monitor estrous cycling. No adverse effects were observed during the course of the experiment. Administration of this powder resulted in a statistically-significant, dose-dependent increase in the length of the estrous cycle in the treated rats. In the control group, the mean number of days of the estrous cycle was 4.3 ± 0.96 days compared to 5.4 ± 1.7 days in the low-dose group and 5.9 ± 1.9 days in the high-dose group. Treatment with this powder caused an overall 100% increase in the mean length of the diestrus phase of the estrous cycle. The mean number of days in diestrus was 0.97 ± 0.22 among the controls compared to 1.4 ± 0.54 in the low-dose group and 2.1 ± 0.88 days in the high-dose group. Treatment had no significant effect on the number of days in estrus, proestrus, or metestrus during the mean estrous cycle. After treatment was stopped, five rats stopped normal estrous cycling; one remained in estrus and four in diestrus.

Blood samples were collected from female Sprague-Dawley rats (n = 19) before treatment with dried *Fucus vesiculosus* powder, and at 2 and 4 weeks of the oral administration of this powder (0 or 175 mg/kg/d) on apple wedges.[107] At 2 weeks, mean serum 17β-estradiol levels were reduced from 48.9 ± 4.5 to 40.2 ± 3.2 ng/l and, after 4 weeks, reduced the levels from baseline to 36.7 ± 2.2 ng/l (25% decrease), suggesting an effect of dosing over time. Serum progesterone levels between controls and the treatment groups did not differ.

Blood samples were collected from female Sprague-Dawley rats (n = 8), that had naturally high circulating estradiol levels (≥ 50 µg/l), before, and after 1 week of the oral administration of *Fucus vesiculosus* powder (350 mg/kg/day) on apple wedges.[107] Median serum 17-β-estradiol levels decreased by 38%. The range in reduction of serum 17-β-estradiol levels in 6 of the rats was 25% to 58%, whereas 2 rats had levels similar to their baseline levels. Progesterone levels were not significantly affected following this treatment. This could be due to the fact that in the studies with rats the blood samples were collected in the morning, and in the morning the 17-β-estradiol levels were at their peak but the progesterone levels were not.

Photoprotection

Sargassum muticum

The effect of the ethyl acetate fraction of *Sargassum muticum* extract against cell death induced by mid-wavelength ultraviolet (UVB) radiation was studied.[109] Cells were seeded in a 96-well plate at a concentration of $1x10^{-5}$ cells/mL. Sixteen hours after plating, 100 µg/mL of *Sargassum muticum* extract were added to the cells and exposed to UVB radiation at a dose of 150 mJ/cm^2. Cell viability was 61% in UVB (150 mJ/cm^2) irradiated cells and 70% in UVB-irradiated cells treated with *Sargassum muticum* extract. Decreased numbers of apoptotic bodies as well as DNA fragmentation was apparent in cells exposed to *Sargassum muticum* extract and UVB versus UVB exposure alone.

DERMAL IRRITATION AND SENSITIZATION STUDIES

The dermal irritation and sensitization studies summarized below are presented in Table 30.

Irritation

In Vitro

Undaria Pinnatifida Cell Culture Extract

A trade name mixture containing 24% Undaria Pinnatifida Cell Culture Extract in water was used in an in vitro dermal irritation study.[110] The test substance (30 µL (liquid), 25 mg (solid)) was applied to reconstructed human epidermis and incubated for 60 minutes. The mixture was reported to be non-irritating. In a different dermal irritation assay, 10 µL of the test substance (trade name mixture containing Undaria Pinnatifida Extract (0.5 - 2%) in caprylic/capric triglycerides) was applied to human skin cell models for 15 minutes.[111] The trade name mixture was considered to be non-irritating.

Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract

A trade name mixture containing Laminaria Japonica (7%), Nereocystis Leutkeana (7%), Macrocystis Pyrifera Extract (7%), and pentaerythrityl tetraethylhexanoate (79%) was applied in doses of either 30 µL (liquid (solvent was not stated)) or 25 mg (solid) to a reconstructed human epidermis and incubated for 60 minutes.[112] The trade name mixture was non-irritating.

Sargassum Filipendula Extract

A trade name mixture containing Sargassum Filipendula Extract (1.3%), water (81.78%), sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), methylparaben (0.2%), and propylparaben (0.025%), was applied to a reconstructed human epidermis (30 µL (liquid), 25 mg (solid)), and incubated for 60 minutes. The test substance was considered to be non-irritating.[113]

Animal

Ascophyllum Nodosum Extract

Ascophyllum Nodosum Extract was non-irritating in a dermal irritation study performed according to OECD TG 404.[87] Species of test animal was not provided. The test substance (0.5 mL (liquid) or 0.5 g (solid), was applied to a small area of skin for 4 hours. In a different study, Ascophyllum Nodosum Extract (0.5 g) was applied to the backs of 3 rabbits, over an area of 12 - 20 cm^2, under semi-occlusion.[6] No irritation was observed after 1, 24, 48, or 72 hours.

Laminaria Digitata Extract

Two trade name mixtures containing either Laminaria Digitata Extract, water, and dipropylene glycol or Laminaria Digitata Extract, water, and sea salt, were considered non-irritating when applied to the skin of rabbits.[49] No other details regarding these studies were provided.

Human

Alaria Escuenta Extract

A 24-hour patch test was performed on 10 subjects over an area of 50 mm^2.[114] The test substance (trade name mixture containing Alaria Esculenta Extract (< 5%) and in caprylic/capric triglycerides) was applied in a dose of 20 µL. No signs of irritation occurred.

Ascophyllum Nodosum Extract

When the irritation potential of a trade name mixture containing 4.7% Ascophyllum Nodosum Extract in water was studied, no irritation was observed.[68]

Cystoseira Amentacea/Caespitosa/Brachycarpa Extract

A test substance containing 52% water and 48% Cystoseira Amentacea/Caespitosa/Brachycarpa Extract was used in a dermal irritation assay.[101] The product (0.02 mL) was applied to the back of 11 subjects, under an occlusive patch, for 48 hours. The test substance was considered to be non-irritating.

Cystoseira Baccata Extract

Cystoseira Baccata Extract in water was non-irritating in a 24-hour patch test performed on 10 subjects (concentration of the extract was not provided).[49] The same test substance was considered non-irritating in a different study involving repeated cutaneous applications on 50 subjects (concentration of extract and duration of dosing was not provided).[49]

Cystoseira Tamaricifolia Extract

A trade name mixture containing Cystoseira Tamaricifolia Extract and caprylic/capric triglycerides was considered non-irritating in a 24-hour patch test (10 subjects; concentration of the extract was not provided).[49]

Fucus Spiralis Extract

A 24-hour patch test was performed on 12 subjects using 20 µL of a trade name mixture consisting of Fucus Spiralis Extract (1 - 3%) in butylene glycol and water (application over an area of 50 mm^2).[115] No signs of irritation were reported. When 20 µL of a trade name mixture consisting of Fucus Spiralis Extract (< 5%) in caprylic/capric triglycerides was applied to the skin of 10 subjects, slight irritation was observed at the 30-minute reading; however, no irritation was reported at the 24-hour reading.[116]

Halidrys Siliquosa Extract

Halidrys Siliquosa Extract (48%) in water (52%) was diluted to 5%, and applied to the backs of 13 subjects at a dose of 0.02 mL. The application lasted for 48 hours.[64] No irritation was reported.

Himanthalia Elongata Extract

A trade name mixture consisting of Himanthalia Elongata Extract, water, and dipropylene glycol was considered non-irritating when applied to the skin of 10 subjects, under an occlusive patch, for 24 hours (concentration of the extract was not provided).[49]

Himanthalia Elongata Extract and Undaria Pinnatifida Extract

A dose of 0.02 mL of the test substance (Himanthalia Elongata Extract (20%), Undaria Pinnatifida Extract (37%), water (43%)) was applied to the backs of 10 subjects.[63] The concentration of the test substance was not provided. The test substance was considered to be very slightly irritating.

Laminaria Digitata Extract

A test substance consisting of Laminaria Digitata Extract and water was applied to the skin of 10 subjects for 24 hours (concentration of the extract was not provided).[32] No signs of irritation were reported. The irritation potential of a trade name mixture containing Laminaria Digitata Extract (<5%) in caprylic/capric triglycerides was studied in 12 subjects.[117] The test substance was applied in a dose of 20 µL over an area of 50 mm^2. The trade name mixture was considered to be non-irritating. The same procedure was performed in a different study using a test substance consisting of Laminaria Digitata Extract (1.5 - 2.5%) in water and propylene glycol.[118] Moderate irritation was observed after 30 minutes, and slight irritation was observed after 24 hours.

Laminaria Hyperborea Extract

Ten subjects had 20 µL of a trade name mixture consisting of Laminaria Hyperborea Extract (1 - 3%) in water applied to the skin for 24 hours under an occlusive patch.[119] No irritation was reported.

Laminaria Japonica Extract

A skin cream containing a 50/50 aqueous propylene glycol extract of Laminaria Japonica was diluted to 10% and applied to the skin of 25 subjects for 48 hours (20 mg).[51] The test substance was non-irritating.

Laminaria Ochroleuca Extract

A trade name mixture (Laminaria Ochroleuca Extract (< 5%) in caprylic/capric triglycerides) was applied to 11 subjects, at a concentration of 2%, under an occlusive patch, for 24 hours.[120] No signs of irritation were observed.

Laminaria Saccharina Extract

A trade name mixture consisting of Laminaria Saccharina Extract (1 - 3%) in water and propylene glycol was applied at concentrations of 8, 16, and 100% (20 µL; 10 subjects).[121] Slight irritation occurred at the 100% dose level, and no irritation occurred at the 8% or 16% dose levels.

Macrocystis Pyrifera (Kelp) Extract

A 48-hour occlusive single patch test was performed on 10 healthy adult volunteers.[97] No other details regarding this study were provided. The test substance, containing approximately 4% Macrocystis Pyrifera (Kelp) Extract, was considered as "very well tolerated" as regards to its cutaneous primary tolerance.

Pelvetia Canaliculata Extract

A trade name mixture containing Pelvetia Canaliculata Extract (1 - 3%) in butylene glycol and water was applied to the skin of 12 subjects under an occlusive patch for 30 minutes and 24 hours (20 µL).[122] Moderate irritation was reported at the 30 minute reading, and slight irritation was reported at the 24 hour reading. The same procedure was performed when studying the irritation potential of a trade name mixture containing Pelvetia Canaliculata Extract (1 - 3%) in propylene glycol and water. The test substance was moderately irritating after 30 minutes, and slightly irritating after 24 hours.[123] A 24-hour patch test was performed using a trade name mixture containing Pelvetia Canaliculata Extract (0.5 - 3%) in water (11 subjects; 20 µL). No signs of irritation occurred.[124]

Sargassum Glaucescens Extract

Ten subjects had a test substance (Sargassum Glaucescens Extract (20%), water (79%), and phenoxyethanol (1%)) applied to the skin under an occlusive patch at a concentration of 10%.[125] Details regarding duration and dosing amounts were not provided. The test substance was left on for 48 hours. No irritation was reported.

Sphacelaria Scoparia Extract

A trade name mixture consisting of Sphacelaria Scoparia Extract, water, and dipropylene glycol was applied to the skin of 11 subjects, under an occlusive dressing, for 24 hours. [49] The concentration of the extract was not provided. The test substance was reported to be non-irritating.[49]

Undaria Pinnatifida Extract

The test substance (Undaria Pinnatifida Extract (< 5%) in water and propylene glycol; 20 µL; 12 subjects) was applied to the skin over an area of 50 mm^2 under an occlusive patch.[126] Moderate irritation was observed after 30 minutes,

and mild irritation was observed after 24 hours. A different study using the same procedures was performed on 10 subjects.[49] The test substance (trade name mixture containing Undaria Pinnatifida Extract in water and dipropylene glycol) was considered to be non-irritating (concentration and dosing details were not provided).

Pelvetia Canaliculata Extract and Laminaria Digitata Extract

The test substance (20 µL; trade name mixture containing Pelvetia Canaliculata Extract and Laminaria Digitata Extract extracted in propylene glycol with panthenol (the amount of dry extract of both extracts combined is estimated to be 5.5 - 9.0%)) was applied to the skin of 10 subjects, under occlusive patches, at concentrations of 5, 10, and 100%, for 24 and 48 hours. Mild irritation was observed at the 100% concentration, minimal concentration was observed at the 10% concentration, and no concentration was reported at the 5% concentration.[127]

Sensitization

In Vitro

Undaria Pinnatifida Cell Culture Extract

An antioxidant response elements-nuclear factor-erythroid 2-related factor (ARE-Nrf2) Luciferase Test was performed according to OECD TG 442 D.[128] The test substance (trade name mixture containing Undaria Pinnatifida Cell Culture Extract (24%) and water) was applied to human keratinocyte cells at concentrations up to 2000 µM. The test substance was considered to be non-sensitizing.

A direct peptide reactivity assay (DPRA) was performed according to OECD TG 442 C. [129] The test chemical consisted of Undaria Pinnatifida Cell Culture Extract (24%), water, and acetonitrile (5 mM or 25 mM). The test substance was considered to be non-sensitizing.

Sargassum Filipendula Extract

A trade-name mixture consisting of Sargassum Filipendula Extract (1.3%), water (81.78%), sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), methylparaben (0.2%), propylparaben (0.025% was used in an ARE-Nrf2 Luciferase Test performed according to OECD TG 442 D.[130] Twelve test concentrations ranging from 0.98 – 2000 µM were assayed in triplicate. The test substance was not predicted to be a skin sensitizer.

Animal

Ascophyllum Nodosum Extract

The sensitizing potential of the test substance (Ascophyllum Nodosum Extract (0.1 – 400 µL; 25% - 75% water solutions) was studied in guinea pigs (20 test, 10 negative control) according to OECD 406 guidelines. [87] The test substance was considered to be non-sensitizing.

Human

Alaria Esculenta Extract

The sensitization potential of a test substance (25 µL of a trade name mixture consisting of Alaria Esculenta Extract (0.5 - 2.5%) in butylene glycol and water) was studied in a human repeat insult patch test (HRIPT), using 50 subjects.[131] The trade name mixture was considered to be non-irritating and non-sensitizing. The same procedure was performed in two different studies using a trade name mixture consisting of Alaria Esculenta Extract (< 5%) in capyrlic/capric triglycerides (100%; 25 µL). No signs of sensitization or irritation were reported.[132,133] An HRIPT was performed using a night cream containing 0.05% Alaria Esculenta Extract as the test substance (0.2 g; 105 subjects).[134] No indication of dermal sensitization was observed.

Fucus Spiralis Extract

An HRIPT was performed using 200 µL of a test substance (trade name mixture consisting of Fucus Spiralis Extract (1 - 3%) in butylene glycol and water) on 50 subjects.[135] The test substance was non-sensitizing. A similar study was performed using a trade name mixture consisting of Fucus Spiralis Extract (< 5%) in caprylic/capric triglycerides as the test substance (52 subjects).[116] No indication of dermal sensitization was observed.

Fucus vesiculosus extract

A gel formulation containing 1% of an aqueous extract of *Fucus vesiculosus* (0.2 mL) was tested in a double-blind, placebo-controlled experiment.[44] Female subjects (n = 10) applied the gel to one cheek at least twice per day (morning and evening) for 5 weeks. The same gel, without the extract, was applied to the other cheek. The skin was examined before the experiment began, daily, and after the experiment ended. There were no signs of erythema or edema during the experiment.

Halidrys Siliquosa Extract

An HRIPT was performed on 103 subjects using a test substance consisting of Halidrys Siliquosa Extract (48%) in water (52%). The concentration of the test substance was not provided. The test substance was considered to be non-sensitizing.[64]

Macrocystis Pyrifera (Kelp) Extract

An HRIPT was performed on 53 healthy adult subjects.[97] The test substance contained approximately 4% Macrocystis Pyrifera (Kelp) Extract. No other details regarding this study were provided. There was no indication of dermal irritation or allergic contact sensitization induced by the test substance.

Laminaria Digitata Extract

An HRIPT was performed using 20 µL of the test substance (trade name mixture consisting of Laminaria Digitata Extract (< 5%) in caprylic/capric triglycerides) on 46 subjects.[136]

Laminaria Saccharina Extract

The sensitizing potential of the test substance (trade name mixture containing Laminaria Saccharina Extract (1 - 3%) in water and propylene glycol) was studied in an HRIPT.[137] The test substance was used on 50 subjects at a concentration of 20% and a dose of 25 µL. No indication of sensitization was observed.

Pelvetia Canaliculata Extract

A trade name mixture containing Pelvetia Canaliculata Extract (0.5 - 3%) was used in an HRIPT.[124] Fifty-five subjects were dosed with 200 µL of the test substance. The test substance was considered to be non-sensitizing and non-irritating.

Sargassum Filipendula Extract

An HRIPT was performed on 206 subjects using 0.2 g of the test substance (face cream containing 1.2% Sargassum Filipendula Extract).[138] The test substance was applied over an area of 4 cm^2, under an occlusive patch. No signs of sensitization were observed.

Sargassum Muticum Extract

An eye cream containing 0.076% Sargassum Muticum Extract (0.2 g) was used as the test substance in an HRIPT on 103 subjects.[139] The test material was applied to a 1 $inch^2$ absorbent pad portion of a clear adhesive dressing and applied to the skin. No indication of sensitization was observed. The same procedure was performed in a different study analyzing the sensitization potential of a skin care product containing 0.076% Sargassum Muticum Extract (0.2 g; 104 subjects). The test substance was considered to be non-sensitizing.[140]

Sphacelaria Scoparia Extract

Fifty volunteers were subjected to repeated epicutaneous applications of the test substance (Sphacelaria Scoparia Extract, water, and dipropylene glycol). The concentration of the extract was not stated. No other details regarding this study were provided. The test substance was considered to be hypoallergenic.[49]

Undaria Pinnatifida Extract

The sensitization potential of 50 µL of a trade name mixture containing Undaria Pinnatifida Extract (< 5%) in caprylic/capric triglycerides was studied in 100 subjects according in an HRIPT.[141] The test substance was considered to be non-sensitizing.

Phototoxicity

In Vitro

Ascophyllum Nodosum Extract

A phototoxicity study was performed according to OECD TG 432 (3T3 NRU phototoxicity test) using a trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water.[68] No additional details were provided. No phototoxic activity was reported.

OCULAR IRRITATION STUDIES

The studies summarized below are presented in Table 31.

In Vitro

A trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water was practically non-irritating when used in a Het-Cam test (no other details were provided).[68] A test substance containing 52% water and 48% Cystoseira Amentacea/Caespitosa/Brachycarpa Extract was was considered to be slightly irritating in a Het-Cam test.[101] Slight irritation was noted in a Het-Cam test using a test substance (at 5%) consisting of Halidrys Siliquosa Extract (48%) in water (52%),

performed according to the same procedures as above.[64] A test substance containing Himanthalia Elongata Extract (20%), Undaria Pinnatifida Extract (37%) and water (43%), was considered non-irritating in a Het-Cam test.[63] A Het-Cam test was also performed on Macrocystis Pyrifera (Kelp) Extract (4%) using hen's egg chorion-allantoic membrane (no other details were provided).[97] The test substance was mildly irritating. A test substance containing 24% Undaria Pinnatifida Cell Culture Extract in water was used in an in vitro ocular irritation assay.[110] The test substance (50 µL (liquid) or 50 mg (solid)) was applied to reconstructed cornea epithelium. After application, tissue inserts dosed with the liquid test substance were incubated for 30 minutes, and tissue inserts dosed with the solid test substance were incubated for 90 minutes. The test substance was considered to be non-irritating. A trade name mixture containing Laminaria Japonica Extract (7%), Nereocystis Leutkeana Extract (7%), Macrocystis Pyrifera Extract (7%), and pentaerythrityl tetraethylhexanoate, was used in an ocular irritation assay using the same procedure and dosing as above.[112] The test substance was considered to be non-irritating. An ocular irritation assay according to the same procedure as above was performed using a test substance consisting of Sargassum Filipendula Extract (1.3%), water (81.78%), sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), methylparaben (0.2%), and propylparaben (0.025%).[113] The test substance was considered to be non-irritating.

Animal

An ocular irritation test was conducted on an *Ascophyllum nodosum* extract (100 mg) in accordance with OECD TG 405 (Acute Eye Irritation/Corrosion) using New Zealand White rabbits (n = 3 males).[6] The test substance was instilled into one eye of each rabbit; the other eye served as the control. After 1 h, both eyes were washed with water. The eyes were examined at 1, 24, 48, and 72 h and 7 days after instillation. The maximum irritation score was 6.7 out of 8 at 1 h post-instillation; the score decreased to 0 by day 7, which indicated that the induced changes were reversible, and thus, the effects of the test substance were classified as 'irritation' and not as 'corrosion.' The test substance was rated as a mild ocular irritant. In a different study performed according to OECD TG 405, Ascophyllum Nodosum Extract was slightly irritating.[87] No other details were provided for this study.

Human

The ophthalmic irritation potential of an eye cream containing 0.076% Sargassum Muticum Extract was tested in 31 subjects, approximately 50% of which wore soft contact lenses.[142] Subjects were directed to apply the cream twice daily at the eye contour, excluding eyelids. A comprehensive ocular examination was performed after 4 weeks of test material usage. The test material did not indicate a potential for ophthalmologic irritation and was considered safe for use by both contact and non-contact lens wearers.

CLINICAL STUDIES

Case Reports

Oral case reports regarding brown algae-derived supplements are presented in Table 29. Decreased platelet count and an increased amount of arsenic in the blood was noted in subjects taking kelp supplements. [143,144]

Clinical Trials

Oral

Clinical trials summarized below are presented in Table 32.

In an oral clinical trial in which an *Ascophyllum nodosum* powder (0.5g/d) was administered to healthy female subjects, median urinary iodine concentrations increased from 78 mg/l to 140 mg/l, and thyroid-stimulating hormone (TSH) concentrations increased slightly, but remained within the normal range.[145] There were no adverse events reported. Administration of an alcohol extract of *Ecklonia cava* (400 mg/day) to subjects with hypercholesterolaemia for 12 weeks did not have an effect on hematology, clinical chemistry, or urinalysis parameters; however, one instance (2.2%) each of nausea, dyspepsia, diarrhea, and alopecia were reported.[9,146] A phlorotannin-rich extract of *Ecklonia cava* (0, 72, or 144 mg/day) was administered for 12 weeks to overweight patients in a randomized, double-blind study. Hematological and clinical chemistry did not reveal any adverse effects; the 144 mg/d group showed decreases in serum glucose and systolic blood pressure (SBP).[9] Administration of capsules containing a desalinated *Undaria pinnatifida* powder (average intake estimated to be 3.3 g per day) to hypertensive subjects for 8 weeks resulted in a decrease in the average SBP, diastolic blood pressure (DBP), and total cholesterol; adverse effects included two cases of indigestion and one case of diarrhea, both of which resolved quickly without treatment.[66]

Three pre-menopausal women with irregular menstrual cycles were administered a *Fucus vesiculosus* powder.[147] Subject number 1 was 43 years old with hypermenorrhea, polymenorrhea, dysmenorrhea, luteal phase deficiency, and endometriosis. Subject number 2 was 42 years old with hypermenorrhea, polymenorrhea, and dysmenorrhea. Subject number 3 was 21 years old with hypermenorrhea, dysmenorrhea, and endometriosis. Menstrual cycles were tracked for three cycles and serum 17β-estradiol and progesterone levels were measured before treatment started. Then the women were

administered this powder in capsules (700 mg/day) for two menstrual cycles. Serum 17-β-estradiol and progesterone levels were measured again. Subject 2 stopped treatment at this point and subjects 1 and 3 continued treatment with a greater dose of this powder (1400 mg/day) for two more cycles. This powder increased the menstrual cycle length and reduced the days of menstruation in a dose-dependent manner (Table 33). In subject 1, the plasma estradiol levels were decreased (before: 626 ± 91 pg/mL; low dose: 164 ± 30 pg/mL; high dose: 92.5 ± 3.5 pg/mL) and the progesterone levels were increased (before: 0.58 ± 0.14 ng/mL; low-dose: 8.4 ± 2.6 ng/mL; high-dose: 16.8 ±0.7 ng/mL).[147]

SUMMARY

This is a review of the safety of 82 brown algae-derived ingredients as used in cosmetics. The ingredients in this review are extracts, powders, juices, or waters derived from one or multiple species of brown algae and may be derived from the whole or a defined part of the seaweed. "Brown algae" is a common name for seaweeds of the class *Phaeophyceae*, which have an abundance of xanthophyll pigments and are a known source of alginate. These ingredients are a highly complex group, with intricate chemistry and compositions that vary depending on species type, harvesting, and method of manufacture. The most frequently reported function of brown algae ingredients in cosmetics is as a skin-conditioning agent; other reported functions include absorbent, antioxidant, binder, hair conditioning agent, oxidizing agent, and viscosity increasing agent.

Extraction methods and solvents vary, depending on the desired composition of the final ingredient. Powders, however, are generally the dried algae pulverized by milling. Inorganic arsenic, usually in the form of arsenosugars, is a natural constituent of brown algae and the amount in harvested algae can be reduced by several methods. In addition to arsenic, brown algae exhibit an affinity for heavy metals and uptake is strongly dependent on environmental parameters.

According to VCRP survey data received in 2018, Fucus Vesiculosus Extract is reported to be used in 287 formulations (201 in leave-on formulations, 75 in rinse-off formulations, and 11 diluted for the bath). Laminaria Digitata Extract is reported to be used in 235 formulations and Macrocystis Pyrifera (Kelp) Extract in 188 formulations. All other in-use ingredients are reported to be used in 132 formulations or fewer. The results of the concentration of use surveys conducted by the Council in 2015 and 2016 indicate Laminaria Digitata Powder has the highest reported maximum concentration of use; it is used at up to 40% in face and neck formulations. Macrocystis Pyrifera (Kelp) Extract is reported to be used at up to 36.4% in eye lotions. The rest of these ingredients are reported to be used at 6% or less.

According to the US FDA, brown algae (i.e., several species of seaweeds that are harvested principally in coastal waters of the northern Atlantic and Pacific oceans) are direct food substances that are generally recognized as safe (GRAS) for human consumption for use as flavor enhancers and flavor adjuvants, when the maximum level in food does not exceed the current good manufacturing practice (GMP). "Kelp" (the dehydrated, ground product prepared from Macrocystis pyrifera, Laminaria digitata, Laminaria saccharina, and Laminaria cloustoni) is approved as a food additive for direct addition to food for human consumption as a source of iodine or as a dietary supplement. In animal drugs, feeds, and related products, brown algae (kelp; *Laminaria* spp. and *Nereocystis* spp.) are GRAS as natural substances and as solvent-free natural extractives used in conjunction with spices and other natural seasonings and flavorings.

Acute oral administration of brown algae extracts was not toxic to mice, rats, and dogs. The LD_{50} was reported to be > 2000 mg/kg when Sprague-Dawley rats were given Ascophyllum Nodosum extract. Cystoseira Compressa Extract was not toxic to mice up to 2000 mg/kg by gavage. Ecklonia Cava Extract was not toxic to rats and dogs up to 3000 mg/kg by gavage. The oral LD_{50}s of two different Fucus Vesiculosus Extracts were 500 mg/kg and greater for mice and rats. There were no signs of toxicity at up to 4000 mg/kg Laminaria Japonica Extract orally administered to rats. Sargassum Fulvellum Extract and Sargassum Thunbergii Extract administered by gavage were not toxic to mice.

In oral short-term and subchronic studies, there were some adverse effects observed. In rats, Cladosiphon Okamuranus Extract (1200 to 4000 mg/kg by gavage) caused a dose-dependent increase in clotting time and decrease in ALP; there were no other adverse effects reported. An enzyme extract of Ecklonia Cava Extract (starting at 2000 mg/kg) administered by gavage for 2 weeks caused reduced ovary and brain weights in female rats. Hepatic effects in rats were observed in an alcohol Ecklonia Cava Extract at 2000 mg/kg/day for 4 weeks and at 1500 mg/kg/day when administered for 13 weeks (the hepatic effects resolved after 4 weeks of recovery). There were increased liver weights in male rats treated with two ethanol Fucus Vesiculosus Extracts (starting at 200 mg/kg/day) administered by gavage for 4 weeks. Vomiting was the only adverse effect when Ecklonia Cava Extract capsules (in increasing amounts up to 1000 mg/kg over 8 days) were orally administered to dogs.

In other oral short-term and subchronic studies, there were no adverse effects observed. Ascophyllum Nodosum was not toxic to pigs for 23 days or to rats for 4 weeks administered in feed at up to 10% and 15%, respectively. While consuming high-fat diets, there were no adverse effects caused by alcohol Ecklonia Cava Extract (up to 5 mg/day) administered to mice by gavage daily for 4 weeks and an ethanol Laminaria Japonica Extract (up to 400 mg/kg) administered by gavage for 6 weeks caused decreased body weight gain, fat-pad weights, and serum and hepatic lipid levels in rats. An *Ecklonia cava* powder (up to 0.15%; inference for Ecklonia Cava Extract and Ecklonia Cava Water) administered in feed for 28 days was not toxic to weanling pigs. An orally administered *Undaria pinnatifida* extract for 28 days was not toxic to rats

up to 1000 mg/kg/day, but ALT and triglyceride levels in males and HDL cholesterol in females increased at 2000 mg/kg/day.

In a chronic oral toxicity study, the NOAEL of a Laminaria Japonica Extract administered to rats by gavage for 6 months was 300 mg/kg/day. In females, a decrease in AST was observed starting at 300 mg/kg/day and, at 2500 mg/kg/day, there was decreased serum glucose concentration; all effects returned to baseline after a 1-month recovery. Laminaria Japonica Powder incorporated into feed did not affect the lifespan of mice at up to 5%. In rats, Undaria Pinnatifida Extract administered as drinking water at 100% for 32 weeks and incorporated into the feed (at up to 5%) for 36 weeks did not cause any toxic effects.

In genotoxicity assays of several of the brown algae-derived ingredients, all results were negative with the exception of an Ascophyllum Nodosum Extract in one mammalian cell gene mutation test in which the extract was genotoxic starting at 1500 µg/mL in CHO cells. Ascophyllum Nodosum Extract was not genotoxic in two Ames assays and a mammalian cell gene mutation test (up to 500 µg/mL), and in chromosome aberration assays (up to 5 mg/mL). Cystoseira Compressa Extract (up to 5 mg/plate) was not genotoxic in an Ames assay. Ecklonia Cava Extract was not genotoxic in Ames assays (up to 5000 µg/plate) and chromosome aberration assays (up to 350 µg/plate). Aqueous Fucus Vesiculosus Extract was not genotoxic in a chromosome aberration assay and a comet assay (up to 1 mg/mL). Halidrys Siliquosa Extract was non-mutagenic in an Ames assay (up to 5 µL/plate). Laminaria Japonica Extract (up to 5000 µg/plate) was not mutagenic in an Ames assay and a chromosome aberration assay. Macrocystis Pyrifera (Kelp) Extract was non-mutagenic in an Ames assay (1 mL test substance in 10 mL 0.9% sodium chloride; concentration of extract not provided). Undaria Pinnatifida Extract was not genotoxic in Ames assays and chromosome aberration assays (up to 5000 µg/mL). In micronucleus assays, Ecklonia Cava Extract (up to 3000 mg/kg), Laminaria Japonica Extract (up to 2000 mg/kg), and Undaria Pinnatifida Extract (up to 2000 mg/kg) were not genotoxic. An Ames test performed using a trade name mixture containing Laminaria Saccharina Extract in sea water and methylpropandiol at up to 5000 µg/plate resulted in negative results. A different Ames test was performed according to OECD TG 471 using a trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water. No mutagenic activity was reported. In a bacterial reverse mutation assay performed according to OECD TG 471, a trade name mixture containing 24% Undaria Pinnatifida Extract was not mutagenic (up to 5000 µg/plate). No genotoxicity was reported in a chemiluminescent 3D assay using water 52% and Cystoseira Amentacea/Caespitosa/ Brachycarpa Extract (48%) as the test substance.

None of the orally or dermally administered brown algae-derived ingredients tested (e.g., Hizikia Fusiforme Extract, Saccharina Angustata Extract (inference from *Saccharina angustata* powder), Undaria Pinnatifida Extract, and Undaria Pinnatifida Powder) were tumor (mammary and colorectal) promoters; instead, decreases in the number, incidence, and/or size of tumors in rats were reported. Rats administered MNNG followed by 8 weeks of Sargassum Pallidum Extract (400 to 800 mg/kg/day) in drinking water exhibited decreased inflammatory responses.

A *Fucus vesiculosus* extract exhibited estrogen effects in several in vitro studies. This extract (50 and 75 µmol/l) reduced 17-β-estradiol levels in human granulosa cells and also competed with estradiol and progesterone for binding to the respective receptors. In another study, a *Fucus vesiculosus* extract competed for, and bound to, ERα (IC_{50} = 42.2 µmol/l), ERβ (IC_{50} = 31.8 µmol/l), and PR-B (IC_{50} = 31.8 µmol/l), with a slightly higher affinity for ERβ. In co-treatments with E2 (12.5 pM; EC_{50}), a *Fucus vesiculosus* extract (2%) reduced the activation of the luciferase reporter by up to 50%, exhibiting potent ER antagonistic effects. ER-dependent and -independent cancer cell lines showed significantly decreased viability with increasing test material concentrations. The cell line-specific sensitivity suggests that *Fucus vesiculosus* extract was not toxic at up to 2%, but instead induces cell death through modulated pathways. In one study, aromatase activity following treatment of hLGCs with a *Fucus vesiculosus* extract (10 to 100 µmol/L) did not change.

In in vivo studies, a *Fucus vesiculosus* powder exhibited estrogenic effects. Oral administration (175 and 350 mg/kg/day) for 4 weeks resulted in a dose-dependent increase in the length of the estrous cycle and an overall 100% increase in the mean length of the diestrus phase of the estrous cycle in the treated rats. Mean serum 17-β-estradiol levels were reduced at 2 weeks and further reduced at 4 weeks. Female rats that had naturally high circulating estradiol had reduced serum 17-β-estradiol (25% to 58% in 2/8 rats) after 1 week oral administration of a *Fucus vesiculosus* powder (350 mg/kg/day). This powder (700 and 1400 mg/day) increased the menstrual cycle length and reduced the days of menstruation in a dose-dependent manner in three female human subjects with hypermenorrhea, dysmenorrhea, and other related ailments. In one subject, the plasma estradiol levels were decreased and the progesterone levels were increased in a dose-dependent manner.

In an in vitro study examining the photo-protection potential involving a *Sargassum muticum* extract, the effect of this extract against cell death induced by UVB radiation was studied. Cell viability was 61% in UVB (150 mJ/cm^2) irradiated cells and 70% in UVB-irradiated cells treated with SME. Decreased numbers of apoptotic bodies as well as DNA fragmentation was apparent in cells exposed to SME and UVB versus UVB exposure alone.

In vitro dermal irritation assays were performed on a mixture containing 24% Undaria Pinnatifida Cell Culture Extract in water; a mixture containing Laminaria Japonica (7%), Nereocystis Leutkeana (7%), Macrocystis Pyrifera Extract (7%), and pentaerythrityl tetraethylhexanoate; and a mixture containing Sargassum Filipendula Extract (1.3%), water (81.78%), sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), methylparaben (0.2%), and propylparaben (0.025). These trade name mixtures were considered to be non-irritating.

Ascophyllum Nodosum Extract and Laminaria Digitata Extract (with dipropylene glycol and water or water and sea salt) were non-irritating in animal dermal irritation studies. Many human irritation studies were provided using test substances containing a brown algae ingredient, or combination of ingredients, along with other substances such as caprylic/capric triglycerides, butylene glycol, water, sea salt, propylene glycol, phenoxyethanol, panthenol, or dipropylene glycol. The majority of these studies resulted in negative results; however, irritation was seen in several studies after treatment with high concentrations or short periods of exposure. In a study using a trade name mixture consisting of Fucus Spiralis Extract (< 5%) in caprylic/capric triglycerides as the test substance, slight irritation was observed after 30 minutes, however, no irritation was reported after 24 hours. A trade name mixture containing 20% Himanthalia Elongata Extract, 37% Undaria Pinnatifida Extract, and 43% water, was considered to be very slightly irritating to human skin. When a test substance consisting of Laminaria Digitata Extract (1.5 - 2.5%) in water and propylene glycol was applied to the skin, moderate irritation was observed after 30 minutes, and slight irritation was observed after 24 hours. In a different study, Laminaria Saccharina Extract (1 - 3%) in water and propylene glycol was applied at concentrations of 8, 16, and 100% to 10 subjects. Slight irritation was observed at the 100% dose level, and no irritation was observed at the lower doses. When a trade name mixture containing Pelvetia Canaliculata Extract (1 - 3%) in propylene glycol and water was applied to the skin, moderate irritation was noted after 30 minutes, and slight irritation was noted after 24 hours. Similar results were observed when a trade name mixture consisting of Undaria Pinnatifida Extract (< 5%) in water and propylene glycol was applied to the skin of 12 subjects. In a different study, the test substance (trade name mixture containing Pelvetia Canaliculata Extract and Laminaria Digitata Extract extracted in propylene glycol with panthenol (the amount of dry extract of both extracts combined is estimated to be 5.5 - 9.0%)) was applied to the skin of 10 subjects at concentrations of 5, 10, and 100%. Mild irritation was observed at the 100% concentration, minimal concentration was observed at the 10% concentration, and no irritation was reported at the 5% concentration.

An ARE-Nfr2 Luciferase Test utilizing human keratinocyte cells at concentrations up to 2000 µM was performed to study the sensitization potential of Undaria Pinnatifida Cell Culture Extract (24%). A DPRA performed testing the sensitizing potential of the same ingredient yielded negative results. An ARE-Nfr2 Luciferase Test was also performed on a trade name mixture containing Sargassum Filipendula Extract (1.3%), water (81.78%), sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), methylparaben (0.2%), and propylparaben (0.025%). No sensitization potential was observed. Ascophyllum Nodosum Extract (25% - 75%), was non-sensitizing when applied to the skin of 20 guinea pigs. All in vivo sensitization studies performed on humans, regarding several brown algae ingredients (Alaria Esculenta Extract (0.5 - 2.5% and < 5%), Ascophyllum Nodosum Extract (25%-75%), Fucus Spiralis (1 -3%), Halidrys Siliquosa Extract (48%), Macrocystis Pyrifera (Kelp) Extract, Sphacelaria Scoparia Extract, Laminaria Digitata Extract (< 5%), Laminaria Saccharina Extract (1 - 3%), Pelvetia Canaliculata Extract (0.5 - 3%), Sargassum Filipendula Extract (1.2%), Sargassum Muticum Extract (0.076%), and Undaria Pinnatifida Extract (<5%)), were negative.

A phototoxicity study was performed according to OECD TG 432 using a trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water. No phototoxic activity was reported.

A trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water was practically non-irritating when used in a Het-Cam test. A test substance containing 52% water and 48% Cytoseira Amentacea/Caespitosa/Brachycarpa was slightly irritating in a Het-Cam test. Slight irritation was also noted in a Het-Cam test peformed using a test substance consisting of Halidrys Siliquosa Extract (48%) in water (52%). In a different study, a test substance containing 24% Undaria Pinnatifida Cell Culture Extract in water was non-irritating when administered to reconstructed corneal epithelium. Macrocystis Pyrifera (Kelp) Extract was moderately irritating when used in a Het-Cam test. A different Het-Cam test assessing the irritation potential of a test substance containing Himanthalia Elongata Extract (20%), Undaria Pinnatida Extract (37%), and water (43%), yielded negative results. A trade name mixture containing Laminaria Japonica Extract (7%), Nereocystis Leutkeana Extract (7%), Macrocystis Pyrifera Extract (7%), and pentaerythrityl tetraethylhexanoate, and a different trade name mixture consisting of Sargassum Filipendula Extract (1.3%), water (81.78%), sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), methylparaben (0.2%), and propylparaben (0.025%), were considered to be non-irritating when administered in the same manner.

An *Ascophyllum nodosum* extract (100 mg) administered to the eyes of rabbits had a maximum irritation score was 6.7 out of 8 at 1 h post-instillation. The score decreased to 0 by day 7 and was rated as a mild ocular irritant. Ascophyllum Nodosum Extract was slightly irritating in an ocular irritation study performed according to OECD TG 405. No other details were provided for this study. The ophthalmic irritation potential of an eye cream containing 0.076% Sargassum Muticum Extract was tested in 31 subjects. The test material did not indicate a potential for ophthalmologic irritation and was considered safe for use by both contact and non-contact lens wearers.

In oral human clinical trials, adverse effects of an *Ascophyllum nodosum* powder (0.5g/d), an *Ecklonia cava* extract (up to 400 mg/day), and an *Undaria pinnatifida* powder (average intake 3.3 g per day) were mild and transient. The adverse effects included nausea, indigestion, dyspepsia, and diarrhea.

DRAFT DISCUSSION

The ingredients in this report, some of which are ingested in common food products, are derived from brown-algae. The Panel noted the widespread oral consumption of some of these ingredients as GRAS food products and nutritional

supplements. Since the ingestion of these ingredients is safe, and systemic exposure resulting from ingestion would be far greater than that due to cosmetic use, the concern for systemic toxicity was mitigated.

Gaps in the available safety data for some of these brown algae-derived ingredients in this safety assessment were noted. The available data on many of the ingredients are sufficient; however, consituent compositions and biologic activities under cosmetic use conditions can be inferred to support the safety of ingredients in the group.

The Panel noted an elevated amount of heavy metals and arsenic, which may be present in these brown algae-derived ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit these impurities. In addition, possible estrogenic effects were noted, however, the concern for these effects were mitigated as they were only seen at concentrations much higher than what would be used in cosmetics.

The Panel discussed the issue of incidental inhalation exposure from formulations that may be aerosolized (e.g., face/neck products at up to 0.79% (Macrocystis Pyrifera (Kelp) Extract. The Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial 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 concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects.

The Panel considered other data available to characterize the potential for these brown algae-derived ingredients to cause systemic toxicity, irritation, sensitization, reproductive and developmental toxicity, and genotoxicity. They noted the lack of systemic toxicity at high doses in acute and chronic oral exposure studies, minimal or no irritation or sensitization in tests of dermal exposure at relevant concentrations, and the absence of relevant genotoxicity in multiple assays. 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.

However, in September 2018, the Panel issued an Insufficient Data Announcement with the following needs:

- Sensitization data for all ingredients, at relevant concentrations
- 28-Day dermal toxicity data for those ingredients that are not GRAS
- Genotoxicity data for those ingredients that are not GRAS
- Composition for each of these brown-algae derived cosmetic ingredients

[to be completed]

CONCLUSION

To be determined.

TABLES

Table 1. Brown algae ingredients included in this assessment

Agarum Cribrosum Extract	Fucus Vesiculosus	Macrocystis Pyrifera (Kelp) Juice
Alaria Esculenta Extract	Fucus Vesiculosus Extract	Macrocystis Pyrifera (Kelp) Protein
Ascophyllum Nodosum	Fucus Vesiculosus Powder	Nereocystis Luetkeana Extract
Ascophyllum Nodosum Extract	Halidrys Siliquosa Extract	Pelvetia Canaliculata Extract
Ascophyllum Nodosum Powder	Halopteris Scoparia Extract	Pelvetia Siliquosa Extract
Cladosiphon Novae-Caledoniae Extract	Himanthalia Elongata Extract	Phyllacantha Fibrosa Extract
Cladosiphon Okamuranus Extract	Himanthalia Elongata Powder	Saccharina Angustata Extract
Cystoseira Amentacea/Caespitosa/ Branchycarpa Extract	Hizikia Fusiforme Extract	Saccharina Japonica Extract
	Hizikia Fusiformis Water	Saccharina Longicruris Extract
Cystoseira Baccata Extract	Hizikia Fusiformis Callus Culture Extract	Sargassum Filipendula Extract
Cystoseira Balearica Extract	Hydrolyzed Ecklonia Cava Extract	Sargassum Fulvellum Extract
Cystoseira Caespitosa Extract	Hydrolyzed Fucus Vesiculosus Extract	Sargassum Fusiforme Extract
Cystoseira Compressa Extract	Hydrolyzed Fucus Vesiculosus Protein	Sargassum Glaucescens Extract
Cystoseira Compressa Powder	Laminaria Cloustoni Extract	Sargassum Horneri Extract
Cystoseira Tamariscifolia Extract	Laminaria Diabolica Extract	Sargassum Muticum Extract
Dictyopteris Polypodioides Extract	Laminaria Digitata Extract	Sargassum Pallidum Extract
Dictyota Coriacea Extract	Laminaria Digitata Powder	Sargassum Siliquastrum Extract
Durvillaea Antarctica Extract	Laminaria Hyperborea Extract	Sargassum Thunbergii Extract
Ecklonia Cava Extract	Laminaria Japonica Extract	Sargassum Vulgare Extract
Ecklonia Cava Water	Laminaria Japonica Powder	Sphacelaria Scoparia Extract
Ecklonia Kurome Extract	Laminaria Longissima Extract	Undaria Peterseniana Extract
Ecklonia Kurome Powder	Laminaria Ochroleuca Extract	Undaria Pinnatifida Extract
Ecklonia/Laminaria Extract	Laminaria Saccharina Extract	Undaria Pinnatifida Cell Culture Extract
Ecklonia Maxima Extract	Lessonia Nigrescens Extract	Undaria Pinnatifida Leaf/Stem Extract
Ecklonia Maxima Powder	Lessonia Nigrescens Powder	Undaria Pinnatifida Powder
Ecklonia Radiata Extract	Macrocystis Pyrifera (Kelp)	Undaria Pinnatifida Root Powder
Eisenia Arborea Extract	Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract	
Fucus Serratus Extract		
Fucus Spiralis Extract	Macrocystis Pyrifera (Kelp) Extract	

Table 2. Current and revised INCI names, definitions, and functions of the brown algae-derived ingredients in this safety assessment[1]

Ingredient	Definition	Function
Agarum Cribrosum Extract	Agarum Cribrosum Extract is the extract of the alga, *Agarum cribrosum.*	Skin-conditioning agent - miscellaneous
Alaria Esculenta Extract	Alaria Esculenta Extract is the extract of the alga, *Alaria esculenta*.	Hair conditioning agent; skin protectant
Ascophyllum Nodosum	Ascophyllum Nodosum is the alga, *Ascophyllum nodosum.*	Skin-conditioning agent - miscellaneous
Ascophyllum Nodosum Extract 84775-78-0	Ascophyllum Nodosum Extract is the extract of the alga, *Ascophyllum nodosum.*	Skin-conditioning agent - miscellaneous
Ascophyllum Nodosum Powder	Ascophyllum Nodosum Powder is the powder obtained from the dried, ground alga, *Ascophyllum nodosum.*	Skin-conditioning agent - miscellaneous
Cladosiphon Novae-Caledoniae Extract	Cladosiphon Novae-Caledoniae Extract is the extract of the alga, *Cladosiphon novae-caledoniae.*	Humectant; skin protectant
Cladosiphon Okamuranus Extract	Cladosiphon Okamuranus Extract is the extract of the alga, *Cladosiphon okamuranus.*	Skin-conditioning agent - miscellaneous
Cystoseira Amentacea/Caespitosa/ Branchycarpa Extract	Cystoseira Amentacea/Caespitosa/Branchycarpa Extract is the extract of the algae, *Cystoseira amentacea*, *Cystoseira caespitosa*, and *Cystoseira branchycarpa*.	Skin-conditioning agent - miscellaneous
Cystoseira Baccata Extract	Cystoseira Baccata Extract is the extract of the alga, *Cystoseira baccata.*	Skin-conditioning agent - miscellaneous
Cystoseira Balearica Extract	Cystoseira Balearica Extract is the extract of the alga, *Cystoseira balearica.* The accepted scientific name for *Cystoseira balearica* is *Cystoseira brachycarpa.*	Skin-conditioning agent - miscellaneous
Cystoseira Caespitosa Extract	Cystoseira Caespitosa Extract is the extract of the alga, *Cystoseira caespitosa.* The accepted scientific name for *Cystoseira caespitosa* is *Cystoseira brachycarpa*.	Skin protectant
Cystoseira Compressa Extract	Cystoseira Compressa Extract is the extract of the alga, *Cystoseira compressa.*	Skin-conditioning agent - miscellaneous
Cystoseira Compressa Powder	Cystoseira Compressa Powder is the dried, ground powder obtained from the alga, *Cystoseira compressa.*	Skin-conditioning agent - miscellaneous
Cystoseira Tamariscifolia Extract	Cystoseira Tamariscifolia Extract is the extract of the alga, *Cystoseira tamarisfolia*.	Skin-conditioning agent - miscellaneous

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Ingredient	Definition	Function
Dictyopteris Polypodioides Extract	Dictyopteris Polypodioides Extract is the extract of the alga, *Dictyopteris polypodioides.*	Skin-conditioning agent – emollient; skin-conditioning agent - miscellaneous
Dictyopteris Membranacea Extract (Retired)	Dictyopteris Membranacea Extract (Retired) is the extract of the alga, *Dictyopteris membranacea.* The INCI Name, Dictyopteris Membranacea Extract, originally published in 2007, was designated with a retired status in 2015. For an interim period of time, trade name assignments formerly published with the INCI Name Dictyopteris Membranacea Extract will be retained in the retired monograph, and also published with the new name assignment based on the current genus and species name, Dictyopteris Polypodioides Extract.	Antioxidant
Dictyota Coriacea Extract	Dictyota Coriacea Extract is the extract of the alga, *Dictyota coriacea.*	Oxidizing agent
Durvillaea Antarctica Extract	Durvillaea Antarctica Extract is the extract of the alga, *Durvillaea antarctica.*	Skin-conditioning agent - miscellaneous
Ecklonia Cava Extract	Ecklonia Cava Extract is the extract of the alga, *Ecklonia cava.*	Humectant; skin-conditioning agent - humectant
Ecklonia Cava Water	Ecklonia Cava Water is the aqueous solution of the steam distillates obtained from the whole ~~plant~~, *Ecklonia cava.*	Skin protectant
Ecklonia Kurome Extract	Ecklonia Kurome Extract is the extract of the alga, *Ecklonia kurome.*	Skin-conditioning agent – humectant; skin-conditioning agent - miscellaneous
Ecklonia Kurome Powder	Ecklonia Kurome Powder is the powder obtained from the dried, ground alga, *Ecklonia kurome.*	Skin-conditioning agent - humectant
Ecklonia/Laminaria Extract	Ecklonia/Laminaria Extract is the extract of a mixture of the algae, *Ecklonia* and *Laminaria.*	Skin-conditioning agent - miscellaneous
Ecklonia Maxima Extract	Ecklonia Maxima Extract is the extract of the alga, *Ecklonia maxima.*	Skin-conditioning agent - miscellaneous
Ecklonia Maxima Powder	Ecklonia Maxima Powder is the powder obtained from the dried, ground alga, *Ecklonia maxima.*	Skin-conditioning agent - miscellaneous
Ecklonia Radiata Extract	Ecklonia Radiata Extract is the extract of the alga, *Ecklonia radiata.*	Hair conditioning agent; skin-conditioning agent - miscellaneous
Eisenia Arborea Extract	Eisenia Arborea Extract is the extract of the alga, *Eisenia arborea.*	Skin-conditioning agent - miscellaneous
Fucus Serratus Extract 94167-02-9	Fucus Serratus Extract is the extract of the alga, *Fucus serratus.*	Skin-conditioning agent - miscellaneous
Fucus Spiralis Extract	Fucus Spiralis Extract is the extract of the alga, *Fucus spiralis.*	Skin-conditioning agent – emollient; skin-conditioning agent - miscellaneous
Fucus Vesiculosus	Fucus Vesiculosus is the alga, *Fucus vesiculosus.*	Skin-conditioning agent - miscellaneous
Fucus Vesiculosus Extract 283-633-7	Fucus Vesiculosus Extract is the extract of the alga, *Fucus vesiculosus.*	Fragrance ingredient; skin-conditioning agent - miscellaneous
Fucus Vesiculosus Powder	Fucus Vesiculosus Powder is the powder obtained from dried, ground *Fucus vesiculosus.*	Skin-conditioning agent - miscellaneous
Halidrys Siliquosa Extract	Halidrys Siliquosa Extract is the extract of the alga, *Halidrys siliquosa.*	Skin-conditioning agent - miscellaneous
Halopteris Scoparia Extract	Halopteris Scoparia Extract is the extract of the alga, *Halopteris scoparia.*	Skin-conditioning agent - miscellaneous
Himanthalia Elongata Extract	Himanthalia Elongata Extract is the extract of the thallus of the alga, *Himanthalia elongata.*	Skin-conditioning agent - miscellaneous
Himanthalia Elongata Powder	Himanthalia Elongata Powder is the powder obtained from the dried, ground alga, *Himanthalia elongata.*	Absorbent; binder; viscosity increasing agent -aqueous
Hizikia Fusiforme Extract	Hizikia Fusiforme Extract is the extract of the alga, *Hizikia fusiforme.* The accepted scientific name for *Hizikia fusiforme* is *Sargassum fusiforme.*	Skin protectant; skin-conditioning agent - miscellaneous
Hizikia Fusiformis Water	Hizikia Fusiformis Water is the aqueous solution of the steam distillates obtained from the alga, *Hizikia fusiformis.*	Skin protectant
Hizikia Fusiformis Callus Culture Extract	Hizikia Fusiformis Callus Culture Extract is the extract of a culture of the callus of *Hizikia fusiformis.* The accepted scientific name for *Hizikia fusiformis* is *Sargassum fusiforme.*	Antifungal agent; antioxidant; hair conditioning agent; skin-conditioning agent - miscellaneous
Hydrolyzed Ecklonia Cava Extract	Hydrolyzed Ecklonia Cava Extract is the hydrolysate of an extract of the alga, *Ecklonia cava* derived by acid, enzyme or other method of hydrolysis.	Skin-conditioning agent - miscellaneous
Hydrolyzed Fucus Vesiculosus Extract 84696-13-9	Fucus Vesiculosus Extract is the extract of the alga, *Fucus vesiculosus.*	Fragrance ingredient; skin-conditioning agent – miscellaneous
Hydrolyzed Fucus Vesiculosus Protein	Hydrolyzed Fucus Vesiculosus Extract is the extract of the hydrolysate of *Fucus vesiculosus* derived by acid, enzyme or other method of hydrolysis.	None reported
Laminaria Cloustoni Extract 90046-11-0 92128-82-0	Laminaria Cloustoni Extract is the extract of the alga, *Laminaria cloustoni.* The accepted scientific name for *Laminaria cloustoni* is *Laminaria hyperborea.*	Fragrance ingredient
Laminaria Diabolica Extract	Laminaria Diabolica Extract is the extract of the alga, *Laminaria diabolica.* The accepted scientific name for *Laminaria diabolica* is *Saccharina japonica.*	Skin-conditioning agent - humectant

Table 2. Current and revised INCI names, definitions, and functions of the brown algae-derived ingredients in this safety assessment[1]

Ingredient	Definition	Function
Laminaria Digitata Extract 90046-12-1 92128-82-0	Laminaria Digitata Extract is the extract of the alga, *Laminaria digitata.*	Fragrance ingredient; skin protectant; skin-conditioning agent - miscellaneous
Laminaria Digitata Powder	Laminaria Digitata Powder is the powder obtained from the dried, ground thallus of the alga, *Laminaria digitata.*	Skin-conditioning agent - miscellaneous
Laminaria Hyperborea Extract 90046-13-2 92128-82-0	Laminaria Hyperborea Extract is the extract of the alga, *Laminaria hyperborea.*	Fragrance ingredient; skin protectant
Laminaria Japonica Extract 92128-82-0	Laminaria Japonica Extract is the extract of the alga, *Laminaria japonica.* The accepted scientific name for *Laminaria japonica* is *Saccharina japonica.*	Fragrance ingredient
Laminaria Japonica Powder	Laminaria Japonica Powder is the powder obtained from the dried, ground alga, *Laminaria japonica.* The accepted scientific name for *Laminaria japonica* is *Saccharina japonica.*	Skin-conditioning agent - miscellaneous
Laminaria Longissima Extract	Laminaria Longissima Extract is the extract of the alga, *Laminaria longissima*. The accepted scientific name for *Laminaria longissima* is *Saccharina longissima*	Skin-conditioning agent - humectant
Laminaria Ochroleuca Extract 92128-82-0	Laminaria Ochroleuca Extract is the extract of the alga, *Laminaria ochroleuca.* The accepted scientific name for *Laminaria ochroleuca* is *Saccharina japonica.*	Fragrance ingredient; skin-conditioning agent - miscellaneous
Laminaria Saccharina Extract 90046-14-3 92128-82-0	Laminaria Saccharina Extract is the extract of the thallus of the alga, *Laminaria saccharina.* The accepted scientific name for *Laminaria saccharina* is *Saccharina latissima.*	Fragrance ingredient; skin-conditioning agent - miscellaneous
Lessonia Nigrescens Extract	Lessonia Nigrescens Extract is the extract of the alga, *Lessonia nigrescens.*	Skin protectant
Lessonia Nigrescens Powder	Lessonia Nigrescens Powder is the powder obtained from the dried, ground alga, *Lessonia nigrescens.*	Binder
Macrocystis Pyrifera (Kelp)	Macrocystis Pyrifera (Kelp) is the alga, *Macrocystis pyriferae.*	Viscosity increasing agent - aqueous
Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract	Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract is the extract of the juice derived from the blade, pneumatocyst and stipe of the alga, *Macrocystis pyrifera.*	Skin-conditioning agent - miscellaneous
Macrocystis Pyrifera (Kelp) Extract 347174-92-9	Macrocystis Pyrifera (Kelp) Extract is the extract of the alga, *Macrocystis pyrifera.*	Skin-conditioning agent - miscellaneous
Macrocystis Pyrifera (Kelp) Juice	Macrocystis Pyrifera (Kelp) Juice is the juice expressed from the alga, *Macrocystis pyrifera.*	Skin-conditioning agent - miscellaneous
Macrocystis Pyrifera (Kelp) Protein	Macrocystis Pyrifera (Kelp) Protein is the protein derived from the alga, *Macrocystis pyrifera.*	Skin-conditioning agent - miscellaneous
Nereocystis Luetkeana Extract	Nereocystis Luetkeana Extract is the extract of the alga, *Nereocystis luetkeana.*	Hair conditioning agent; skin-conditioning agent - miscellaneous
Pelvetia Canaliculata Extract 223751-75-5	Pelvetia Canaliculata Extract is the extract of the alga, *Pelvetia canaliculata.*	Skin protectant; skin-conditioning agent - miscellaneous
Pelvetia Siliquosa Extract	Pelvetia Siliquosa Extract is the extract of the alga, *Pelvetia siliquosa.*	Antioxidant; skin protectant; skin-conditioning agent - humectant
Phyllacantha Fibrosa Extract	Phyllacantha Fibrosa Extract is the extract of the alga, *Phyllacantha fibrosa.* The accepted scientific name for *Phyllacantha fibrosa* is *Cystoseira baccata*.	Skin-conditioning agent - miscellaneous
Saccharina Angustata Extract	Saccharina Angustata Extract is the extract of the alga, *Saccharina angustata*.	Skin-conditioning agent - emollient; skin-conditioning agent - miscellaneous
Laminaria Angustata Extract (Retired)	Laminaria Angustata Extract (Retired) is the extract of the alga, *Laminaria angustata*. The INCI Name, Laminaria Angustata Extract, originally published in 2003, was designated with a retired status in 2015. For an interim period of time, trade name assignments formerly published with the INCI Name Laminaria Angustata Extract will be retained in the retired monograph, and also published with the new name assignment based on the current genus and species name, Saccharina Angustata Extract.	Skin-conditioning agent - miscellaneous
Saccharina Japonica Extract	Saccharina Japonica Extract is the extract of the alga, *Saccharina japonica.*	Skin-conditioning agent - miscellaneous
Laminaria Ochotensis Extract (Retired)	Laminaria Ochotensis Extract (Retired) is the extract of the alga, *Laminaria ochotensis.* The INCI Name, Laminaria Ochotensis Extract, originally published in 2008, was designated with a retired status in 2015. For an interim period of time, trade name assignments formerly published with the INCI Name Laminaria Ochotensis Extract will be retained in the retired monograph, and also published with the new name assignment based on the current genus and species name, Saccharina Japonica Extract.	Skin-conditioning agent - emollient
Saccharina Longicruris Extract	Saccharina Longicruris Extract is the extract of the alga, *Saccharina longicruris.*	Skin-conditioning agent - humectant
Sargassum Filipendula Extract	Sargassum Filipendula Extract is the extract of the brown alga, *Sargassum filipendula*.	Skin-conditioning agent - miscellaneous
Sargassum Fulvellum Extract	Sargassum Fulvellum Extract is the extract of the alga, *Sargassum fulvellum.*	Skin-conditioning agent - miscellaneous

Table 2. Current and revised INCI names, definitions, and functions of the brown algae-derived ingredients in this safety assessment[1]

Ingredient	Definition	Function
Sargassum Fusiforme Extract	Sargassum Fusiforme Extract is the extract of the brown alga, *Sargassum fusiforme.*	Skin-conditioning agent - miscellaneous
Sargassum Glaucescens Extract	Sargassum Glaucescens Extract is the extract of the alga, *Sargassum glaucescens.*	Antioxidant
Sargassum Horneri Extract	Sargassum Horneri Extract is the extract of the alga, *Sargassum horneri.*	Skin-conditioning agent - miscellaneous
Sargassum Muticum Extract	Sargassum Muticum Extract is the extract of the alga *Sargassum muticum.*	Skin-conditioning agent - miscellaneous
Sargassum Pallidum Extract	Sargassum Pallidum Extract is the extract of the alga, *Sargassum pallidum.*	Antifungal agent; antioxidant
Sargassum Siliquastrum Extract	Sargassum Siliquastrum Extract is the extract of the alga, *Sargassum siliquastrum.*	Skin-conditioning agent - miscellaneous
Sargassum Thunbergii Extract	Sargassum Thunbergii Extract is the extract of the alga, *Sargassum thunbergii.*	Antimicrobial agent
Sargassum Vulgare Extract	Sargassum Vulgare Extract is the extract of the alga, *Sargassum vulgare.*	Skin-conditioning agent - miscellaneous
Sphacelaria Scoparia Extract	Sphacelaria Scoparia Extract is the extract of the alga, *Sphacelaria scoparia.* The accepted scientific name for *Sphacelaria scoparia* is *Halopteris scoparia.*	Corn/callus/wart remover
Undaria Peterseniana Extract	Undaria Peterseniana Extract is the extract of the alga *Undaria peterseniana.*	Skin-conditioning agent - miscellaneous
Undaria Pinnatifida Extract	Undaria Pinnatifida Extract is the extract of the alga, *Undaria pinnatifida.*	Skin protectant; skin-conditioning agent - miscellaneous
Undaria Pinnatifida Cell Culture Extract	Undaria Pinnatifida Cell Culture Extract is the extract of a cell culture suspension of *Undaria pinnatifida.*	Hair conditioning agent; skin-conditioning agent - miscellaneous
Undaria Pinnatifida Leaf/Stem Extract	Undaria Pinnatifida Leaf/Stem Extract is the extract of the leaves and stems of *Undaria pinnatifida.*	Skin-conditioning agent – emollient
Undaria Pinnatifida Powder	Undaria Pinnatifida Powder is the powder obtained from the dried, ground alga, *Undaria pinnatifida.*	Absorbent; binder; viscosity increasing agent - nonaqueous
Undaria Pinnatifida Root Powder	Undaria Pinnatifida Root Powder is the powder obtained from the dried, ground root-like structures of the alga, *Undaria pinnatifida.*	Humectant; skin-conditioning agent - humectant

Table 3. Descriptions of major algae groups

Common Name	Kingdom	Class/Phylum	Description	Reference
Brown Algae	Stramenopila	Phaeophyceae	-mostly large, leathery seaweeds -cellulose wall with alginic acid and fucoidan -derived alginic acid is used as a suspending, emulsifying, gel-forming and film-forming agent	12
Green Algae	Plantae	Chlorophyta	-usually green in color -cellulose cell walls -store starch -beta carotene -chlorophyll a & b	12
Diatoms	Stramenopila	Bacillariophyceae	-golden brown in color -silica cell walls -store oil as food reserve -carotenoids -chlorophyll a & c	12
Chrysophytes	Stramenopila	Chrysophyta	-consists of diatoms, golden-brown algae and yellow-green algae -cellulose cell walls with large amounts of silica -chlorophyll a &c	12,148
Blue Green Algae	Monera	Cyanophyta	-phycobilins present -store glycogen -prokaryotic -chlorophyll a -some are toxic	12
Red Algae	Plantae	Rhodophyta	-phycobilins present -store floridean starch -cellulose cell wall -chlorophyll a & d -source of agar -used as a stabilizer and thickener in many products	12
Dinoflagellates	Alveolata	Pyrrhophyta	-some produce toxins -mostly marine	12,149
Euglenoids	Euglenozoa	Euglenophyta	-common in freshwater -can be parasitic	12,150

Table 4. Taxonomy of brown-algae derived ingredients[151]

Subclass	Order	Family	Genus	Ingredient
Dictyotophycidae	Dictyotales	Dictyotacaea	Dictyopteris	Dictyopteris Polypodioides Extract
Dictyotophycidae	Dictyotales	Dictyotacaea	Dictyotaceae	Dictyota Coriacea Extract
Dictyotophycidae	Sphacelariales	Sphacelariaceae	Sphacelaria	Sphacelaria Scoparia Extract
Dictyotophycidae	Sphacelariales	Sphacelariaceae	Stypocaulaceae	Halopteris Scoparia Extract
Fucophycidae	Ectocarpales	Chordariaceae	Cladosiphon	Cladosiphon Novae-Caledoniae Extract
Fucophycidae	Ectocarpales	Chordariaceae	Cladosiphon	Cladosiphon Okamuranus Extract
Fucophycidae	Fucales	Durvillaeaceae	Durvillaea	Durvillaea Antarctica Extract
Fucophycidae	Fucales	Fucaceae	Ascophyllum	Ascophyllum Nodosum
Fucophycidae	Fucales	Fucaceae	Ascophyllum	Ascophyllum Nodosum Extract
Fucophycidae	Fucales	Fucaceae	Ascophyllum	Ascophyllum Nodosum Powder
Fucophycidae	Fucales	Fucaceae	Fucus	Fucus Serratus Extract
Fucophycidae	Fucales	Fucaceae	Fucus	Fucus Spiralis Extract
Fucophycidae	Fucales	Fucaceae	Fucus	Fucus Vesiculosus
Fucophycidae	Fucales	Fucaceae	Fucus	Fucus Vesiculosus Extract
Fucophycidae	Fucales	Fucaceae	Fucus	Fucus Vesiculosus Powder
Fucophycidae	Fucales	Fucaceae	Fucus	Hydrolyzed Fucus Vesiculosus Extract
Fucophycidae	Fucales	Fucaceae	Fucus	Hydrolyzed Fucus Vesiculosus Protein
Fucophycidae	Fucales	Fucaceae	Pelvitia	Pelvetia Canaliculata Extract
Fucophycidae	Fucales	Fucaceae	Pelvitia	Pelvetia Siliquosa Extract
Fucophycidae	Fucales	Himanthaliaceae	Himanthalia	Himanthalia Elongata Extract
Fucophycidae	Fucales	Himanthaliaceae	Himanthalia	Himanthalia Elongata Powder
Fucophycidae	Fucales	Sargassaceae	Cystoseira	Cystoseira Amentacea/Caespitosa/ Branchycarpa Extract
Fucophycidae	Fucales	Sargassaceae	Cystoseira	Cystoseira Baccata Extract
Fucophycidae	Fucales	Sargassaceae	Cystoseira	Cystoseira Balearica Extract
Fucophycidae	Fucales	Sargassaceae	Cystoseira	Cystoseira Caespitosa Extract
Fucophycidae	Fucales	Sargassaceae	Cystoseira	Cystoseira Compressa Extract
Fucophycidae	Fucales	Sargassaceae	Cystoseira	Cystoseira Compressa Powder
Fucophycidae	Fucales	Sargassaceae	Cystoseira	Cystoseira Tamariscifolia Extract
Fucophycidae	Fucales	Sargassaceae	Halidrys	Halidrys Siliquosa Extract
Fucophycidae	Fucales	Sargassaceae	Hizikia	Hizikia Fusiforme Extract
Fucophycidae	Fucales	Sargassaceae	Hizikia	Hizikia Fusiformis Water
Fucophycidae	Fucales	Sargassaceae	Hizikia	Hizikia Fusiformis Callus Culture Extract
Fucophycidae	Fucales	Sargassaceae	Phyllacantha	Phyllacantha Fibrosa Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Filipendula Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Fulvellum Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Fusiforme Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Glaucescens Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Horneri Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Muticum Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Pallidum Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Siliquastrum Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Thunbergii Extract
Fucophycidae	Fucales	Sargassaceae	Sargassum	Sargassum Vulgare Extract
Fucophycidae	Laminariales	Agaraceae	Agarum	Agarum Cribrosum Extract
Fucophycidae	Laminariales	Agaraceae	Alaria	Alaria Esculenta Extract
Fucophycidae	Laminariales	Alariaceae	Undaria	Undaria Peterseniana Extract
Fucophycidae	Laminariales	Alariaceae	Undaria	Undaria Pinnatifida Extract
Fucophycidae	Laminariales	Alariaceae	Undaria	Undaria Pinnatifida Cell Culture Extract
Fucophycidae	Laminariales	Alariaceae	Undaria	Undaria Pinnatifida Leaf/Stem Extract
Fucophycidae	Laminariales	Alariaceae	Undaria	Undaria Pinnatifida Powder
Fucophycidae	Laminariales	Alariaceae	Undaria	Undaria Pinnatifida Root Powder
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Cloustoni Extract
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Diabolica Extract
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Digitata Extract
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Digitata Powder
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Hyperborea Extract
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Japonica Extract
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Japonica Powder
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Longissima Extract
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Ochroleuca Extract
Fucophycidae	Laminariales	Laminariaceae	Laminaria	Laminaria Saccharina Extract

Table 4. Taxonomy of brown-algae derived ingredients[151]

Subclass	Order	Family	Genus	Ingredient
Fucophycidae	Laminariales	Laminariaceae	Macrocystis	Macrocystis Pyrifera (Kelp)
Fucophycidae	Laminariales	Laminariaceae	Macrocystis	Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract
Fucophycidae	Laminariales	Laminariaceae	Macrocystis	Macrocystis Pyrifera (Kelp) Extract
Fucophycidae	Laminariales	Laminariaceae	Macrocystis	Macrocystis Pyrifera (Kelp) Juice
Fucophycidae	Laminariales	Laminariaceae	Macrocystis	Macrocystis Pyrifera (Kelp) Protein
Fucophycidae	Laminariales	Laminariaceae	Nereocystis	Nereocystis Luetkeana Extract
Fucophycidae	Laminariales	Laminariaceae	Saccharina	Saccharina Angustata Extract
Fucophycidae	Laminariales	Laminariaceae	Saccharina	Saccharina Japonica Extract
Fucophycidae	Laminariales	Laminariaceae	Saccharina	Saccharina Longicruris Extract
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Ecklonia Cava Extract
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Ecklonia Cava Water
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Ecklonia Kurome Extract
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Ecklonia Kurome Powder
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Ecklonia/Laminaria Extract
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Ecklonia Maxima Extract
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Ecklonia Maxima Powder
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Ecklonia Radiata Extract
Fucophycidae	Laminariales	Lessoniaceae	Ecklonia	Hydrolyzed Ecklonia Cava Extract
Fucophycidae	Laminariales	Lessoniaceae	Eisenia	Eisenia Arborea Extract
Fucophycidae	Laminariales	Lessoniaceae	Lessonia	Lessonia Nigrescens Extract
Fucophycidae	Laminariales	Lessoniaceae	Lessonia	Lessonia Nigrescens Powder

Table 5. General characteristics and geographic distribution of brown algae species

Species (common name)	Description	Distribution/Habitat/Ecology	References
Agarum cribrosum	-	North Atlantic (Massachusetts to east Greenland) and North Pacific (Washington state to Japan and Russia) Forms thick beds at depths of 10-12 m	151
Alaria esculenta (dabberlocks, badderlocks, winged kelp)	Olive or yellow-brown fronds to 4 m long and 25 cm wide, more often about 1 m and 7.5 cm wide. Attached by a root-like holdfast at the base from which a narrow flexible stipe arises which continues into the leafy part of thealgae as a distinct mid-rib, generally with a yellow-brown color. The reproductive structures, apparent as dark-brown areas, are confined to unbranched leafy appendages borne on the stipe, usually in two rows.	North Atlantic Ocean Generally growing on rock in wave-exposed places, often forming a band at low water and in the shallow subtidal, but also occurring in tidal pools in the lower shore.	151,152
Ascophyllum nodosum (asco, sea whistle, bladderwrack, rockweed)	Closely related to *Fucus*. Up to 3 m in height and is yellow in areas exposed to sunlight and dark green or brown in its shaded parts. Single bladders are central in long, strap-like fronds. Fronds hang downwards. Multiple fronds grow from each basal holdfast;generally regenerates new fronds from base when one of the larger fronds is damaged. Reproduction takes place in spring in yellow receptacles, which develop in response to short days in autumn, mature during winter, and are at their most prolific in spring. Eggs and sperm are released into water, and eggs release a low molecular weight pheromone, finnavarene.	North Atlantic basin (Virginia to Spain) Has been observed in San Francisco Bay, but does not persist there. Sheltered intertidal rocks in shallow (usually where it is exposed at low or extreme low tides)	151-154
Cystoseira baccata (bushy berry wrack)	Thallus to 1 m long, usually solitary, attached by a thick, conical attachment disc. Axis simple or branched, and flattened; apex smooth and surrounded during periods of active growth by incurved young laterals. Lateral branch systems alternate, radially symmetrical, profusely branched in a repeatedly pinnate fashion and bearing sparse, filiform, occasionally bifurcated appendages on the branches; deciduous, leaving decurrent bases which give an irregular, zigzag outline to the axis. Air vesicles present in axes of branches of higher order, sometimes in chains; seasonal, particularly numerous in autumn. Receptacles 1-5 cm long, formed from axes of ultimate ramuli, irregularly nodose and bearing simple, filiform appendages.	S England, W Ireland north to W Scotland. Has been noted down to Morocco and in Mediterranean Sea. Lower intertidal in large sandy pools or lagoons, mostly in persistent stands.	151,152
Cystoseira tamariscifolia (bushy rainbow wrack)	Solitary thalli, up to 1 m long, bushy, with a pronounced greenish or bluish iridescence when submerged or wet; attached by a conical disc. Axis is cylindrical, up to 60 cm long, usually branched and with an inconspicuous apex. Lateral branch systems arising in spiral sequence, up to 60 cm long, profusely branched in a repeatedly pinnate fashion, showing radial symmetry with simple or bifid spine-like appendages: deciduous, leaving prominent scars or stumps. Cryptostomata present on branches and appendages. Ovoid air vesicles often present in axes of ultimate ramuli. Receptacles 1-2 cm long, formed from terminal regions of ultimate ramuli.	Western Mediterranean Sea/northern Africa to Ireland Large intertidal rock pools and lagoons and shallow subtidal shores	151,152
Dictyopteris polypodioides [*Dictyopteris membranacea* (Retired)]	Thallus flat and leaf-like, to 300 mm long and 20-30 mm broad; fronds olive to yellow-brown, translucent, and somewhat regularly dichotomously forked with a prominent midrib extending to the apices. Margins sometimes split to midrib. Has an unpleasant smell shortly after collection, which degenerates quickly.	Ireland (except for east coast), west Scotland, Wales, southwest England, to Portugal and West Africa Large pools at low water and shallow subtidal shores	151,152
Fucus serratus (serrated wrack, saw wrack, toothed wrack)	Dichotomously branched fronds arising from a small disc via a short stipe; distinct midrib.Algae grows to 300 mm with terminal, compressed receptacles with warty conceptacles. It is easily recognized by its saw-toothed frond, and a lack of swollen receptacles.	Widely distributed on all coasts of Britain and Ireland. Baltic Sea to Spain and Canary Islands. Introduced to Nova Scotia and has spread to New Brunswick and Maine. Zone forming on sheltered and semi-exposed shores.	151-153
Fucus spiralis (jelly bags, spiral wrack, flat wrack spiraled wrack)	Fronds lack bladders; elongated air bladders are on either side of the midrib. Fronds have twisted, dichotomous branches. This species is up to 20 cm long, attached to the substratum with a discoid holdfast. Color ranges from dark brown to olive-green.	North Atlantic and North Pacific; Baltic Sea to Morocco/Canary Islands and New York; Alaska to California. Introduced to Mediterranean Sea (France). Uppermost species of *Fucus* that occurs on shore.	153

Table 5. General characteristics and geographic distribution of brown algae species

Species (common name)	Description	Distribution/Habitat/Ecology	References
Fucus vesiculosus (paddy tang, red fucus, dyers fucus, swine tang, sea ware, bladder, rockweed, bladderwrack, popping wrack, wrack)	Paired bladders occur on either side of a prominent midrib. Frond is generally not strongly spiraled and receptacles do not have a sterile rim, and frond does not have a serrated margin. Attached by a small, strong disc which gives rise to a short stipe. This species is 15 to 90 cm long and 0.6 to 2.5 cm wide. Reproductive receptacles are swollen areas at tips of fronds that have many flask-shaped cavities called conceptacles, which house male and female reproductive structures known as antheridia (borne on antheridiophores) and oogonia (containing 8 eggs), respectively. Eggs and sperm are liberated onto surface of receptacles and a pheromone (sex-attracting substance) is released by eggs that attract sperm. Fertilization results in a zygote that forms a new *Fucus* adult.	North Atlantic (Canadian Arctic, Russia, White Sea, Baltic Sea) south to Canary Islands and West Indies Midshore zone A bladderless form occurs on more wave-exposed shores in the NE Atlantic. Grows in various conditions, from saline lagoons to exposed rocky shores, as well as on sheltered rocky shores. Forms dense canopies.	151-153,155
Halidrys siliquosa (podweed, sea oak)	Thallus 30-130 cm long, tawny to yellow-brown ochre, tough and leathery; attached by a large, discoid holdfast, giving rise to compressed, irregularly alternately branched fronds, with several orders of close branching in the same plane. Pod-shaped, segmented air bladders are produced replacing some lateral branches. Reproductive conceptacles forming in swollen conceptacles at apices of branches	Northeast Atlantic (Norway/Baltic Sea to Morocco) Large, mid-intertidal pools, often dominating in very large, sunny pools, but more often forming occasional stands. Occasionally forming extensive forests in shallow subtidal to about 10 m, generally in current-exposed locations. Widespread and common. Halidrys produces meroditerpenoids that seemingly act as antifouling agents preventing other organisms adhering to surface of the algae.	151,152
Halopteris scoparia (sea flax weed)	*Stypocaulon scoparium* may be synonymous	Northwest Atlantic (Baltic Sea to Canary Islands) and Mediterranean Sea	151
Himanthalia elongata (thongweed, buttonweed, sea spaghetti, sea thong, sea haricots)	Long thong-like fronds, basal mushroom-like buttons. Thallus consisting of a button-shaped vegetative thallus to 30 mm wide and 25 mm high, and a long, narrow, strap-like, sparingly branched, light yellow-brown reproductive receptacle to 2 m in length and up to 10 mm in width, on which conceptacles are borne. Buttons, initially club-shaped but later mushroom-like, develop from zygotes in late summer, mature in winter, and begin to form reproductive receptacles in January/February. Some 4-6 dichotomies are produced at this stage, and fronds then elongate and thicken, developing no further branches, and become reproductively mature in July-September.	Northwest Atlantic Ocean (Scandinavia to Spain) Gently sloping rocks, particularly on semi-wave-exposed shore, on which they may form a distinct zone at low water. Sparse populations sometimes develop in sheltered lagoons where thealgae are more yellow and less flattened.	151,152
Laminaria cloustoni [*Laminaria hyperborea*] (kelp, may weed, kelpie, liver weed, mirkle, pennant weed, strapwrack, cuvie, tangle, split whip wrack, sea rods, forest kelp, northern kelp)	Dark brown, to 2 m in length; with a claw-like, conical holdfast, a rough, rigid stipe which generally rises up out of the water, and is covered in epiphytes when older, and a laminate blade to 1.5 m long dividing into finger-like segments. Stipe is rugose (rough) when older, circular in cross-section, and snaps easily when bent; the holdfast is conical.	Northwest Atlantic Ocean (Scandinavia to Spain) Common at extreme low water in wave-exposed areas, and in the subtidal in optically clear water growing on rock to a depth of 32 m. Forms extensive closed communities at depths of 0-24 m. There are usually large quantities of ephytic red algae growing on the older stipes; the old fronds are cast off in spring and new ones grow below for a time.	151,152
Laminaria digitata (kelp)	Dark brown, to 2 m in length; with a claw-like holdfast, a smooth, flexible stipe, and a laminate blade to 1.5 m long split into finger-like segments. The stipe is oval in cross-section, and does not snap easily when bent. Underwater algae are more golden in color in sunlight.	North Atlantic (Arctic Canada/ Baltic Sea/Russia to Spain and New England) Very common in lower intertidal and shallow subtidal growing on rock. May form extensive meadows at low tide.	151,152

Table 5. General characteristics and geographic distribution of brown algae species

Species (common name)	Description	Distribution/Habitat/Ecology	References
Laminaria hyperborea (kelpie, liver weed, mirkle, pennant weed, strapwrack, cuvie, tangle, split whip wrack)	Dark brown, to 2 m in length; with a claw-like, conical holdfast, a rough, rigid stipe which generally sticks up out of the water, and is covered in epiphytes when older, and a laminate blade to 1.5 m long dividing into finger-like segments. Stipe is rugose (rough) when older, circular in cross-section, and snaps easily when bent; the holdfast is conical.	Northeast Atlantic (Scandinavia/Iceland to Spain and Canary Islands) Common at extreme low water in wave-exposed areas, and in subtidal in optically clear water growing on rock to a depth of 32 m. Forms extensive closed communities at depths of 0-24 m; there are usually large quantities of ephytic red algae growing on the older stipes; the old fronds are cast off in spring and new ones grow below for a time.	151,152,156
Laminaria saccharina [The accepted scientific name is *Saccharina latissima*] (sea belt, poor man's weather glass, sweet wrack, sugar wrack, sugar tang, oarweed, tangle, kelp, sugar sea belt, sweet tangle, sugarwrack, zuckertang)	Yellow brown, to 3 m in length; with a claw-like holdfast, a small, smooth, flexible stipe, and an undivided laminate blade to 3 m long with parallel, ruffled sides and a elongated, tongue-like appearance. Frond is characteristically dimpled with regular bullations (depressions). Stipe is relatively small, cylindrical in section and more flexible than those of *Laminaria digitata* and *Laminaria hyperborea*. It is only species in the NE Atlantic Ocean with an undivided frond, distinct bullations, and a frilly margin.	Circumboreal (Atlantic Ocean: Canada, Scandinavia, Greenland, Iceland to Galicia, Spain and Maine, but not known in the Bay of Biscay; Pacific Ocean: Alaska to California, Japan, Korea, Central Polynesia, India, New Zealand) Intertidal pools and occasional in shallow subtidal areas, becoming more abundant at low water in sheltered localities with fast-moving water, such as rapids systems. In subtidal, it is characteristic of intermittently disturbed areas.	151,152
Macrocystis pyrifera (giant kelp, sea ivy, giant pacific kelp)	This species reaches 45 meters long and grow in waters 6-20 (possibly up to 80) m deep, and grow at up to 30 cm per day. Now believed to be a monospecific genera ranging from intertidal to deep water with environments dictating morphology.	Eastern and southern Pacific Ocean in both hemispheres (Alaska to New Zealand and Australia) Dominant canopy-forming algae in southern and central California.	151,157,158
Pelvetia canaliculata (channeled wrack, cow tang)	This species is 80-120 mm long, yellow-brown in color, turning black when dry, and often so dry that fronds disintegrate when trodden upon; regularly dichotomously branched with a distinct channel on underside (side nearest rock), which holds moisture and apparently helps algae survive at very high levels on shore. Reproduction in conceptacles visible as dots on warty terminal receptacles. Usually infected by a fungus which may assist in allowing it to survive high in intertidal zone.	NE Atlantic from the Faroe Islands to Portugal Occurring very high on shore, generally above mean high water neap tides, on wave-exposed and sheltered shores, but absent from very exposed rocky shores.	151-153
Sargassum muticum	Thallus bushy, elongated, yellowish-tawny to dark brown, generally to 4 m long;tough, cylindrical, repeatedly alternately pinnately branched to the third or fourth order; whorls of distinctly flattened sculpted leaves at the base (resembling the leaves of Holly); with characteristic rounded-elliptical air bladders above and below, formed terminally. Reproductive receptacles below, formed in the axils of spiny leaves; spectacularly fecund. Basal holdfast penetrating and conical, persisting for several years. Reproductive plants detach easily, and continue to reproduce while drifting, and spreading the reproductive zygotes that develop on the surfaces of the receptacles. Terminal air bladders below; receptacles in the axils of spiny leaves.	Native to Japan; spread to China and Korea. Invasive in France, Spain and Portugal; western Mediterranean; Alaska south to Mexico. Throughout the intertidal in pools, but largest and commonest at low water.	151,152

Table 5. General characteristics and geographic distribution of brown algae species

Species (common name)	Description	Distribution/Habitat/Ecology	References
Undaria pinnatifida (sea mustard, precious sea grass, wakame)	Thallus laminate, yellowish to dark brown, usually 1-2 m, occasionally 3 m or more in length; holdfast spreading, dichotomously branched and claw-like, giving rise to a flattened oar-like stipe with a "fried-egg" like margin with small proliferations and basally with beautifully lobed sporophylls that coil around it when mature; stipe continuing into the fond as a flattened midrib that bears broadly lobed laciniate fronds with a roughly pyramidal shape. Frilly sporophylls coiling around the base of the flattened stipe at the base. A similar flattened midrib is not found in any other kelp in the Atlantic. *Alaria esculenta* has a midrib which is not flattened and the frond of *Alaria* is not lobed, although it may be similarly laciniate.	Native to Pacific Russia, Japan, China and Korea. NE Ireland, S England, NW France, NW Spain, Mediterranean Lower intertidal and very shallow subtidal (no more than a few m), particularly in sheltered locations, growing particularly on marinas, buoys, and similar floating structures in harbors. Often occurring on boat-hulls.	[151]

Table 6. Chemical and physical properties of brown algae-derived ingredients

Property	Value	Reference
Ascophyllum Nodosum Extract		
Physical Form	Liquid	159,160
	Viscous liquid	161
	Solid flakes	6
Color	Black	6,159
	Dark brown	160
	Dark brown (aq. ext)	161
Odor	Marine-like/Fish-like	159,160
	Characteristic, seaweed (aq. ext)	161
	Odorless	6
Density/Specific Gravity	1.17	159
	1.1 (aq. ext.)	161
Bulk Density (g/mL)	0.58	6
Viscosity kg/(s m)	< 0.1	159
Melting Point ^{o}C	0 (aq. ext.)	161
	> 300	6
Boiling Point ^{o}C	100	159
	100 (aq. ext.)	161
	65 – 96	160
Water Solubility g/L @ 20 ^{o}C & pH 7.4 – 7.5	> 10,000	6
@ 20 ^{o}C	100%	159,160
	100%	161
Other Solubility g/L		
Acetone @ 22 ^{o}C	0.007	6
Ethyl acetate @ 22^{o}C	0.009	6
Methanol @ 22 ^{o}C	0.251	6
log P_{ow}	-3.3 est.	5,6
Particle size	> 0.250 mm, 93.5%	6
	< 0.045 mm, none	
Ascophyllum Nodosum Powder		
Physical Form	Flakes or powder	162
	Powder	163
Color	Olive green	162
	Green	163
Odor	Marine-like	162
	Characteristic, fish-like	163
Water Solubility g/L	Insoluble	162
Ecklonia Cava Extract		
Physical Form	Powder (alcohol ext)	9
Color	Brown (alcohol ext)	9

aq. = aqueous; ext. = extract

Table 7. Methods of manufacture for brown algae-derived ingredients

Ingredient (characterization)	Method of Manufacture	Reference
Alaria Esculenta Extract	trade name mixture consisting of Alaria Esculenta Extract in butylene glycol and water: harvesting/identification → washing → grinding → extraction with the solvents and butylene glycol and water → filtration → quality control → packaging → quality control	19
Alaria Esculenta Extract	trade name mixture consisting of Alaria Esculenta Extract in butylene glycol and water – dried before extraction: harvesting/identification → washing → drying → grinding → extraction with the solvents butylene glycol and water → filtration → quality control → packaging → quality control	19
Alaria Esculenta Extract	trade name mixture containing Alaria Esculenta Extract in Caprylic/Capric Triglycerides: harvesting/identification → drying → grinding → extraction with solvent caprylic/capric triglyceride → filtration → quality control → packaging → quality control	20
Ascophyllum Nodosum Extract	A trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water, reported a manufacturing process consisting of grinding the algae, extracting the water, fucoidan purification and ultrafiltration.	21
Ascophyllum Nodosum Extract	The species *Ascophyllum nodosum* is grinded, extracted by water, then undergoes fucoidan purification and ultrafiltration.	22
Cladosiphon Okamuranus Extract (high in fucoidan)	*Cladosiphon okamuranus* is hydrolyzed in 0.05 M or 0.5 M hydrochloric acid at 80°C for 30 min and then is neutralized with sodium hydroxide. Salt is removed by electrodialysis and then hydrolysate is lyophilized.	47
Cystoseira Tamaricifolia Extract	Cystoseira Tamaricifolia Extract and Caprylic/Capric Triglycerides: extraction with supercritical carbon dioxide	49
Dictyopteris Polypodioides Extract (high fractions of C_{11} hydrocarbons and sulfur compounds)	Air-dried algae material is extracted with diethyl ether. Solvent is removed vacuum distillation leaving a crude concrete extract. Crude extract is treated with hydrodistillation followed by liquid-liquid extraction with diethyl ether to obtain the essential oil.	23
Dictyopteris Polypodioides Extract (high fraction of sulfur compounds)	Air-dried algae material is extracted with diethyl ether. Solvent is removed by vacuum distillation leaving a crude concrete extract. Crude extract is then subjected to supercritical fluid (CO_2) extraction.	23
Dictyopteris Polypodioides Extract (high fractions of sesquiterpenes)	Air-dried algae material is extracted with diethyl ether. Solvent is removed vacuum distillation leaving a crude concrete extract. Crude extract is mixed with water and irradiated in a microwave oven (focused microwave-assisted hydrodistillation).	23
Ecklonia Cava Extract	Fresh, semidried *Ecklonia cava* seaweed is dried and crushed followed by alcohol (i.e., food-grade ethanol) extraction, purification, filtration, and concentration steps.	9
Ecklonia Cava Extract	Small pieces of *Ecklonia cava* fronds (~ 5 cm; 30 kg) are placed in 750 L of distilled water in the presence of enzymes (300 g pectinase and 300 g cellulase). Suspension is stirred for 24 h at 50°C, centrifuged at 3000 g for 20 min at 4°C, and vacuum filtered. Three volumes of 60% ethanol are then added for 18 h of extraction. Solution is filtered and concentrated using a rotary evaporator. Concentrated solution is made into powder using a spray dryer.	91
Ecklonia Cava Extract (high in polyphenols)	Dried *Ecklonia cava* powder is extracted with ethanol, concentrated, and freeze-dried.	24
Fucus Spiralis Extract	trade name mixture containing Fucus Spiralis Extract ("1 - 3% dry extract" (further details not provided)) in butylene glycol and water: harvesting/identification → washing → grinding → extraction with the solvents butylene glycol and water → addition of phenyllactic acid → filtration → quality control → packaging → quality control	25
Fucus Vesiculosus Extract	trade name mixture containing water, alcohol and Fucus Vesiculosus Extract: dried raw material → extract with 30% ethanolic solution → filtrate → concentration → filtrate → packaging	26
Fucus Vesiculosus Extract	trade name mixture containing sodium sulfate and Fucus Vesiculosus Extract: dried raw material → extract with 30% ethanolic solution → filtrate → concentration → add anhydrous sodium sulfate → packaging	26
Fucus Vesiculosus Extract	trade name mixture containing Fucus Vesiculosus Extract in caprylic/capric triglyceride: harvesting/identification → washing → grinding → extraction with the solvent caprylic/capric triglyceride → filtration → quality control → packaging → quality control	27
Fucus Vesiculosus Extract (28.8% polyphenols)	Ethanol (30% - 35% aq.) extraction of *Fucus vesiculosus* (10% w/w) is performed at room temperature under mechanical stirring for 4 h. After filtration on a filter press, liquid phase undergoes an initial purification step to remove alginates by precipitation in presence of excess calcium chloride. Liquid phase undergoes a second purification step involving diafiltration to remove iodine and low molecular weight compounds. Extract is freeze-dried to obtain a powder extract.	89
Fucus Vesiculosus Extract (18% polyphenols plus 0.0012% fucoxanthin)	Ethanol (50% - 70% aq.) extraction of *Fucus vesiculosus* (10% w/w) is performed to solubilize a greater amount of carotenoids at room temperature under mechanical stirring for 2 h. After filtration on a filter press, liquid phase undergoes an initial purification step to remove alginates by precipitating them in presence of excess calcium chloride. After solid-liquid separation, a second extraction is performed under same conditions. Two liquid phases are then blended, submitted to diafiltration to remove iodine and low molecular weight compounds, and freeze-dried to obtain a powder extract.	89
Fucus Vesiculosus Extract	Dried algae material is extracted with water for 24 h, with stirring at room temperature. Residue is then removed by filtration to give a slightly brown colored extract.	44
Hizikia Fusiforme Extract	trade name mixture containing water butylene glycol and Hizikia Fusiforme Extract: dried raw material → extract with 80% ethanolic	26

Table 7. Methods of manufacture for brown algae-derived ingredients

Ingredient (characterization)	Method of Manufacture	Reference
	solution → filtrate → concentration → add 50% 1,3-butylene glycolic solution → filtrate → packaging	
Laminaria Digitata Extract (high in oligosaccharides)	An aqueous extraction is conducted followed by enzymatic depolymerization that breaks the polysaccharide into oligosaccharides (e.g., smaller polymers with 3 to 10 sugar components). Final process involves chelating oligosaccharide with zinc sulfate (0.1% zinc-pyrrolidone).	29
Laminaria Digitata Extract	trade name mixture containing Laminaria Digitata Extract in caprylic/capric triglyceride: harvesting/identification → washing → drying → grinding → extraction with the solvent caprylic/capric Triglyceride → filtration → quality control → packaging → quality control	28
Laminaria Digitata Extract	trade name mixture containing Laminaria Digitata Extract in water and propylene glycol: harvesting/identification → washing → grinding → extraction with the solvents water and propylene glycol → addition of methylparaben and propylparaben → filtration → quality control → packaging → quality control	30
Laminaria Hyperborea Extract	trade name mixture containing Laminaria Hyperborea Extract in water: harvesting/identification → washing → grinding → extraction with water → addition of benzylic alcohol and dehydroacetic alcohol → filtration → quality control → packaging → packaging → quality control	31
Laminaria Japonica Extract (low-molecular weight fucoidan)	Enzyme hydrolysis	52
Laminaria Japonica Extract	Algae is rinsed with tap water to remove salt and dried in an air dryer at 60°C for 40 h. Dried material is ground with a hammer mill, and powder stored at −20°C until used. Dried powder (2.5 kg) is extracted 3 times with 96% (v/v) ethanol for 3 h at 70°C. Combined extracts are filtered and concentrated under reduced pressure to obtain ethanol extracts	46
Laminaria Japonica Extract	Freshly collected algae material is air dried with a fan for 24 h then ground into a fine powder. 5 g of powder is added to 100 mL of 1:1 water:propylene glycol at room temperature for 1 day. This procedure is repeated 2 times, and the combined extracts were stored at −20°C until use.	51
Laminaria Japonica Extract, Nereocystis Leutkeana, and Macrocystis Pyrifera	trade name mixture containing Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract: test of acceptance → processing (mechanical grinding/milling) → extraction with pentaerythrityl tetraethylhexanoate at specific pH and temperature for specific duration → filtration → batch adjustments (refiltration) → sample for QC → pack → sample for Micro → shipping	32
Laminaria Japonica Powder	Dried algae is pulverized to desired size.	48
Laminaria Ochroleuca Extract	trade name mixture consisting on Laminaria Ochroleuca extract in Caprylic/Capric Triglyceride: harvesting/identification → washing → grinding → extraction with the solvent caprylic/capric triglyceride → filtration → quality control → packaging → quality control	33
Laminaria Saccharina Extract	trade name mixture containing Laminaria Saccharina Extract in water and propylene glycol: harvesting/identification → washing → grinding → extraction with solvents: water + propylene glycol → mixture (addition of preservatives) → filtration → quality control	34
Laminaria Saccharina Extract	trade name mixture containing Laminaria Saccharina Extract ("1-2.5% dry extract" (no other details provided)) in water and butylene glycol: harvesting/identification → washing → grinding → extraction with the solvents water and butylene glycol → mixture → addition of preservatives → filtration → quality control	34
Macrocystis Pyrifera Extract	Macrocystis Pyrifera Extract ("1-3% dry extract (no other details provided)) – extracted in water with added methylpropanediol: harvesting → washing → grinding → extraction (water) → centrifugation → filtration → addition of 20% Methylpropanediol → filtration	35
Pelvetia Canaliculata Extract	trade name mixture containing Pelvetia Canaliculata Extract ("1 - 3% dry extract" (no other details provided)) in butylene glycol and water: harvesting/identification → washing → drying → grinding → extraction with the solvents vegetable butylene glycol and water → filtration → quality control → packaging → quality control	36
Pelvetia Canaliculata Extract	trade name mixture containing Pelvetia Canaliculata Extract ("1 - 3% dry extract" (no other details provided)) in water and propylene glycol: harvesting/identification → washing → grinding → extraction with the solvents water and propylene glycol → addition of methylparaben and propylparaben → filtration → quality control → packaging → quality control	36
Pelvetia Canaliculata Extract	trade name mixture containing Pelvetia Canaliculata Extract ("0.5 - 3% dry extract" (no other details provided)) in water: harvesting/identification → washing → grinding → extraction with water → addition of benzylic alcohol and dehydroacetic acid → filtration → addition of trisodium citrate dehydrate → filtration → quality control → packaging → quality control	37
Pelvetia Canaliculata Extract	trade name mixture containing Pelvetia Canaliculata Extract in water: harvesting/identification → washing → grinding → extraction with water → addition of phenoxyethanol and sorbic acid → filtration → quality control → packaging → quality control	38
Pelvetia Canaliculata and Laminaria Digitata Extract	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in propylene glycol with panthenol: harvesting/identification → washing → grinding → extraction with the solvent propylene glycol → filtration → quality control → mixture → filtration → quality control → packaging → quality control	39
Pelvetia Canaliculata and Laminaria Digitata Extract	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in butylene glycol with preservatives: harvesting/identification → washing → grinding → extraction with butylene glycol → filtration → quality control → mixture → filtration → quality control → packaging → quality control	39

Table 7. Methods of manufacture for brown algae-derived ingredients

Ingredient (characterization)	Method of Manufacture	Reference
Pelvetia Canaliculata and Laminaria Digitata Extract	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in butylene glycol without preservatives: harvesting/identification → washing → grinding → extraction with butylene glycol → filtration → quality control → mixture → filtration → quality control → packaging → quality control	40
Sargassum Fusiforme Extract and Undaria Pinnatifida Extract (high in fucosterol and phytol)	Microwave-assisted extraction coupled with high-speed countercurrent chromatography.	41
Sargassum Fusiforme Extract and Undaria Pinnatifida Extract (high in lipids and antioxidant compounds)	Supercritical fluid extraction and subcritical water extraction.	41
Sargassum Glaucescens Extract	trade name mixture containing 20% Sargassum Glaucescens Extract, 79% water and 1% phenoxyethanol: grinding → extraction → preservative addition → sterilization → filtration → packaging → storage	125
Unidaria Pinnatifida Extract (high in fucoidan)	Algae material is hydrolyzed in 0.05 or 0.5 M hydrochloric acid at 80°C for 30 min then neutralized with 1 M sodium hydroxide. Resulting material is desalted by gel filtration and hydrolysate lyophilized.	65
Undaria Pinnatifida Extract	trade name mixture containing Undaria Pinnatifida Extract in water and propylene glycol: harvesting/identification → drying → grinding → extraction with solvents water and propylene glycol, and addition of preservatives (methylparaben and propylparaben) → filtration → quality control → packaging → quality control	43
Undaria Pinnatifida Extract	trade name mixture containing Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride: harvesting of fertile sporophytes → fragment isolation of gametophyte → culture in liquid medium → gametophyte separation → freeze-dried gametophyte -→ quality control → extraction with the solvent caprylic/capric triglyceride → filtration → quality control → packaging → quality control	42

Abbreviations: aq. = aqueous; HPLC = high-performance liquid chromatography

Table 8. Constituents in brown algae

Constituent(s)	Description
Alkaloids	Tyramine (TYR, 4-hydroxyphenylethylamine) has been detected in *Laminaria saccharina*.[164] The alkaloids found in marine algae may be divided into three groups: phenylethylamine alkaloids, indole and halogenated indole alkaloids, and other alkaloids.
Amino acids	Brown algae contain all of the essential amino acids and are greater in threonine, valine, leucine, lysine, glycine, and alanine than are the green and blue algaes.[41] *Fucus spiralis* was reported to contain 63.5% essential amino acids per total protein, containing leucine (5.5 mg/g protein), isoleucine (15.3 mg/g protein), lysine (12.5 mg/g protein), glutamic acid (12.1 mg/g protein), arginine (11.7 mg/g protein), serine (11.5 mg/g protein), valine (11.1 mg/g protein), and threonine (10.9 mg/g protein).[165]
Betaines	Glycinebetaine, γ-aminobutyric acid betaine, and/or trigonelline have been found in Alaria esculenta, Ecklonia maxima, Ecklonia radiata, Eisenia arborea, Laminaria digitata Macrocystis pyrifera, Nereocystis luetkeana, Saccharina angustata, Saccharina japonica, and Undaria pinnatifida.[166]
Iodine	The concentration of iodine in *Alaria esculenta* was reported to have a range of approximately 200 mg/kg (dry wt) to approximately 700 mg/kg (dry wt) depending on year, season, location, and whether it was collected in the wild, a monoculture, or an integrated culture.[167] *Fucus vesiculosus* contains between 0.03% and 0.2% iodine in dried material.[168] The iodine content is highest in the spring in freshly cut young blades. In *Laminaria digitata*, iodine content is highest in late autumn and winter (0.75% to 1.20% dry wt) and lowest in summer (0.25% to 0.60% dry wt).[169] Iodine content for *Fucus spiralis* and *Laminaria ochroleuca* have been reported to be 232.7 and 883.5 mg/kg dry wt.[165]
Laminarins	Laminarins are basically a class of low molecular weight storage β-glucans. These are composed of (1,3)-β-D-glucan and can be up to 35% of the dry weight of brown algae.[170]
Lipids	Fucosterol and fucosterol derivatives are present in brown algae.[41] Fucoxanthine, tocopherols, and sterols are also found in brown algae.
Omega-3 fatty acids	Omega-3 fatty acids include stearidonic acid and hexadecatetraenoic acid.[171] These make up to 40% of the total fatty acid content in *Undaria pinnatifida*.
Phenolic compounds, polyphenols, and phlorotannins	Phlorotannins are found in brown algae.[41] Flavonoids are integral structural components of cell walls (e.g., eckol, phlorofucofuroeckol A, dieckol, catechin, and epigallocatechin).
Pheromones	The pheromones include lamoxirene 4 (e.g., Agarum cribrosum, Ecklonia radiata, Eisenia arborea, Laminaria digitata, Laminaria hyperborea, Laminaria japonica, Laminaria saccharina, Saccharina angustata, Undaria pinnatifida, Macrocystis pyrifera, and Nereocystis luetkeana), fucoserratene 6 (e.g., Fucus serratus, Fucus spiralis, and Fucus vesiculosus), hormonsirene 8 (e.g., Durvillaea antarctica), and finavarrene 12 (Ascophyllum nodosum). The major constituents of the essential oil of Dictyopteris polypodioides are C_{11} hydrocarbons sulfur products such as 3-hexyl-4,5-dithiacycloheptanone.[23]
Phytohormones	Auxins (plant hormones that cause the elongation of cells in shoots and are involved in regulating plant growth), such as indoleacetic acid are found in the genera *Macrocystis*, *Laminaria*, *Fucus*, *Ascophyllum*).[41,172] Cytokinins (genera *Fucus*, *Ascophyllum*, *Sargassum*, *Macrocystis*), gibberellins (genus *Fucus*), abscisic acid (genera *Ascophyllum*, *Laminaria*), and polyamines (genus *Dyctiota*) are also found.
Pigments	Carotenoids including fucoxanthin, β-carotene, zeaxanthin, violaxanthin, and antheraxanthin are found in brown algae.[41] These vary with season.

Table 8. Constituents in brown algae

Constituent(s)	Description
Protein	The protein content of algae varies according to species and season.[14,41] In general, the protein fraction of brown algae is low (1% to 24% dry wt.) compared with that of green or red algae (4% to 50% dry wt). Except for the species *Undaria pinnatifida*, which has a protein content between 11% and 24% (dry wt.), most commercial brown algae have a protein content lower than 15% (dry wt; e.g., *Ascophyllum nodosum*, 3% to 15%; *Fucus vesiculosus*, *Himanthalia elongate*, and *Laminaria digitata*, 8% to 15%). The protein content of *Fucus* sp. tend to range from 3% to 11% (e.g., *Fucus spiralis*, 9.71% dry weight).[165]
Sterols	Sterols found in brown algae include desmosterol, ergosterol, fucosterol, cholesterol, campesterol, stigmasterol, and β-sterol.[59,60]
Terpenoids	Terpenes, phenolic compounds, and meroterpenes make up the three major classes of secondary metabolites in brown seaweed.[41]

Table 9. Constituents in *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Laminaria digitata*

	Ascophyllum nodosum (ppm) [173]	*Fucus vesiculosus* (ppm) [174]	*Fucus vesiculosus* (ppm) [173]	*Laminaria digitata* (ppm) [29]
Algin	NR	41300 – 500000	NR	
Alginic acid	NR	NR	NR	200000 – 450000
Aluminum	NR	75.0 - 631.0	NR	
Arsenic	NR	68.0	NR	
Ascorbic-acid	NR	30.0 - 258.0	NR	
Bromine	NR	150.0	NR	
Calcium	9847	3587 – 30400	11600	
Carbohydrates	NR	77290 – 655000	NR	10000 – 20000
β-carotene	NR	5.0 – 40.0	NR	
Chromium	NR	0.1 – 0.7	NR	
Cobalt	NR	0.2 – 1.6	NR	
Fat	NR	3540 – 30000	NR	10000 – 20000
Fiber	NR	98000	NR	
Fiber(crude)	NR	98000	NR	
Fiber(dietary)	NR	482000	NR	
Fucinicacid	NR	1000	NR	
Fucoidin	NR	600000	NR	20000 – 40000
Fucose	NR	240000	NR	
Iodine	NR	64.0 – 540.0	NR	3000 – 1100
Iron	133.4	2.0 – 16.0	189.9	
Kilocalories	NR	2490	NR	
Lead	NR	91.0	NR	
γ-Linolenic acid	NR	NR	NR	
Magnesium	8678	1023 – 8670	7320	5000 – 8000
Mannitol	NR	NR	NR	40000 – 160000
Manganese	19.6	0.9 – 7.6	82.8	
Mercury	NR	40.0	NR	
Niacin	NR	6.0 – 47.0	NR	
Phosphorus	NR	294.0 -2490	1935.7	
Potassium	37810	2490 – 21,100	37450	13000 – 38000
Selenium	NR	0.2 – 1.7	NR	
Silicon	NR	0.9 – 7.6	NR	
Sodium	45757	6620 – 56,100	21875	9000 – 22000
Sugars	NR	2360 – 20000	NR	
Tin	NR	3.0 – 24.0	NR	
Water	NR	882000	NR	730000 – 900000
Zinc	NR	0.1 – 0.6	NR	

NR = not reported

Table 10. Sterols in several brown algae

Species	Desmosterol (mg/kg)	Ergosterol (mg/kg)	Fucosterol (mg/kg)	Cholesterol (mg/kg)	Campesterol + Stigmasterol (mg/kg)	β-Sterol (mg/kg)	Brassicasterol (mg/kg)	Ssaringosterol (mg/kg)	24-ketocholesterol (mg/kg)	Total[a] (mg/kg)	Reference
Cystoseira tamariscifolia	44.1 ± 3.4	-	5260.2 ± 14.9	500.4 ± 2.6	680.9 ± 21.4	17.0 ± 0.3	NR	NR	NR	6502.6	[60]
Fucus spiralis	37.6 ± 3.8	-	3815.1 ± 329.5	325.1 ± 13.5	183.4 ± 0.3	-	NR	NR	NR	4361.0	[60]
Sargassum vulgare	47.2 ± 0.2	5.6 ± 0.4	4451.5 ± 16.7	406.3 ± 13.2	303.3 ± 18.9	15.2 ± 2.8	NR	NR	NR	5229.1	[60]

NR = not reported; - = not found

[a] Total may not be exact due to rounding.

Table 11. Constituents of ethanol extracts of *Fucus spiralis* and *Sargassum vulgare*[62]

	Range (if provide; ppm)	
Constituent	***Fucus spiralis* extract**	***Sargassum vulgare* extract**
Arachidic Acid	ND	ND
Arachidonic Acid	465.6 ± 29.0	ND
Cholesterol	ND	127.4 ± 11.6
Eicosapentaenoic Acid	217.0 ± 11.4	ND
Fucosterol	317.6 ± 9.4	257.6 ± 43.6
γ-Linolenic Acid	ND	2413.6 ± 57.6
Mannitol (Total)	1273.8 ± 34.8	394.6 ± 15.2
Myristic Acid	69.8 ± 2.7	ND
Palmitic Acid	606.0 ± 20.6	340.4 ± 95.0
Phloroglucinol	< LOD	ND
Proline	396.8 ± 96.8	117.4 ± 11.0
β-Sitosterol	ND	ND
Stearic Acid	208.4 ± 21.4	204.0 ± 26.0
Vaccenic Acid	21,690.6 ± 1667.6	2848.6 ± 71.2

LOD = limit of detection; ND = not detected

Table 12. Composition of a 50/50 water/propylene glycol extract of *Laminaria japonica*[51]

Constituent	**Amount**
Constituent Groups (mg/g)	
Carbohydrate	6
Sugars	5
Proteins	2
Crude fat	2
Saturated fatty acid	1
Unsaturated fatty acid	None detected
Amino Acids (mg/L)	
Alanine	42.3
Ammonium chloride	16.2
Arginine	20.3
Aspartic acid	424.7
Glutamic acid	689.4
Glycine	1.7
Hydroxyproline	381.4
Phosphoserine	3.7
Serine	8.6
Threonine	4.2
Minerals (mg/g)	
Sodium	404
Calcium	300
Potassium	1022
Magnesium	35
Iron	0.5
Zinc	0.2

Table 13. Composition of enzyme hydrolysis extracts of *Laminaria japonica*[52]

Constituent	Concentration (% w/w)
	***Laminaria japonica* extract[52]**
Ash	4.1 ± 0.1
Fat	0.6 ± 0.1
Fucose	85.9
Moisture	3.9 ± 0.8
Monosaccharides (neutral)	NR
Protein	4.3 ± 0.3%
Sulfate	28.4 ± 2.1

NR = not reported

Table 14. Specifications of an alcohol extract of *Ecklonia cava* for use as a food supplement[9]

Parameter	Specification
Phlorotannin	90 ± 5.0%
Dieckol	6.6% – 9.9%
Moisture content	< 5%
Ash	< 5%
Insoluble substances	Negative
Substances not originating from *E. cava*	Negative
Viable cell count	< 3000 CFU/g
Staphylococcus aureus	Negative
Molds and yeasts	< 300 CFU/g
Salmonella spp.	Negative
Coliforms	Negative
Lead	< 3 mg/kg
Mercury	< 0.1 mg/kg
Cadmium	< 3 mg/kg
Arsenic	< 25 mg/kg
Iodine	150.0 – 650.0 mg/kg
Sieving size	> 60 (0.250 mm)

CFU = colony-forming unit

Table 15. Constituents of desalinated Undaria pinnatifida powder[66]

Constituent	Amount (mg/g)
Ash	147
Calcium	13.6
Copper	0.00130
Dietary fiber	532
Iron	0.107
Lipid	14
Magnesium	13.4
Protein	209
Sodium	25.4
Zinc	0.02

Table 16. Concentration of arsenic found in several brown algae species[54]

Species	Arsenic Concentration (mg/kg wet wt.)	Arsenic Concentration (mg/kg dry wt.)
Ecklonia radiate	10[54]	-
Hizikia fusiforme	10[54]	-
Laminaria japonica	4[54]	-
Laminaria ochroleuca	-	56.8 ± 2.4[67]
Laminaria saccharina	-	52.4 ± 2.1[67]
Saccharina (spp)	-	< 0.3[175]
Sargassum fusiforme	-	67 - 96[175]
Sargassum thunbergii	4[54]	-
Unidaria pinnatifida	2.8 – 4.5[54]	< 0.3[175] 115 ± 9[67]

- = no data

Table 17. Arsenic -containing moieties found in various brown algae[67]

	Amount (mg/kg)			
Arsenic-Containing Moiety	***Laminaria ochroleuca***	***Laminaria saccharina***	***Sargassum fulvellum***	***Undaria pinnatifida***
Arsenic III	ND	ND	ND	ND
Arsenic V	ND	ND	69.9 ± 1.0	0.29 ± 0.03
Methylarsonate	ND	0.21 ± 0.03	ND	ND
Dimethylarsinate	0.26 ± 0.08	0.67 ± 0.02	2.1 ± 0.1	0.13 ± 0.03
Trimethylarsine oxide	ND	ND	ND	ND
Arsenobetaine	0.20 ± 0.02	0.09 ± 0.02	ND	ND
Phospate-sug po4	6.2 ± 0.1	6.9 ± 0.1	2.2 ± 0.1	0.30 ± 0.02
Sulfonate-sug so3	39.4 ± 1.6	30.7 ± 1.2	1.80 ± 0.10	ND
Sulfate-sug so4	ND	ND	9.0 ± 0.7	ND
Glycerol-sug gly	2.71 ± 0.04	2.9 ± 0.1	1.2 ± 0.2	0.87 ± 0.03
Arsenocholine	ND	ND	ND	ND
Inorganic arsenic	ND	ND	69.9	0.29

ND = not detected

Table 18. Arsenic species found in *Laminaria japonica* and an extract of *Laminaria japonica*[52]

	Amount (mg/kg)	
Arsenic Species	*Laminaria japonica*	*Laminaria japonica* extract[a]
Arsenic III	ND	ND
Arsenic V	ND	ND
Monomethylarsonic Acid	9.27 ± 0.96	1.35 ± 0.63
Dimethylarsinic Acid	9.23 ± 0.83	ND
Arsenobetaine	34.31 ±1.21	4.77 ± 0.88
Arsenocholine	6.19 ± 2.17	ND
Arsenic (sum)	59.00 ± 1.65	6.12 ± 2.005

ND = not detected

[a] Extracted by enzyme hydrolysis, high in low-molecular-weight fucoidan

Table 19. Heavy metals and arsenic in brown algae

	Concentration of heavy metals and arsenic (mg/kg dry weight)							Reference
Species	**Cadmium**	**Lead**	**Mercury**	**Copper**	**Zinc**	**Arsenic**	**Inorganic Arsenic**	
Alaria esculenta	0.22 – 7.9	0.2 – 1.9	< 0.005 - <0.071	0.39 - 4	7 - 45	<0.074 - 100	-	[176]
Fucus vesiculosus	1.7	11	-	12.7	89	13.5	-	[155]
Himanthalia elongata	0.310 – 0.326	0.203 – 0.259	0.008 – 0.016	1.14 – 1.25	48.5 – 48.7	32.9 – 36.7	0.166 – 0.245	[69]
Hizikia fusiforme	0.988 – 2.50	< 0.008[a] – 0.531	0.015 – 0.050	1.78 – 7.70	4.72 – 19.5	103 – 147	32.1 – 69.5	[69]
Laminaria spp.	0.085 – 1.83	< 0.008[a] – 0.460	0.001 – 0.005	0.91 – 2.50	10.3 – 23.2	51.7 – 68.3	0.052 – 0.443	[69]
Undaria pinnatifida	0.267 – 4.82	< 0.008[a] – 1.28	0.010 – 0.057	1.07 – 1.70	8.25 – 26.6	42.1 – 76.9	0.045 – 0.346	[69]

[a] Limit of detection.

spp. = multiple species

Table 20. Heavy metal and arsenic impurities in trade name mixtures containing brown algae species

	Concentration of heavy metals (ppm)							Reference
Trade name mixture	**Arsenic**	**Cadmium**	**Lead**	**Nickel**	**Silver**	**Iodine**	**Mercury**	
Alaria Esculenta Extract in butylene glycol and water	< 5	< 3	< 5	< 2	< 5	< 10	-	[177]
Alaria Esculenta Extract in butylene glycol and water – dried before extraction	< 5	< 3	< 5	< 2	< 5	< 10	-	[178]
Alaria Esculenta Extract in Caprylic/Capric Triglycerides	< 2	< 3	< 5	< 2	< 5	< 1	< 1	[179]
Cystoseira Amentacea/ Caespitosa/Brachycarpa Extracts	7.303	< 0.010	< 0.010	-	-	-	< 0.010	[101]
Cystoseira Tamaricifolia Extract and Caprylic/Capric Triglycerides	-	-	-	-	-	1	-	[49]
Fucus Vesiculosus Extract, water and alcohol	< 10	-	-	-	-	-	-	[180]
Fucus Vesiculosus Extract and sodium sulfate	< 10	-	-	-	-	-	-	[180]

Table 20. Heavy metal and arsenic impurities in trade name mixtures containing brown algae species

	Concentration of heavy metals (ppm)							Reference
Trade name mixture	**Arsenic**	**Cadmium**	**Lead**	**Nickel**	**Silver**	**Iodine**	**Mercury**	
Fucus Vesiculosus Extract in caprylic/capric triglyceride	< 2	< 3	< 5	< 2	< 5	< 1	-	181
Fucus Spiralis Extract, butylene glycol, water	< 2	< 3	< 5	< 2	< 5	< 10	-	182
Halidrys Siliquosa Extract in water	0.01	< 0.010	< 0.010	-	-	-	< 0.010	64
Himanthalia Elongata Extract, water, and dipropylene glycol	-	-	-	-	-	< 9	-	49
Himanthalia Elongata Extract, Undaria Pinnatifida Extract, and water	0.510	0.010	-	-	-	-	0.010	63
Hizikia Fusiforme Extract, water, and butylene glycol	<10	-	-	-	-	-	-	26
Laminaria Digitata Extract, water, and sea salt	1.5	-	-	-	-	62	-	49
Laminaria Digitata Extract, water, dipropylene glycol	2.37	-	-	-	-	87	-	49
Laminaria Digitata Extract and water	< 10	-	-	-	-	550 ± 150	-	49
Laminaria Digitata Extract and water	19.06	-	-	-	-	192	-	49
Laminaria Digitata Extract in caprylic/capric triglyceride	< 2	< 3	< 5	< 2	< 5	< 300	-	183
Laminaria Digitata Extract in water and propylene glycol	< 5	< 10	< 5	< 2	< 5	< 400	-	184
Laminaria Japonica Extract, Nereocystis Leutkeana Extract, and Macrocystis Pyrifera Extract	< 2	< 1	<10	-	-	-	-	185
Laminaria Hyperborea Extract	< 2	< 3	< 5	< 2	< 5	< 320	-	186
Laminaria Saccharina, water, and propylene glycol	< 2	< 3	< 5	< 2	< 5	< 200	< 1	187
Laminaria Saccharina Extract in water and propylene glycol	< 2	< 3	< 5	< 2	< 5	< 200	< 1	187
Laminaria Saccharina Extract in water and butylene glycol	< 2	< 3	< 5	< 2	< 5	< 200	< 1	188
Macrocystis Pyrifera in water and methylpropanediol	< 5	< 10	< 5	< 2	< 5	< 5	-	35
Pelvetia Canaliculata Extract in butylene glycol and water	< 3	< 3	< 5	< 2	< 5	< 10	-	189
Pelvetia Canaliculata Extract in propylene glycol and water	< 2	< 3	< 5	< 2	< 5	< 10	-	190
Pelvetia Canaliculata and Laminaria Digitata extracted in propylene glycol with panthenol	< 5	< 3	< 5	< 2	< 5	< 100	-	191
Pelvetia Canaliculata and Laminaria Digitata extracted in butylene glycol with preservatives	< 5	< 10	< 5	< 2	< 5	< 100	-	192
Pelvetia Canaliculata and Laminaria Digitata extracted in butylene glycol without preservatives	< 5	< 10	< 5	< 2	< 5	< 100	-	189
Phyllacantha Fibrosa Extract and water	11.35	-	-	-	-	140	-	49
Sargassum Glaucescens Extract, water, phenoxyethanol	< 2.5	-	< 1	< 230	-	-	-	193
Undaria Pinnatifida Cell Culture Extract	< 2	< 1	< 10	-	-	-	-	194
Sphacelaria Scoparia Extract	0.73	-	-	-	-	15	-	49
Undaria Pinnatifida Extract in water and propylene glycol	< 5	< 10	< 5	< 2	< 5	< 1	< 1	195
Undaria Pinnatifida Extract in caprylic/capric triglyceride	< 2	< 3	< 5	< 2	< 5	< 1	< 1	196

Table 21. Frequency of use according to duration and exposure of brown algae-derived ingredients[73-75]

Use type	# Uses	Max. Conc. (%)	Uses	Max. Conc.(%)	Uses	Max. Conc.(%)	Uses	Max.Conc. (%)
	Agarum Cribrosum Extract		**Alaria Esculenta Extract**		**Ascophyllum Nodosum Extract**		**Ascophyllum Nodosum Powder**	
Total/range	**NR**	**0.012**	**37**	**0.0005-0.05**	**120**	**0.0000004-0.2**	**4**	**NR**
Duration of use[a]								
Leave-on	*NR*	*0.012*	*37*	*0.0005-0.05*	*91*	*0.0000004-0.2*	*3*	*NR*
Rinse-off	*NR*	*NR*	*NR*	*0.0015*	*29*	*0.00004-0.0032*	*NR*	*NR*
Diluted for (bath) use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*1*	*NR*
Exposure type								
Eye area	NR	NR	11	NR	16	0.025-0.2	NR	NR
Incidental Ingestion	NR	NR	3	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	3[a]; 6[b]	0.0005[a]	23[a]; 44[b]	0.002[a]	2[a]	NR
Incidental Inhalation-Powder	NR	NR	6[b]	0.0015-0.05[c]	1; 44[b]	0.0000004-0.03[c]	NR	NR
Dermal Contact	NR	0.012	33	0.0005-0.05	104	0.0000004-0.2	4	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Non-Coloring	NR	NR	1	NR	13	0.00005-0.002	NR	NR
Hair- Coloring	NR	NR	NR	NR			NR	NR
Nail	NR	NR	NR	NR	3	0.000065-0.02	NR	NR
Mucous Membrane	NR	NR	3	NR	6	0.00004	1	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

	Cladosiphon Okamuranus Extract		**Dictyopteris Polypodioides Extract**[d]		**Durvillaea Antarctica Extract**		**Ecklonia Radiata Extract**	
Total/range	**9**	**0.005-0.05**	**1**	**0.01**	**NR**	**0.0001**	**NR**	**0.005-0.0051**
Duration of use								
Leave-on	*8*	*0.025-0.05*	*1*	*0.01*	*NR*	*0.0001*	*NR*	*0.0051*
Rinse-off	*1*	*0.005*	*NR*	*NR*	*NR*	*NR*	*NR*	*0.005*
Diluted for (bath) use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure type								
Eye area	1	0.025	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	0.01	NR	NR	NR	NR
Incidental Inhalation-Spray	3[a]; 3[b]	NR	1[b]	NR	NR	NR	NR	0.0051
Incidental Inhalation-Powder	3[b]	0.025[b]	1[b]	NR	NR	NR	NR	NR
Dermal Contact	9	0.005-0.05	1	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Non-Coloring	NR	NR	NR	NR	NR	NR	NR	0.0051
Hair- Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	0.001	NR	NR
Mucous Membrane	NR	NR	NR	0.01	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

	Fucus Serratus Extract		**Fucus Vesiculosus**		**Fucus Vesiculosus Extract**		**Fucus Vesiculosus Powder**	
Total/range	**8**	**0.00001-0.05**	**NR**	**0.0003-0.0051**	**287**	**0.00002-5**	**3**	**NR**
Duration of use								
Leave-on	*8*	*0.05*	*NR*	*0.00098-0.0051*	*201*	*0.000032-5*	*1*	*NR*
Rinse-off	*NR*	*0.00001-0.05*	*NR*	*0.0003*	*75*	*0.00002-5*	*2*	*NR*
Diluted for (bath) use	*NR*	*NR*	*NR*	*NR*	*11*	*0.0001-5*	*NR*	*NR*
Exposure type								
Eye area	8	0.05	NR	NR	6	0.01-5	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	0.0005	NR	NR
Incidental Inhalation-Spray	3[a]; 4[b]	NR	NR	0.00098	1; 90[a]; 82[b]	0.00018-0.12; 0.0001-0.1[a]	1[b]	NR
Incidental Inhalation-Powder	4[b]	0.05[c]	NR	NR	82[b]	0.000032-.05[c]	1[b]	NR
Dermal Contact	8	NR	NR	0.00098-0.0051	262	0.00002-5	3	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair- Non-Coloring	NR	0.000010	NR	0.0003	22	0.0001-5	NR	NR
Hair- Coloring	NR	NR	NR	NR	NR	0.0001-0.001	NR	NR
Nail	NR	NR	NR	NR	NR	0.02	NR	NR
Mucous Membrane	NR	NR	NR	NR	30	0.00002-5	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

Table 21. Frequency of use according to duration and exposure of brown algae-derived ingredients[73-75]

Use type	# Uses	Max. Conc. (%)	Uses	Max. Conc.(%)	Uses	Max. Conc.(%)	Uses	Max.Conc. (%)
	Himanthalia Elongata Extract		**Laminaria Cloustoni Extract**		**Laminaria Digitata Extract**		**Laminaria Digitata Powder**	
Total/range	**9**	**0.2**	**14**	**NR**	**235**	**0.00004-5**	**21**	**40**
Duration of use								
Leave-on	*7*	*0.2*	*10*	*NR*	*162*	*0.0001-5*	*2*	*40*
Rinse-off	*2*	*NR*	*4*	*NR*	*66*	*0.00004-5*	*16*	*NR*
Diluted for (bath) use	*NR*	*NR*	*NR*	*NR*	*7*	*0.1-5*	*3*	*NR*
Exposure type								
Eye area	1	NR	1	NR	15	0.0035-0.5	NR	NR
Incidental Ingestion	NR	NR	NR	NR	2	NR	NR	NR
Incidental Inhalation-Spray	1[a]; 4[b]	NR	4[a]; 4[c]	NR	1; 44[a]; 67[c]	0.0007; 0.0035-5[a]	1[b]	NR
Incidental Inhalation-Powder	4[b]	NR	4[c]	NR	3; 67[c]	0.0001-0.1[c]	1[b]	40[b]
Dermal Contact	7	0.2	14	NR	191	0.0001-5	15	40
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair- Non-Coloring	2	NR	NR	NR	36	0.0007-5	6	NR
Hair- Coloring	NR	NR	NR	NR	1	0.00004-0.0007	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	20	0.06-5	4	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

	Laminaria Hyperborea Extract		**Laminaria Japonica Extract**		**Laminaria Ochroleuca Extract**		**Laminaria Saccharina Extract**	
Total/range	**2**	**0.03**	**77**	**0.005-5**	**26**	**0.000024-0.63**	**132**	**0.00001-0.54**
Duration of use								
Leave-on	*2*	*0.03*	*62*	*0.0005-5*	*22*	*0.00017-0.63*	*85*	*0.000092-0.54*
Rinse-off	*NR*	*NR*	*15*	*0.0005-5*	*4*	*0.000024-0.017*	*47*	*0.00001-0.51*
Diluted for (bath) use	*NR*	*NR*	*NR*	*0.011-5*	*NR*	*NR*	*NR*	*NR*
Exposure type								
Eye area	NR	NR	3	0.0005-0.007	2	0.0034-0.63	NR	0.000092-0.019
Incidental ingestion	NR	NR	1	NR	1	NR	NR	NR
Incidental Inhalation-Spray	2[a]	NR	7[a]; 28[b]	0.3-5[a]	3[a]; 5[b]	0.017; 0.017[a]	40[a]; 19[c]	0.001-0.005
Incidental Inhalation-Powder	NR	0.03[c]	3; 1[c]; 28[b]	0.0035; 0.0055-5[c]	3; 5[b]	0.0005-0.17[c]	19[c]	0.0008; 0.000092-0.1[c]
Dermal Contact	2	0.03	72	0.0005-5	25	0.000024-0.63	120	0.000092-0.54
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	0.15[e]
Hair- Non-Coloring	NR	NR	1	0.0005-0.3	NR	0.017	12	0.00001-0.045
Hair- Coloring	NR	NR		NR	NR	0.017	NR	
Nail	NR	NR	2	NR	NR	NR	NR	0.001
Mucous Membrane	NR	NR	3	0.011-5	3	NR	4	0.51
Baby Products	NR	NR	1	NR	NR	NR	NR	NR

	Lessonia Nigrescens Extract		**Macrocystis Pyrifera (Kelp)**		**Macrocystis Pyrifera (Kelp) Extract**		**Macrocystis Pyrifera (Kelp) Protein**	
Total/range	**NR**	**0.032**	**2**	**NR**	**188**	**0.00005-36.4**	**3**	**NR**
Duration of use								
Leave-on	*NR*	*NR*	*1*	*NR*	*106*	*0.0002-36.4*	*1*	*NR*
Rinse-off	*NR*	*0.032*	*1*	*NR*	*78*	*0.00005-5*	*2*	*NR*
Diluted for (bath) use	*NR*	*NR*	*NR*	*NR*	*4*	*0.0051-1*	*NR*	*NR*
Exposure type								
Eye area	NR	NR	NR	NR	5	0.007-36.4	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	0.079	NR	NR
Incidental Inhalation-Spray	NR	NR	1[a]	NR	9; 38[a]; 26[b]	0.042-0.79; 0.0036-5[a]; 0.17[b]	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	2; 26[b]	0.0035; 0.001-33.3[c]; 0.17[b]	NR	NR
Dermal Contact	NR	0.032	2	NR	123	0.00005-36.4	3	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair- Non-Coloring	NR	NR	NR	NR	56	0.001-5	NR	NR
Hair- Coloring	NR	NR	NR	NR	4		NR	NR
Nail	NR	NR	NR	NR	5	0.0002-0.0011	NR	NR
Mucous Membrane	NR	NR	1	NR	34	0.0051-5	1	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

Table 21. Frequency of use according to duration and exposure of brown algae-derived ingredients[73-75]

Use type	# Uses	Max. Conc. (%)	Uses	Max. Conc.(%)	Uses	Max. Conc.(%)	Uses	Max.Conc. (%)
	Pelvetia Canaliculata Extract		**Sargassum Filipendula Extract**		**Sargassum Fusiforme Extract**		**Sargassum Muticum Extract**	
Total/range	**47**	**0.00002-0.018**	**46**	**0.0001-1.2**	**7**	**NR**	**1**	**0.01-0.076**
Duration of use								
Leave-on	*34*	*0.00002-0.018*	*14*	*0.0001-1.2*	*4*	*NR*	*NR*	*0.076*
Rinse-off	*13*	*0.00004-0.0018*	*32*	*0.002-0.29*	*3*	*NR*	*1*	*0.01*
Diluted for (bath) use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure type[a]								
Eye area	6	0.00002-0.0007	2	NR	NR	NR	NR	0.076
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	1; 18[a]; 8[b]	0.00004-0.0007; 0.002-0.0035[a]	3; 5[a]; 1[b]	0.0001[a]	2[a]; 2[b]	NR	NR	NR
Incidental Inhalation-Powder	8[b]	0.002-0.018[c]	1[b]	0.8[c]	2[b]	NR	NR	NR
Dermal Contact	19	0.00002-0.018	16	0.002-1.2	7	NR	1	0.076
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair- Non-Coloring	24	0.00004-0.0025	7	0.0001-0.29	NR	NR	NR	NR
Hair- Coloring	1	0.0000-0.0007	23	0.011-0.29	NR	NR	NR	NR
Nail			NR	NR	NR	NR	NR	NR
Mucous Membrane	1	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

	Sargassum Vulgare Extract		**Sphacelaria Scoparia Extract**		**Undaria Pinnatifida Extract**		**Undaria Pinnatifida Powder**	
Total/range	**NR**	**0.0075-0.016**	**8**	**0.016**	**74**	**0.00001-5**	**NR**	**0.1**
Duration of use								
Leave-on	*NR*	*0.009-0.016*	*6*	*0.016*	*64*	*0.00001-5*	*NR*	*NR*
Rinse-off	*NR*	*0.0075*	*2*	*NR*	*10*	*0.0001-5*	*NR*	*0.1*
Diluted for (bath) use	*NR*	*NR*	*NR*	*NR*	*NR*	*0.0001*	*NR*	*NR*
Exposure type								
Eye area	NR	0.011	NR	NR	4	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	0.009[a]	1[a]; 4[c]	NR	12[a]; 38[b]	0.002[a]	NR	NR
Incidental Inhalation-Powder	NR	0.011[c]	4[c]	NR	3; 38[b]	0.00001-5; 0.00001-5[c]	NR	NR
Dermal Contact	NR	0.011-0.016	8	0.016	68	0.00001-5	NR	0.1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair- Non-Coloring	NR	0.0075-0.009	NR	NR	6	0.002-5	NR	NR
Hair- Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	2	NR	4	0.0001	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

	Kelp[f]		**Kelp Extract**[f]		**Laminaria Extract**[f]	
Total/range	**24**	**NS**	**15**	**NS**	**4**	**NS**
Duration of use						
Leave-on	*9*	*NS*	*10*	*NS*	*2*	*NS*
Rinse-off	*10*	*NS*	*4*	*NS*	*2*	*NS*
Diluted for (bath) use	*5*	*NS*	*1*	*NS*	*NR*	*NS*
Exposure type						
Eye area	NR	NS	1	NS	NR	NS
Incidental Ingestion	1	NS	NR	NS	NR	NS
Incidental Inhalation-Spray	3[a]; 3[b]	NS	5[a]; 1[b]	NS	1[b]	NS
Incidental Inhalation-Powder	3[b]	NS	1[b]	NS	1[b]	NS
Dermal Contact	19	NS	8	NS	2	NS
Deodorant (underarm)	NR	NS	NR	NS	NR	NS
Hair- Non-Coloring	4	NS	7	NS	2	NS
Hair- Coloring	NR	NS	NR	NS	NR	NS
Nail	NR	NS	NR	NS	NR	NS
Mucous Membrane	11	NS	2	NS	NR	NS
Baby Products	NR	NS	NR	NS	NR	NS

NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on + Diluted for Bath Product Uses.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.
[a] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
[c] It is possible these products may be powders, but it is not specified whether the reported uses are powders.
[d] Frequency of use and concentration of use were reported under the INCI name Dictyopteris Membranacea Extract (Retired).
[e] Not spray.
[f] Reported in the VCRP under a non-INCI name and presented here for information purposes.

Table 22. Brown algae-derived ingredients with no reported uses in the VCRP or the Council survey[73-75]

Ascophyllum Nodosum		Cladosiphon Novae-Caledoniae Extract
Cystoseira Amentacea/Caespitosa / Branchycarpa Extract	Cystoseira Baccata Extract	Cystoseira Balearica Extract
Cystoseira Caespitosa Extract	Cystoseira Compressa Extract	Cystoseira Compressa Powder
Cystoseira Tamariscifolia Extract	Dictyota Coriacea Extract	Ecklonia Cava Extract
Ecklonia Cava Water	Ecklonia Kurome Extract	Ecklonia Kurome Powder
Ecklonia/Laminaria Extract	Ecklonia Maxima Extract	Ecklonia Maxima Powder
Eisenia Arborea Extract	Fucus Spiralis Extract	Halidrys Siliquosa Extract
Halopteris Scoparia Extract		Himanthalia Elongata Powder
Hizikia Fusiforme Extract	Hizikia Fusiformis Water	Hizikia Fusiformis Callus Culture Extract
Hydrolyzed Ecklonia Cava Extract	Hydrolyzed Fucus Vesiculosus Extract	Hydrolyzed Fucus Vesiculosus Protein
Laminaria Diabolica Extract	Laminaria Japonica Powder	Laminaria Longissima Extract
Lessonia Nigrescens Powder	Macrocystis Pyrifera (Kelp) Blade/ Pneumatocyst/Stipe Juice Extract	Macrocystis Pyrifera (Kelp) Juice
Nereocystis Luetkeana Extract	Pelvetia Siliquosa Extract	Phyllacantha Fibrosa Extract
	Saccharina Angustata Extract [Laminaria Angustata Extract (Retired)]	Saccharina Japonica Extract [Laminaria Ochotensis Extract (Retired)]
Saccharina Longicruris Extract	Sargassum Fulvellum Extract	Sargassum Glaucescens Extract
Sargassum Horneri Extract	Sargassum Pallidum Extract	Sargassum Siliquastrum Extract
Sargassum Thunbergii Extract		Undaria Peterseniana Extract
Undaria Pinnatifida Cell Culture Extract	Undaria Pinnatifida Leaf/Stem Extract	Undaria Pinnatifida Root Powder

Table 23. GRAS brown algae-derived ingredients

Ingredient	Functional Use in Food	CFR Citation
Hizikia fusiforme	Spices, seasoning, flavoring	21CFR184.1120
Laminaria spp.	Natural substances; solvent-free natural extractives	21CFR582.30; 21CFR582.40
Laminaria claustonia	Spices, seasoning, flavoring; dietary supplement	21CFR184.1120; 21CFR172.365
Laminaria digitata	Spices, seasoning, flavoring; dietary supplement	21CFR184.1120; 21CFR172.365
Laminaria japonica	Spices, seasoning, flavoring	21CFR184.1120
Laminaria longissima	Spices, seasoning, flavoring	21CFR184.1120
Laminaria saccharina	Spices, seasoning, flavoring; dietary supplement	21CFR184.1120; 21CFR172.365
Nereocystis spp.	Natural substances; solvent-free natural extractives	21CFR582.30; 21CFR582.40
Macrocystis pyrifera	Spices, seasoning, flavoring; dietary supplement	21CFR184.1120; 21CFR172.365
Undaria pinnatifida	Spices, seasoning, flavoring	21CFR184.1120

Table 24. Brown algae species used in food products[16]

Brown Algae Ingredient	Methods of consumption
Alaria esculenta	Eaten either fresh or cooked
Cladosiphon okamuranus	Eaten fresh and in seaweed salads
Ecklonia cava	Used in addition to *Hizikia* as pigment replacer; typically cooked into stir fries
Hizikia fusiforme	Steamed to remove phlorotannins, and cooked into stir fries; used as a spice
Laminaria angustata	Typically cooked in soups; can be powdered and added to sauces and soups; used in tea
Laminaria japonica	Typically cooked in soups; can be powdered and added to sauces and soups; used in tea
Laminaria longissima	Typically cooked in soups; can be powdered and added to sauces and soups; used in tea
Laminaria ochotensis	Typically cooked in soups; can be powdered and added to sauces and soups; used in tea
Macrocystis pyrifera	Used as spices, seasonings
Undaria pinnatifida	Eaten raw in dehydrated form; used in instant foods such as noodles and soups; used as spice, seasoning

Table 25. Acute oral toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/Results	Reference
ORAL						
Ascophyllum Nodosum Extract	Sprague-Dawley rats	NR	NR	OECD TG 401	LD_{50} > 2000 mg/kg	87
Cystoseira Compressa Extract (methanol, hexane, and chloroform extracts)	Albino mice	2	Not specified	Up to 2000 mg/kg by gavage. Observed for 24 h.	There were no mortalities or clinical signs for any of the extracts.	61
Ecklonia Cava Extract (alcohol extract)	Sprague-Dawley (Crl:DC(DS)) rats	10/sex	Not specified	2000 mg/kg by gavage. Observed for 2 weeks.	There were no mortalities. Clinical signs were soft stools, diarrhea, mucus stools, compound-colored feces, and soiled perineal region from the day of administration until day 2.	9
Ecklonia Cava Extract (enzyme extract)	SD rats	5/sex	Distilled water	0 or 3000 mg/kg by oral gavage. Rats were observed for 14 days.	No abnormal changes in body weights, clinical signs, or mortalities were observed. Necropsy results showed no macroscopic lesions in any organs of treatment group.	91
Ecklonia Cava Extract (enzyme extract)	Beagle dogs	2/sex	Distilled water	3000 mg/kg by oral gavage in two equally divided doses approximately 6 h apart. Dogs were observed for 14 days.	No abnormal changes in body weights, clinical signs, or mortalities were observed. Necropsy results showed no macroscopic lesions in any organs of treatment group.	91
Fucus Vesiculosus Extract (28.8% polyphenols)	Swiss mice	7/sex	1% carboxymethyl-cellulose	1000 - 2000 mg/kg OECD TG 425 Administered by gavage. An Irwin test (determines the general effects of a test substance on the central nervous system and physiological functions) was performed at 1 and 5 h after administration of the extracts to detect motor, respiratory, temperature, circulatory, behavior, or other alterations. Mice were observed for 7 days.	LD_{50}: Males = 1000 mg/kg; females = between 1000 and 2000 mg/kg	89
Fucus Vesiculosus Extract (18% polyphenols plus 0.0012% fucoxanthin)	Swiss mice	7/sex	1% carboxymethyl-cellulose	1000 - 2000 mg/kg OECD TG 425 Administered by gavage. Irwin test was performed at 1 and 5 h after administration of the extracts to detect motor, respiratory, temperature, circulatory, behavior, or other alterations. Mice were observed for 7 days.	LD_{50}: Males = 500 mg/kg; females = < 750 mg/kg	89
Fucus Vesiculosus Extract (28.8% polyphenols)	Sprague-Dawley rats	7/sex	1% carboxymethyl-cellulose	1000 - 2000 mg/kg OECD TG 425 Administered by gavage. Irwin test was performed at 1 and 5 h after administration of the extracts to detect motor, respiratory, temperature, circulatory, behavior, or other alterations. Rats were observed for 7 days.	LD_{50}: Males and females = between 1000 and 2000 mg/kg	89

Table 25. Acute oral toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/Results	Reference
Fucus Vesiculosus Extract (18% polyphenols plus 0.0012% fucoxanthin)	Sprague-Dawley rats	7/sex	1% carboxymethyl-cellulose	1000 - 2000 mg/kg OECD TG 425 Administered by gavage. Irwin test was performed at 1 and 5 h after administration of the extracts to detect motor, respiratory, temperature, circulatory, behavior, or other alterations. Rats were observed for 7 days.	LD_{50}: Males and females = > 2000 mg/kg	89
Sargassum Fulvellum Extract (dichloromethane, ethanol, and water extracts)	BALB/c mice	5	Tween-80 (5%)	5000 mg in 10 mL vehicle by gavage. Observed for 2 weeks.	There were no mortalities. Most of the mice reacted immediately by perpetual gagging, jumping, sleeping, scaling, and writhing for 5–10 min.	50
Sargassum Thunbergii Extract	BALB/c mice	5	Tween-80 (5%)	5000 mg in 10 mL vehicle by gavage. Observed for 2 weeks.	There were no mortalities. Most of the mice reacted immediately by perpetual gagging, jumping, sleeping, scaling, and writhing for 5–10 min.	50

OECD GL = Organisation for Economic Co-operation and Development Guidelines

Table 26. Oral repeated dose studies

Test Article	Extraction Solvent/Method or Composition	Animals (n)	Study Duration	Vehicle	Dose / Concentration	Results	Reference
			Short-Term				
Ascophyllum nodosum	Dried	Topigs Hybrid X Piétrain weanling pigs (20)	23 days	Feed	0, 2.5, 5.0, or 10.0 g/kg feed (0.25%, 0.5%, or 1.0%)	There were no adverse effects from treated feed. There were no effects on weight gain, feed consumption. Digestion characteristics were similar to controls (pH, fresh matter weight, and dry matter content), except for pH of part of the intestine was increased in the high-dose group (6.28 vs.5.96).	88
Ascophyllum nodosum	Freeze-dried and powdered	Male Sprague-Dawley rats (6)	4 weeks	Feed	0, 5%, 10%, or 15% in feed	Food intake, weight gain, and serum enzyme (alanine transaminase and aspartate transaminase) levels indicated that seaweed diets were well tolerated.	45
Cladosiphon Okamuranus Extract	hydrolyzing in HCl	Wistar Rats (12/group)	3 months	Water	300, 600, 1299, 2400, 4000 mg/kg bw/d	A dose-dependent increase in clotting time and decrease in alkaline phosphatase (ALP) was noted in high doses. No significant differences compares to control. No treatment-related changes in organ weights reported. No abnormalities is morphology of brain, thymus, lungs, heart, spleen, liver, adrenal glands, kidneys, testes, thyroids, prostate gland, uterus or ovaries.	47
Ecklonia Cava Extract	Alcohol extract	Male ICR mice (10)	4 weeks	None	0, 1.25, 2.5 or 5 mg/day Mice were fed high fat diet (20% fat) or normal diet (5% to 10% fat). After 1 week, mice in high fat diets were administered Ecklonia Cava Extract by gavage while continuing on the high fat diet.	There were no mortalities. There was a dose-dependent lower body weight of ~ 12% - ~ 16% in the mice administered the extract compared to control group. Triglycerides, total cholesterol and LDL cholesterol were decreased in all treated groups. Liver enzymes (GPT and GOT), BUN, and creatinine values in serum were similar to controls. No data on feed consumption provided.	92

Table 26. Oral repeated dose studies

Test Article	Extraction Solvent/Method or Composition	Animals (n)	Study Duration	Vehicle	Dose / Concentration	Results	Reference
Ecklonia Cava Extract	Enzyme extract	SD rats (5/sex)	14 days	Water	0, 1000, 2000, or 5000 mg/kg by gavage	- There were no mortalities. No dose-related clinical abnormalities or body weight changes. - Macroscopic examination did not reveal any treatment-related abnormal lesions in males or females at necropsy; although redness in thymus, red spot in lung, and congestion and red spot in cervical lymph node were sporadically observed without a dose-dependent relationship. - Females in the 2000 and 5000 mg/kg groups had decreases in absolute and relative left ovary weights relative to control group and decreases in absolute brain weights were observed in females in 5000 mg/kg group.	91
Ecklonia Cava Extract	Alcohol extract	Sprague-Dawley (Crl:CD(SD)) rats (5/sex)	4 weeks	None	0, 500, 1000, or 2000 mg/kg/day by gavage.	- Compound-colored stools were observed in all rats in all dosing groups starting from day 1 of dosing. Salivation after dosing was observed sporadically in 1 female in the 1000 mg/kg/day group and in 2 males and 2 females in the 2000 mg/kg/day group on days 5 to 17 of dosing. - In clinical chemical investigations in 2000 mg/kg/day group, increases in ALT, and decreases in total protein, triglycerides and glucose were observed in males. Absolute and relative liver weights and absolute kidney weights were increased in males in 2000 mg/kg/day group. In females, relative heart weights were decreased in 1000 and 2000 mg/kg/day groups. There were no differences between study groups concerning body weight. Histopathologically, atrophy of periportal hepatocytes in livers was detected in male rats in 2000 mg/kg/day group.	9
Ecklonia Cava Extract	Alcohol extract	Beagle dogs (2/sex)	8 days 2-week observation period	Capsule	Day 1, 100 mg/kg; Day 4, 300 mg/kg; and Day 8, 1000 mg/kg	There were no mortalities. Compound-colored stools were observed in all dogs in 300 and 1000 mg/kg groups. Vomiting was observed in 1 male and 1 female dog when treated at 1000 mg/kg.	9
Ecklonia cava powder (inference for Ecklonia Cava Extract and Ecklonia Cava Water)	Freshly collected fronds were dried and powdered	Landrace x Yorkshire x Duroc weanling pigs (50)	28 days	In feed (growing-finishing diet)	0%, 0.05%, 0.1%, or 0.15% in feed	No mortalities. Weight gain was similar to controls. No significant effect on serum level of IgG, IgA, and IgM.	93
Fucus Vesiculosus Extract (28.8% polyphenols)	Ethanol (30% - 35% aq)	Sprague-Dawley rats (7/sex)	4 weeks	1% CMC	0, 200, or 750 mg/kg/day by gavage	- There were no mortalities. -Males: body and most organ weights were similar to controls. Livers had an increase weight (21%) at necropsy. - Females: body and organ weights were similar to controls.	89
Fucus Vesiculosus Extract (18% polyphenols plus 0.0012% fucoxanthin)	Ethanol (50% - 70% aq.)	Sprague-Dawley rats (7/sex)	4 weeks	1% CMC	0, 200, or 750 mg/kg/day by gavage	- There were no mortalities. - Males: body and most organ weights were similar to controls. Livers had an increase weight (25%) at necropsy. - Females: body and organ weights were similar to controls.	89
Laminaria Japonica Extract	Ethanol extract	Sprague-Dawley rats (6)	6 weeks	Not clear (probably daily gavage)	0, 100, 200, or 400 mg/kg starting after 6 weeks of a 12-week high-fat diet	- There were no mortalities. - Treatment groups had decreased the body weight gain, fat-pad weights, and serum and hepatic lipid levels in high-fat-induced obese rats. Histological analysis showed that treated groups had decreased number of lipid droplets and size of adipocytes compared to untreated high-fat diet group.	46

Table 26. Oral repeated dose studies

Test Article	Extraction Solvent/Method or Composition	Animals (n)	Study Duration	Vehicle	Dose / Concentration	Results	Reference
					Subchronic Oral		
Ecklonia Cava Extract	Alcohol extract	Sprague–Dawley (Crl:CD(SD)) rats (10/sex;5 additional in control and high-dose groups)	13 weeks 4-week recovery period for 5 rats in control and high-dose group	Water	0, 375, 750, or 1500 mg/kg/day	- Compound-colored stools in all dose levels; not considered to be of toxicological significance. -At 750 and 1500 mg/kg/day, BUN was decreased in males, glucose was decreased in females, and neutrophil counts were increased in females, compared to controls. Sporadic salivation occurred in females. - At 1500 mg/kg/day, incidence of salivation in females increased and occurred in male rats. Salivation was mainly observed after gavage, but to some degree also before. It was considered by authors to be a temporary sign caused by the test substance, since it was no longer evident later in the day. Number of rats with salivation increased with study duration. -At 1500 mg/kg/day, males and females had a lower body weight (11.7% and 8.7%, respectively) at end of study compared to controls (not statistically significant). This effect was dose related, appearing to a minor degree also at lower dose levels. Body weight effects were more pronounced in recovery group in both sexes. Feed consumption was not decreased. Blood chemistry analyses showed increases of phosphorus and ALT concentrations and a decrease of triglycerides in males, and a decrease of glucose in females, compared to controls. Prothrombin time was increased in males compared to controls. These changes were not evident after recovery period. There were no compound related findings in histopathological investigations including liver.	[9]
Ecklonia Cava Extract	Enzyme extract	SD rats (5/sex)	13 weeks	Water	0, 500, 1000, 2000, or 3000 mg/kg by gavage	- There were no mortalities. None of groups had any dose-related clinical abnormalities or body weight changes. - Urinalysis and hematological analysis showed no treatment-related adverse effects. - Serum biochemistry and organ weights showed sporadic changes. However, sporadic changes might not have any relationship with treatment because these changes were very minimal within physiologically acceptable ranges without consistency between male and female rats. - Gross visual and macroscopic changes were not observed in organs of treated rats. Histopathological examination of sampled organs revealed a few spontaneous lesions which might be unrelated to treatment because there was no difference in incidence between control and treatment groups.	[91]
					Chronic Oral		
Laminaria Japonica Powder	Dried and powdered	Male CDF1 mice (6)	Life time	Feed	0, 2%, 5%	Mean lifespans were similar in all groups: 907 ± 135, 746 ± 183, and 851 ± 225 days for 0, 2%, and 5%, respectively.	[48]
Undaria Pinnatifida Extract	Filtered aqueous extract of powdered stems and thick leaves	Female Sprague-Dawley (SD) rats (12)	32 weeks	Drinking water	1.5 g in 1000 mL water	There were no mortalities. Body weight changes were similar between groups.	[94]
Undaria Pinnatifida Powder	Dried and ground	Female SD rats (5)	36 weeks	Feed	0, 1.0%, or 5.0%	There were no mortalities. Body weight changes, thyroid weights, and T4 levels were similar between groups.	[106]

Table 26. **Oral repeated dose studies**

Test Article	Extraction Solvent/Method or Composition	Animals (n)	Study Duration	Vehicle	Dose / Concentration	Results	Reference

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AMP = adenosine monophosphate; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CMC = carboxymethylcellulose; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; HDL = high-density lipoprotein; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; LDL = low-density lipoprotein; MCHC = mean corpuscular hemoglobin concentration; T4 = thyroxin

Table 27. **Genotoxicity studies**

Ingredient/Test Article	Extraction Solvent/ Method	Concentration/ Vehicle	Procedure	Test System	Results	Reference
			In Vitro			
Ascophyllum Nodosum Extract	Not specified	Not specified	Ames assay performed in according to OECD TG 471. No other details provided.	Not specified	Non-mutagenic.	[87]
Ascophyllum Nodosum Extract	Not specified	50, 150, 500, 1500, or 5000 µg/plate; in water	Ames assay, with and without metabolic activation in accordance with OECD TG 471 (bacterial reverse mutation test). Negative control: histidine; positive control: 4-nitroquinoline-N-oxide, 3-methylmethane sulphonate, 2-aminoanthracene, and sodium azide. There was no solvent control.	*S. typhimurium* (strains TA97, TA98, TA100, TA102, and TA1535)	Not genotoxic in all strains	[6]
Ascophyllum Nodosum Extract	Not specified	150, 500, 1500, or 5000 µg/mL; in water	Mammalian cell gene mutation test accordance with OECD TG 476 (in vitro mammalian cell gene mutation test) with and without metabolic activation. Positive control without metabolic activation: ethylmethanesulphonate, with metabolic activation: BaP	CHO; K1 sub clone CHO K1	Increased mutant frequencies at 1500 and 5000 µg/mL without metabolic activation; no increase in mutation frequencies at lower concentrations. No increase in mutation frequencies at any concentration with metabolic activation.	[6]
Ascophyllum Nodosum Extract	Not specified	With metabolic activation: 0.63, 1.25, 2.5, or 5 mg/mL; without metabolic activation: 1.25, 2.5, or 5 mg/mL	Chromosome aberration assay in accordance with OECD TG 487 (in vitro mammalian chromosome aberration test) with and without metabolic activation. Negative control: medium (serum free cell culture medium); positive controls: CPA, MMC, and colchicine	Human lymphocytes	Not genotoxic	[6]
Ascophyllum Nodosum Extract	Not specified	Experiment I: With metabolic activation: 1.25, 2.5, or 5 mg/mL; without metabolic activation: 1.25, 2.5, or 5 mg/mL Experiment II: without metabolic activation: 0.63, 1.25, 2.5, or 5 mg/mL Serum free cell culture medium	Chromosome aberration assay in accordance with OECD TG 487 with and without metabolic activation. Negative control: solvent (serum free cell culture medium); Positive control: CPA, MMC, colchicine	Human peripheral lymphocytes	Not genotoxic or cytotoxic	[6]

Table 27. Genotoxicity studies

Ingredient/Test Article	Extraction Solvent/ Method	Concentration/ Vehicle	Procedure	Test System	Results	Reference
Ascophyllum Nodosum Extract	Not specified	4.7% Ascophyllum Nodosum Extract	An Ames test was performed using a trade name mixture containing 4.7% Ascophyllum nodosum extract in 94.5% water. The procedure was done in accordance to OECD TG 471.	Not specified	Not mutagenic or pro-mutagenic activity	68
Cystoseira Compressa Extract	n-Hexane, chloroform, and methanol	1, 2.5, or 5 mg/plate	Ames Assay with and without metabolic activation. Negative control: DMSO. Positive controls: BaP, 2-nitrofluorene, and sodium azide.	*S. typhimurium* (strains TA 98 and TA 100)	Not mutagenic	61
Ecklonia Cava Extract	Enzymatic extraction	911 - 3500 µg/plate; distilled water	Ames assay, with and without metabolic activation. OECD TG 471	*S. typhimurium* (strains TA 98, TA 100, TA 1535, and TA 1537) and *E. coli* (WP2uvrA)	Not genotoxic	91
Ecklonia Cava Extract	Alcohol	Up to 5000 µg/plate; vehicle not specified	Ames assay, with and without metabolic activation	*S. typhimurium* (strains TA 98, TA 100, TA 1535, and TA 1537) and *E. coli* (WP2uvrA(pKM101))	Not genotoxic or cytotoxic	9
Halidrys Siliquosa Extract (48%) in water (52%)	Water	0.06 µL – 5 µL/plate	Ames assay; OECD TG 471; with and without metabolic activation	*S. typhimurium* (strains TA 98, TA 100, TA 102, TA 1535)	Non-mutagenic; Non-promutagenic	64
Laminaria digitata	Not specified	Not specified	Ames assay, with and without metabolic activation	*S. typhimurium*	No evidence of mutagenicity	197
Laminaria Saccharina Extract	NR	50, 150, 500, 1500 and 5000 µg/plate; sea water and methylpropandiol	Ames test with and without metabolic activation	*S. typhimurium* (TA 1535, TA 1537, TA 102, TA98, and TA 100)	Non-mutagenic	96
Macrocystis Pyrifera (Kelp) Extract	Water	1 mL extract in 10 mL 0.9% sodium chloride (concentration of extract was approximately 4%)	Ames test with and without metabolic activation	*S. typhimurium* (TA 98, TA 100, TA 1535, TA 1537, TA1538)	Non-mutagenic	97
Ecklonia Cava Extract	Alcohol	Up to 290 µg/mL	Chromosome aberration test, with and without metabolic activation	CHL cells	Not genotoxic	9
Ecklonia Cava Extract	Enzymatic extraction	87.5 – 350 µg/plate; distilled water	Chromosome aberration test, with and without metabolic activation. OECD TG 473	CHL cells	Not genotoxic	91
Fucus Vesiculosus Extract	Aqueous	0, 0.25, 0.5, or 1 mg/mL; cell medium	Chromosome aberration assay OECD TG 487	Human peripheral lymphocytes	Frequency of chromosome aberrations, mitotic index and extent of DNA damage in cells treated with extract were similar to controls at all concentrations.	95
Fucus Vesiculosus Extract	Aqueous	0, 0.25, 0.5, or 1 mg/mL; cell medium	Comet assay	Human peripheral lymphocytes	Extent of DNA damage in cells treated with extract was similar to controls at all concentrations.	95

Table 27. Genotoxicity studies

Ingredient/Test Article	Extraction Solvent/ Method	Concentration/ Vehicle	Procedure	Test System	Results	Reference
Trade name mixture containing 24% Undaria Pinnatifida Cell Culture Extract	Aqueous	1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate	Bacterial reverse mutation assay performed with and without metabolic activation; OECD TG 471	*S. typhimurium* (strains TA 98, TA 100, TA 1537, TA 1535) and tryptophan-dependent *E. coli* (strain WPRuvrA)	Non-mutagenic	198
Cystoseira Amentacea/ Caespitosa/ Brachycarpa Extract (48%), Water (52%)	Water	0.01, 0.1, 1, and 10%	A chemiluminescent 3D Assay was performed by using plasmid DNA adsorbed on sensitized microplates as the substrate	NR	No direct genotoxicty.	101
			In Vivo			
Ecklonia Cava Extract	Alcohol	0 or 2000 mg/kg	Micronucleus assay. Test substance administered via oral gavage. Bone marrow (2,000 erythrocytes) was checked for frequency of micronuclei, after 24, 48, and 72 h.	Male Crlj:CD1(ICR) mice (n = 3)	There was no increase in frequency of micronuclei in any of the time points.	9
Ecklonia Cava Extract	Alcohol	0, 500, 1000, or 2000 mg/kg	Micronucleus assay. Test substance administered via oral gavage. Bone marrow (2,000 erythrocytes) was checked for the frequency of micronuclei, after 24 h.	Male Crlj:CD1(ICR) mice (n = 5)	There was no increase in frequency of micronuclei polychromatic erythrocytes (PCE)/(PCE + normochromatic erythrocytes (NCE)) ratio was not significantly different between treatment groups and control groups. No evidence of genotoxicity.	9
Ecklonia Cava Extract	Enzymatic extraction	1000, 2000, or 3000 mg/kg; distilled water	Mouse micronucleus assay. The number of mice used in the study was not provided. Administered by gavage. Saline and MMC were the controls. OECD TG 474	Male ICR mice	There were no mortalities or abnormal clinical signs in any group. There were no increases in structural or numerical chromosomal aberrations at any dose compared to the negative control.	91

BaP = benzo(a)pyrene; CHL = Chinese hamster lung; CHO = Chinese hamster ovary; CPA = cyclophosphamide; HCl = hydrochloric acid; MMC = mitomycin C; MNPCE = micronucleated polychromatic erythrocyte; NCE = normochromatic erythrocyte; NR = Not Reported; PBS = phosphate-buffered saline; PCE = polychromatic erythrocytes

Table 28. Tumor promotion studies

Test Article	Extraction/solvent/ method	Dose/Exposure Route	Species (n)	Tumor Type	Carcinogenicity Model	Results	Reference
				Dermal			
Undaria Pinnatifida Extract	Dichloromethane extract	1 mg	Female ICR mice (n not specified)	Skin	- Initiation: a single dermal dose of DMBA (50 µg) - 1 week later, mice were dermally treated twice per week with TPA (1 µg) or Undaria Pinnatifida Extract (1 mg) 1 h prior to treatment with TPA for 15 weeks	TPA: tumors > 1 mm were observed after week 8; average number of tumors was 3.7. Undaria Pinnatifida Extract and TPA: mice did not show 1-mm tumors until week 14 (< 5%); average number of tumors was 0.2.	102

Table 28. Tumor promotion studies

Test Article	Extraction/solvent/ method	Dose/Exposure Route	Species (n)	Tumor Type	Carcinogenicity Model	Results	Reference
				Oral			
Hizikia Fusiforme Extract	95% Ethanol aq.	0, 2%, or 6% in feed	Male F344 rats (10, control, 8)	Colorectal	- Group 1 – standard diet - Group 2 – injected with AOM (15 mg/1 mL/kg once a week for 2 weeks) and standard diet - Group 3 – Injected with AOM and diet with 2% Hizikia Fusiforme Extract - Group 4 – Injected with AOM and diet with 6% Hizikia Fusiforme Extract - After 8 weeks, the rats were killed and necropsied.	- Body weights were similar among groups at 11 weeks. - No tumors were found in the negative control group and 58 tumors were found in the positive control group. Treatment groups had reduced number of tumors (21 each). - Immuno-histochemistry analysis of PCNA expression, a marker of tumor cell proliferation and apoptosis, was lower in treatment groups than in treated control group.	[103]
Saccharina Angustata Extract (inference from *Saccharina angustata* powder)	Dried and milled	0 or 5% in feed	Female Sprague-Dawley rats (54)	Mammary	- After 50 days on respective diets, 4 rats in each group were killed and examined for abnormalities. None were found. - At 55 days treatment groups were administered DMBA by gavage after fasting. - Rats were palpated weekly for tumors. - The rats were killed at 181 - 188 days after DMBA administration and necropsied.	- Weight gains were similar among groups. - First tumors in the control group appeared at 11.0 weeks and 19.8 in the treatment group. - 41 of 54 rats (76%) in control group and 34 of 54 rats (63%) in the treatment group had 1 or more adenocarcinomas at necropsy. - During treatment, 13 rats (8 control and 5 experimental) were euthanized between 74 and 170 days post- DMBA. 10 of these rats had developed large (~ 4 cm in diameter) mammary tumors, 2 developed malignant lymphomas, and 1 developed a large necrotic ear gland tumor (Zymbal's gland carcinoma). There were no other deaths. - 12 tumor-free rats (6 from each group) were found to have small nonpalpable mammary masses; 11 of these were found to be adenocarcinomas and 1 to be an adenoma. 93% of all tumors found in the mammary gland region at autopsy were adenocarcinomas; 5 tumors, which were mostly fibroadenoma but which had focal proliferations of malignant epithelial cells. Other tumors consisted of 7 fibroadenomas, 5 adenomas, 3 epidermal inclusion cysts, and 1 adenocarcinoma of sebaceous glands.	[104]

Table 28. Tumor promotion studies

Test Article	Extraction/solvent/ method	Dose/Exposure Route	Species (n)	Tumor Type	Carcinogenicity Model	Results	Reference
Sargassum Pallidum Extract	Aqueous. Boiled under reflux and filtered.	400, 600 or 800 mg/kg/day	Male Wistar rats (10)	Gastric	- Group 1 – distilled water - Group 2 – 800 mg/kg/day Sargassum Pallidum Extract - Group 3 - 6 – MNNG (25 mg/mL) in drinking for 25 weeks; then 0, 400, 600, or 800 mg/kg Sargassum Pallidum Extract for 8 weeks - All rats were killed at 33 weeks, blood analyzed, and stomachs examined.	- There were no mortalities. - Compared to group 1 (control), Sargassum Pallidum Extract increased serum IL-2, IL-4, and IL-10 levels in group 2; serum IL-2, IL-4, and IL-10 levels in group 3 were decreased. - Compared to group 1, Sargassum Pallidum Extract decreased serum IL-6, IL-1β, and TNF-α levels in group 2; serum IL-6, IL-1β, and TNF-α levels in group 3 were increased. - Compared with group 3, Sargassum Pallidum Extract dose-dependently decreased serum IL-6, IL-1β, and TNF-α levels in groups 4, 5, and 6. - Concentration of serum and gastric mucosa MDA decreased in a dose-dependent manner in groups 4, 5, and 6. - Concentration of serum and gastric mucosa GSH and antioxidant enzyme activities increased in a dose-dependent manner in groups 4, 5, and 6. - Sargassum Pallidum Extract could decrease inflammatory response and improve immunity function partly through stimulating inflammatory cytokines (IL-2, IL-4, IL-10) production and inhibiting pro-inflammatory cytokines production.	[105]
Undaria Pinnatifida Powder	Not specified	0, 1.0% or 5.0% in feed	Female Sprague-Dawley (SD) rats (11)	Mammary	- Initiation: a single dose of DMBA (20 mg) by gastric intubation - Once tumors reached 1 cm, rats were dived between 3 treatment groups for 8 weeks - Rats were then killed and all mammary tumors were histologically examined and thyroid glands, ovaries, and adrenal glands were weighed. Blood samples collected for measurement of serum total iodine concentration and serum T4 levels.	No differences in body weight gains between groups. Tumors in control group increased by more than 450%; tumor growth was suppressed in the 1% group and there was almost no change in tumor size in the 5% group. Mean combined weight of all mammary tumors of each rat in treatment groups was lower than that in the control group (~ 7 vs 20 g) at end of experiment. Weights of thyroid glands, ovaries, and adrenal glands did not differ among groups. Concentration of serum iodine was greater in treatment groups compared to controls. Serum iodine concentration had a positive relationship with concentration of Undaria Pinnatifida Powder in diet. Serum T4 levels showed no differences among groups. Test substance did not promote mammary tumors and suppressed tumor growth after a single dose of DMBA.	[106]
Undaria Pinnatifida Extract	Filtered aqueous extract of powdered stems and thick leaves	1.5 g in 1000 mL water	Female Sprague-Dawley (SD) rats (12)	Mammary	- Initiation: a single dose of DMBA (20 mg) by gastric intubation - 1 week later, treatment began for 32 weeks - Mammary tumors were removed and measured	- Body weight gains were similar in both groups - Incidence of tumors at end of experiment was 22% vs 100% (controls) - The number of tumors was an average of < 1 vs. ~ 7 (controls) - Total tumor diameters was < 250 vs > 5000 mm - Histologically, mammary tumors were cystic adenocarcinoma, and tumors in treatment group had a decreased density of epithelial cells and fibrosis.	[94]

AOM = azoxymethane; DMBA = 7,12-dimethylbenz(*a*)anthracene; GSH = glutathione; MDA = malondialdehyde; MNNG = *N*-methyl-*N′*-nitro-N-nitrosoguanidine; PCNA = proliferating cell nuclear antigen; T4 = thyroxin; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

Table 29. Case Reports of brown algae

Ingredient/substance (dose, if known)	Details	Reference
Fucus vesiculosus supplement (1200 mg 3 times per day)	18-year-old female presented with polyuria, polydipsia, extreme faintness, and a general poor condition. She had been on a hypocaloric diet for 3 months and taking Fucus vesiculosus supplements. Renal biopsy showed widespread tubular degeneration, and diffuse lymphomonocytic infiltrate; the glomeruli displayed scarce and focal mesangial proliferation, but the basal membrane appeared intact. The supplement was tested for heavy metals: arsenic, 21.3 mg/kg; cadmium, 0.3 ppm; mercury, 0.06 ppm; and chrome, 4 ppm. The patient recovered within 1 year.	[199]
Kelp tablets	54-year-old female developed thrombocytopenia with mucocutaneous bleeding after ingesting kelp tablets (that contained 1.3 µg/g arsenic) twice daily for 6 weeks. Marrow aspirate demonstrated normal megakaryocytes and dyserythropoiesis. After discontinuation of the supplements and treatment with steroids and azathioprine, her platelet count recovered after 3 months.	[143]
Kelp supplements	A 54-year-old woman presented with a 2-year history of worsening alopecia and memory loss. She also had a rash, increasing fatigue, nausea, and vomiting to the point of disablement. She took daily kelp supplements. A urine sample showed an arsenic level of 83.6 µg/g creatinine (normal < 50 µg/g creatinine). A sample from her kelp supplements contained 8.5 mg/kg arsenic. Within weeks of discontinuing the supplements, her symptoms resolved and arsenic blood and urine levels were undetectable.	[144]

Table 30. Dermal irritation and sensitization

Ingredient	Test Substance	Concentration/Dose of the test substance	Test Population	Procedure	Results	Reference
Irritation						
IN VITRO						
Sargassum Filipendula Extract	Trade name mixture containing Sargassum Filipendula Extrac (1.3%), water (81.78%), Sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), Methylparaben (0.2%), Propylparaben (0.025%)	100%	3	30 µL (liquid) or 25 mg (solid) of the test substance was applied to 3 tissue inserts and incubated for 60 minutes; inserts were then washed, transferred to fresh media	Non-irritating	[113]
Undaria Pinnatifida Cell Culture Extract	Trade name mixture containing Undaria Pinnatifida Cell Culture Extract (24%) with water as solvent	30 µL (liquid); 25 mg (solid)	3 per test concentration	The test substance, either liquid or solid, was applied to reconstructed human epidermis and incubated for 60 minutes. These tissue inserts were then washed and cell viability was measured.	Non-irritating	[110]
Undaria Pinnatifida Extract	Trade name mixture containing Undaria Pinnatifida Extract (0.5-2%) in caprylic/capric triglycerides	100%; 10 µL	3	OECD TG 439; 3 replicates of human skin cell models were treated with the test substance for approximately 15 minutes; time of recovery was 42 hours ± 1 hour	Non-irritating	[111]
Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract	Trade name mixture containing Laminaria Japonica (7%), Nereocystis Leutkeana (7%), Macrocystis Pyrifera Extract (7%), and Pentaerythrityl Tetraethylhexanoate (79%)	100%; 30 µL (liquid) or 25 mg (solid)	3	Reconstructed human epidermal model; 3 tissues treated with test substance and incubated for 60 minutes	Non-irritating	[112]
ANIMAL						
Ascophyllum nodosum Extract	Ascophyllum Nodosum extract	0.5 mL (liquid); 0.5 g (solid)	NR	Dermal irritation assay performed according to OECD TG 404 guidelines; application for 4 hours	Non-irritating	[87]

Table 30. Dermal irritation and sensitization

Ingredient	Test Substance	Concentration/Dose of the test substance	Test Population	Procedure	Results	Reference
Ascophyllum nodosum Extract	Ascophyllum nodosum extract	0.5 g; concentration not stated	3 male rabbits	A dermal irritation assay was performed according to OECD TG 404 guidelines. The test substance was administered in three patches on areas of 12-20 cm^2 to the shaved backs of the rabbits under semi-occlusion for 3 min (patch 1), 1 h (patch 2), and 4 h (patch 3). There were no signs of irritation after the removal of patch 1 from one rabbit; patch 2 was then applied to the same rabbit. There were no signs of irritation after patch 2 was removed; patch 3 was then applied to all three rabbits. The test site was examined at 1, 24, 48, and 72 hours after removal of the last patch.	Non-irritating	6
Laminaria Digitata Extract	Trade name mixture containing Laminaria Digitata Extract, water, and dipropylene glycol	NR	Rabbits (# not stated)	Dermal irritation assay	Non-irritating	49
Laminaria Digitata Extract	Trade name mixture containing Laminaria Digitata Extract, water, and sea salt	NR	Rabbits (# not stated)	Dermal irritation assay	Non-irritating	49
HUMAN						
Alaria Esculenta Extract	Trade name mixture containing Alaria Esculenta Extract (<5%) and in caprylic/capric triglycerides	100%; 20 µL	10	24-hour patch test; occlusive patch; over a surface of 50 mm^2	Non-irritating	114
Ascophyllum Nodosum Extract	Trade name mixture containing 4.7% Ascophyllum Nodosum Extract in 94.5% water	NR	NR	A cutaneous irritation test was performed according to OECD TG 439. No additional details were provided.	Non-irritating	68
Cystoseira Amentacea/Caespitosa/Brachyc-arpa Extract	52% water; 48% Cystoseira Amentacea/Caespitosa/Brachyc-arpa Extract	NR	11	0.02 mL of test substance applied to back under an occlusive patch for 48 hours	Non-irritating	101
Cystoseira Baccata Extract	Cystoseira Baccata Extract in water	100%	10	24-hour patch test; occlusive dressing	Non-irritating	49
Cystoseira Baccata Extract	Cystoseira Baccata Extract in water	100%	50	Repeated cutaneous applications	Non-irritating	49
Cystoseira Tamaricifolia Extract	Trade name mixture containing Cystoseira Tamaricifolia Extract and caprylic/capric triglycerides	100%	10	24-hour patch test; occlusive patch	Non-irritating	49
Fucus Spiralis Extract	Trade name mixture consisting of Fucus Spiralis Extract (1 - 3%) in butylene glycol and water	100%; 20 µL	12	24-hour patch test; occlusive patch; application over an area of 50 mm^2	Non-irritating	115
Fucus Spiralis Extract	Trade name mixture consisting of Fucus Spiralis Extract (< 5%) in caprylic/capric triglycerides	100%; 20 µL	10	Test substance applied to an area of 50 mm^2 under an occlusive patch for 30 minutes and 24 hours	Slightly irritating at the 30 minute reading and non-irritating at the 24 hour reading	116
Fucus vesiculosus extract	Aqueous extract of *Fucus vesiculosus* extract	1%; 0.2 mL	10	The test substance was a gel formulation that was applied to the cheek twice a day for 5 weeks. The same gel, without the extract, was pplied to the other cheek.	Non-irritating	44
Halidrys Siliquosa Extract	Halidrys Siliquosa Extract (52%) in water (48%)	5%; 0.02 mL	13	Test substance was diluted to 5% and applied to the back under an occlusive patch for 48 hours	Non-irritating	64

Table 30. Dermal irritation and sensitization

Ingredient	Test Substance	Concentration/Dose of the test substance	Test Population	Procedure	Results	Reference
Himanthalia Elongata Extract	Trade name mixture containing Himanthalia Elongata Extract, water, and dipropylene glycol	100%	10	24-hour patch test; occlusive patch	Non-irritating	49
Himanthalia Elongata Extract and Undaria Pinnatifida Extract	Himanthalia Elongata Extract (20%), Undaria Pinnatifida Extract (37%), and water (43%)	NR	10	Test substance (0.02 mL) applied to the back under an occlusive patch for 48 hours	Very Slightly Irritating (average irritant score of 0.10)	63
Laminaria Digitata Extract	Laminaria Digitata Extract and water	NR	10	24-hour patch test; occlusive patch	Non-irritating	49
Laminaria Digitata Extract	Trade name mixture containing Laminaria Digitata Extract (<5%) in caprylic/capric triglycerides	100%; 20 µL	12	24-hour patch test; test substance applied to an area of 50 mm^2; occlusive patch	Non-irritating	117
Laminaria Digitata Extract	Laminaria Digitata Extract (1.5-2.5%) in water and propylene glycol	100%; 20 µL	12	Test substance applied under an occlusive patch for 30 minutes or 24 hours over an area of 50 mm^2	Moderately irritating at the 30 minute reading; Slightly irritating at the 24 hour reading	118
Laminaria Hyperborea Extract	Trade name mixture containing Laminaria Hyperborea Extract (1-3%) in water	100%; 20 µL	10	24-hour patch test; occlusive patch	Non-irritating	119
Laminaria Japonica Extract	Skin cream containing a 50/50 aqueous propylene glycol extract of Laminaria japonica	10%; 20 mg	25	Patches were applied to the forearms of subjects using Finn chambers for up to 48 h and scored for irritation 6 h after patch removal.	Non-irritating	51
Laminaria Ochroleuca Extract	Trade name mixture consisting of Laminaria Ochroleuca Extract (<5%) in caprylic/capric triglycerides	2%; 20 µL	11	Single 24 hour application over an area of 50 mm^2; occlusive patch	Non-irritating	120
Macrocystis Pyrifera (Kelp) Extract	Macrocystis Pyrifera (Kelp) Extract (water extract)	4%	10	48-hour occlusive single path test	Non-irritating	97
Laminaria Saccharina Extract	Trade name mixture containing Laminaria Saccharina Extract (1 -3%) in water and propylene glycol	8, 16, or 100%; 20 µL	10	Six occlusive patches (drenched with test substance) per concentration were applied to the arms over a 50 mm^2 surface for 24 and 48 hours	100% dose was slightly irritating; minimal erythema in 5/10 subjects; 16% dose was non-irritating; 8% dose was non-irritating	121
Pelvetia Canaliculata Extract	Trade name mixture containing Pelvetia Canaliculata Extract (1 -3%) in butylene glycol and water	100%; 20 µL	12	Test substance was applied to skin under occlusive patches over a 50 mm^2 surface for 30 minutes and 24 hours	Non-irritating at the 30 minute reading; Slightly irritating at the 24 hour reading	122
Pelvetia Canaliculata Extract	Trade name mixture containing Pelvetia Canaliculata Extract (1 -3%) in propylene glycol and water	100: 20 µL	12	Test substance was applied to skin under occlusive patches over a 50 mm^2 surface for 30 minutes and 24 hours	Moderately irritating at the 30 minute reading; slightly irritating at the 24 hour reading	123

Table 30. Dermal irritation and sensitization

Ingredient	Test Substance	Concentration/Dose of the test substance	Test Population	Procedure	Results	Reference
Pelvetia Canaliculata Extract	Trade name mixture containing Pelvetia Canaliculata Extract (0.5 - 3%) in water	100%; 20 µL	11	24-hour patch test; occlusive patch	Non-irritating	124
Sargassum Glaucescens Extract	Trade name mixture containing 20% Sargassum Glaucescens Extract, 79% water, and 1% phenoxyethanol	10%	10	Test substance was applied under an occlusive patch for 48 hours	Non-irritating	125
Sphacelaria Scoparia Extract	Sphacelaria Scoparia Extract, water, and dipropylene glycol	100%	11	24-hour patch test; occlusive dressing	Non-irritating	49
Undaria Pinnatifida Extract	Trade name mixture containing Undaria Pinnatifida Extract (< 5%) in water and propylene glycol	100%; 20 µL	12	Test substance applied to the skin over an area of 50 mm^2 for either 30 minutes or 24 hours; occlusive patch	Moderately irritating after 30 minutes; Mildly irritating after 24 hours	126
Undaria Pinnatifida Extract	Trade name mixture containing Undaria Pinnatifida Extract in water and dipropylene glycol	NR	10	24-hour patch test; occlusive dressing	Non-irritating	49
Pelvetia Canaliculata Extract and Laminaria Digitata Extract	Trade name mixture containing Pelvetia Canaliculata Extract and Laminaria Digitata Extract extracted in propylene glycol with panthenol (the amount of dry extract of both extracts combined is estimated to be 5.5-9.0%)	5, 10, and 100%; 20 µL	10	Test substance was applied to skin under occlusive patches over a 50 mm^2 surface for 24 and 48 hours	Mild irritation at the 100% concentration; Minimal irritation at the 10% concentration; No irritation at the 5% concentration	127
Sensitization						
IN VITRO						
Undaria Pinnatifida Cell Culture Extract	Trade name mixture containing Undaria Pinnatifida Cell Culture Extract (24%) with water as solvent	0.98 – 2000 µM	3 per test concentration	ARE-Nrf2 Luciferase Test performed according to OECD TG 442D; immortalized adherent human keratinocyte cell line; 12 test concentrations ranging from 0.98 to 2000 µM were used	Non-sensitizing	128
Undaria Pinnatifida Cell Culture Extract	Undaria Pinnatifida Cell Culture Extract (24%) with water as solvent in Acetonitrile	5 mM or 25 mM	3 per test concentration	Direct Peptide Reactivity Assay (DPRA) performed according to OECD TG 442C; 1:10 ratio of Cysteine Peptide (0.5 mM) and test chemical (5 mM)) and 1:50 ratio of Lysine peptide (0.5 mM) and test chemical (25 mM)	Non-sensitizing	129
ANIMAL						
Ascophyllum nodosum Extract	Ascophyllum Nodosum extract	0.1 to 400 µL of 25% to 75% water solutions	20 test and 10 control guinea pigs	Magnusson and Kligman (guinea pig maximization test); OECD TG 406	Non-sensitizing	87
HUMAN						
Alaria Esculenta Extract	Trade name mixture consisting of Alaria Esculenta Extract (<5%) in capyrlic/capric triglycerides – dried before extraction	100%; 25 µL	50	The sensitizing potential of the test substance was studied according to an HRIPT. *	Non-irritating; Non-sensitizing	133
Alaria Esculenta Extract	Night cream containing 0.05% Alaria Esculenta Extract	0.2 g	105	A HRIPT * was performed. The test material was applied to the 1 $inch^2$ absorbent pad portion of a clear adhesive dressing.	Non-sensitizing	134

Table 30. Dermal irritation and sensitization

Ingredient	Test Substance	Concentration/Dose of the test substance	Test Population	Procedure	Results	Reference
Alaria Esculenta Extract	Trade name mixture consisting of Alaria Esculenta Extract (0.5-2.5%) in butylene glycol and water	100%; 25 µL	50	The sensitizing potential of the test substance was studied according to an HRIPT.**	Non-irritating; Non-sensitizing	131
Fucus Spiralis Extract	Trade name mixture consisting of Fucus Spiralis Extract (1-3%) in butylene glycol and water	100%; 200 µL	50	An HRIPT* was performed.	Non-sensitizing	135
Fucus Spiralis Extract	Trade name mixture consisting of Fucus Spiralis Extract (<5%) in caprylic/capric triglycerides	100%; 200 µL	52	An HRIPT* was performed.	Non-sensitizing	116
Halidrys Siliquosa Extract	Halidrys Siliquosa Extract (48%) and water (52%)	100%	107	An HRIPT* was performed. Dosing details were not provided.	Non-sensitizing	64
Laminaria Digitata Extract	Laminaria Digitata Extract (<5%) in caprylic/capric triglycerides	100%; 20 µL	46	An HRIPT* was performed.	Non-sensitizing	136
Laminaria Saccharina Extract	Trade name mixture containing Laminaria Saccharina Extract (1-3%) in water and propylene glycol	20%; 25 µL	50	The sensitizing potential of the test substance was studied according to an HRIPT.**	Non-irritating; Non-sensitizing	137
Macrocystis Pyrifera (Kelp) Extract	Macrocystis Pyrifera (Kelp) Extract (water extract)	4%	53	An HRIPT* was performed.	Non-irritating; Non-sensitizing	97
Pelvetia Canaliculata Extract	Trade name mixture containing Pelvetia Canaliculata Extract (0.5-3%) in water	100%; 200 µL	55	A HRIPT* was performed.	Non-irritating; Non-sensitizing	124
Sargassum Filipendula Extract	Face cream containing 1.2% Sargassum Filipendula Extract	0.2 g	206	A HRIPT* was performed. A 4 cm^2 occlusive patch was used.	Non-sensitizing	138
Sargassum Filipendula Extract	Trade name mixture containing Sargassum Filipendula Extract (1.3%), water (81.78%), Sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), Methylparaben (0.2%), Propylparaben (0.025%)	0.98-2000 µM	2 per test conncentration	ARE-Nrf2 Luciferase Test performed according to OECD TG 442D; immortalized adherent human keratinocyte cell line; 12 test concentrations ranging from 0.98 to 2000 µM were used		130
Sargassum Muticum Extract	Eye cream containing 0.076% Sargassum Muticum Extract	0.2 g	103	A HRIPT* was performed. The test material was applied to the 1 $inch^2$ absorbent pad portion of a clear adhesive dressing.	Non-sensitizing	139
Sargassum Muticum Extract	Skin care product containing 0.076% Sargassum Muticum Extract	0.2 g	104	A HRIPT* was performed. The test material was applied to the 1 $inch^2$ absorbent pad portion of a clear adhesive dressing.	Non-sensitizing	140
Sphacelaria Scoparia Extract	Sphacelaria Scoparia Extract, water, and dipropylene glycol	100%	50	Repeated epicutaneous applications	Hypoallergenic	49
Undaria Pinnatifida Extract	Trade name mixture containing Undaria Pinnatifida Extract (<5%) in caprylic/capric triglycerides	100%; 50 µL	100	HRIPT	Non-irritating; Non-sensitizing	141

ARE = Antioxidant Response Elements; HRIPT = Human Repeat Insult Patch Test; Nrf2 = Nuclear factor-erythroid 2-related factor; NR = Not Reported

*The following is the general procedure in which an HRIPT is performed. The test material is applied to the upper back under a semi-occlusive patch. During the induction phase, patches are applied 3 times per week for 3 weeks, for a total of 9 applications. If the test substance caused a moderate reaction (2-level), the application is moved to an adjacent area. If 3-level or 4-level reactions were noted, applications are discontinued. Two weeks after the final induction application, a challenge patch is applied to a previously untested site adjacent to the original patch site. Patches are removed and sites were scored 24 and 72 hours after application.

**The Marzulli-Maibach method is as follows: The test substance is applied (under an occlusive patch) 3 times a week during the induction phase and once a week during challenge phase. The induction phase lasts for 3 weeks, followed by a latent phase which lasts for 2 weeks.

Table 31. Ocular irritation studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO					
Trade name mixture containing Ascophyllum Nodosum Extract (4.7%) in water (94.5%)	NR	NR	Het-Cam test	Non-irritating	68
Cystoseira Amentacea/Caespitosa/Brachycarpa Extract (48%), water (52%)	100%	NR	Het-Cam test on hen's egg chorion-allantoic membrane; incubation for 11 days	Slightly irritating	101
Halidrys Siliquosa Extract (48%) in water (52%)	5%	NR	Het-Cam test on hen's egg chorion-allantoic membrane; incubation for 11 days	Slightly irritating	64
Himanthalia Elongata Extract (20%), Undaria Pinnatifida Extract (37%), water (43%)	10%	NR	Het-Cam test on hen's egg chorion-allantoic membrane	Slightly irritating	63
Macrocystis Pyrifera (Kelp) Extract	4%	NR	Het-Cam test on hen's egg chorion-allantoic membrane	Mildly irritating	97
Trade name mixture containing Sargassum Filipendula Extrac (1.3%), water (81.78%), Sorbitol (14%), Hypnea Musciformis Extract (1.4%), Gellidiela Acerosa Extract (1.3%), Methylparaben (0.2%), Propylparaben (0.025%)	100%, 50 µL (liquid) or 50 mg (solid)	2	Test substance was applied to reconstructed cornea epithelium and allowed to incubate for 90 minutes	Non-irritating	113
Undaria Pinatifida Cell Culture Extract (24%) in water	50 µL (liquid) or 50 mg (solid)	NR	Test substance was applied to reconstructed cornea epithelium; after application, epithelia was incubated for 30 (liquid) or 90 (solid) minutes	Non-irritating	110
Laminaria Japonica Extract (7%), Nereocystis Leutkeana Extract (7%), Macrocystis Pyrifera Extract (7%), and pentaerythrityl tetraethylhexanoate	50 µL (liquid) or 50 mg (solid)	NR	Test substance was applied to reconstructed cornea epithelium; after application, epithelia was incubated for 30 (liquid) or 90 (solid) minutes	Non-irritating	112
ANIMAL					
Ascophyllum Nodosum Extract	100 mg	3	OECD TG 405; New Zealand White rabbits; test substance was instilled into one eye of each rabbit and rinsed after 1 hour; examination occurred 1, 24, 48, and 72 hours, and 7 days after administration	The maximum irritation score was 6.7 out of 8 at 1 h post-instillation; the score decreased to 0 by day 7, which indicated that the induced changes were reversible, and thus, the effects of the test substance were classified as 'irritation' and not as 'corrosion.' The test substance was rated as a mild ocular irritant.	6
Ascophyllum Nodosum Extract	NR	NR	OECD TG 405; no other details were provided for this study	Slightly irritating	87
HUMAN					
Eye cream containing 0.076% Sargassum Muticum Extract	100%	31	Test substance was applied to the eye contour of 31 subjects. Half of the subjects were soft-contact lens wearers. Exam was performed 4 weeks after usage.	Non-irritating	142

NR = Not Reported

Table 32. Oral clinical trials

Test Article	Extraction/ Solvent Method or Characterization	Study group	Study Details	Results	Reference
Ascophyllum Nodosum Powder (0.5 g/day)	Powdered plant	Healthy female subjects (n = 42)	After a 4-day period of keeping a food diary, subjects were administered capsules containing extract or potassium iodide daily for 14 days, then repeated 4-day food diary. All-day urine sample was collected on fourth day of run-in period and last day of treatment period (day 19) and fasted blood samples were collected on fourth day of run-in period and on day after treatment period (day 20).	There was an increase in urinary iodine concentrations (median 140 mg/l vs78 mg/l) in the treatment group. TSH increased slightly but within normal range 2 subjects. Increase in TSH concentrations may be associated with iodine-induced hypothyroidism, especially in those subjects with low iodine stores, although no change in the concentrations of thyroid hormones was observed. There were no adverse events reported during this experiment.	145
Ecklonia Cava Extract (400 mg/day)	Alcohol	Subjects with hyper-cholesterolaemia (n = 52)	Uncontrolled, open-label, single-arm study for 12 weeks	Hematological, clinical chemistry, and urinalysis did not reveal any adverse effects. There was one instance (2.2%) each of nausea, dyspepsia, diarrhea, and alopecia reported.	9,146
Ecklonia Cava Extract (0, 72, or 144 mg/day)	Phlorotannin-rich	Overweight subjects (n = 32 or 33)	Randomized, double-blind, three-arm, parallel trial for 12 weeks	Hematological and clinical chemistry did not reveal any adverse effects. Only high-dose group showed significant decreases in serum glucose and systolic blood pressure. No adverse signs were observed during the trial.	9
Ecklonia Cava Extract (0 or 400 mg/day)	Alcohol	Overweight subjects (n = 40)	Randomized, double-blind, and placebo-controlled trial for 12 weeks. Administered as 200 mg twice per day in capsules	There were no adverse events reported that were related to the test substance.	24
Undaria Pinnatifida Powder (desalinated; 5040 mg/day)	Powdered	Hypertensive subjects (n = 18)	Subjects were gender and age matched to control group. Capsules (420 mg/capsule; 4 capsules/dose) 3 times/day with meals. Examined for body weight, BP, and blood chemistry parameters prior to experiment, at 4 weeks, and at 8 weeks. 1 subject in treatment group left study for personal reasons, so final number of paired subjects was 18, (some of her data (e.g., adverse effects) were used).	Compliance was not consistent; 6 subjects followed protocol; 1 ingested 9 capsules/day, 2 ingested 8 capsules/day, 6 ingested 6 capsules/day, and 3 ingested 3capsules/day. Average intake was estimated to be 7.9 capsules or 3.3 g/day. Average SBP in treatment group decreased by 13 mmHg from the baseline after 4 weeks, and was reduced by 8 mmHg below baseline after 8 weeks. Average DBP decreased by 9 mmHg from baseline after 4 weeks and by 8 mmHg after 8 weeks. There were no significant changes in either SBP or DBP in control group. However, the differences in reductions in SBP and DBP were significant between the treatment group and control group. Hypercholesterolemia subjects in treatment group had decreased total cholesterol by 8% after 4 weeks; no changes were observed in subjects with normal cholesterol levels. Adverse effects included 2 cases of indigestion and 1 case of diarrhea, all of which resolved quickly without treatment.	66

BP = blood pressure; DBP = diastolic blood pressure; SBP = systolic blood pressure; TSH = thyroid-stimulating hormone

Table 33. Change in menstrual cycle with the oral administration of Fucus Vesiculosus Powder[147]

	Menstrual cycle length			Days of Menstruation		
Subject	Baseline	Low-Dose	High-Dose	Baseline	Low-Dose	High-Dose
1	16.3 ± 0.6 days	26.0 ± 1.4 days	31.2 ± 1.1 days	9.3 ± 0.6 days	6.3 ± 1.8 days	4.5 ± 0.7 days
2	23.0 ± 1.7 days	28.5 ± 0.7 days	-	8.0 ± 1.0 days	5.3 ± 2.5 days	-
3	27.3 ± 0.6 days	31.5 ± 0.7 days	36.0 ± 2.8 days	6.3 ± 1.5 days	5.8 ± 0.4 days	3.5 ± 0.7 days

- = no data

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2018 VCRP Data for Brown Algae-Derived Ingredients

03C - Eye Shadow	ALARIA ESCULENTA EXTRACT	8
03D - Eye Lotion	ALARIA ESCULENTA EXTRACT	2
03G - Other Eye Makeup Preparations	ALARIA ESCULENTA EXTRACT	1
05I - Other Hair Preparations	ALARIA ESCULENTA EXTRACT	1
07A - Blushers (all types)	ALARIA ESCULENTA EXTRACT	6
07B - Face Powders	ALARIA ESCULENTA EXTRACT	5
07C - Foundations	ALARIA ESCULENTA EXTRACT	1
07E - Lipstick	ALARIA ESCULENTA EXTRACT	3
07I - Other Makeup Preparations	ALARIA ESCULENTA EXTRACT	1
12C - Face and Neck (exc shave)	ALARIA ESCULENTA EXTRACT	4
12D - Body and Hand (exc shave)	ALARIA ESCULENTA EXTRACT	2
12F - Moisturizing	ALARIA ESCULENTA EXTRACT	3
		37

03D - Eye Lotion	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT (84775780)	8
03G - Other Eye Makeup Preparations	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	8
05A - Hair Conditioner	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	6
05F - Shampoos (non-coloring)	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	3
05G - Tonics, Dressings, and Other Hair Grooming Aids	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	4
07B - Face Powders	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	1
07F - Makeup Bases	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	2
08B - Cuticle Softeners	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	1
08G - Other Manicuring Preparations	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	2
10A - Bath Soaps and Detergents	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	6
11A - Aftershave Lotion	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	1
11E - Shaving Cream	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	1
12A - Cleansing	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	5
12C - Face and Neck (exc shave)	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	40
12D - Body and Hand (exc shave)	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	4
12F - Moisturizing	ASCOPHYLLUM NODOSUM (SEAWEED)	16

	EXTRACT	
12G - Night	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	2
12H - Paste Masks (mud packs)	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	8
12I - Skin Fresheners	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	1
12J - Other Skin Care Preps	ASCOPHYLLUM NODOSUM (SEAWEED) EXTRACT	1
		120

02A - Bath Oils, Tablets, and Salts	ASCOPHYLLUM NODOSUM POWDER	1
12F - Moisturizing	ASCOPHYLLUM NODOSUM POWDER	2
12J - Other Skin Care Preps	ASCOPHYLLUM NODOSUM POWDER	1
		4

03G - Other Eye Makeup Preparations	CLADOSIPHON OKAMURANUS EXTRACT	1
07C - Foundations	CLADOSIPHON OKAMURANUS EXTRACT	1
12A - Cleansing	CLADOSIPHON OKAMURANUS EXTRACT	1
12C - Face and Neck (exc shave)	CLADOSIPHON OKAMURANUS EXTRACT	3
12F - Moisturizing	CLADOSIPHON OKAMURANUS EXTRACT	1
12G - Night	CLADOSIPHON OKAMURANUS EXTRACT	2
		9

12C - Face and Neck (exc shave)	DICTYOPTERIS MEMBRANACEA EXTRACT (RETIRED)	1

03D - Eye Lotion	FUCUS SERRATUS EXTRACT	1
12C - Face and Neck (exc shave)	FUCUS SERRATUS EXTRACT	4
12F - Moisturizing	FUCUS SERRATUS EXTRACT	2
12G - Night	FUCUS SERRATUS EXTRACT	1
		8

02A - Bath Oils, Tablets, and Salts	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	3
02B - Bubble Baths	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	2
02D - Other Bath Preparations	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	6
03D - Eye Lotion	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	2
03F - Mascara	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	3
03G - Other Eye Makeup Preparations	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
04E - Other Fragrance Preparation	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
05A - Hair Conditioner	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	7
05C - Hair Straighteners	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
05F - Shampoos (non-coloring)	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	7
05G - Tonics, Dressings, and Other Hair Grooming Aids	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	6
05I - Other Hair Preparations	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
07F - Makeup Bases	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
07I - Other Makeup Preparations	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
10A - Bath Soaps and Detergents	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	14
10E - Other Personal Cleanliness Products	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	5
11A - Aftershave Lotion	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
11E - Shaving Cream	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
11F - Shaving Soap	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
11G - Other Shaving Preparation Products	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
12A - Cleansing	FUCUS VESICULOSUS	12

	(BLADDERWRACK) EXTRACT	
12B - Depilatories	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
12C - Face and Neck (exc shave)	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	48
12D - Body and Hand (exc shave)	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	33
12E - Foot Powders and Sprays	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
12F - Moisturizing	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	36
12G - Night	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
12H - Paste Masks (mud packs)	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	25
12I - Skin Fresheners	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	4
12J - Other Skin Care Preps	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	18
13B - Indoor Tanning Preparations	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	42
13C - Other Suntan Preparations	FUCUS VESICULOSUS (BLADDERWRACK) EXTRACT	1
		287

12C - Face and Neck (exc shave)	FUCUS VESICULOSUS POWDER	1
12H - Paste Masks (mud packs)	FUCUS VESICULOSUS POWDER	2
		3

03G - Other Eye Makeup Preparations	HIMANTHALIA ELONGATA EXTRACT	1
05A - Hair Conditioner	HIMANTHALIA ELONGATA EXTRACT	1
05I - Other Hair Preparations	HIMANTHALIA ELONGATA EXTRACT	1
12C - Face and Neck (exc shave)	HIMANTHALIA ELONGATA EXTRACT	1
12D - Body and Hand (exc shave)	HIMANTHALIA ELONGATA EXTRACT	3
12F - Moisturizing	HIMANTHALIA ELONGATA EXTRACT	1
12H - Paste Masks (mud packs)	HIMANTHALIA ELONGATA EXTRACT	1
		9

07F - Makeup Bases	KAPPAPHYCUS ALVAREZII EXTRACT	1
10E - Other Personal Cleanliness Products	KAPPAPHYCUS ALVAREZII EXTRACT	2
12C - Face and Neck (exc shave)	KAPPAPHYCUS ALVAREZII EXTRACT	2
		5

03D - Eye Lotion	LAMINARIA CLOUSTONI EXTRACT	1
07F - Makeup Bases	LAMINARIA CLOUSTONI EXTRACT	1
12A - Cleansing	LAMINARIA CLOUSTONI EXTRACT	3
12C - Face and Neck (exc shave)	LAMINARIA CLOUSTONI EXTRACT	4
12F - Moisturizing	LAMINARIA CLOUSTONI EXTRACT	2
12G - Night	LAMINARIA CLOUSTONI EXTRACT	1
12H - Paste Masks (mud packs)	LAMINARIA CLOUSTONI EXTRACT	1
12I - Skin Fresheners	LAMINARIA CLOUSTONI EXTRACT	1
		14

02A - Bath Oils, Tablets, and Salts	LAMINARIA DIGITATA EXTRACT	2
02B - Bubble Baths	LAMINARIA DIGITATA EXTRACT	3
02D - Other Bath Preparations	LAMINARIA DIGITATA EXTRACT	2
03D - Eye Lotion	LAMINARIA DIGITATA EXTRACT	3
03E - Eye Makeup Remover	LAMINARIA DIGITATA EXTRACT	2
03F - Mascara	LAMINARIA DIGITATA EXTRACT	4
03G - Other Eye Makeup Preparations	LAMINARIA DIGITATA EXTRACT	6
05A - Hair Conditioner	LAMINARIA DIGITATA EXTRACT	4
05B - Hair Spray (aerosol fixatives)	LAMINARIA DIGITATA EXTRACT	1
05F - Shampoos (non-coloring)	LAMINARIA DIGITATA EXTRACT	12
05G - Tonics, Dressings, and Other Hair Grooming Aids	LAMINARIA DIGITATA EXTRACT	18
05I - Other Hair Preparations	LAMINARIA DIGITATA EXTRACT	1
06H - Other Hair Coloring Preparation	LAMINARIA DIGITATA EXTRACT	1
07B - Face Powders	LAMINARIA DIGITATA EXTRACT	3
07C - Foundations	LAMINARIA DIGITATA EXTRACT	3
07E - Lipstick	LAMINARIA DIGITATA EXTRACT	1
07F - Makeup Bases	LAMINARIA DIGITATA EXTRACT	1
07I - Other Makeup Preparations	LAMINARIA DIGITATA EXTRACT	2
09A - Dentifrices	LAMINARIA DIGITATA EXTRACT	1
10A - Bath Soaps and Detergents	LAMINARIA DIGITATA EXTRACT	6

10C - Douches	LAMINARIA DIGITATA EXTRACT	1
10E - Other Personal Cleanliness Products	LAMINARIA DIGITATA EXTRACT	4
11A - Aftershave Lotion	LAMINARIA DIGITATA EXTRACT	4
12A - Cleansing	LAMINARIA DIGITATA EXTRACT	18
12C - Face and Neck (exc shave)	LAMINARIA DIGITATA EXTRACT	36
12D - Body and Hand (exc shave)	LAMINARIA DIGITATA EXTRACT	31
12F - Moisturizing	LAMINARIA DIGITATA EXTRACT	20
12G - Night	LAMINARIA DIGITATA EXTRACT	3
12H - Paste Masks (mud packs)	LAMINARIA DIGITATA EXTRACT	17
12I - Skin Fresheners	LAMINARIA DIGITATA EXTRACT	3
12J - Other Skin Care Preps	LAMINARIA DIGITATA EXTRACT	22
		235

02A - Bath Oils, Tablets, and Salts	LAMINARIA DIGITATA POWDER	1
02D - Other Bath Preparations	LAMINARIA DIGITATA POWDER	2
05A - Hair Conditioner	LAMINARIA DIGITATA POWDER	2
05F - Shampoos (non-coloring)	LAMINARIA DIGITATA POWDER	4
10E - Other Personal Cleanliness Products	LAMINARIA DIGITATA POWDER	1
12C - Face and Neck (exc shave)	LAMINARIA DIGITATA POWDER	1
12H - Paste Masks (mud packs)	LAMINARIA DIGITATA POWDER	9
12J - Other Skin Care Preps	LAMINARIA DIGITATA POWDER	1
		21

12F - Moisturizing	LAMINARIA HYPERBOREA EXTRACT	2

01B - Baby Lotions, Oils, Powders, and Creams	LAMINARIA JAPONICA EXTRACT	1
03D - Eye Lotion	LAMINARIA JAPONICA EXTRACT	2
03F - Mascara	LAMINARIA JAPONICA EXTRACT	1
05F - Shampoos (non-coloring)	LAMINARIA JAPONICA EXTRACT	1
07A - Blushers (all types)	LAMINARIA JAPONICA EXTRACT	2

07B - Face Powders	LAMINARIA JAPONICA EXTRACT	3
07C - Foundations	LAMINARIA JAPONICA EXTRACT	10
07E - Lipstick	LAMINARIA JAPONICA EXTRACT	1
07F - Makeup Bases	LAMINARIA JAPONICA EXTRACT	2
08G - Other Manicuring Preparations	LAMINARIA JAPONICA EXTRACT	2
10A - Bath Soaps and Detergents	LAMINARIA JAPONICA EXTRACT	1
10E - Other Personal Cleanliness Products	LAMINARIA JAPONICA EXTRACT	1
12A - Cleansing	LAMINARIA JAPONICA EXTRACT	3
12C - Face and Neck (exc shave)	LAMINARIA JAPONICA EXTRACT	26
12D - Body and Hand (exc shave)	LAMINARIA JAPONICA EXTRACT	2
12F - Moisturizing	LAMINARIA JAPONICA EXTRACT	5
12G - Night	LAMINARIA JAPONICA EXTRACT	2
12H - Paste Masks (mud packs)	LAMINARIA JAPONICA EXTRACT	9
12J - Other Skin Care Preps	LAMINARIA JAPONICA EXTRACT	3
		77

03C - Eye Shadow	LAMINARIA OCHROLEUCA EXTRACT	2
07B - Face Powders	LAMINARIA OCHROLEUCA EXTRACT	3
07C - Foundations	LAMINARIA OCHROLEUCA EXTRACT	2
07E - Lipstick	LAMINARIA OCHROLEUCA EXTRACT	1
07I - Other Makeup Preparations	LAMINARIA OCHROLEUCA EXTRACT	2
10E - Other Personal Cleanliness Products	LAMINARIA OCHROLEUCA EXTRACT	2
12A - Cleansing	LAMINARIA OCHROLEUCA EXTRACT	1
12C - Face and Neck (exc shave)	LAMINARIA OCHROLEUCA EXTRACT	3
12D - Body and Hand (exc shave)	LAMINARIA OCHROLEUCA EXTRACT	2
12F - Moisturizing	LAMINARIA OCHROLEUCA EXTRACT	2
12H - Paste Masks (mud packs)	LAMINARIA OCHROLEUCA EXTRACT	1
12J - Other Skin Care Preps	LAMINARIA OCHROLEUCA EXTRACT	4
13B - Indoor Tanning Preparations	LAMINARIA OCHROLEUCA EXTRACT	1
		26

05A - Hair Conditioner	LAMINARIA SACCHARINA EXTRACT reported as SACCHARINA LATISSIMA (KELP) EXTRACT*	4
05F - Shampoos (non-coloring)	SACCHARINA LATISSIMA (KELP) EXTRACT	4
05G - Tonics, Dressings, and Other Hair Grooming Aids	SACCHARINA LATISSIMA (KELP) EXTRACT	4
07C - Foundations	SACCHARINA LATISSIMA (KELP) EXTRACT	9
07I - Other Makeup Preparations	SACCHARINA LATISSIMA (KELP) EXTRACT	2
10A - Bath Soaps and Detergents	SACCHARINA LATISSIMA (KELP) EXTRACT	2
10E - Other Personal Cleanliness Products	SACCHARINA LATISSIMA (KELP) EXTRACT	2
11A - Aftershave Lotion	SACCHARINA LATISSIMA (KELP) EXTRACT	4
11D - Preshave Lotions (all types)	SACCHARINA LATISSIMA (KELP) EXTRACT	1
11E - Shaving Cream	SACCHARINA LATISSIMA (KELP) EXTRACT	1
12A - Cleansing	SACCHARINA LATISSIMA (KELP) EXTRACT	27
12C - Face and Neck (exc shave)	SACCHARINA LATISSIMA (KELP) EXTRACT	19
12F - Moisturizing	SACCHARINA LATISSIMA (KELP) EXTRACT	33
12G - Night	SACCHARINA LATISSIMA (KELP) EXTRACT	1
12H - Paste Masks (mud packs)	SACCHARINA LATISSIMA (KELP) EXTRACT	6
12I - Skin Fresheners	SACCHARINA LATISSIMA (KELP) EXTRACT	2
12J - Other Skin Care Preps	SACCHARINA LATISSIMA (KELP) EXTRACT	11
		132

* The accepted scientific name for *Laminaria saccharina* is *Saccharina latissima.*

10A - Bath Soaps and Detergents	MACROCYSTIS PYRIFERA (KELP)	1
12F - Moisturizing	MACROCYSTIS PYRIFERA (KELP)	1
		2

02A - Bath Oils, Tablets, and Salts	MACROCYSTIS PYRIFERA (KELP) EXTRACT	3
02B - Bubble Baths	MACROCYSTIS PYRIFERA (KELP) EXTRACT	1
03D - Eye Lotion	MACROCYSTIS PYRIFERA (KELP) EXTRACT	1
03E - Eye Makeup Remover	MACROCYSTIS PYRIFERA (KELP) EXTRACT	1
03G - Other Eye Makeup Preparations	MACROCYSTIS PYRIFERA (KELP) EXTRACT	3
04E - Other Fragrance Preparation	MACROCYSTIS PYRIFERA (KELP) EXTRACT	6
05A - Hair Conditioner	MACROCYSTIS PYRIFERA (KELP) EXTRACT	10
05B - Hair Spray (aerosol fixatives)	MACROCYSTIS PYRIFERA (KELP) EXTRACT	3
05F - Shampoos (non-coloring)	MACROCYSTIS PYRIFERA (KELP) EXTRACT	12
05G - Tonics, Dressings, and Other Hair Grooming Aids	MACROCYSTIS PYRIFERA (KELP) EXTRACT	20

05H - Wave Sets	MACROCYSTIS PYRIFERA (KELP) EXTRACT	1
05I - Other Hair Preparations	MACROCYSTIS PYRIFERA (KELP) EXTRACT	10
06H - Other Hair Coloring Preparation	MACROCYSTIS PYRIFERA (KELP) EXTRACT	4
07A - Blushers (all types)	MACROCYSTIS PYRIFERA (KELP) EXTRACT	2
07B - Face Powders	MACROCYSTIS PYRIFERA (KELP) EXTRACT	2
07C - Foundations	MACROCYSTIS PYRIFERA (KELP) EXTRACT	3
08A - Basecoats and Undercoats	MACROCYSTIS PYRIFERA (KELP) EXTRACT	2
08E - Nail Polish and Enamel	MACROCYSTIS PYRIFERA (KELP) EXTRACT	2
08G - Other Manicuring Preparations	MACROCYSTIS PYRIFERA (KELP) EXTRACT	1
10A - Bath Soaps and Detergents	MACROCYSTIS PYRIFERA (KELP) EXTRACT	16
10E - Other Personal Cleanliness Products	MACROCYSTIS PYRIFERA (KELP) EXTRACT	14
11A - Aftershave Lotion	MACROCYSTIS PYRIFERA (KELP) EXTRACT	2
11E - Shaving Cream	MACROCYSTIS PYRIFERA (KELP) EXTRACT	1
12A - Cleansing	MACROCYSTIS PYRIFERA (KELP) EXTRACT	6
12B - Depilatories	MACROCYSTIS PYRIFERA (KELP) EXTRACT	8
12C - Face and Neck (exc shave)	MACROCYSTIS PYRIFERA (KELP) EXTRACT	13
12D - Body and Hand (exc shave)	MACROCYSTIS PYRIFERA (KELP) EXTRACT	13
12F - Moisturizing	MACROCYSTIS PYRIFERA (KELP) EXTRACT	14
12G - Night	MACROCYSTIS PYRIFERA (KELP) EXTRACT	1
12H - Paste Masks (mud packs)	MACROCYSTIS PYRIFERA (KELP) EXTRACT	5
12I - Skin Fresheners	MACROCYSTIS PYRIFERA (KELP) EXTRACT	3
12J - Other Skin Care Preps	MACROCYSTIS PYRIFERA (KELP) EXTRACT	5
		188

10A - Bath Soaps and Detergents	MACROCYSTIS PYRIFERA (KELP) PROTEIN	1
12H - Paste Masks (mud packs)	MACROCYSTIS PYRIFERA (KELP) PROTEIN	1
12J - Other Skin Care Preps	MACROCYSTIS PYRIFERA (KELP) PROTEIN	1
		3

03D - Eye Lotion	PELVETIA CANALICULATA EXTRACT	1
03F - Mascara	PELVETIA CANALICULATA EXTRACT	3
03G - Other Eye Makeup Preparations	PELVETIA CANALICULATA EXTRACT	2
05A - Hair Conditioner	PELVETIA CANALICULATA EXTRACT	4
05B - Hair Spray (aerosol fixatives)	PELVETIA CANALICULATA EXTRACT	1
05F - Shampoos (non-coloring)	PELVETIA CANALICULATA EXTRACT	6
05G - Tonics, Dressings, and Other Hair Grooming Aids	PELVETIA CANALICULATA EXTRACT	12

05I - Other Hair Preparations	PELVETIA CANALICULATA EXTRACT	1
06H - Other Hair Coloring Preparation	PELVETIA CANALICULATA EXTRACT	1
10E - Other Personal Cleanliness Products	PELVETIA CANALICULATA EXTRACT	1
12A - Cleansing	PELVETIA CANALICULATA EXTRACT	1
12C - Face and Neck (exc shave)	PELVETIA CANALICULATA EXTRACT	8
12F - Moisturizing	PELVETIA CANALICULATA EXTRACT	4
12G - Night	PELVETIA CANALICULATA EXTRACT	2
		47

03D - Eye Lotion	SARGASSUM FILIPENDULA EXTRACT	2
05A - Hair Conditioner	SARGASSUM FILIPENDULA EXTRACT	1
05B - Hair Spray (aerosol fixatives)	SARGASSUM FILIPENDULA EXTRACT	3
05F - Shampoos (non-coloring)	SARGASSUM FILIPENDULA EXTRACT	2
05G - Tonics, Dressings, and Other Hair Grooming Aids	SARGASSUM FILIPENDULA EXTRACT	1
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	SARGASSUM FILIPENDULA EXTRACT	23
07I - Other Makeup Preparations	SARGASSUM FILIPENDULA EXTRACT	1
11F - Shaving Soap	SARGASSUM FILIPENDULA EXTRACT	1
12A - Cleansing	SARGASSUM FILIPENDULA EXTRACT	2
12C - Face and Neck (exc shave)	SARGASSUM FILIPENDULA EXTRACT	1
12F - Moisturizing	SARGASSUM FILIPENDULA EXTRACT	4
12H - Paste Masks (mud packs)	SARGASSUM FILIPENDULA EXTRACT	3
12J - Other Skin Care Preps	SARGASSUM FILIPENDULA EXTRACT	2
		46

12C - Face and Neck (exc shave)	SARGASSUM FUSIFORME EXTRACT	2
12F - Moisturizing	SARGASSUM FUSIFORME EXTRACT	2
12H - Paste Masks (mud packs)	SARGASSUM FUSIFORME EXTRACT	3
		7

12H - Paste Masks (mud packs)	SARGASSUM MUTICUM EXTRACT	1

10A - Bath Soaps and Detergents	SPHACELARIA SCOPARIA EXTRACT	2
12D - Body and Hand (exc shave)	SPHACELARIA SCOPARIA EXTRACT	4
12F - Moisturizing	SPHACELARIA SCOPARIA EXTRACT	1
12J - Other Skin Care Preps	SPHACELARIA SCOPARIA EXTRACT	1
		8

03D - Eye Lotion	UNDARIA PINNATIFIDA EXTRACT	4
05A - Hair Conditioner	UNDARIA PINNATIFIDA EXTRACT	1
05F - Shampoos (non-coloring)	UNDARIA PINNATIFIDA EXTRACT	3
05I - Other Hair Preparations	UNDARIA PINNATIFIDA EXTRACT	2
07B - Face Powders	UNDARIA PINNATIFIDA EXTRACT	3
07C - Foundations	UNDARIA PINNATIFIDA EXTRACT	3
07I - Other Makeup Preparations	UNDARIA PINNATIFIDA EXTRACT	2
10A - Bath Soaps and Detergents	UNDARIA PINNATIFIDA EXTRACT	1
10E - Other Personal Cleanliness Products	UNDARIA PINNATIFIDA EXTRACT	3
12A - Cleansing	UNDARIA PINNATIFIDA EXTRACT	1
12C - Face and Neck (exc shave)	UNDARIA PINNATIFIDA EXTRACT	26
12D - Body and Hand (exc shave)	UNDARIA PINNATIFIDA EXTRACT	12
12F - Moisturizing	UNDARIA PINNATIFIDA EXTRACT	8
12G - Night	UNDARIA PINNATIFIDA EXTRACT	4
12H - Paste Masks (mud packs)	UNDARIA PINNATIFIDA EXTRACT	1
		74

There were no reported uses in the 2018 VCRP:

Agarum Cribrosum Extract
Ascophyllum Nodosum
Asterionellopsis Glacialis Extract
Cladosiphon Novae-Caledoniae Extract
Cystoseira Amentacea/Caespitosa/Branchycarpa Extract
Cystoseira Baccata Extract
Cystoseira Balearica Extract
Cystoseira Caespitosa Extract
Cystoseira Compressa Extract
Cystoseira Compressa Powder
Cystoseira Tamariscifolia Extract
Dictyopteris Polypodioides Extract
Dictyota Coriacea Extract
Durvillaea Antarctica Extract

Ecklonia Cava Extract
Ecklonia Cava Water
Ecklonia Kurome Extract
Ecklonia Kurome Powder
Ecklonia/Laminaria Extract
Ecklonia Maxima Extract
Ecklonia Maxima Powder
Ecklonia Radiata Extract
Eisenia Arborea Extract
Fucus Spiralis Extract
Fucus Vesiculosus
Halidrys Siliquosa Extract
Halopteris Scoparia Extract
Himanthalia Elongata Powder
Hizikia Fusiforme Extract
Hizikia Fusiformis Water
Hizikia Fusiformis Callus Culture Extract
Hydrolyzed Ecklonia Cava Extract
Hydrolyzed Fucus Vesiculosus Extract
Hydrolyzed Fucus Vesiculosus Protein
Laminaria Diabolica Extract
Laminaria Japonica Powder
Laminaria Longissima Extract
Lessonia Nigrescens Extract
Lessonia Nigrescens Powder
Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract
Macrocystis Pyrifera (Kelp) Juice
Nereocystis Luetkeana Extract
Pelvetia Siliquosa Extract
Phyllacantha Fibrosa Extract
Rissoella Verruculosa Extract
Saccharina Angustata Extract
Laminaria Angustata Extract (Retired)
Saccharina Japonica Extract
Laminaria Ochotensis Extract (Retired)
Saccharina Longicruris Extract
Sargassum Fulvellum Extract
Sargassum Glaucescens Extract
Sargassum Horneri Extract
Sargassum Pallidum Extract
Sargassum Siliquastrum Extract
Sargassum Thunbergii Extract
Sargassum Vulgare Extract
Sahel Scenedesmus Extract
Undaria Peterseniana Extract
Undaria Pinnatifida Cell Culture Extract
Undaria Pinnatifida Leaf/Stem Extract
Undaria Pinnatifida Powder
Undaria Pinnatifida Root Powder

02A - Bath Oils, Tablets, and Salts	KELP	1
02D - Other Bath Preparations	KELP	4
05E - Rinses (non-coloring)	KELP	1
05F - Shampoos (non-coloring)	KELP	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	KELP	2
09C - Other Oral Hygiene Products	KELP	1
10A - Bath Soaps and Detergents	KELP	2
10E - Other Personal Cleanliness Products	KELP	3
12C - Face and Neck (exc shave)	KELP	3
12F - Moisturizing	KELP	1
12H - Paste Masks (mud packs)	KELP	2
12J - Other Skin Care Preps	KELP	3
		24

02D - Other Bath Preparations	KELP EXTRACT	1
03D - Eye Lotion	KELP EXTRACT	1
05F - Shampoos (non-coloring)	KELP EXTRACT	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	KELP EXTRACT	1
05H - Wave Sets	KELP EXTRACT	2
05I - Other Hair Preparations	KELP EXTRACT	3
10E - Other Personal Cleanliness Products	KELP EXTRACT	1
12C - Face and Neck (exc shave)	KELP EXTRACT	1
12F - Moisturizing	KELP EXTRACT	1
12G - Night	KELP EXTRACT	3
		15

05C - Hair Straighteners	LAMINARIA EXTRACT	1
05F - Shampoos (non-coloring)	LAMINARIA EXTRACT	1
12D - Body and Hand (exc shave)	LAMINARIA EXTRACT	1
12J - Other Skin Care Preps	LAMINARIA EXTRACT	1
		4

05A - Hair Conditioner	PHAEOPHYCEAE (BROWN ALGAE)	4

02D - Other Bath Preparations	SEAWEED EXTRACT	1
03D - Eye Lotion	SEAWEED EXTRACT	3
03G - Other Eye Makeup Preparations	SEAWEED EXTRACT	1
05A - Hair Conditioner	SEAWEED EXTRACT	1
05F - Shampoos (non-coloring)	SEAWEED EXTRACT	7
05G - Tonics, Dressings, and Other Hair Grooming Aids	SEAWEED EXTRACT	1
05I - Other Hair Preparations	SEAWEED EXTRACT	4
07B - Face Powders	SEAWEED EXTRACT	1
07E - Lipstick	SEAWEED EXTRACT	1
07F - Makeup Bases	SEAWEED EXTRACT	2
07I - Other Makeup Preparations	SEAWEED EXTRACT	1
10A - Bath Soaps and Detergents	SEAWEED EXTRACT	3
12A - Cleansing	SEAWEED EXTRACT	7
12C - Face and Neck (exc shave)	SEAWEED EXTRACT	18
12D - Body and Hand (exc shave)	SEAWEED EXTRACT	2
12F - Moisturizing	SEAWEED EXTRACT	9
12G - Night	SEAWEED EXTRACT	4
12H - Paste Masks (mud packs)	SEAWEED EXTRACT	4
12I - Skin Fresheners	SEAWEED EXTRACT	1
12J - Other Skin Care Preps	SEAWEED EXTRACT	11
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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: September 13, 2018

SUBJECT: Pelvetia Canaliculata Extract and Laminaria Digitata Extract

Biotech Marine. 2014. Manufacturing Process- Bioenergizer P (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Propylene Glycol with Panthenol).

Biotech Marine. 2016. Bioenergizer™ P (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Propylene Glycol with Panthenol) Physico-chemical data.

Palmer Research. 1996. Etude de la tolérance cutanée aiguë chez 10 volontaires adultes Patch-tests 24 et 48 heures, uniques (Bioenergizer P mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Propylene Glycol with Panthenol).

Biotech Marine. 2014. Manufacturing Process- Bioenergizer P BG (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Butylene Glycol with Panthenol, with preservatives).

Biotech Marine. 2014. Bioenergizer P BG (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Butylene Glycol with Panthenol with preservatives) Physico-chemical data.

Biotech Marine. 2014. Manufacturing Process- Bioenergizer P BG/PF (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Butylene Glycol with Panthenol - no preservatives).

Biotech Marine. 2014. Bioenergizer P BG/PF (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Butylene Glycol with Panthenol - no preservatives) Physico-chemical data.

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SYNOPSIS DE FABRICATION/
MANUFACTURING PROCESS
BIOENERGIZER P

RECOLTE / IDENTIFICATION
(Pelvetia Canaliculata)
HARVESTING / IDENTIFICATION
↓
LAVAGE
WASHING
↓
BROYAGE
GRINDING
↓
EXTRACTION AVEC LE SOLVANT
PROPYLENE GLYCOL
EXTRACTION WITH THE SOLVENT
PROPYLENE GLYCOL
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL

RECOLTE / IDENTIFICATION
(Laminaria Digitata)
HARVESTING / IDENTIFICATION
↓
LAVAGE
WASHING
↓
BROYAGE
GRINDING
↓
EXTRACTION AVEC LE SOLVANT
PROPYLENE GLYCOL
EXTRACTION WITH THE SOLVENT
PROPYLENE GLYCOL
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL

↓
MELANGE — Ajout panthenol / *Panthenol addition*
MIXTURE
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL
↓
CONDITIONNEMENT
PACKAGING
↓
CONTRÔLE QUALITE
QUALITY CONTROL

Responsable production
Production Manager
Jean-Marc CAPROUX

BIOTECHMARINE (02/10/2014)

Date de mise à jour / *Updated date* : 01/12/2016

BIOENERGIZER™ P

INCI NAME : Propylene Glycol - Aqua / Water – Panthenol - Pelvetia Canaliculata Extract – Laminaria Digitata Extract
CAS N°: 57-55-6 – 7732-18-5 – 81-13-0– 223751-75-5 - 90046-12-1
EC N°: 200-338-0(EINECS) – 231-791-2(EINECS) –201-327-3 (EINECS) – 607-055-5 - 289-980-0 (EINECS)

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide à légèrement opalescent** *Limpid liquid to slightly opalescent*
Couleur *Colour*	MO PHY 002	**Brun orange à brun rouge** *Orange brown to red brown*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	5,5 – 7,0
Teneur en eau *Water content*	MO PHY 018	43,0 – 47,0 %
Densité (20°C) *Density*	MO PHY 024	1,040 – 1,060
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1{,}395_0$ – $1{,}405_0$
Extrait sec *Dry extract*	MO PHY 017	5,5 – 9,0 %
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Conservateurs *Preservatives*		
- **POB méthyle** *Methyl Paraben*	MO PHY 020	0,15 – 0,20 %
- **POB propyle** *Propyl Paraben*	MO PHY 020	0,03 – 0,06 %

BIOENERGIZER™ P

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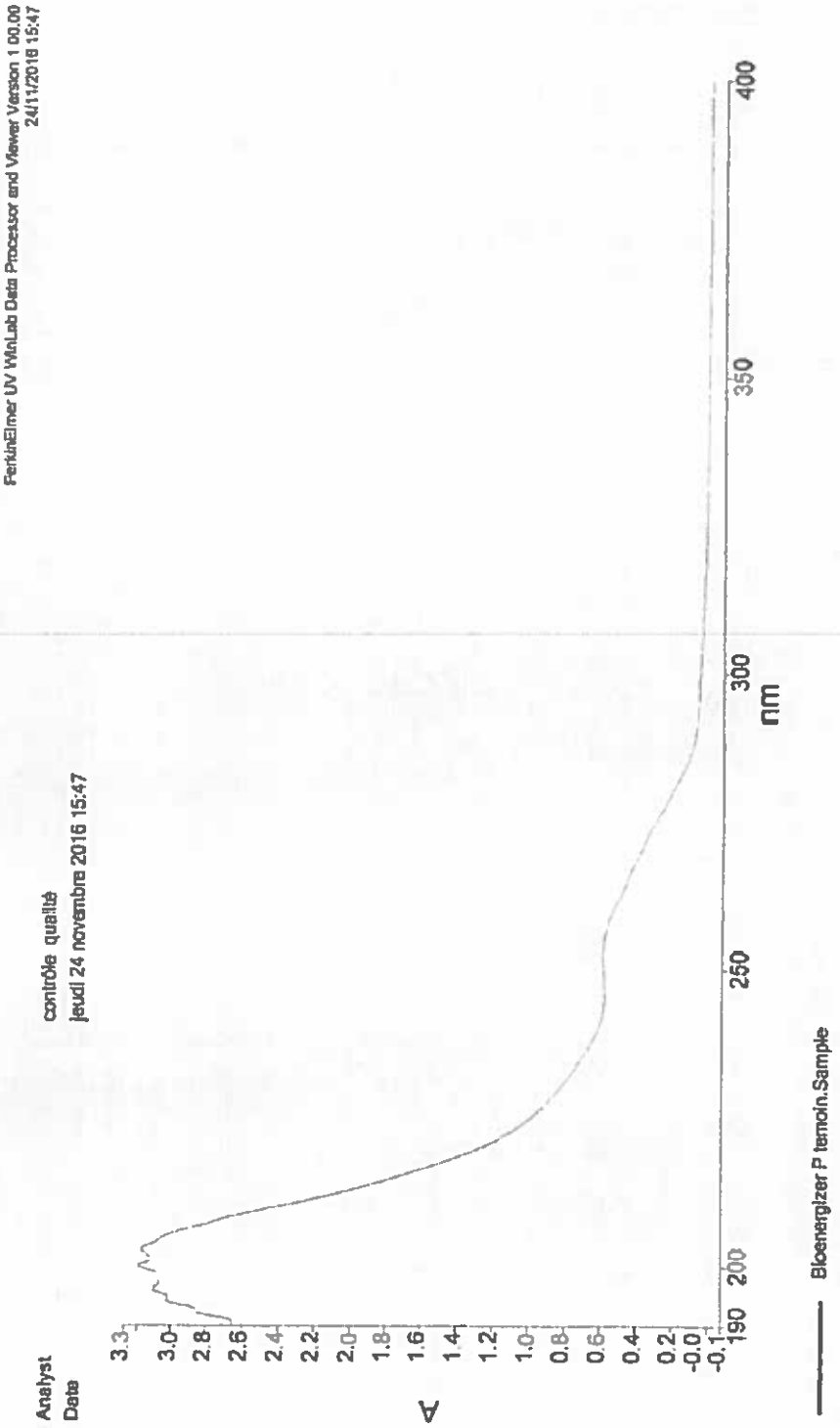
EC N°: 200-338-0(EINECS) – 231-791-2(EINECS) –201-327-3 (EINECS) – 607-055-5 - 289-980-0 (EINECS)

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds* (mg/kg) *Heavy metals* (mg/kg)*	
- Arsenic *- Arsenic*	< 5
- Cadmium *- Cadmium*	< 3
- Plomb *- Lead*	< 5
- Nickel *- Nickel*	< 2
- Argent *- Silver*	< 5

Zodine L100 ppm

* Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*

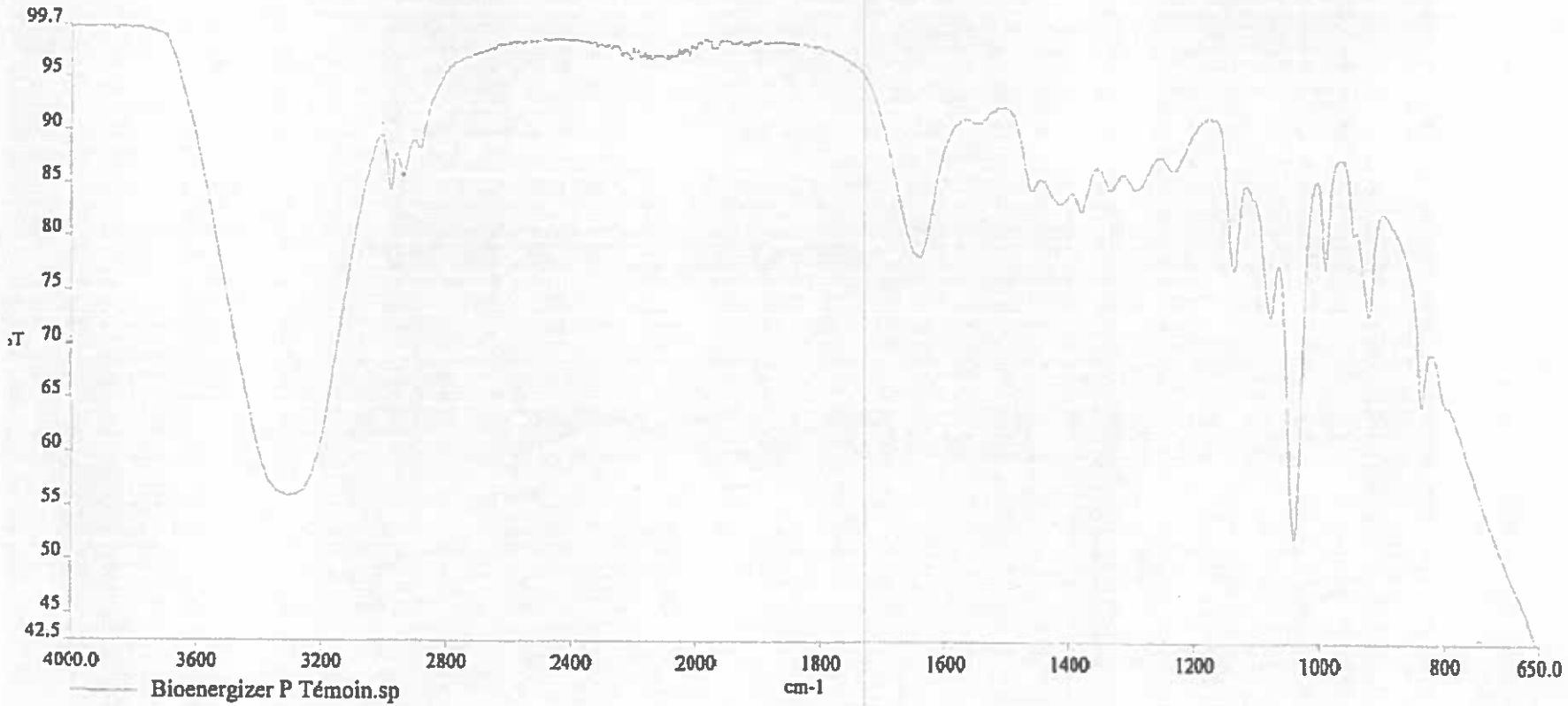


Date: jeudi 24 novembre 2016

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



BIOENERGIZER™ P

INCI NAME : Propylene Glycol - Aqua / Water – Panthenol - Pelvetia Canaliculata Extract – Laminaria Digitata Extract
CAS N°: 57-55-6 – 7732-18-5 – 81-13-0– 223751-75-5 - 90046-12-1
EC N°: 200-338-0(EINECS) – 231-791-2(EINECS) –201-327-3 (EINECS) – 607-055-5 - 289-980-0 (EINECS)

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Germes totaux** *Total germs***	MO MIC 002 / NF EN ISO 21149	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures** *Yeasts / Moulds***	MO MIC 021 / NF EN ISO 16212	**< 100**

** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
01 DEC. 2016

CONFORME
CERTIFIED TRUE AND CORRECT
COORDINATRICE ASSURANCE QUALITE : **M. TANNIOU**
QUALITY ASSURANCE COORDINATOR
01 DEC. 2016

PALMER Research

Etude de la tolérance cutanée aiguë chez 10 volontaires adultes Patch-tests 24 et 48 heures,uniques

Etude référence TC.aiguë.5-SEC/B/PALM 95
réalisée du 19/12/95 au 21/12/95

Société : **SECMA Biotechnologies Marines**
B.P. 65
22260 PONTRIEUX

Produit : **BIOENERGIZER P (3 concentrations)**

Propylene Glycol / Water / Pantenol /
Pelvetia Canaliculata Extrat and Laminaria
Digitata Extract

Arbanats, Février 1996

SOCIÉTÉ DE CONSEIL-EXPERTISE PHARMACEUTIQUE & COSMÉTOLOGIQUE

18 , RUE DE COULON - B.P. 15 - 33640 ARBANATS - TÉL : 56 67 33 02 – FAX : 56 67 05 60
S.A.R.L. AU CAPITAL DE 120.000 FRANCS – APE 7312 – SIRET 384 324 141 00017

1 - INTRODUCTION

A la demande de la société **SECMA Biotechnologies Marines - B.P. 65 ; 22260 PONTRIEUX** -, nous avons évalué sur 10 volontaires adultes la tolérance cutanée aiguë du produit **BIOENERGIZER** aux 3 concentrations suivantes :

- **B : BIOENERGIZER à 100 %**
- **B1 : 5 % de BIOENERGIZER**
- **B2 : 10 % de BIOENERGIZER**

après application unique sur la peau de la face antérieure d'un bras, sous pansements occlusifs maintenus pendant 24 et 48 heures (Patch-Tests 24 et 48 heures).

Cet essai a été réalisé "en ouvert" selon la méthodologie des tests épicutanés sous occlusion.

Pour réaliser cette étude, nous avons reçu le 18/12/95 trois échantillons de 60 ml de chaque concentration du produit BIOENERGIZER.

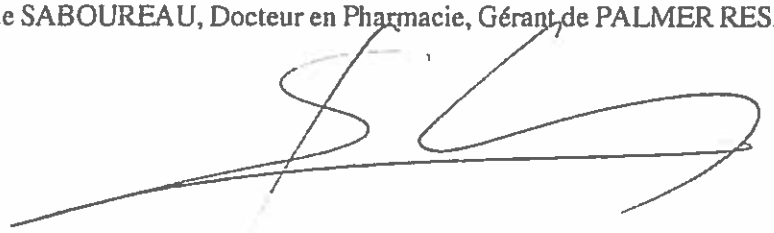
L'essai a commencé le19/12/95 pour s'achever le 21/12/95.

2 - AUTHENTIFICATION DES RESULTATS

L'étude faisant l'objet du présent rapport a été conduite sous ma responsabilité, en conformité avec le protocole expérimental et dans le respect des règles des Bonnes Pratiques Cliniques.
Toutes les observations et les données numériques recueillies au cours de cet essai sont rapportées dans le présent document.

Après relecture, je certifie ces données conformes à la réalité des résultats obtenus.
Docteur Pascale DENIS, Investigateur et Directeur de l'Etude.

En tant que Moniteur de l'Etude, je certifie avoir relu ce rapport et je suis en accord avec son contenu.
Dominique SABOUREAU, Docteur en Pharmacie, Gérant de PALMER RESEARCH.



3 - PROTOCOLE EXPERIMENTAL

3.1 - Volontaires

3.1.1 - Caractéristiques des sujets inclus

- 10 sujets, dont 5 de sexe masculin et 5 de sexe féminin, ont été inclus dans l'essai,
- âgés de21 à 46 ans.

Tous les sujets devaient répondre aux critères d'inclusion et ne présenter aucun critère de non inclusion.

3.1.2 - Critères d'inclusion

- aucun antécédent d'intolérance ou d'allergie à un produit cosmétique,
- acceptation de signature du consentement éclairé de participation.

3.1.3 - Critères de non inclusion

- pathologie cutanée, quelque soit son site,
prise d'un traitement interférant avec le métabolisme cutané, en particulier isotrétinoïne, acitrétine et étrétinate.

3.2 - Méthodologie

3.2.1 - Matériel, dose, durée

Le produit a été appliqué aux 3 concentrations suivantes :

- **B : BIOENERGIZER à 100 %**
- **B1 : 5 % de BIOENERGIZER**
- **B2 : 10 % de BIOENERGIZER**

une seule fois, sur une surface d'environ 50mm de peau de la face antérieure d'un bras de chaque volontaire, à la dose d'environ 0,02ml imbibant la rondelle de papier filtre.

Nota : La raison du choix de la dose est conditionnée par la capacité de la cupule, indiquée par le fabricant dans "Finn Chambers".

Six pansements occlusifs, correspondant à chaque concentration et à chaque temps de contact, ont été posés et maintenus en contact avec la peau pendant respectivement 24 et 48 heures consécutives.

Ces applications sont effectuées parallèlement et dans les mêmes conditions avec un patch-test seul (sans produit) en tant que témoin négatif.

3.2.2 - Lectures

Les examens macroscopiques cutanés ont été réalisés immédiatement, 30 minutes et 24 heures après l'enlèvement des patch.

L'évaluation des réactions cutanées (érythème, oedème, ...) a été effectuée selon la nomenclature proposée par l'International Contact Dermatitis Research Group (I.C.D.R.G) :

NT	:	Non testé.
?+	:	Réaction douteuse. Léger érythème seulement.
+	:	Réaction positive faible (non vésiculeuse) : érythème, infiltration, parfois quelques papules.
++	:	Forte réaction positive : présence d'érythème, de papules, de vésicules.
+++	:	Réaction positive violente, avec présence de bulles.
-	:	Réaction négative.
IR	:	Réaction d'irritation = Erythème (E) **E = 0,5 érythème très léger** **E = 1 érythème léger** **E = 2 érythème net** **E = 3 érythème important**

Nota : En l'absence de toute réaction cutanée locale à la lecture de 24 heures, l'essai est arrêté. Dans le cas de réactions nettes ou douteuses, une lecture est effectuée 48 heures et si nécessaire 72 heures après la dépose des patchs.

3.2.3 - Interprétation des résultats

Référence bibliographique :"Les essais cliniques en dermatologie", Thérapie, 1991, Tome 46, page 183-7.

L'indice d'irritation moyen à chaque temps de lecture est calculé selon le rapport :

$$IM = \frac{\sum \text{des cotations érythémateuses}}{\text{nombre de sujets}}$$

Le barême d'interprétation de l'irritation cutanée est le suivant :

Si $IM \leq 0,20$ non irritant
Si $0,20 \leq IM \leq 0,5$ légèrement irritant
Si $0,50 \leq IM \leq 1$ moyennement irritant
Si $IM > 1$ irritant

4 - RESULTATS

Les résultats individuels des lectures à chaque expérimental et à chacune des 3 concentrations du produit sont regroupés dans les tableaux ci-dessous.

Tableau 1 : BIOENERGIZER à 100 %

SUJETS		Produit à l'essai : BIOENERGIZER 100% Patch 24 heures		Produit à l'essai : BIOENERGIZER 100% Patch 48 heures		Témoin Négatif
Identification	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	E1	E0,5	-
CR.FR	28 M	E1	-	-	-	-
RA.AM	30 F	E0,5	-	-	-	-
RI.JP	46 M	E1	E0,5	-	-	-
KA.LA	21 F	-	-	-	-	-
AH.RO	31 F	-	-	E0,5	-	-
EN.RA	31 M	-	-	E0,5	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	-	-	E1	-	-
KA.TH	37 M	E2	-	-	-	-
IM Résultats		0,45 légèrement irritant	0,05 non irritant	0,30 légèrement irritant	0.05 non irritant	0 non irritant

Tableau 2 : 10 % de BIOENERGIZER

SUJETS		Produit à l'essai : 10 % BIOENERGIZER Patch 24 heures		Produit à l'essai : 10 % BIOENERGIZER Patch 48 heures		Témoin Négatif
Identification	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	-	-	-
CR.FR	28 M	-	-	E0,5	-	-
RA.AM	30 F	-	-	E0,5	-	-
RI.JP	46 M	E1	-	E0,5	-	-
KA.LA	21 F	-	-	-	-	-
AH.RO	31 F	E1	E0,5	-	-	-
EN.RA	31 M	-	-	-	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	-	-	-	-	-
KA.TH	37 M	-	-	-	-	-
IM		0,20	0.05	0,15	0	0
Résultats		non irritant	non irritant	non irritant	non irritant	non irritant

***Tableau 3* : 5 % de BIOENERGIZER**

SUJETS		Produit à l'essai : 5 % BIOENERGIZER Patch 24 heures		Produit à l'essai : 5 % BIOENERGIZER Patch 48 heures		Témoin Négatif
Identification	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	-	-	-
CR.FR	28 M	-	-	-	-	-
RA.AM	30 F	-	-	-	-	-
RI.JP	46 M	E0,5	-	-	-	-
KA.LA	21 F	-	-	-	-	-
AH.RO	31 F	-	-	-	-	-
EN.RA	31 M	-	-	-	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	-	-	-	-	-
KA.TH	37 M	-	-	-	-	-
IM		**0,05**	**0**	**0**	**0**	**0**
Résultats		**non irritant**	**non irritant**	**non irritant**	**non irritant**	**non irritant**

5 - CONCLUSION

Dans les conditions expérimentales retenues, le produit **BIOENERGIZER** s'est révélé :

- appliqué pur, après 24 et 48 heures de contact avec la peau, ***Légèrement Irritant*** , induisant chez 4 sujets sur 10, à chaque temps de contact, un érythème très léger à léger. La réversibilité des réactions a été globalement bonne et aucun effet secondaire n'a été observé.

- appliqué dilué à 10 %, après 24 et 48 heures de contact avec la peau, ***Très Faiblement Irritant***, induisant à chaque temps respectivement, chez 2 sujets un érythème léger et chez 3 autres un très léger érythème. La réversibilité a été bonne et aucun effet secondaire n'a été observé.

- appliqué dilué à 5 %, après 24 et 48 heures de contact avec la peau, ***Non Irritant***, aucun des sujets n'ayant présenté de réaction d'irritation significative d'une intolérance cutanée. Aucun effet secondaire n'a été observé.

D. SABOUREAU
Docteur en Pharmacie

P. DENIS
Docteur en Médecine

SYNOPSIS DE FABRICATION/ *MANUFACTURING PROCESS*

BIOENERGIZER P BG

RECOLTE / IDENTIFICATION
(Pelvetia Canaliculata)
HARVESTING / IDENTIFICATION

↓

LAVAGE
WASHING

↓

BROYAGE
GRINDING

↓

EXTRACTION AVEC LE SOLVANT BUTYLENE GLYCOL
EXTRACTION WITH THE SOLVENT BUTYLENE GLYCOL

↓

FILTRATION

↓

CONTRÔLE QUALITE
QUALITY CONTROL

RECOLTE / IDENTIFICATION
(*Laminaria Digitata*)
HARVESTING / IDENTIFICATION

↓

LAVAGE
WASHING

↓

BROYAGE
GRINDING

↓

EXTRACTION AVEC LE SOLVANT BUTYLENE GLYCOL
EXTRACTION WITH THE SOLVENT BUTYLENE GLYCOL

↓

FILTRATION

↓

CONTRÔLE QUALITE
QUALITY CONTROL

↓

MELANGE
MIXTURE

Ajout panthenol
Panthenol addition

↓

FILTRATION

↓

CONTRÔLE QUALITE
QUALITY CONTROL

↓

CONDITIONNEMENT
PACKAGING

↓

CONTRÔLE QUALITE
QUALITY CONTROL

Responsable production
Production Manager
Jean-Marc CAZROUX

BIOTECHMARINE (02/18/2014)

Date de mise à jour : 18/02/2014

BIOENERGIZER P BG

CTFA / INCI NAME : Aqua / Water - Butylene Glycol – Panthenol – Propylene glycol - Pelvetia Canaliculata Extract – Laminaria Digitata Extract
CAS N° : 7732-18-5 - 107.88.0 – 81-13-0 – 57-55-6 – 223751.75.5 – 90046.12.1/92128.82.0
EINECS N°: 231-791-2 – 203-529-7 – 201-327-3 – 200-338-0 – 607-055-5 – 289-980-0 – 202-785-7

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide** *Limpid liquid*
Couleur *Colour*	MO PHY 002	**Brun orange à brun rouge** *Orange brown to red brown*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	6 – 7
Teneur en eau *Water content*	MO PHY 018	43 – 49 %
Densité (20°C) *Density*	MO PHY 024	1,030 – 1,040
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1{,}397_0$ – $1{,}405_0$
Extrait sec (MA 40 - 1g - 10 min à 160°C) *Dry extract*	MO PHY 017	5 – 9 %
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Conservateur *Preservative*		
- POB méthyle *Methyl Paraben*	MO PHY 019	0,16 – 0,20 %

BIOENERGIZER P BG

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DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds (mg/kg) *Heavy metals (mg/kg)*	
- Arsenic mineral *- Mineral arsenic*	< 5
- Cadmium *- Cadmium*	< 10
- Plomb *- Lead*	< 5
- Nickel *- Nickel*	< 2
- Argent *- Silver*	< 5

Iodine
< 100 ppm

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE PHYSICO-CHIMIE : C. AUBRY
PHYSICOCHIMICAL LABORATORY MANAGER

- BiotechMarine Z.I. – B P 72 – 22260 Pontrieux - FRANCE Tél : +33 (0) 2 96 95 31 32

Analyst
Date

contrôle qualité
mercredi 19 février 2014 15:54

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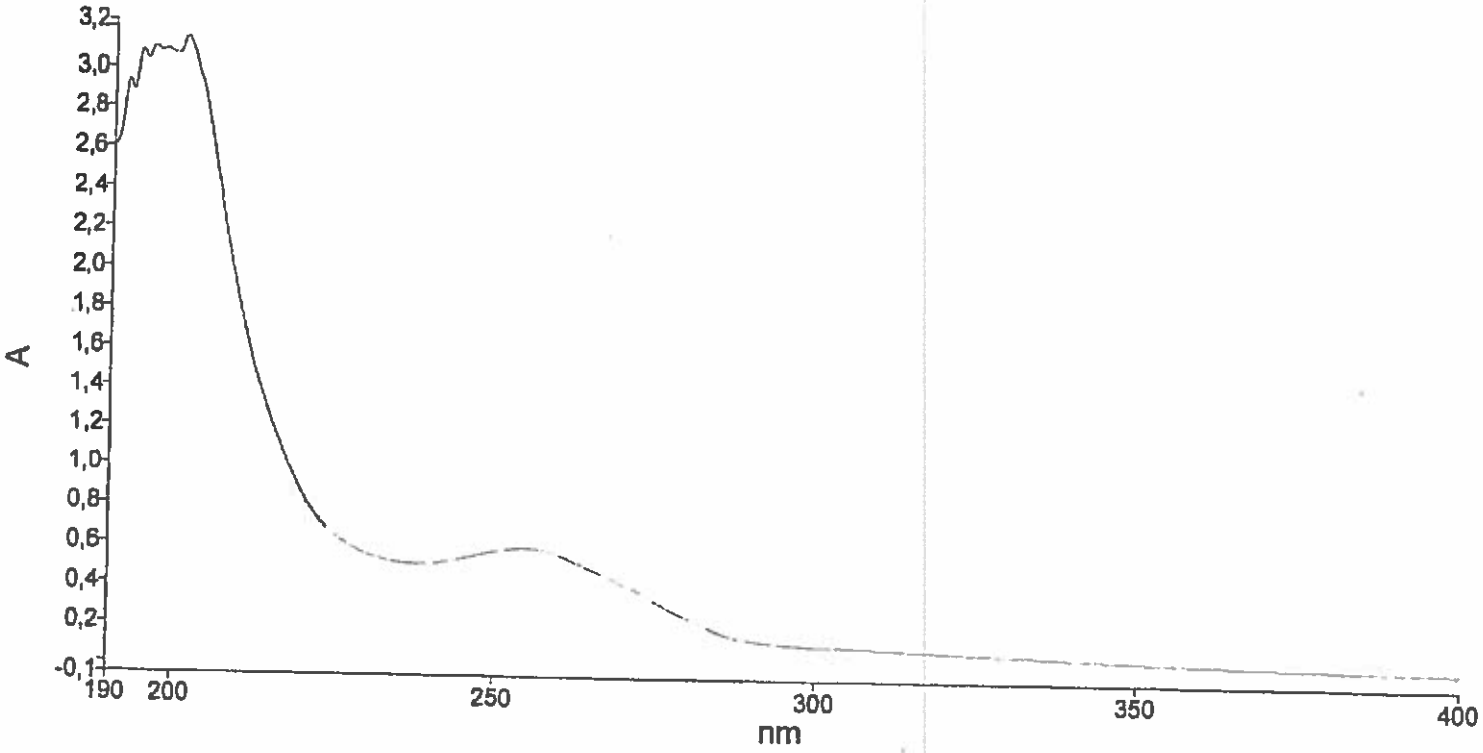

Bioenergizer P BG.Sample Temoin

blanc = H_2O

SPECTRE IRFT

Date: mardi 18 février 2014

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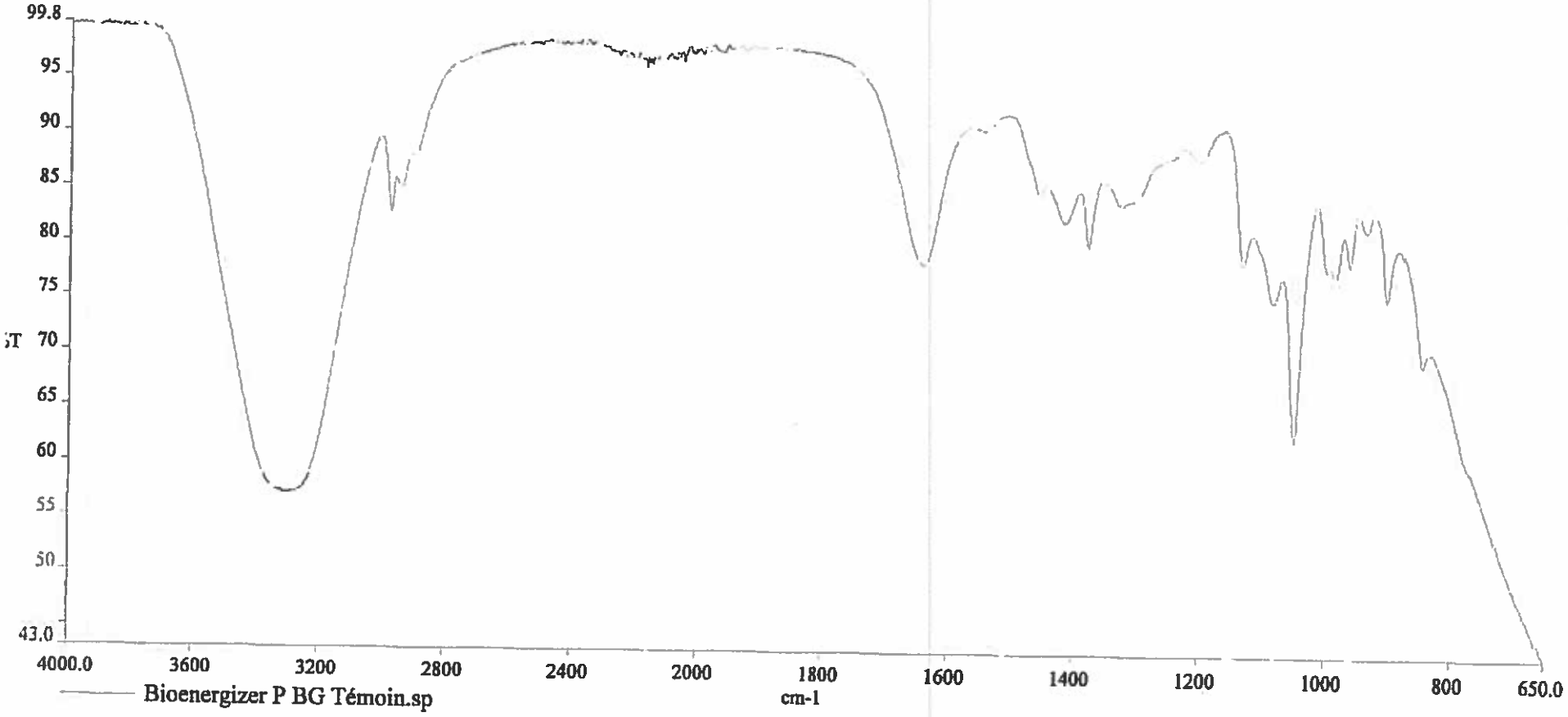
Accessoire ATR Universel N° 7031330



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DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux * *Total germs **	MO MIC 002	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	**Absence** *None*
- *Candida albicans*	MO MIC 010	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011	**Absence** *None*
- *Enterobacteriaceae*	MO MIC 020	**Absence** *None*
Levures / Moisissures* *Yeasts / Moulds**	MO MIC 021	**< 100**

*Les résultats sont indiqués en UFC/ml pour les liquides et en UFC/g pour les solides

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE MICROBIOLOGIE : M.TANNIOU
MICROBIOLOGICAL LABORATORY MANAGER

Le 25/02/14

- BiotechMarine Z.I. – B.P.72 – 22260 Pontrieux - FRANCE Tél : +33 (0) 2 96 95 31 32

SYNOPSIS DE FABRICATION/ *MANUFACTURING PROCESS* BIOENERGIZER P BG/PF

RECOLTE / IDENTIFICATION
(Pelvetia Canaliculata)
HARVESTING / IDENTIFICATION
↓
LAVAGE
WASHING
↓
BROYAGE
GRINDING
↓
EXTRACTION AVEC LE SOLVANT BUTYLENE GLYCOL
EXTRACTION WITH THE SOLVENT BUTYLENE GLYCOL
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL

RECOLTE / IDENTIFICATION
(*Laminaria Digitata*)
HARVESTING / IDENTIFICATION
↓
LAVAGE
WASHING
↓
BROYAGE
GRINDING
↓
EXTRACTION AVEC LE SOLVANT BUTYLENE GLYCOL
EXTRACTION WITH THE SOLVENT BUTYLENE GLYCOL
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL

↓
MELANGE
MIXTURE
← Ajout panthenol / *Panthenol addition*
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL
↓
CONDITIONNEMENT
PACKAGING
↓
CONTRÔLE QUALITE
QUALITY CONTROL

Responsable production
Production Manager
Jean-Marc GATROUX

Date de mise à jour : 18/02/2014

BIOENERGIZER P BG/PF

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DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD PROVISOIRE

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide** *Limpid liquid*
Couleur *Colour*	MO PHY 002	**Brun orange à brun rouge** *Orange brown to red brown*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	6 – 7
Teneur en eau *Water content*	MO PHY 018	43 – 49 %
Densité (20°C) *Density*	MO PHY 024	1,030 – 1,040
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1{,}397_0$ – $1{,}405_0$
Extrait sec (MA 40 - 1g - 10 min à 160°C) *Dry extract*	MO PHY 017	5 – 9 %
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*

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DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence/ *Reference number* **: STANDARD PROVISOIRE**

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds *Heavy metals* (ppm)	
• **Arsenic mineral** *Mineral Arsenic*	< 5
• **Cadmium** *Cadmium*	< 10
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5

Iodine < 100 ppm

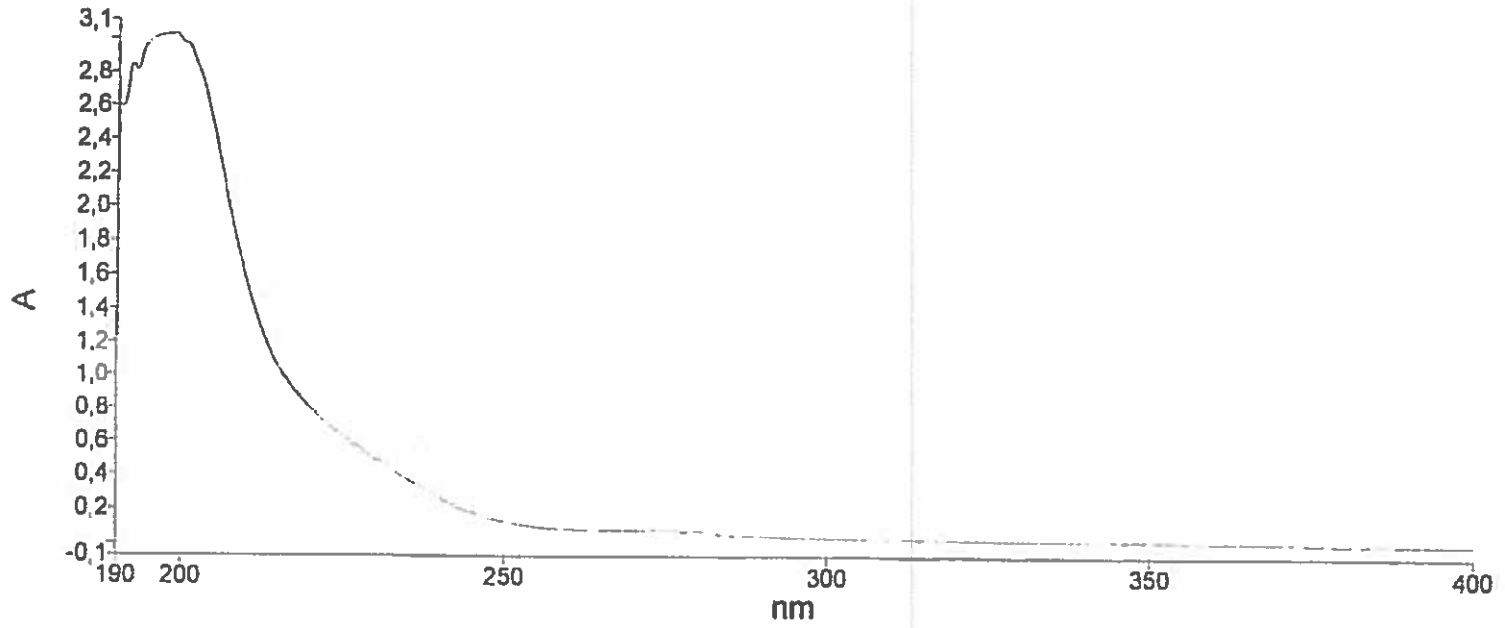
CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE PHYSICO-CHIMIE : **C. AUBRY**
PHYSICOCHIMICAL LABORATORY MANAGER

- BiotechMarine Z.I. – B.P.72 – 22260 Pontrieux - FRANCE Tél : +33 (0) 2 96 95 31 32 -

PerkinElmer UV WinLab Data Processor and Viewer Version 1.00.00
18/02/2014 13:41

Analyst
Date

contrôle qualité
mardi 18 février 2014 13:41



Sample Name	Description
Bioenergizer P BG PF .Sample	

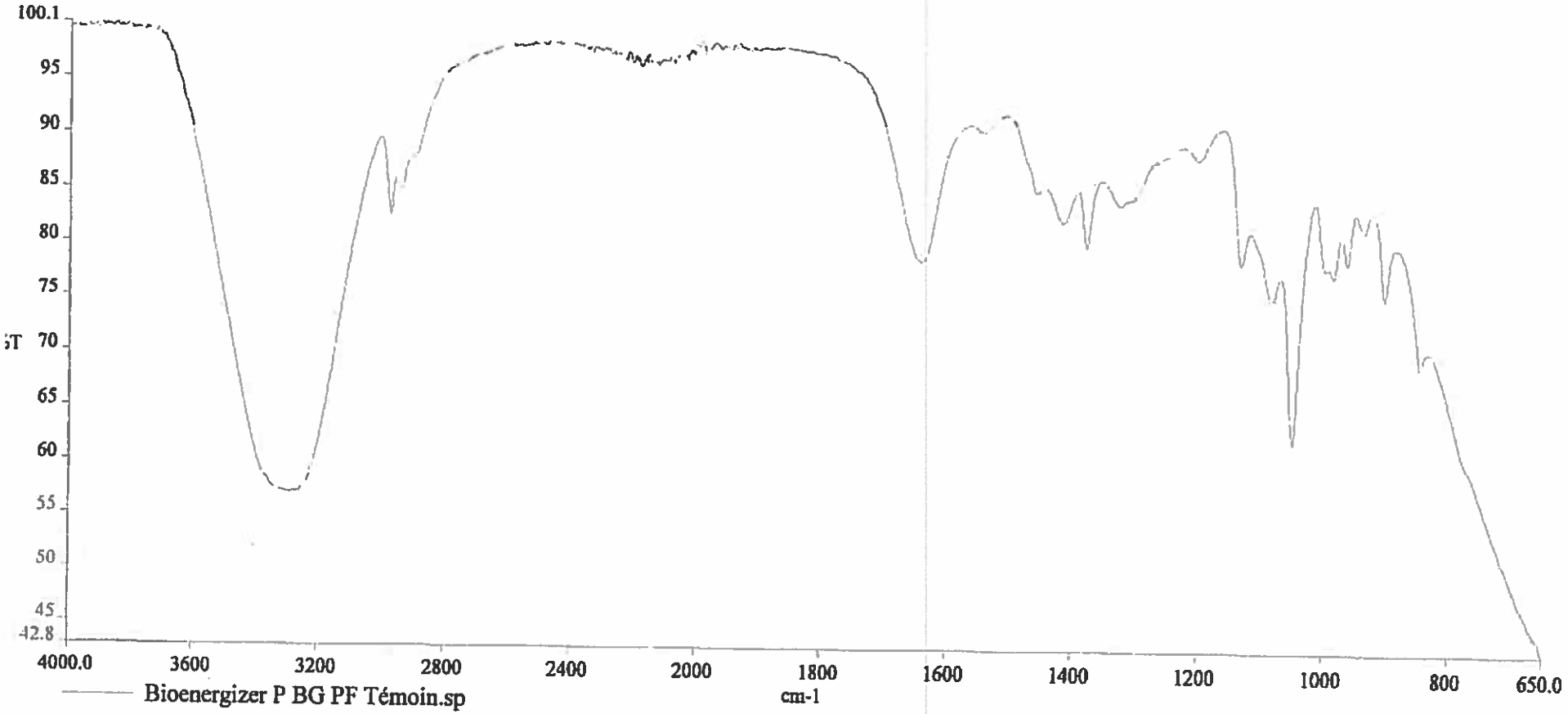
blanc = H_2O P_e = 0,2g /100ml H_2O

SPECTRE IRFT

Date: mardi 18 février 2014

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



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DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD PROVISOIRE

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux * *Total germs **	MO MIC 002	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	**Absence** *None*
- *Candida albicans*	MO MIC 010	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011	**Absence** *None*
- *Enterobacteriaceae*	MO MIC 020	**Absence** *None*
Levures / Moisissures* *Yeasts / Moulds**	MO MIC 021	**< 100**

***Les résultats sont indiqués en UFC/ml pour les liquides et en UFC/g pour les solides**

CERTIFIE CONFORME
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RESPONSABLE DU LABORATOIRE DE MICROBIOLOGIE : **M.TANNIOU**
MICROBIOLOGICAL LABORATORY MANAGER

Le 25/02/14

- BiotechMarine Z.I. - B.P.72 - 22260 Pontrieux - FRANCE Tél. : +33 (0) 2 96 95 31 32 -

Groupe Dermscan

EVALUATION DU POTENTIEL SENSIBILISANT D'UN PRODUIT COSMETIQUE : TEST CLINIQUE FINAL DE SECURITE SOUS CONTRÔLE DERMATOLOGIQUE

ASSESSMENT OF THE SENSITIZING POTENTIAL OF A COSMETIC PRODUCT: FINAL CLINICAL SECURITY TEST UNDER DERMATOLOGICAL CONTROL

Rapport / *Report:*	**14E0898 (version 1)**
Référence etude / *Study reference:*	**DN-1344**
Produit / *Product* **:**	**LCA14027 - 14P0898-1** Ephemer Undaria Pinnat. F. da Extract in Caprylic / capric Triglyceride
Promoteur / *Sponsor* **:**	**SEPPIC** **Biotechmarine** **Z.I.** **22260 Pontrieux** **FRANCE**
C.R.O.	**DERMSCAN** Domaine Scientifique de la Doua 56, Boulevard Niels Bohr **69623 VILLEURBANNE Cedex - FRANCE**
Moniteur de l'étude / *Study Monitor*	**LISKIN - Dr. Bogdan WICHROWSKI** IMMEUBLE FONTENAY AFFAIRES 91, rue Boucicaut **92260 FONTENAY-AUX-ROSES - FRANCE**
Investigateur / *Investigator*	**PROCOS - Dr Marlena NOWAKOWSKA**

Lyon,16/07/2014

RAPPORT / *REPORT*

REFERENCE ETUDE / *STUDY REFERENCE*	**DN-1344/14E0898**
PRODUIT / *PRODUCT*	**«LCA14027 - 14P0898-1»**
NOMBRE DE SUJETS / *NUMBER OF SUBJECTS*	**100**
PROMOTEUR / *C.R.O*	**Groupe DERMSCAN**
MONITEUR / *MONITOR*	**LISKIN** **IMMEUBLE FONTENAY AFFAIRES** **91, rue Boucicaut** **92260 FONTENAY-AUX-ROSES** **FRANCE** **☎ : 33 (0)9 50 27 08 28** **🖷 : 33 (0)1 49 73 66 80**
INVESTIGATEUR / *INVESTIGATOR*	**Dr Marlena NOWAKOWSKA,** **Médecin Dermatologue / *Dermatologist***

Document comportant 28 pages / 28 pages document

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RESUME DE L'ETUDE - *STUDY SUMMARY*

TITRE : TEST CLINIQUE FINAL DE SECURITE : ETUDE DU POUVOIR SENSIBILISANT D'UN PRODUIT, SELON LA METHODE DE MARZULLI-MAIBACH SUR 100 SUJETS PENDANT 6 SEMAINES.

TITLE : CLINICAL FINAL SECURITY TEST: *SENSITIZING POTENTIAL STUDY OF A PRODUCT ACCORDING TO MARZULLI-MAIBACH METHOD ON 100 SUBJECTS DURING 6 WEEKS*

PRODUIT / PRODUCT: LCA14027 - 14P0898-1

REALISATION DE L'ETUDE : L'étude a été réalisée et les valeurs numériques saisies par l'Unité Clinique PROCOS, localisée en Pologne ; ul. Slowackiego 27/33 lok. 33/34 ; 01-592 Varsovie.
STUDY IMPLEMENTATION: The study was carried out and all test values recorded by the Clinical Unit PROCOS, localized in Poland, ul. Slowackiego 27/33 lok. 33/34 01-592 Warsaw.

INVESTIGATEUR / INVESTIGATOR: Dr Marlena NOWAKOWSKA

PROTOCOLE : TEST DE MAXIMALISATION SELON MARZULLI-MAIBACH
PROTOCOL: SENSITIZATION TEST ACCORDING TO MARZULLI-MAIBACH METHOD.

BUT DE L'ETUDE : Evaluer sous contrôle dermatologique le potentiel irritant et sensibilisant d'un produit dans les conditions prévues par le promoteur de l'étude.
AIM OF THE STUDY: To evaluate the sensitizing potential of a product under dermatological control and under the conditions defined by study's sponsor.

SUJETS : 100 volontaires à peau normale correspondant aux critères d'inclusion et de non-inclusion déterminés par le Groupe DERMSCAN.
SUBJECTS: 100 healthy volunteers with normal skin corresponding to the inclusion and non-inclusion criteria defined by the DERMSCAN Group.

PERIODE DE L'ETUDE / STUDY DATE: 02/06/14 - 11/07/14 / *June 2nd to July 11th, 2014*

PLAN EXPERIMENTAL : Etude monocentrique en simple aveugle.
STUDY DESIGN: *Monocentric and simple blind study.*

PRINCIPAUX PARAMETRES DE TOLERANCE :

- Potentiel irritant (phase d'induction)
  Erythème, œdème, sécheresse, vésicules évalués par le dermatologue selon un score de 0 à 3
- Potentiel sensibilisant (phase de révélation)
  Réaction évaluée par le dermatologue selon un score de 0 à 3 établis par l'ICDRG (International Contact Dermatitis Research Group)

MAIN TOLERANCE PARAMETERS:

- *Irritation potential (Induction Phase)*
  Erythema, edema, desquamation, vesicles rated from 0 to 3 by the dermatologist
- *Sensitizing potential (Challenge Phase)*
  Reaction rated from 0 to 3 by the dermatologist according to ICDRG (International Contact Dermatitis Research Group)

RESULTATS - *RESULTS:*

Dénomination du produit - *Product name*	**POTENTIEL IRRITANT** *IRRITATION POTENTIAL*	**POTENTIEL SENSIBILISANT** *SENSITIZING POTENTIAL*
LCA14027 - 14P0898-1	non irritant *non-irritating*	Aucune réaction de type allergique *No allergic reaction*

CONCLUSION :
Dans les conditions de cette étude, le produit «LCA14027 - 14P0898-1» s'est avéré **non irritant** et **non sensibilisant.**

CONCLUSION :
*Under these study conditions, the product «LCA14027 - 14P0898-1» can be considered **non-irritating** and **non-sensitizing**.*

1. ASSURANCE QUALITE / *QUALITY ASSURANCE*

L'étude a été réalisée selon les règles des Bonnes Pratiques Cliniques définies par les ICH Topic E6 "Note for Guidance and good clinical practice" (CPMP/ICH/135/95), par la Déclaration d'Helsinki (1964, WMA) et ses mises à jours successives), par la CEE (Directives n° 91/507 et III 3976/88 EN du 11/07/1990) et par le Ministère de la Santé de la République Française.

The described study has been conducted according to the Good Clinical Practice defined by the ICH Topic E6 "Note for Guidance and good clinical practice" (CPMP/ICH/135/95), the Helsinki Declaration (1964, WMA) and its succesives updates, the EEC (Directives n° 91/507 and III 3976/88 of 11/07/1990) and to the Ministry of Health of the French Republic.

Il est de la responsabilité de l'industriel, fabricant du produit testé, de justifier qu'aucune substance constituant ce produit n'est sensibilisante.

The first evaluation of sensitization risks for all ingredients depends on the responsibility of the tested product manufacturer.

L'étude a été menée selon les Procédures Opératoires Standards et selon le protocole de l'étude défini par le promoteur.
Les cahiers d'observation et les journaux de suivi ont été vérifiés ainsi que l'exactitude des données.

The study has been conducted according to Standard Operating Procedures and to the study protocol defined by the sponsor. All study events recorded during the study are reported.

L'authenticité et la véracité des données expérimentales recueillies ont été confirmées par les personnes ayant participé à l'étude (ANNEXE II).

Controls on data veracity and conformity with the protocol have been performed and confirmed by persons participating in the study (APPENDIX II).

2. CERTIFICAT DE CONFORMITE / *CERTIFICATE OF CONFORMITY*

A ma connaissance, l'étude DN-1344/14E0898 a été conduite en accord avec l'**«Assurance qualité»** précitée.
I am aware that the study DN-1344/14E0898 *has been conducted according to the «**Quality Assurance**» described before.*

Il ne s'est pas produit d'événement susceptible d'affecter la qualité ou l'intégrité des données.
There was no event which may have affected the quality or integrity of the data.

Mme Charlotte OEHMICHEN
Directeur technique / Technical director

16/07/2014
date

3. METHODOLOGIE / *METHOD*

3.1 PRODUIT A L'ETUDE / *STUDY PRODUCT*

Le produit fourni par le Groupe DERMSCAN, présentait les caractéristiques suivantes :
The product supplied by Group DERMSCAN, had the following characteristics :

Dénomination du produit - *Product name*	Aspect du produit Product aspect	Code du produit *Product code*
LCA14027 - 14P0898-1	liquide jaune transparent *transparent yellow liquid*	KA

Le produit a été réceptionné le 26/05/2014.
The product was receipted on May 26th, 2014.

3.2 METHODES CLINIQUES / CLINICAL METHODS

3.2.1 Objectif de l'étude / Aim of the study

Evaluer le pouvoir irritant et sensibilisant du produit par la méthode de Marzulli-Maibach.
To evaluate irritating and the sensitizing potential of a product by Marzulli-Maibach method.

3.2.2 Plan expérimental / *Experimental design*

L'étude a été réalisée en ouvert.
This was an open study

3.2.3 Sujets de l'étude / *Study subjects*

Critères d'inclusion

- Volontaire sain d'origine caucasienne
- Age compris entre 18 et 70 ans
- Phototype II, III ou IV
- Personne ne présentant ni cicatrice, ni tatouage, ni tache pigmentaire d'aucune sorte, ni pilosité trop importante, ni lésion dermatologique, ni traces d'un maillot de bain au niveau du dos
- Personne ayant donné par écrit son consentement libre, éclairé et exprès
- Sujet coopérant, averti de la nécessité et de la durée des contrôles permettant d'espérer une parfaite adhésion au protocole mis en place par le Groupe DERMSCAN.

Inclusion criteria

- *Healthy volunteer of Caucasian origin*
- *Age between 18 and 70*
- *Phototype II, III or IV*
- *Volunteer without scars, active dermal lesions, tattoos, any pigmentary marks, excessive pilosity and uneven skin tones of the areas of the back to be tested.*
- *Subjects having given their informed, written consent*
- *Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by the DERMSCAN Group could have been expected.*

Critères d'exclusion

- Femme enceinte ou qui allaite
- Exposition au soleil ou aux U.V. depuis 15 jours avant le début et pendant l'étude et /ou ayant reçu des photopatch-tests depuis moins de 2 mois
- Peau hyper irritable ou pathologie cutanée,
- Allergique ou sensibilité connues au sparadrap et /ou aux produits cosmétiques
- Pathologie cutanée, cicatrices, grains de beauté, tache de rousseur ou toute anomalie sur la zone d'expérience et/ou présentant une lucite
- Maladie grave ou évolutive
- Sujet suivant un traitement médicamenteux topique ou systémique :
  - anti-inflammatoires et/ou antihistaminiques pendant la semaine qui précède et durant l'étude
  - substances photosensibilisantes et /ou phototoxiques depuis moins d'un mois et pendant l'étude
  - immunosuppresseurs et /ou corticoïdes pendant les 4 semaines qui précèdent et durant l'étude
  - rétinoïdes pendant les 6 mois précédant l'étude et durant l'étude
- Troubles dus à l'absorption excessive d'alcool ou de substances toxiques.

Non-inclusion criteria

- *Pregnant or nursing women*
- *Sun exposure or UV exposure 15 days before or during the study and/or photopatch-tests from less than 2 months*
- *Hyperirritable skin or cutaneous pathology*
- *Known allergies or sensitivities to adhesive plaster and/or cosmetics products*
- *History of abnormal responses to sunlight or presence of active dermal lesions, Scars, beauty spots, freckle or any abnormality, on the back*
- *History of cancer or other important disease*
- *Volunteers undergoing a topical or systemic treatment:*
  - *anti-inflammatories and/or anti-histamines during the previous week and during the study*
  - *photo-allergic and/or phototoxic substances from less than 1 month and during the study*
  - *immuno-suppressors and/or corticoids during the four previous weeks and during the study*
  - *retinoids during the six previous months and during the study*
- *Excessive use of alcohol, tobacco and toxic substances.*

Inclusion

100 sujets volontaires ont été choisis en accord avec les critères d'inclusion et les critères d'exclusion, et 100 sujets ont réalisé la totalité de l'étude. Le tableau suivant regroupe les informations concernant la participation à l'étude de tous les sujets sélectionnés.

100 healthy volunteers were selected according to the inclusion and the non-inclusion criteria, and 100 subjects completed study. The table below presents the information concerning all the included volunteers.

	Non inclus *Non included*	Inclus *Included*	Arrêt en cours d'étude *Drop out*	Perdus de vue *Untraceable*
Nombre de sujets *Number of subjects*	0	100	0	0

Caractéristiques des sujets / *Subjects characteristics*

Le tableau récapitulatif ci-dessous présente une synthèse des observations concernant uniquement les volontaires inclus dans l'analyse des données.
The summary table below presents a synthesis of the observations concerning exclusively the volunteers taken into account for data analysis

Nombre de Volontaires *Number of subjects*	Sexe *Sex*	Age (moy±SEM) *Age (mean±SEM)*	Phototype	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
				avant l'étude *Before the study*	pendant l'étude *During the study*
100	81 F 19 M	43 ± 1	II : 100 III : 0 IV : 0	cf. Tableaux en ANNEXE II *cf. Tables in the APPENDIX II*	

3.3 MATERIEL / MATERIAL

Les patch-tests utilisés sont des bandes de Webril ® ($4cm^2$) qui assure une semi occlusion. Après application du produit sur le patch, ce dernier est appliqué au niveau de la zone scapulaire des volontaires.

The semi-occlusive patch-tests used are Band of Webril® ($4cm^2$) ensuring a semi occlusion. The product is applied on the semi-occlusive patch test which is then placed on the volunteer's back.

4 APPLICATION DU PRODUIT / *PRODUCT APPLICATION*

Zones d'application	Zones scapulaires : homolatérale (zone d'induction) et controlatérale (zone de révélation)
Quantité et concentration appliquée	50 µl pur
Fréquence	Phase d'induction : 3 fois par semaine pendant 48 heures Phase de révélation : 1 fois pendant 48 heures
Durée	Phase d'induction : 3 semaines Phase de latence : 2 semaines Phase de révélation : 1 semaine
Conditions d'application	Avant application, la peau a été préalablement nettoyée et séchée. Le produit «LCA14027 - 14P0898-1» a été déposé dans un patch semi-occlusif (avec papier filtre), et appliqué sur le dos du volontaire. Un patch ne contenant aucun produit a été appliqué dans les mêmes conditions et a servi de témoin non traité. Durant toute la phase d'induction, la zone homolatérale n'a pas été mouillée. Les volontaires se sont douchés le dimanche après le retrait des patchs en faisant attention à ne pas mettre de produit détergent sur les sites. Lors de la Phase de Révélation, aucun lavage ni aucune application de quelconque produit n'ont été effectués sur la zone controlatérale.

Application area	*Scapular zones: homolateral (induction zone) and controlateral (challenge zone)*
Quantity and Concentration applied	*50 µl* *pure*
Frequency	*Induction Phase: 3 times a week during 48 hours* *Challenge Phase: once during 48 hours*
Contact time	*Induction Phase: 3 weeks* *Rest Phase: 2 weeks* *Challenge Phase: 1 week*
Application conditions	*Before application, the skin was cleaned and dried. The product «LCA14027 - 14P0898-1» was applied in a semi-occlusive patch with filter paper and applied to the volunteer's back.* *The patch containing no product was applied under the same conditions to serve as a non-treated control.* *During the whole induction phase, the homolateral zone was not wet.* *Volunteers took a shower on Sunday, after patches removing, and paid attention not to put a detergent product on all tested zones. During all the challenge phase, no washing and no product application took place on the controlateral zone.*

5 DEROULEMENT DE L'ETUDE / *STUDY SCHEDULE*

Phase d'induction - trois semaines (S1, S2, S3)
Induction phase – 3 weeks (W1, W2, W3)
S1 / *W1:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J1 *D1*	J2 *D2*	J3 *D3*	J4 *D4*	J5 *D5*	J6 *D6*	J7 *D7*
Application du produit *Product application*	↓		↓		↓		

S2 / *W2:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J8 *D8*	J9 *D9*	J10 *D10*	J11 *D11*	J12 *D12*	J13 *D13*	J14 *D14*
Application du produit *Product application*	↓		↓		↓		

S3 / *W3:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J15 *D15*	J16 *D16*	J17 *D17*	J18 *D18*	J19 *D19*	J20 *D20*	J21 *D21*
Application du produit *Product application*	↓		↓		↓		

Après enlèvement du dernier patch de la phase d'induction à leur domicile, il est demandé aux volontaires de se présenter à la clinique à J22 en cas d'apparition d'un nouveau signe (ou de dégradation d'un signe existant à J19)
After having removed the last patch of the induction phase at home, it was asked to the subjects, to come at the clinical unit D22 if a new sign appeared (or deterioration of an existing sign D19).

Phase de latence - deux semaines (S4, S5) *Rest Phase - 2 weeks (W4, W5)*
Pas de lecture – *No reading*
S4/ *W4* :

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J22 *D22*	J23 *D23*	J24 *D24*	J25 *D25*	J26 *D26*	J27 *D27*	J28 *D28*

S5 / *W5:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J29 *D29*	J30 *D30*	J31 D31	J32 *D32*	J33 *D33*	J34 *D34*	J35 *D35*

Phase de révélation (double challenge test) - une semaine (S6)
Challenge Phase - 1 week (W6)
S6 / *W6* :

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*
Jour d'étude *Study day*	J36 D36	J37 D37	J38 D38	J39 D39	J40 D40
Application du produit *Product application*	↓				
Jour d'étude *Study day*			L		L

6 CRITERES D'EVALUATION / *ASSESSMENT CRITERIA*

6.1 CRITERES CLINIQUES CONCERNANT LE POTENTIEL IRRITANT (PHASE D'INDUCTION)
CLINICAL CRITERIA REGARDING THE IRRITATING POTENTIAL (INDUCTION PHASE)

Après chaque application, le patch est enlevé et la lecture est effectuée 30 minutes plus tard pour éliminer l'effet de pression, d'occlusion et d'arrachement dû au matériel.
Le test est négatif si la peau garde un aspect normal.
Les quatre critères suivants sont évalués par le dermatologue selon une cotation de 0 à 3 :

After each application, the patch is removed and the clinical examination is performed by the investigator 30 minutes later in order to eliminate the pressure and the occlusion effects.
The result of examination is negative if the skin looks normal.
The clinical examination is made on the back using the following criteria and scale (Quotation 0 to 3):

Score	Quotation	CRITERES : description *CRITERIA : description*			
		ERYTHEME *ERYTHEMA*	OEDEME *EDEMA*	SECHERESSE *DRYNESS*	VESICULES *VESICLES*
0	absent	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*
1	Léger *slight*	Coloration rosée discrète de toute la surface testée ou bien visible sur une partie de la surface testée *Discreet pink coloration of the whole tested area or rather visible on part of the tested area*	Plus palpable que visible *More palpable than visible*	Desquamation fine discrète, aspect dépoli *Discreet thin desquamation, tarnished aspect*	Vésicules plus palpables que visibles *More palpables than visible vesicles*
2	Net *obvious*	Erythème net couvrant toute la surface testée *Marked erythema covering the whole tested area*	Œdème visible *Visible edema*	Desquamation visible, aspect écailleux *Visible desquamation, flaky aspect*	Vésicules visibles *Visible vesicles*
3	Important *important*	Erythème intense couvrant toute la surface testée ou érythème diffusant en dehors de la surface testée *Severe erythema covering the whole tested area or erythema diffusing beyond the tested area*	Pouvant déborder de la surface testée *Edema diffusing beyond the tested area*	Desquamation importante, fissuration *Important desquamation, cracking*	Vésicules débordant de la zone testée ou bulles *Vesicles diffusing beyond the tested area or blisters.*

6.2 CRITERES CLINIQUES CONCERNANT LE POTENTIEL SENSIBILISANT (PHASE DE REVELATION)

CLINICAL CRITERIA REGARDING THE SENSITIZING POTENTIAL (CHALLENGE PHASE)

Les réactions allergiques ont été évaluées selon l'échelle suivante :
The allergic reactions were evaluated according to the following scale:

Critère - *Criterion*	Cotation ICDRG* *ICDRG (*)Quotation*	Cotation "notée" *Numeric score Quotation*
Absence de réaction *No reaction*	0	0
Réaction douteuse *Doubtful reaction*	?	?
Erythème et œdème *Erythema and edema*	+	1
Erythème, œdème et vésicules *Erythema, edema and vesicles*	++	2
Réaction forte avec présence de bulles ou d'ulcérations post-bulbeuses *Severe reaction with blisters*	+++	3

* (International Contact Dermatitis Research Group)

6.3 MODE D'EVALUATION / *ASSESSMENT METHOD*

6.3.1 Pouvoir irritant - Phase d'induction / *Irritating potential - Induction Phase*

A l'issue des 8 lectures de la phase d'induction, le score moyen de chaque volontaire a été calculé en additionnant les scores obtenus à chacune des lectures et en divisant cette somme par le nombre effectif de lectures effectuées à la clinique (une lecture n'était pas prise en compte s'il y avait réaction au témoin ou irritation globale).
Le pouvoir irritant du produit a été évalué lors de la phase d'induction, en faisant la moyenne des réactions survenues.
Le pouvoir irritant du produit a été déterminé selon la formule suivante :

At the conclusion of the 8 readings of the induction phase, the average score of every volunteer was calculated by adding the scores obtained for each of the readings and by dividing this sum by the actual number of readings made at the clinical unit (a reading was not taken into account if there was reaction of the control or global irritation).
The irritating power of the product was estimated, by calculating the mean of the reactions observed.
The irritating power of the product was determined according to the following formula

$$\text{Score moyen} = \frac{[(\sum \text{scores J1...J19 / nb de lectures) vol1} + ... + (\sum \text{scores J1...J19 / nb de lectures) volN}]}{\text{nb de volontaires (N)}}$$

$$\textit{Average score} = \frac{[(\sum \textit{scores D1...D19 / nb of readings) vol1} + ... + (\sum \textit{scores D1...D19 / nb of readings) volN}]}{\textit{nb of volunteers (N)}}$$

Score moyen *Average score*	Pouvoir irritant *Irritating Potential*
0,000 – 0,080	Non irritant *Non-irritating*
0,081 – 0,160	Très légèrement irritant *Very slightly-irritating*
0,161 – 0,560	Légèrement irritant *Slightly-irritating*
0,561 – 1,000	Modérément irritant *Moderately-irritating*
1,001 – 1,600	Fortement irritant *Strongly-irritating*
> 1,600	Très fortement irritant *Very strongly-irritating*

6.3.2 Pouvoir sensibilisant - Phase de révélation
Sensitizing potential - Challenge Phase

Une réaction allergique éventuelle au cours des Phases d'Induction ou de Révélation était notée de 0 à 3 selon les critéres de l'ICDRG (International Contact Dermatitis Research Group) – voir le tableau en paragraphe 6.2.
Lors de la révélation, une lecture sera faite 30 minutes après enlèvement des patch-tests puis 48h plus tard

The possible allergic reaction, during the Induction or Challenge Phase, was rated from 0 to 3 according to ICDRG (International Contact Dermatitis Research Group) – see the table paragraph 6.2.
During the Challenge Phase, the reading took place 30 minutes after patch-tests removal and 48 hours later.

Le pouvoir sensibilisant du produit a été évalué lors des lectures à J38 et J40 (phase de révélation) en fonction des critères suivants : réaction ++ (2) ou +++ (3) en l'absence de phénomène d'irritation surajouté.

The sensitizing potential of the product was assessed by the readings on D38 and D40 (Challenge Phase) according to the following criteria: reaction ++ (2) or +++ (3) in the absence of added irritation phenomenon.

La survenue d'un seul cas de sensibilisation active **(score supérieur ou égale à ++ (2))** du côté controlatérale conduit à la conclusion : « Produit potentiellement sensibilisant ».

*The presence of only one case of active sensitizing **(upper or equal score in ++ (2))** on controlateral side leads to the conclusion "Potentially sensitive product".*

7 ARRET PREMATURE / *PREMATURE STUDY TERMINATION*

Les sujets avaient le droit de sortir de l'essai à tout moment pour quelle que raison que ce soit.

The subjects had the right to leave the study at any time whatever the reason.

L'arrêt prématuré peut être du à des multiples raisons :
- non respect du calendrier des visites par le sujet
- événements indésirables (incluant les maladies intercurrentes)
- violations et déviations au protocole

- sorties après retrait du consentement du sujet.

The premature study termination could be due to multiple reasons:
- *non-compliance with the visits schedule,*
- *adverse events (including intercurrent diseases),*
- *protocol non-adherence/departures from protocol,*
- *withdrawal of subject consent*

Le médecin investigateur peut interrompre l'essai en cours, soit sur certains sujets, soit sur l'ensemble du panel, notamment, si le produit entraine des réactions cutanées importantes ou anormales, ou s'il juge que la poursuite de l'essai peut nuire à la santé du ou des sujets concernés.

The doctor investigator can interrupt the essay either on certain subjects or on the the whole panel, if the product induces important or abnormal cutaneous reactions or if he considers that the continuation of the essay can damage health of one or several concerned subjects.

8 AMENDEMENTS AU PROTOCOLE / *PROTOCOL AMENDMENT*

Néant / *None.*

9 RESULTATS / *RESULTS*

9.1 POUVOIR IRRITANT: PHASE D'INDUCTION / *IRRITATING POTENTIAL: INDUCTION PHASE*

Le TABLEAU DES LECTURES durant la phase d'induction est présenté en ANNEXE III.
The TABLE OF READINGS regarding the Induction Phase is presented in the APPENDIX III.

Ces lectures effectuées 30 minutes après le retrait des patch-tests ont montré les résultats suivants :
The readings done 30 minutes after having removed the patch-tests showed the following results:

Produit *Product*	score	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*		Conclusion
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
KA	T+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	non irritant *non-irritating* (IRR = 0.000)
	0 :	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	
	1 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

T+ = Témoin positif / *Positive control*
VM = valeur manquante / *missing value*
% = % of subjects / *% of subjects*
IRR = irritation globale / *global irritation*
n = nombre de sujet / *number of subjects*

Dans les conditions de cette étude, le produit «LCA14027 - 14P0898-1» a montré un score inférieur à 0,080.
Il peut donc être considéré comme non irritant.

Under these study conditions, the product «LCA14027 - 14P0898-1» showed a score lower than 0.080.
It can thus be considered as non-irritating.

9.2 POTENTIEL SENSIBILISANT : PHASE DE REVELATION
SENSITIZING POTENTIAL: CHALLENGE PHASE

Le TABLEAU DES LECTURES durant la phase de révélation est présenté en ANNEXE IV.
Les lectures effectuées 30 minutes et 48 heures après le retrait des patch-tests de révélation ont donné les résultats suivants :

The TABLE OF READING regarding the Challenge Phase is presented in APPENDIX IV.
These reading made 30 minutes and 48 hours after having removed the patch-tests showed the following results:

Code Produit : KA *Product Code : KA*	Zones	score	Jour de lecture *Day of the reading*				Résultat global *Global result*
			J38 / *D38*		J40 / *D40*		
			n	%	n	%	
LCA14027 - 14P0898-1	Lectures zone homolatérale *Homolateral zone readings*	T+ :	0	0	0	0	**non sensibilisant** *non-sensitizing*
		0 :	100	100	100	100	
		? :	0	0	0	0	
		1 :	0	0	0	0	
		2 :	0	0	0	0	
		3 :	0	0	0	0	
	Lectures zone controlatérale *Controlateral Zone readings*	T+ :	0	0	0	0	
		0 :	100	100	100	100	
		? :	0	0	0	0	
		1 :	0	0	0	0	
		2 :	0	0	0	0	
		3 :	0	0	0	0	

KA = LCA14027 - 14P0898-1

T+ = Témoin positif / Positive control
IRR = irritation globale / global irritation
VM = valeur manquante / missing value
n = nombre de sujet / *number of subjects*
% = % of subjects / *% of subjects*

Dans les conditions de cette étude, aucune réaction ++ (2) ou +++ (3) ont été constatées. Le produit «LCA14027 - 14P0898-1» peut donc être considéré comme non sensibilisant.

Under these study conditions no reaction ++ (2) nor +++ (3) were observed, so the product «LCA14027 - 14P0898-1» can be considered non-sensitizing.

10 CONCLUSION

Dans les conditions de cette étude, le produit «LCA14027 - 14P0898-1» s'est avéré non irritant et non sensibilisant.

Under these study conditions, the product «LCA14027 - 14P0898-1» can be considered non-irritating and non-sensitizing.

ANNEXE / *APPENDIX*

ANNEXE I / *APPENDIX I*

FEUILLE D'AUTHENTIFICATION DES RESULTATS

AUTHENTIFICATION PAGE

IROCOS

KARTA AUTENTYCZNOŚCI REZULTATÓW
FICHE D'AUTHENTIFICATION DES RESULTATS
AUTHENTIFICATION PAGE

Według posiadanych przeze mnie informacji, badanie Nr :
A ma connaissance l'étude N° :
I am aware that the study N° :

DN – 1344

było przeprowadzone zgodnie PROTOKOŁEM oraz KARTĄ PARAMETRÓW TESTU.
a été conduite en accord avec le PROTOCOLE et la FICHE DES PARAMETRES D'ETUDE.
has been conducted according to the PROTOCOL and to the STUDY PARAMETERS PAGE.

Mgr inż. Barbara WAŁEJKO Odpowiedzialna za badania *Responsable d'unité* Unit head	podpis / *signature*	*11/07/2014* data *date*
Dr Marlena NOWAKOWSKA Lekarz dermatolog *Médecin dermatologue* Dermatologist	podpis / *signature*	*11/07/2014* data *date*
Mgr Magdalena KUREK Odpowiedzialna za jakość *Responsable qualité* Responsible for quality control	podpis / *signature*	*11/07/2014* data *date*
Barbara SZULC Asystentka *Assistante*	podpis / *signature*	*11/07/2014* data *date*

ANNEXE II / *APPENDIX II*

CARACTERISTIQUES DES VOLONTAIRES

SUBJECTS CHARACTERISTICS

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *during the study*
1	OLEIR	53	M	II	N	-	-
2	CHWKR	23	M	II	N	-	-
3	ZGOMA	36	F	II	N	-	-
4	ARKEL	40	F	II	N	-	-
5	GESLU	29	M	II	N	-	-
6	MIEAL	44	F	II	N	-	-
7	DLUKA	34	F	II	N	-	-
8	TABST	20	M	II	N	-	-
9	GILAN	47	F	II	N	-	-
10	SZARY	58	M	II	N	-	-
11	BRUMA	48	F	II	N	-	-
12	PTAPI	49	M	II	N	-	-
13	NIEIR	34	F	II	N	-	-
14	JAGJA	58	M	II	N	-	-
15	BRZKR	51	F	II	N	-	-
16	GLAJO	61	F	II	N	-	-
17	GORBO	31	M	II	N	-	-
18	SUSAN	60	M	II	N	-	-
19	ADARO	48	M	II	N	-	-
20	STEST	27	F	II	N	-	-
21	MARBA	53	F	II	N	-	-
22	WNUCZ	52	F	II	N	-	-
23	PIEDO	41	F	II	N	-	-
24	ABREM	27	F	II	N	-	-
25	KISDO	42	F	II	N	-	-
26	DABWE	21	F	II	N	-	-
27	OKRKR	34	M	II	N	-	-
28	KROMA	36	F	II	N	-	-
29	CZAJA	50	M	II	N	-	-
30	JANBO	41	F	II	N	-	-
31	MAJAN	23	F	II	N	-	-
32	WIKIW	55	F	II	N	-	-
33	KUTDA	27	F	II	N	-	-
34	CHOMA	51	F	II	N	-	-
35	ORKDO	49	F	II	N	-	-
36	MELUR	60	F	II	N	-	-
37	OLEDA	62	F	II	N	-	-
38	KOZAN	21	F	II	N	-	-
39	TRYED	37	F	II	N	-	-
40	WROAU	61	F	II	N	-	-

N : Normale / *normal*
S : Sensible / *sensitive*

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *dunng the study*
41	KROZB	57	M	II	N	-	-
42	GRUAN	29	F	II	N	-	-
43	JANJO	30	M	II	N	-	-
44	KALWI	47	F	II	N	-	-
45	KRUED	42	F	II	N	-	-
46	MAJJA	34	M	II	N	-	-
47	BAREW	47	F	II	N	-	-
48	KIEHE	38	F	II	N	-	-
49	BOCUR	41	F	II	N	-	-
50	GAJAN	22	F	II	N	-	-
51	CZOST	34	F	II	N	-	-
52	RUTBE	46	F	II	N	-	-
53	RUDMI	48	F	II	N	-	-
54	CEGHA	58	F	II	N	-	-
55	GRAIR	54	F	II	N	-	-
56	POPKA	29	F	II	N	-	-
57	PIEBA	63	F	II	N	-	-
58	DUDAG	36	F	II	N	-	-
59	SIEIW	34	F	II	N	-	-
60	KOWAL	20	F	II	N	-	-
61	SEPZO	54	F	II	N	-	-
62	PECBO	63	F	II	N	-	-
63	KIEEW	27	F	II	N	-	-
64	SIESA	58	F	II	N	-	-
65	PELBE	52	F	II	N	-	-
66	JANEM	43	F	II	N	-	-
67	ZOCSY	27	F	II	N	-	-
68	ZALGR	59	F	II	N	-	-
69	HACBO	55	F	II	N	-	-
70	HACMA	32	M	II	N	-	-
71	ZWIAP	45	F	II	N	-	-
72	GORMA	53	F	II	N	-	-
73	SKOBR	50	M	II	N	-	-
74	BAKAG	41	F	II	N	-	-
75	KURHE	49	F	II	N	-	-
76	JEDRE	53	F	II	N	-	-
77	ZIEDA	56	F	II	N	-	-
78	JANMA	52	F	II	N	-	-
79	ZAREW	28	F	II	N	-	-
80	GRAAN	49	F	II	N	-	-

N : Normale / *normal*
S : Sensible / *sensitive*

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *during the study*
81	RYLHA	38	F	II	N	-	-
82	POLJA	28	M	II	N	-	-
83	SZUEL	53	F	II	N	-	-
84	SENEL	56	F	II	N	-	-
85	RODKR	50	F	II	N	-	-
86	WAGAN	27	F	II	N	-	-
87	PASJA	29	F	II	N	-	-
88	PSZBE	23	F	II	N	-	-
89	DANEL	47	F	II	N	-	-
90	KURDA	40	F	II	N	-	-
91	KIEWA	52	F	II	N	-	-
92	MROMO	50	F	II	N	-	-
93	ZARBE	50	F	II	N	-	-
94	WYSSY	33	F	II	N	-	-
95	BULJO	42	F	II	N	-	-
96	KOBTO	44	M	II	N	-	-
97	LONMA	50	F	II	N	-	-
98	KANEW	54	F	II	N	-	-
99	CZAWI	27	F	II	N	-	-
100	BOGAG	38	F	II	N	-	-

N : Normale / *normal*
S : Sensible / *sensitive*

ANNEXE III / *APPENDIX III*

TABLEAUX DES LECTURES - PHASE D'INDUCTION

TABLES OF THE READINGS – INDUCTION PHASE

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / control
KA = LCA14027 - 14P0898-1

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / *control*
KA = LCA14027 - 14P0898-1

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA
81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / control
KA = LCA14027 - 14P0898-1

ANNEXE IV / *APPENDIX IV*

TABLEAUX DES LECTURES - PHASE DE REVELATION

TABLES OF THE READINGS -- CHALLENGE PHASE

TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	KA	T	KA	T	KA	T	KA
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0

T = témoin / control
KA = LCA14027 - 14P0898-1

TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	KA	T	KA	T	KA	T	KA
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
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46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0

T = témoin / control
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TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	KA	T	KA	T	KA	T	KA
81	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0

T = témoin / control
KA = LCA14027 - 14P0898-1

Institut Pasteur de Lille

Fondation reconnue d'utilité publique

Docteur Fabrice NESSLANY
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e-mail : fabrice.nesslany@pasteur-lille.fr

Final Study Report

***IN VITRO* SKIN IRRITATION :**
RECONSTRUCTED HUMAN EPIDERMIS TEST METHOD

Study Number
FSR-IPL 140410

Study Completion
10 July 2014

Test Item Ephemer Undaria Pinnatifida Extract in Caprylic / Capric Triglyceride
LCA14026

Study Director
Mrs. Gwendoline DEWAELE

Sponsor
BiotechMarine

TEST FACILITY
INSTITUT PASTEUR DE LILLE
Genetic Toxicology Laboratory
1, rue du Professeur Calmette - BP. 245
59019 LILLE CEDEX

SPONSOR
BiotechMarine
Z.I.
22260 Pontrieux

SPONSOR REPRESENTATIVE
Mr. Mickaël PUGINIER
SEPPIC
127 Chemin de la poudrerie
81100 Castres

TABLE OF CONTENTS

STUDY INFORMATION

STUDY	***In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method**
TEST ITEM	**LCA14026**

I. TEST FACILITY INFORMATION:

Test facility	**Institut Pasteur de Lille**
Test facility address	Genetic Toxicology Laboratory 1, rue du Professeur Calmette - B.P. 245 59019 LILLE CEDEX France
Study director	**Mrs. Gwendoline DEWAELE** **Institut Pasteur de Lille** Genetic Toxicology Laboratory 1, rue du Professeur Calmette - B.P. 245 59019 LILLE CEDEX France
Deputy Study director	**Dr. Sophie SIMAR**
Quality Assurance	**Mrs. Frédérique LOBEZ**
Test facility management	**Dr. Fabrice NESSLANY** Head of Toxicology Department

II. SPONSOR INFORMATION:

Sponsor	**BiotechMarine**
Sponsor's address	Z.I. 22260 Pontrieux
Sponsor representative	**Mr. Mickaël PUGINIER** SEPPIC 127 Chemin de la poudrerie 81100 Castres

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT AND REPORT AUTHENTICATION

* * *

The work described in this report was performed according to the agreed study plan and with the Standard Operating Procedures of the test facility, unless otherwise stated, and was conducted in accordance with:

- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17;
- Article Appendix II to the article D523-8 of the French environmental code;
- EC Commission Directive 2004/10/EC of 11th February 2004 (Official Journal No. L050).

I consider the data generated and reported to be valid and I declare that this report is a true and accurate record of the results obtained.

As described in the Study Plan, the sponsor certifies that the test item to be tested sponsored by **BiotechMarine** is identical to the test item described in the Final Study Plan and in the Analytical Certificate.

NB : The certificate of analysis sponsored by the Sponsor was not compliant with GLP, GMP or ISO regulations.

The study was performed at the Toxicology Department of Institut Pasteur de Lille for irritation assay.

The following software, used during the study phase, Excel®, was not fully validated in accordance with GLP regulations. However, calculations were double checked within this study. Consequently, the use of the software was judged not to have impaired the integrity of the study.

Submitted by:

Study director **Mrs. Gwendoline DEWAELE** 10.07.2014 Date  Signature

STUDY	***In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method**
TEST ITEM	**LCA14026**
SPONSOR	**BiotechMarine**

This report was reviewed and approved by:

Test facility management

Dr. Fabrice NESSLANY
Head of Toxicology Department 10/09/2014

Date Signature

Deputy Study director **Dr. Sophie SIMAR**

QUALITY ASSURANCE STATEMENT

* * *

STUDY — ***In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method**

TEST ITEM — **LCA14026**

SPONSOR — **BiotechMarine**

TEST FACILITY — **INSTITUT PASTEUR DE LILLE**
Genetic Toxicology Laboratory
1, rue du Professeur Calmette
B.P. 245
59019 LILLE CEDEX FRANCE

This study was inspected by the Quality Assurance Unit, employing methods detailed in Standard Operating Procedures used at the Toxicology Department of Institut Pasteur de Lille.

- **STUDY PLAN AND AMENDMENT AUDITS**

Audit	Date of audit	Approved by the Study Director on	Approved by the Test Facility Management on
Study Plan	**22/05/2014**	**23/05/2014**	**27/05/2014**
Amendment No. 1	**30/06/2014**	**30/06/2014**	**30/06/2014**

The data presented in the report accurately reflect data collected during the conduct of the study. Any data supplied by or under the responsibility of the Sponsor were not reviewed.

- **IN STUDY AUDIT**

	Inspections / audits on critical phases of this study			Inspections / audits on critical phases associated with this type of study		
Phase audited	Dates of Inspection/ Audit	Approved by the Study director on	Approved by the Test facility Management on	Dates of Inspection/ Audit	Approved by the Study director on	Approved by the Test facility Management on
Receipt-preculture of epidermis	-	-	-	03/06/14	13/06/14	13/06/14
Treatment	04/06/14	13/06/14	13/06/14	-	-	-
MTT technique	-	-	-	06/06/14	13/06/14	13/06/14
Reading of OD	-	-	-	06/06/14	13/06/14	13/06/14

In addition, process and facility based audits are carried out according to the annual quality assurance program.

- **REPORT AUDITS**

Audit	Date of audit	Approved by the Study Director on	Approved by the Test Facility Management on
Draft report	13/06/2014	19/06/2014	18/06/2014
Final report	10/07/2014	10/07/2014	10/07/2014

- **CONCLUSION**

Based on these audits, I declare that the data presented in this report accurately reflect the raw data collected during the current study.

le 10/07/2014

Mrs. Frédérique LOBEZ*
Quality Assurance Unit

** In absence of Mrs. Aurélie RIZZA, Mrs. Brigitte GOREZ has audited and signed the Amendment No. 1 to the Final Study Plan FSP-IPL140410 and Mrs. Frédérique LOBEZ has audited and signed the Final Study Report.*

ARCHIVE STATEMENT

* * *

Test Facility archives

The following study materials are retained in the archives of the Toxicology Laboratory of the Institut Pasteur de Lille (1, rue du Pr Calmette – BP 245 – 59019 Lille Cedex – France) for at least 10 years after the end of the study:

- Study plan and amendment,
- Raw data or authenticated copies thereof,
- Correspondence,
- Final report and possible amendments.

After the end of this period, they should be returned to the Sponsor or destroyed at Sponsor's written request. In addition, raw data not specific to the study, including but not limited to equipments calibration, are also archived at Institut Pasteur de Lille for at least 20 years.

According to OECD Guideline Number 7 (as revised in 1997) point II 6.2.6 relative to the application of the Good Laboratory Practice Principles to short-term studies, **the test item does not need to be preserved at the end of the study**.

After the finalisation of the whole package of studies, at Sponsor Representative's request and in accordance with the Final Study Plan, Institut Pasteur de Lille should destroy the remaining test item.

In Vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method

SUMMARY

SPONSOR	:	**BiotechMarine**
TEST ITEM	:	**LCA14026**
BATCH NUMBER	:	**13.11.401**
STUDY LOCATION	:	**INSTITUT PASTEUR DE LILLE** Genetic Toxicology Laboratory 1, rue du Professeur Calmette - B.P. 245 59019 LILLE CEDEX FRANCE

THIS STUDY WAS CARRIED OUT IN COMPLIANCE WITH GOOD LABORATORY PRACTICE REGULATIONS

Study initiation date (date Study Director signed Study Plan):	27/05/2014
Experimental start date :	03/06/2014
Experimental completion date:	06/06/2014
Study completion:	10/07/2014

AIM OF THE STUDY

The skin irritant potential of **LCA14026 (batch 13.11.401)** sponsored by **BiotechMarine** was investigated by using the Episkin® culture system.

The *In Vitro* Skin Irritation using the Reconstructed Human *Epidermis* Test Method was designed to predict and classify the skin irritant potential of items according to chemical safety regulations, using the reconstructed human epidermis model Episkin™ small model. This method does not require the use of live animals or animal tissue for the assessment of skin irritant potential.
This test provided an *in vitro* procedure that may be used for the hazard identification of skin irritant chemicals (substances and mixtures) in accordance with OECD Guideline No. 439 and for the classification and Labelling of Chemicals according to the regulation United Nations Globally Harmonized System (2009).

METHOD

- Reconstructed human Epidermises	:	Human skin cells small model (Episkin® batch 14-EKIN-020)
- Culture media	:	Episkin® 'maintenance' culture medium (Episkin® batch 14-MAIN3-024) Episkin® 'assay' culture medium (Episkin® batch 14-ESSC-021)
- Negative control	:	phosphate Buffered Saline (PBS) (GIBCO batch 1376272)
- Positive control	:	sodium Dodecyl Sulfate 5% (SDS 5%, Biorad batch 210010817)

SEARCH FOR INTERFERENCE WITH THE TEST SYSTEM

- Possible direct MTT reduction	:	no interference
- coloring potential	:	no interference

IRRITATION ASSAY:

- Number of assays	:	1
- Number of replicates treated	:	3 for test item and for positive and negative controls
- Time exposure	:	15 ± 0.5 minutes
- Time of recovery period	:	42 hours ± 1 hour
- Dose tested	:	**10 µL of LCA14026 as supplied / epidermis**

RESULTS :

	Mean[1] of viability (%)	SD
Negative control	100	7.7
Test Item	106.1	8.6

SD : Standard deviation
[1] from 3 replicates

After 15 minutes treatment followed by 42 hours of recovery, the mean of cell viability was of 106.1%, when compared to the negative control, *i.e.* clearly higher than 50%.
Therefore, the test item **LCA14026** was considered as **not irritant** to skin under these experimental conditions.

CONCLUSION

The skin irritant potential of LCA14026 (batch 13.11.401) sponsored by BiotechMarine was investigated by using the *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method in compliance with the OCDE Guideline No. 439.

The validity criteria for the assay were fulfilled. The current study is thus considered as valid.

Under these experimental conditions, LCA14026 is considered as not irritant to skin according to OECD Guideline No. 439 and have not to be classified according to UN GHS (2009).

In Vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method

* * *

1. PURPOSE OF THE STUDY

Skin irritation refers to the production of reversible damage to the skin following the application of a test item.
The *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method was designed to predict and classify the skin irritant potential of items according to chemical safety regulations, using the small model reconstructed human epidermis model Episkin™.
This test provided an *in vitro* procedure that may be used for the hazard identification of skin irritant chemicals (substances and mixtures) in accordance with OECD Guideline No. 439 and for the classification and Labelling of Chemicals according to the regulation United Nations Globally Harmonized System (UN GHS, 2009).

The current study was performed in accordance with the Final Study Plan FSP-IPL 140410 (see Appendix No. 5) and amendment (see Appendix No. 6).

Experimental phase:
Initiated – completed : 03/06/2014 – 06/06/2014

2. PRINCIPLE

The test item is applied topically to a three-dimensional RhE model, Episkin™. After a 15-minutes exposure time with the test item, epidermis are rinsed and then incubated for 42 additional hours.

Cell viability determination is based on cellular mitochondrial dehydrogenase activity, measured by MTT reduction and conversion into a blue formazan salt that is quantitatively measured after extraction from tissues (Faller C. *et al.*, 2002, Mosmann T., 1983). The reduction of cell viability in treated tissues is compared to negative controls and expressed as a percentage. The percentage reduction in viability is used to predict the irritation potential.

Irritant chemicals are identified by their ability to decrease cell viability below defined threshold levels (*i.e.* ≤ 50%). Chemicals that induce cell viabilities above the defined threshold level, may be considered non-irritant (*i.e.* > 50%).

3. MATERIALS AND METHOD

3.1. Reason of the choice of the reactive system

Human skin cells from Episkin™ is used for these different reasons:

- Episkin™ reconstitutes a model that is very close to human skin (histological structure, metabolic activity…).
- The production of Episkin™ is standardized and it is more reproducible than human skin.
- Test results on human skin cells are more pertinent in the assessment of possible human hazard.
- Cells are more easily isolated from Episkin™ than from human skin.
- Episkin® model is recommended by OECD Guideline No. 439.

3.2. Cells and culture media

Episkin® cells (batch 14-EKIN-020, see Appendix No. 2 for the Quality Certificate), which are 0.38 cm^2 skin equivalent and the culture media (batches 14-MAIN3-024 and 14-ESSC-021) were purchased from Episkin SNC (Lyon, France).

4. TEST ITEM AND VEHICLE INFORMATION

4.1. Test item

TEST ITEM	:	**LCA14026**
OTHER NAME / CODE	:	-
IPL REGISTRATION NUMBER	:	140410
BATCH NUMBER	:	13.11.401
EXPIRY DATE	:	10/2016
APPEARANCE	:	green liquid
PURITY	:	100% commercial product
COMPOSITION	:	9.5 mg/kg of fucoxanthine
WATER CONTENT	:	unknown
QUANTITY SUPPLIED	:	50 mL
STORAGE CONDITIONS	:	room temperature, protected from light
STABILITY UNDER STORAGE CONDITIONS	:	stable up to expiry date, *i.e.* 10/2016 for batch 13.11.401

Storage conditions: Immediately upon receipt, the test item was registered, then stored at room temperature, protected from light in accordance with the Sponsor's instructions. The complete description of the chemical and physical properties of the test item including stability is the responsibility of the Sponsor.

This test item, the characteristics of which are given in Appendix No. 3, was tested in accordance with the Final Study Plan.

4.2. Check-method for possible direct MTT reduction with test item:

Prior to experiments the test item was put in contact with the MTT solution. A 12-well plate was filled with 2 mL of MTT solution (0.3 mg/mL). A volume of 10 µL of the test item was added and the plate was incubated for 2 hours and 30 minutes at 37°C protected from light. The MTT solution color became yellow, *i.e.* neither blue nor purple and it was concluded that the test item did not interact with MTT in a manner that it would have disturb the test system.

4.3. Check-method to detect the coloring potential of test item.

Prior to treatment, the test item was also evaluated for its intrinsic color or ability to become colored in contact with water). A volume of 100 µL of the test item was added to 900 µL of water, mixed and let for 15 minutes at room temperature. At the end of the period, the test item appeared as a layer above water, *i.e.* not miscible. Nevertheless, the Optical Densities (OD) at 550 nm were measured :

Item	OD at 550 nm	Mean of OD	ΔOD at 550 nm
Distilled water	0.036	0.035	-
	0.034		
Test item (100 µL+900 µL distilled water)	0.077	0.077	+ 0.042
	0.077		

The coloring potential of the test item did not interfere with the test system.

4.4. Formulation of the test item

A sufficient amount of test item should be applied to uniformly cover the epidermis surface while avoiding an infinite dose. As the test item **LCA14026** was a liquid, a volume of 10 µL was used (*i.e.* 26 µL/cm²), while a nylon mesh was used to improve the uniformity of treatment.

5. CONTROLS

Concurrent negative (PBS, GIBCO batch 1376272) and positive (SDS 5%, Biorad batch 210010817) controls were used in each run to demonstrate that viability, barrier function and resulting sensitivity of the tissues were within a defined historical acceptance range (see Appendix No. 4 for Historical Data).

6. PRECULTURE AND TREATMENTS

Upon receipt, the reconstituted skin inserts were transferred into 12-well plates containing 2 mL of Episkin® "maintenance" medium and placed in a cell incubator at 37°C under 5% CO_2 with 95% humidity, up to the treatment day within 18 to 24 hours.
Three replicates were used for test item and for positive and negative controls in each run.

After 15±0.5 minutes of exposure at room temperature, the epidermis were rinsed twice with bath and with 25 mL of pre-warmed PBS at 37°C.

The epidermis were then transferred into 12-well plates containing 2 mL of "maintenance" medium and incubated at +37°C for 42±1 h (under 5% CO_2, with 95% of humidity).

7. MTT TECHNIQUE

After incubation, the inserts were transferred into a 12-well plate containing 2 mL of MTT medium (0.3 mg/mL in "assay" medium). The plates were then incubated for 2 hours and 30 minutes to 3 hours in a CO_2 incubator at 37°C. After this contact time, the epidermises were dried and then placed in Eppendorf tubes containing 0.5 mL of acidified isopropanol. The Eppendorf tubes were then placed at room temperature, protected from light, for 4 hours. At the end of this period, the tubes were agitated, and 2 x 0.2 mL per well were transferred into a flat bottom plate. Optical densities (OD) were determinated using a spectophotometer at 550 nm.
For each epidermis, the mean optical densities values (OD) and the percentage of viability were calculated :

$$\textbf{Corrected OD} = OD_{treated} - OD_{blank*}$$

** : extraction solvent alone (acidified isopropanol)*

The mean corrected OD of the 3 epidermis of negative control corresponds to 100 % of cell viability.

With the corrected $OD_{treated}$, the percentage of cell viability was calculated for each treated epidermis.

$$\textbf{\% cell viability} = (\textbf{Corrected } OD_{treated} / \textbf{ Mean corrected } OD_{control}) \times 100$$

The mean cell viability and the standard deviation was then determinated for the 3 epidermis and was used to determine the irritation potential.

8. ACCEPTANCE CRITERIA FOR THE RESULTS

The study was accepted as the following criteria were fulfilled :

- the optical density of the extraction solvent alone (OD_{blank}) was inferior to 0.1.
- the mean corrected $OD_{control}$ was within the acceptability range from 0.6 to 1.5 and the standard deviation was inferior or equals to 18.
- the mean viability of the positive control was below 40% and the standard deviation was inferior or equal to 18.

9. INTERPRETATION OF THE RESULTS

The test item is considered to be irritant to skin in accordance with OECD Guideline No. 439 if the tissue viability after exposure and post-treatment incubation is less than or equal (≤) to 50%. The test item is thus classified as irritant to skin Category 2 according to UN GHS (2009).
The test item may be considered as non-irritant to skin in accordance with OECD Guideline No. 439 if the tissue viability after exposure and post-treatment incubation is more than (>) 50%. The test item is thus not classified as irritant to skin according to UN GHS (2009).

10. RESULTS FOR THE IRRITATION ASSAY ON RECONSTRUCTED EPIDERMIS

The test results are given in Appendix No. 1 (Table 1).

No decrease of cells viability was noted after 15 minutes exposure followed by 42 hours recovery period, with percentage of relative survival of 106.1%, when compared to the solvent control.

Therefore, the test item **LCA14026** was considered as **not irritant** to skin under these experimental conditions.

11. STUDY PLAN ADHERENCE

11.1. Deviations

This study was performed in accordance with the Final Study Plan FSP-IPL 140410 and the Amendment No. 1.

There were no deviations from the Final Study Plan.

11.2. Notes

-

12. CONCLUSION

The skin irritant potential of LCA14026 (batch 13.11.401) sponsored by BiotechMarine was investigated by using the *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method in compliance with the OCDE Guideline No. 439.

The validity criteria for the assay were fulfilled. The current study is thus considered as valid.

Under these experimental conditions, LCA14026 is considered as not irritant to skin according to OECD Guideline No. 439 and have not to be classified according to UN GHS (2009).

13. REFERENCES

EpiSkin™ SOP, Version 1.8 (February 2009), ECVAM Skin Irritation Validation Study: Validation of the EpiSkin™ test method 15 min - 42 hours for the prediction of acute skin irritation of chemicals.

Faller C., Bracher M., Dami N. and Roguet R. (2002) Predictive activity of reconstructed human epidermis equivalents for assessment of skin irritation of cosmetics.

Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunological Methods* 65, 55-62.

O.E.C.D. Guideline for the Testing of Chemicals No. 439: *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method, 26 July 2013.

UN (2009), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Third revised edition, UN New York and Geneva.

Appendix No. 1: Results

TABLE 1 : Results

***In vitro* skin irritation : reconstructed human epidermis test method**

Reading of Optical Density at 550 nm and Viability calculation

Test item	LCA14026
Sponsor	BiotechMarine
MTT reduction	yellow solution
Coloring potential	non miscible
Beginning of the study	03/06/2014

Item	Epidermis No.	OD	Corrected OD*	Mean of corrected OD	Mean per item	Viability (%)	Mean of viability (%)	Standard deviation
Extraction solvent	-	*0.034*						
		0.032						
		0.034						
		0.035						
		0.034						
		0.035						
Negative Control (PBS)	1	*0.900*	0.866	0.863	0.803	107.5	100	7.7
		0.894	0.860					
	2	*0.827*	0.793	0.806		100.4		
		0.853	0.819					
	3	*0.767*	0.733	0.739		92.1		
		0.779	0.745					
Test Item	1	*0.900*	0.866	0.869	0.852	108.2	106.1	8.6
		0.905	0.871					
	2	*0.810*	0.776	0.776		96.7		
		0.810	0.776					
	3	*0.944*	0.910	0.911		113.5		
		0.946	0.912					
Positive control (SDS 5%)	1	*0.212*	0.178	0.157	0.146	19.5	18.2	4.0
		0.169	0.135					
	2	*0.190*	0.156	0.173		21.5		
		0.223	0.189					
	3	*0.149*	0.115	0.110		13.7		
		0.139	0.105					

OD : optical density

* with mean OD for extraction solvent = 0.034

% cell viability = (Corrected ODtreated / Mean corrected ODcontrol) x100

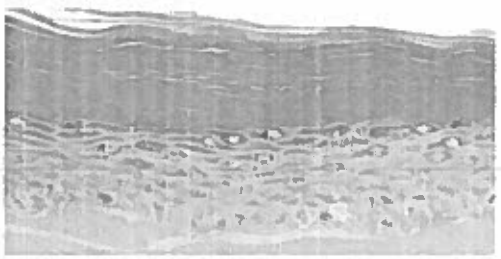
Mean cell viability (%) = (% cell viability epidermis 1 + % cell viability epidermis 2% +cell viability epidermis 3) / 3

Appendix No. 2: Quality Certificate of epidermis

SkinEthic
laboratories

TECHNICAL DATA, SAFETY SHEET AND CERTIFICATE OF ANALYSIS
RECONSTRUCTED HUMAN EPIDERMIS

CCL-037/14

Description:	**Episkin Small Model**		
	0.38 cm² reconstructed epidermis of normal human keratinocytes. Cells are grown on a collagen matrix, for 13 days.		
Usage:	**FOR SCIENTIFIC USE ONLY - PRODUCT OF HUMAN ORIGIN**		
Storage:	This product was prepared and packaged using aseptic techniques. Store in an incubator at 37° C, 5% CO_2 with saturated humidity.		
Passage:	Second (Strains n° : 09-KERA-006, 09-KERA-003, 10-KERA-001, 11-KERA-001)		
Batch N°:	**14-EKIN-020**		
Origin:	Adult donors.		
Histology:	 **Control n° E140388**		
Quality Controls:	**Test**	**Specification**	**Result**
	Histology scoring (HES stained vertical paraffin sections, n = 6)	≥ 19.5	21.8 ± 0.5 (CV = 2.4 %)
		Well-differentiated epidermis consisting of a basal layer, several spinous and granular layers and a thick stratum corneum	
	IC 50 determination (SDS concentration, MTT test, n = 14)	≥ 1.5 mg/ml	2.2 mg/ml
	Statistical Analysis : → Histology : probability 0.95 that 100 % of the batch > 20 → IC 50 : probability 0.95 that IC 50 ≥ 2.2 mg/ml (threshold value)		
Biological safety:	On blood of the same donors, we have verified: . the absence of HIV1 and 2 antibodies (Architect Abbott) . the absence of hepatitis C antibodies (Architect Abbott) . the absence of hepatitis B antigen HBs (Architect Abbott) On epidermal cells of the same donors, we have verified: . the absence of bacteria, fungus and mycoplasma		
Expiration date	June 9, 2014.		

Lyon, June 3, 2014.
Certified and released by
Julie BIDOGGIA, Quality Control Manager

Manufactured in accordance to the ISO9001 quality system of Episkin.

EPISKIN
4, rue Alexander Fleming - 69366 Lyon Cedex 07 - France - Tél. (33) 04 37 28 22 00 - Fax (33) 04 37 28 22 01
au capital de 13 888 807 € - 412 127 585 R.C.S. Lyon - N° TVA intracommunautaire FR 46 412 127 585
Email : sales@skinethic.com

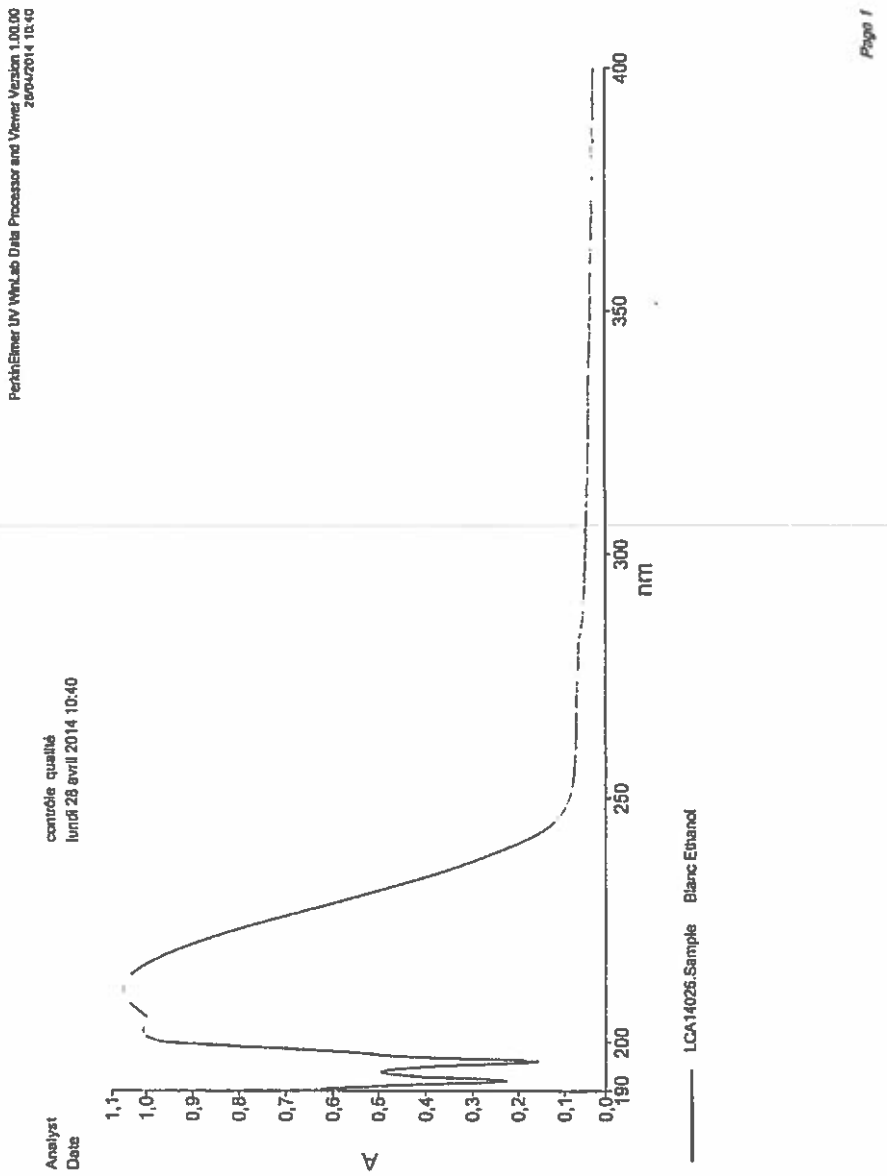
Appendix No. 3: Certificate of analysis of the test item

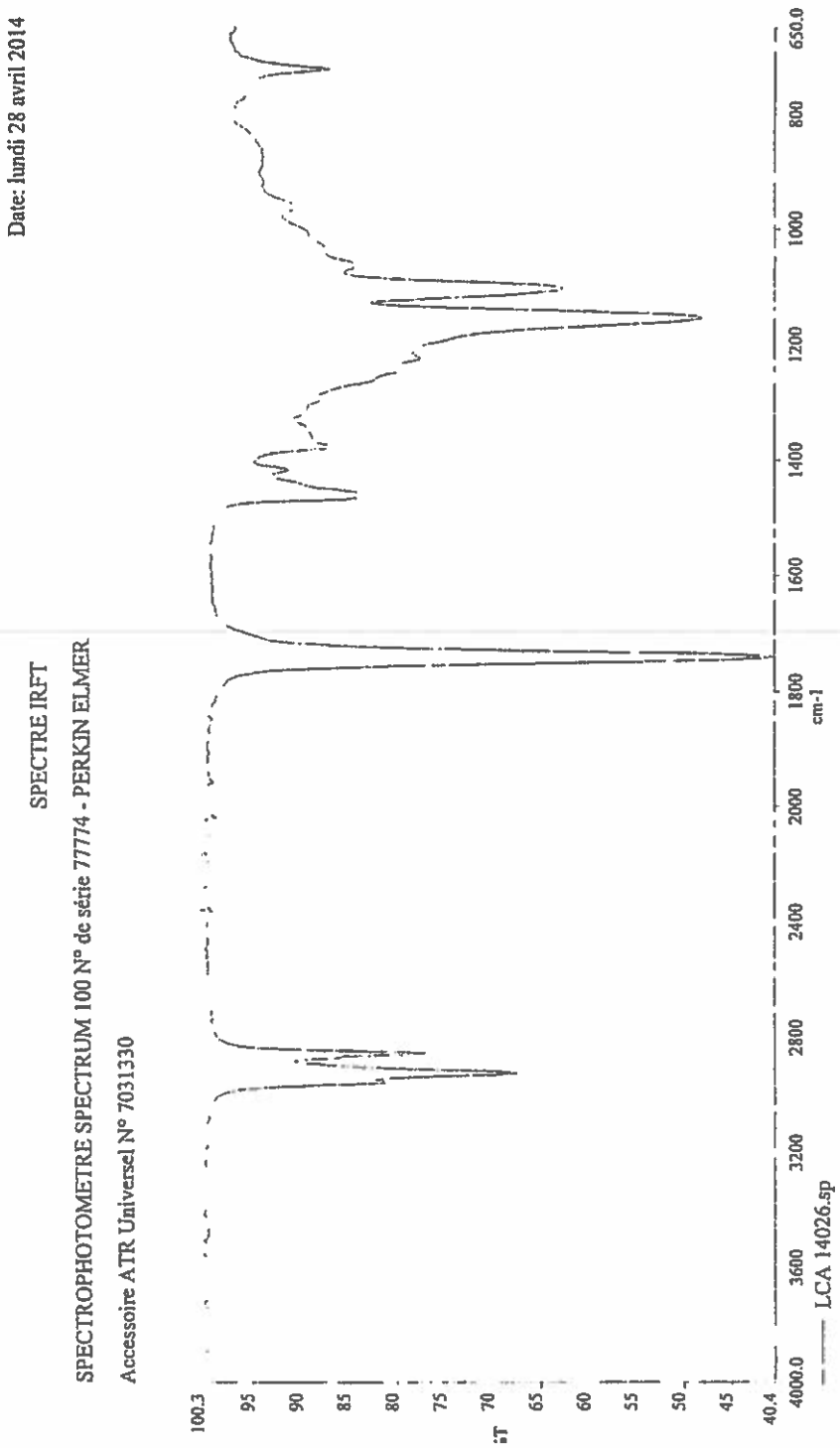
Biotech Marine

LCA14026

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence (fabrication 10/2013-expiration 10/2016): 13.11.401
Reference number (manufacturing 10/2013-expiration 10/2016)

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	RESULTATS *RESULTS*
Aspect *Aspect*	MO PHY 002	Liquide limpide *limpid liquid*
Couleur *Colour*	MO PHY 002	Vert *Green*
Odeur *Odour*	MO PHY 002	Faible *Slight*
Densité (20°C) *Density*	MO PHY 024	0,941
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	1,450$_9$
Teneur en fucoxanthine *Fucoxanthine content*	MO PHY 034	9,5 mg/kg
Spectre UV *UV spectrum*	MO PHY 013	Enregistré *Registered*
Spectre IR *IR spectrum*	MO PHY 011	Enregistré *Registered*





LCA14026

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence (fabrication 10/2013-expiration 10/2016): 13.11.401
Reference number (manufacturing 10/2013-expiration 10/2016)

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux* *Total germs**	MO MIC 002	< 100
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	Absence *None*
- *Candida albicans*	MO MIC 010	Absence *None*
- *Pseudomonas aeruginosa*	MO MIC 011	Absence *None*
- *Enterobacteriaceae*	MO MIC 020	Absence *None*
Levures / Moisissures* *Yeasts / Moulds**	MO MIC 021	< 100

*Les résultats sont exprimés en UFC/ml pour les liquides et UFC/g pour les solides

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
COORDINATRICE ASSURANCE QUALITE : M. TANNIOU
QUALITY ASSURANCE COORDINATOR

Le 28/04/14

Appendix No. 4: Historical data

In vitro skin irritation : reconstructed human epidermidis test method

HISTORICAL DATA FROM FEBRUARY 2013 TO MAY 2013

Mean of corrected OD					
Negative control (PBS)			Positive control (SDS 5%)		
Mean ± *sd* (extreme deviations)			Mean ± *sd* (extreme deviations)		
0.855	**±**	0.137	**0.137**	**±**	0.115
0.765	-	1.013	0.054	-	0.269

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: September 13, 2018

SUBJECT: Undaria Pinnatifida Extract

Biotech Marine. 2016. Manufacturing Process- Phycol UP (Undaria Pinnatifida Extract in Water and Propylene Glycol).

Biotech Marine. 2008. Phycol® UP (Undaria Pinnatifida Extract in Water and Propylene Glycol) Physico-chemical data.

Palmer Research. 2004. Etude de la tolérance cutanée aiguë d'une matière première chez le volontaire adulte: Patch-test 24 heures occlusif sous contrôle dermatologique (Phycol UP - Undaria Pinnatifida Extract in Water and Propylene Glycol).

Biotech Marine. 2014. Manufacturing Process- Ephemer™ (Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride).

Biotech Marine. 2016. Ephemer™ (Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride) Physico-chemical data.

Groupe Dermscan. 2014. Assessment of the sensitizing potential of a cosmetic product (Ephemer Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride).

Institut Pasteur de Lille. 2014. *In vitro* skin irritation: Reconstructed human epidermis test method Ephemer™ (Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

MANUFACTURING PROCESS
PHYCOL UP

HARVESTING / IDENTIFICATION (Undaria pinnatifida)

↓

DRYING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENTS WATER AND PROPYLENE GLYCOL

Addition of preservatives :
- *Methylparaben*
- *Propylparaben*

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

24/2/16

Date de mise à jour : 19/03/2008

PHYCOL® UP

CTFA/INCI NAME : Propylene Glycol – Aqua / Water – Undaria Pinnatifida Extract
CAS : 57.55.6 – 7732.18.5 – 223751.81.3
EINECS : 200.338.0 – 231.791.2 -

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide** *Limpid liquid*
Couleur *Colour*	MO PHY 002	**Jaune (≤ 7 UG)** *Yellow*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	4,5 - 6,5
Densité (20°C) *Density*	MO PHY 024	1,030 - 1,050
Indice de réfraction (20°C) *Refractive index*	MO PHY 008	$1,382_0$ - $1,396_0$
Extrait sec (1g – 4 heures à 105°C) *Dry extract*	MO PHY 033	0,5 - 2 %
Propylène glycol *Propylene glycol*	MO PHY 001	48 - 52 %
Teneur en eau *Water content*	MO PHY 018	48 - 52 %
Acide alginique (réaction colorée) *Alginic acid (colored reaction)*	MO PHY 004	**Positive** *Positive*
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Conservateurs *Preservatives*		
- **POB méthyle** *Methyl Paraben*	MO PHY 020	0,16 - 0,20 %
- **POB propyle** *Propyl Paraben*	MO PHY 020	0,03 - 0,06 %

PHYCOL® UP

CTFA/INCI NAME : Propylene Glycol – Aqua / Water – Undaria Pinnatifida Extract
CAS : 57.55.6 – 7732.18.5 – 223751.81.3
EINECS : 200.338.0 – 231.791.2 -

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux* *Total germs**	MO MIC 002	< 100
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	**Absence** *None*
- *Candida albicans*	MO MIC 010	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011	**Absence** *None*
- *Enterobacteriaceae*	MO MIC 020	< 100
Levures / Moisissures* *Yeasts / Moulds**	MO MIC 021	< 100

*Les résultats sont exprimés en (UFC/ml) pour les liquides et (UFC/g) pour les solides

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE MICROBIOLOGIE : **M. QUINTIN**
MICROBIOLOGICAL LABORATORY MANAGER

le 19.03.08

PHYCOL® UP

CTFA/INCI NAME : Propylene Glycol – Aqua / Water – Undaria Pinnatifida Extract
CAS : 57.55.6 – 7732.18.5 – 223751.81.3
EINECS : 200.338.0 – 231.791.2 -

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds *Heavy metals (ppm)*	
• **Arsenic mineral** *Mineral Arsenic*	< 5
• **Cadmium** *Cadmium*	< 10
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5

Iodine < 1ppm

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE PHYSICO-CHIMIE : **M. LE BRETON**
PHYSICOCHIMICAL LABORATORY MANAGER

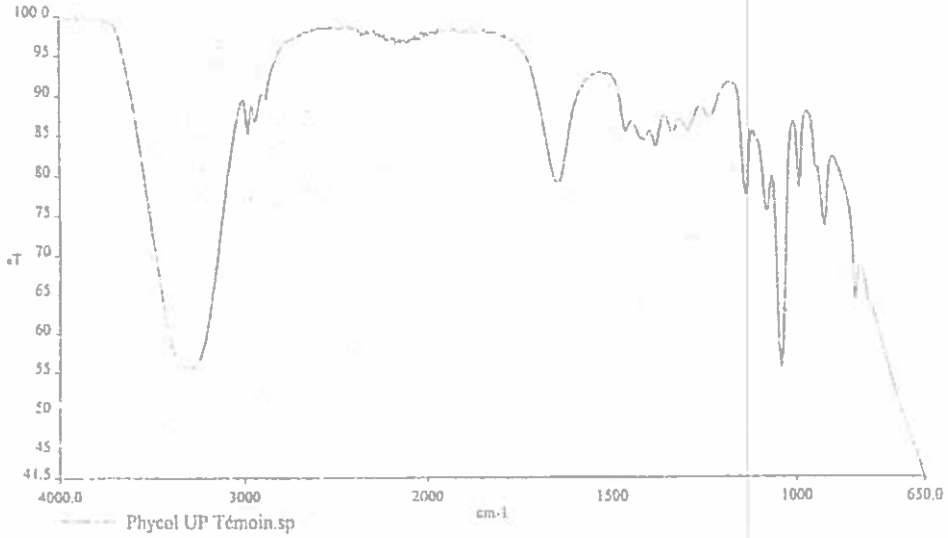
Date: lundi 28 avril 2008

SPECTRE IRFT

PERKIN ELMER

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774

Accessoire ATR Universel N° 7031330



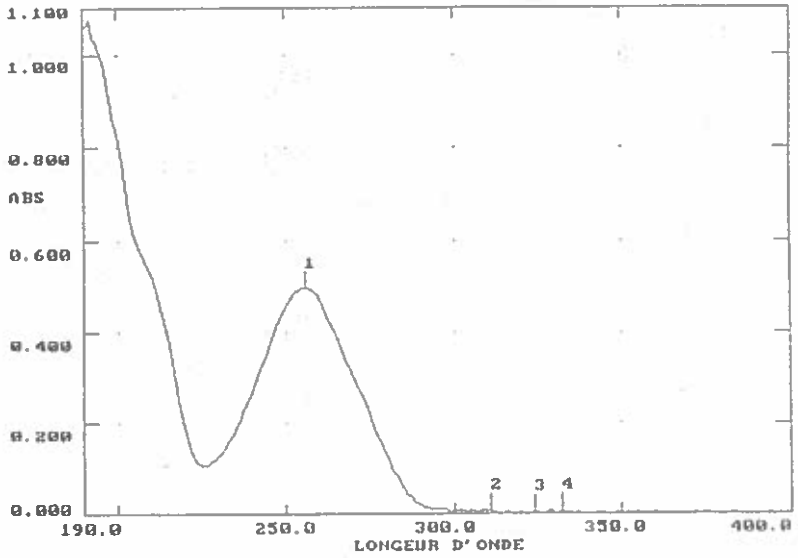
UV310 SPECTROPHOTOMÈTRE UV-VISIBLE v6.56 PAGE 1

DATE :10/06/08 SÉRIE No :104601 ID :SPECTRE UV
HEURE:13:27:04 OPÉRATEUR: N°ÉCHANTILLON:TEMOIN

TYPE SPECTRE:INTELLIGENTVITESSE:NORMAL ENTRE 2 VAL:1.0nm
LIGNE BASE:OPÉRATEUR FENTE:1.5nm CHANGER LAMPE:315nm

PICS

	1	2	3	4	5	6	7	8	9	10
λnm	256.0	311.0	324.0	332.0						
ABS	0.498	0.008	0.006	0.008						



*Blanc : H_2O

10 Jun 2008
Modèle balance
Plate-forme SAP:
Terminal SAP:
Lot Phycol UP
Signature EF

PALMER RESEARCH

Etude de la tolérance cutanée aiguë d'une matière première chez le volontaire adulte : Patch-test 24 heures occlusif sous contrôle dermatologique

Version n° 01/004 du 16 janvier 2004

GROUPE DERMSCAN

SIEGE SOCIAL - LYON
27, bd du 11 Novembre 1918
B.P. 2132
69603 VILLEURBANNE Cedex
FRANCE
Tél. : 33 (0)4 72 82 60 88
Fax : 33 (0)4 72 82 60 83

BORDEAUX
Parc Innolin - 3, rue du Golf
33700 MERIGNAC
FRANCE
Tél. : 33 (0)5 56 34 75 56
Fax : 33 (0)5 56 34 75 54

e-mail : [illegible]
Internet : www.palmer-research.com

Etude : 1030478PA

Matière première : PHYCOL UP LOT 3.01.017 (58359)

Promoteur: **SECMA BIOTECHNOLOGIE MARINE**
ZI – BP 65
22260 PONTRIEUX
FRANCE

Lyon, le 16 janvier 2004

SOMMAIRE

RESUME DU RAPPORT D'ETUDE

Promoteur : **SECMA BIOTECHNOLOGIE MARINE** **Adresse : ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Matière première:** **PHYCOL UP LOT 3.01.017** **Code PALMER Research : 58359**

ETUDE DE LA TOLERANCE CUTANEE AIGUE D'UNE MATIERE PREMIERE CHEZ LE VOLONTAIRE ADULTE : PATCH-TEST 24 HEURES OCCLUSIF SOUS CONTRÔLE DERMATOLOGIQUE

Numéro d'étude :	1030478PA	
Dates de l'étude :	du 7 au 9 janvier 2004.	
Lieu de l'étude :	PALMER RESEARCH - Groupe DERMSCAN Immeuble le CEI 2 – B.P.2132 27 Bd du 11 novembre 1918 69603 VILLEURBANNE CEDEX – FRANCE	
Objectif :	Déterminer le potentiel irritant primaire d'une matière première après application unique sous pansement occlusif pendant 24 heures chez le volontaire adulte.	
Méthodologie :	Etude en ouvert.	Nombre de sujets : 12.
Critères d'inclusion :	Peau indemne de toute lésion dermatologique, sujet non allergique.	• Durée de l'application : 24 heures. • Condition d'utilisation : pure.
Critères d'évaluation :	Détermination du score d'irritation moyen : I.I.M = score total des réactions (érythème + œdème) / nombre total de volontaires Les réactions sont cotées de 0 à 3.	
Méthodes d'analyse :	Classement de la matière première en fonction de son I.I.M : Si I.I.M < 0,20 : Non Irritante Si 0,20 ≤ I.I.M < 0,50 : Légèrement Irritante Si 0,50 ≤ I.I.M < 1 : Moyennement Irritante Si I.I.M ≥ 1 : Irritante	
Conclusion :	L'indice d'irritation moyen de la matière première **PHYCOL UP LOT 3.01.017** est égal à **0.71 (moyennement irritante)** à la lecture 30 minutes et à **0.29 (légèrement irritante)** à la lecture 24 heures.	
Investigateur : **Dr Yvette WELTERT,** ***Dermatologue***		

1 - INTRODUCTION

A la demande de la société **SECMA BIOTECHNOLOGIE MARINE - ZI - BP 65 - 22260 PONTRIEUX - FRANCE**, nous avons évalué sur 12 volontaires adultes, la tolérance cutanée aiguë ou potentiel irritant de la matière première:

PHYCOL UP LOT 3.01.017

après application unique sur la peau du dos (zone scapulaire), sous pansement occlusif maintenu pendant 24 heures (patch-test 24 heures).

Cet essai a été réalisé "en ouvert" selon la méthodologie des essais épicutanés sous occlusion.

Pour réaliser cette étude, nous avons reçu le 5 décembre 2003 un échantillon de la matière première que nous avons référencé sous le code PALMER Research 58359.

L'essai a commencé le 7 janvier pour s'achever le 9 janvier 2004.

2 - CERTIFICAT D'AUTHENTICITE DES RESULTATS

L'étude faisant l'objet du présent rapport a été conduite sous ma responsabilité, en conformité avec le protocole expérimental et dans le respect des règles des Bonnes Pratiques Cliniques.
Toutes les observations et les données numériques recueillies au cours de cet essai sont rapportées dans le présent document.

Après relecture et en tant qu'Investigateur, je certifie ces données conformes
à la réalité des résultats obtenus.
Docteur Yvette WELTERT, *Dermatologue*.

Date : 30 01 04 Signature :

Ce rapport a été audité par la personne en charge du Contrôle Qualité.
Il est considéré comme étant le reflet exact des données générées et des procédures en vigueur en rapport avec les Bonnes Pratiques Cliniques.

Date : 03.02.04 Nom : BRUNET-DUNAND Séverine

Signature :

3 - PROTOCOLE EXPERIMENTAL

L'essai a été réalisé conformément au mode opérationnel référencé « Patch test simple».

3.1 - Volontaires

3.1.1 - Caractéristiques des sujets inclus

- ✓ 12 sujets ont été inclus dans l'essai,
- ✓ dont dix de sexe féminin et deux de sexe masculin,
- ✓ âgés de 19 à 57 ans (moyenne d'âge: 36 ans).

Tous les sujets devaient répondre aux critères d'inclusion et ne présenter aucun critère de non-inclusion, dont en particulier :

3.1.2 - Critères d'inclusion

- ✓ aucun antécédent d'intolérance ou d'allergie à une matière première,
- ✓ acceptation de signature du consentement éclairé de participation,
- ✓ phototype I à III.

3.1.3 - Critères de non-inclusion

- ✓ femme enceinte ou qui allaite ou prévoyant un début de grossesse en cours d'étude,
- ✓ pathologie cutanée sur la zone d'expérience (psoriasis, eczéma, vitiligo, pytiriasis versicolor, acné, etc...),
- ✓ présence d'un traitement médicamenteux per os:
  - antihistaminiques, anti-inflammatoires et/ou antibiotiques < 1 semaine,
  - anti-tussifs et/ou corticoïdes < 4 semaines,
  - immunosuppresseur, rétinoïde et/ou anti-cancéreux < 6 mois,
- ✓ début, arrêt ou changement de traitement hormonal (y compris pilule contraceptive) < 1 mois et demi,
- ✓ exposition au soleil ou aux UV < 1 mois au niveau du dos,
- ✓ personne présentant une peau hyper irritable,
- ✓ personne présentant une pilosité importante, des taches de rousseur, des grains de beauté ou un tatouage au niveau du dos,
- ✓ sujet atteint d'une maladie grave ou évolutive,
- ✓ usage immodéré de l'alcool ou du tabac.

3.2 - Méthodologie

3.2.1 - Matériel, dose, durée

La matière première a été appliquée dans les conditions suivantes :

	PHYCOL UP LOT 3.01.017
Zone:	zone scapulaire
Type de Patch tests:	Finn Chamber® 8mm (50mm²) occlusif
Dose*:	approximativement 0.02ml
Condition de l'application:	pure, imprégnant une rondelle de papier filtre
Durée de l'application:	24 heures
Control:	patch sans produit

* Note: La raison du choix de la dose est conditionnée par la capacité de la cupule, indiquée par le fabricant des "Finn Chambers®".

3.2.2 - Lectures

Les examens macroscopiques cutanés ont été réalisés dans les mêmes conditions, en particulier au niveau de l'éclairage (lampe « lumière du jour »), 30 minutes après l'enlèvement des patchs. En l'absence de toute réaction cutanée locale à la lecture de 30 minutes après enlèvement du pansement, l'essai a été arrêté. Cependant, il a été demandé à chaque volontaire de vérifier le lendemain l'absence de réaction. Dans le cas d'une réaction visible, le sujet devait revenir au centre, des lectures pouvant être effectuées jusqu'à réversibilité des réactions cutanées.

Les cotations des éventuelles réactions d'irritation sur chaque site ayant reçu la matière première étudiée ont été réalisées comparativement au site sans produit, selon les échelles numériques suivantes :

Erythème « E » :

E = 0 : absence d'érythème.
E = 0.5: érythème très léger (à peine perceptible : coloration rosée discrète d'une partie de la surface testée).
E = 1 : érythème léger (coloration rosée discrète de toute la surface testée ou bien visible sur une partie de la surface testée).
E = 2 : érythème net (érythème net couvrant toute la surface testée).
E = 3 : érythème important (érythème intense couvrant toute la surface testée ou érythème diffusant en dehors de la surface testée

Œdème « O » :

O = 0 : absence d'œdème
O = 0.5: œdème très léger (palpable, à peine visible)
O = 1 : œdème léger (palpable et visible)
O = 2 : œdème net avec ou sans présence de papule(s) ou vésicule(s)
O = 3 : œdème important (surface débordant la zone d'application) avec ou sans présence de vésicules ou de bulle(s).

Les modifications de structure cutanée (dessèchement, rugosité, épaississement, réflectivité) pouvant être liées à la nature même de la matière première étudiée ou à l'un des ingrédients, ont fait l'objet d'une description clinique dont l'intensité de chaque modification a été appréciée selon le barème :

0,5 = douteux
1 = léger
2 = net
3 = important

3.2.3 – Interprétation des résultats

L'analyse et l'interprétation des résultats ont été réalisées en fonction des données obtenues dans les conditions expérimentales, à chaque temps de lecture.

Elles sont descriptives et complétées par le calcul d'un indice d'irritation moyen (I.I.M) à chaque temps de lecture, selon le rapport :

$$\text{I.I.M} = \frac{\sum \text{des cotations (érythème + œdème)}}{\text{Nombre de sujets}}$$

Cet indice ainsi obtenu (maximum 12), permet de classer arbitrairement la matière première étudiée selon le barème d'interprétation suivant :

I.I.M	Classe
I.I.M < 0.20	**Non irritante (NI)**
0.20 ≤ I.I.M < 0.50	**Légèrement irritante(LI)**
0.50 ≤ I.I.M < 1	**Moyennement irritante (MI)**
I.I.M ≥ 1	**Irritante (I)**

Les valeurs individuelles et la catégorie de matières premières à laquelle appartient la matière première étudiée ont également été prises en compte pour une conclusion adaptée dans les conditions de l'essai (24 heures sous pansement occlusif).

**Références bibliographiques :*

- *« Les essais cliniques en dermatologie », Thérapie, 1991, Tome 46, pages 183 à 187*
- *« Dermato-allergologie de contact », G. DUCOMBS, Editions MASSON, 1988 pages 13 à 16 ; 36-37*
- *« Dermatotoxicology Methods : The laboratory worker's VADEMECUM » ; N. MARZULLI – H. MAIBACH. Ed. Taylor & Francis, 1998.*

4 - RESULTATS

Les résultats individuels des lectures à chaque temps expérimental sont regroupés dans le tableau ci-dessous.

PHYCOL UP LOT 3.01.017
(patch test 24 heures occlusif – pure)

SUJETS					LECTURES									
N°	Identification	Age	Sexe (1)	Type de peau	Lecture 30 minutes après enlèvement du patch					Lecture 24 heures après enlèvement du patch				
					Témoin		Matière première		Modification de structure	Témoin		Matière première		Modification de structure
					E	O	E	O		E	O	E	O	
16S02	FEV Mi	55	F	Normale	0	0	1	0	-	0	0	0	0	-
18S02	LAR Cl	55	F	Normale	0	0	0.5	0	-	0	0	0	0	-
19S02	SCA La	21	F	Normale	0	0	1	0	-	0	0	0.5	0	-
20S02	SAN Va	30	F	Normale	0	0	0	0	-	0	0	0	0	-
21S02	GUI Al	19	F	Normale	0	0	0.5	0	-	0	0	0	0	-
23S02	PIC Pa	57	F	Normale	0	0	0	0	-	0	0	0	0	-
24S02	TAN So	21	F	Normale	0	0	0.5	0	-	0	0	0	0	-
25S02	SER Fr	45	F	Normale	0.5	0	0.5	0	-	0	0	0.5	0	-
26S02	BER So	21	F	Normale	0	0	0	0	-	0	0	0	0	-
27S02	FOR Gi	51	M	Normale	0	0	2	1	-	0	0	1	0	-
30S02	LOM Fr	38	F	Normale	0	0	1	0.5	-	0	0	1	0	-
31S02	KHA Gr	19	M	Normale	0	0	0.5	0	-	0	0	0.5	0	-
Age moyen		36	I.I.M		0.04		0.75		-	0		0.29		-

I.I.M	0.71	0.29
Résultats	**moyennement irritante**	**légèrement irritante**

(1) : M = masculin
F = féminin

Remarque : Le volontaire n°25S02 a présenté une très légère réaction érythémateuse au niveau de la cupule témoin 30 minutes après le retrait des patchs. Cette réaction ayant disparu à 24 heures, le sujet est inclus dans le calcul.
Le calcul de l'I.I.M est effectué par différence entre le score de la matière première et le score témoin.

5 - CONCLUSION

30 minutes après l'enlèvement du patch occlusif, huit volontaires (n°16S02, n°18S02, n°19S02, n°21S02, n°24S02, n°27S02, n°30S02 et n°31S02) ont présenté un très léger à net érythème accompagné d'un très léger à léger œdème pour les volontaires n°27S02 et n°30S02.
A la lecture 24 heures, un très léger à léger érythème était toujours observé chez les sujets n°19S02, n°27S02, n°30S02 et n°31S02. Un très léger érythème retardé était noté chez les sujets n°17S02 et n°25S02.
A la lecture 4 jours, plus aucune réaction n'était constatée.

Par ailleurs, aucun effet secondaire n'a été observé.

Dans les conditions expérimentales retenues, on peut donc conclure que la matière première **PHYCOL UP LOT 3.01.017** testée sous contrôle dermatologique, et appliquée pure et localement sous pansement occlusif pendant 24 heures, sur la peau de 12 volontaires adultes, est classée **movennement irritante** à la lecture 30 minutes et **légèrement irritante** à la lecture 24 heures selon la cotation de l'IIM.

Dr Yvette WELTERT
Dermatologue

STUDY SUMMARY REPORT

Sponsor: **SECMA BIOTECHNOLOGIE MARINE** **Address: ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Raw material: PHYCOL UP LOT 3.01.017** **PALMER Research code: 58359**

EVALUATION OF THE ACUTE CUTANEOUS TOLERANCE OF A RAW MATERIAL ON ADULT VOLUNTEERS: 24-HOUR SINGLE PATCH TEST UNDER DERMATOLOGICAL CONTROL

Study number:	1030478PA	
Study dates:	from January 7 to January 9, 2004.	
Study place:	PALMER RESEARCH - Groupe DERMSCAN Immeuble le CEI 2 – B.P.2132 27 Bd du 11 novembre 1918 69603 VILLEURBANNE Cedex - FRANCE	
Objective:	Determination of the acute skin tolerance of a raw material by application under occlusive patch over a 24-hour period on the adult volunteer.	
Methodology:	Open Study.	Number of subjects: 12.
Included criteria:	Skin without any dermatological lesion, non allergic volunteer.	• Application duration: 24 hours. • Condition of application: pure.
Evaluation criteria:	Calculation of the mean irritation index: $M.I.I. = \frac{\text{total cutaneous reactions score (erythema + edema)}}{\text{number of volunteers}}$ Skin responses are scored from 0 to 3.	
Analysis:	Classification of the raw material according to its M.I.I: if M.I.I. < 0.20 : Non irritating if 0.20 ≤ M.I.I. < 0.50 : Slightly irritating if 0.50 ≤ M.I.I. < 1 : Moderately irritating if M.I.I. ≥ 1 : Irritating	
Conclusion:	The irritation index of the raw material **PHYCOL UP LOT 3.01.017** is equal to **0.71 (moderately irritating)** at the 30-minute reading and to **0.29 (slightly irritating)** at the 24-hour reading.	
Dr Yvette WELTERT, *Dermatologist*		

MANUFACTURING PROCESS

EPHEMER™

HARVESTING OF
FERTILE SPOROPHYTES OF *Undaria Pinnatifida* / IDENTIFICATION

↓

FRAGMENT ISOLATION OF GAMETOPHYTE OF *Undaria Pinnatifida*

↓

CULTURE IN LIQUID MEDIUM

↓

GAMETOPHYTE SEPARATION OF *Undaria Pinnatifida* / CULTURE MEDIUM

↓

FREEZE-DRIED GAMETOPHYTE OF *Undaria Pinnatifida*

↓

GRINDING OF GAMETOPHYTE OF *Undaria Pinnatifida* / SIEVING

↓

QUALITY CONTROL

↓

EXTRACTION WITH THE SOLVENT
CAPRYLIC/CAPRIC TRIGLYCERIDE

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

BIOTECHMARINE (10/14/2014)

Date de mise à jour / *Updated date* : 01/12/2016

EPHEMER™

INCI NAME : Caprylic/Capric Triglyceride – Undaria Pinnatifida Extract
CAS N°: 73398-61-5 – 223751-81-3 caprylic / capric triglyceride 795%
EINECS N°: 277-452-2 –
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant*
(Roundtable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497

Undaria Pinnatifida
<5% Extra

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA

Numéro de référence / *Reference number* : Standard provisoire / *Temporary standard*

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD PROVISOIRE *TEMPORARY STANDARD*
Conformité Mass Balance *Mass Balance conformity*	PO-HSE-004	**Conforme** *Conform*
Aspect *Aspect*	MO PHY 002	**Liquide limpide huileux** *Oily limpid liquid*
Couleur *Colour*	MO PHY 002	**Jaune-vert pâle à vert foncé** *Pale yellow green to dark green*
Odeur *Odour*	MO PHY 002	**Faible** *Slight*
Densité (20°C) *Density*	MO PHY 024	0,920 - 0,950
Indice de réfraction (20°C) *Refractive index*	MO PHY 008	$1{,}440_0$ - $1{,}460_0$
Teneur en fucoxanthine *Fucoxanthine content*	MO PHY 084	≥ 5 mg/kg
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Solvant *Solvent*	**Mélange de triglycérides d'acides gras saturés (C8C10) d'origine végétale** *Mixture of triglycerides of saturated fatty acids (C8C10) of vegetal origin*	
Indice d'iode* *Iodine value**		≤ 1 g I_2/100g
Indice de saponification* *Saponification value**		320 – 350 mg KOH / g

* Indices du Caprylic/capric triglycérides (C8C10) garantis par notre fournisseur
* *Caprylic/capric triglycérides (C8C10) values guaranteed by our supplier*

BiotechMarine Z.I. – B.P. 72 – 22260 Pommeux – FRANCE Tél. +33 (0) 2 96 95 31 32

EPHEMER™

INCI NAME : Caprylic/Capric Triglyceride – Undaria Pinnatifida Extract
CAS N°: 73398-61-5 – 223751-81-3
EINECS N°: 277-452-2 –
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant (Roumtable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA

Numéro de référence / *Reference number* : Standard provisoire / *Temporary standard*

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD PROVISOIRE *TEMPORARY STANDARD*
Métaux lourds (ppm)** *Heavy metals (ppm)***	
• **Arsenic mineral** *Mineral Arsenic*	< 2
• **Cadmium** *Cadmium*	< 3
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5
• **Mercure** *Mercury*	< 1

Iodine < 1 ppm

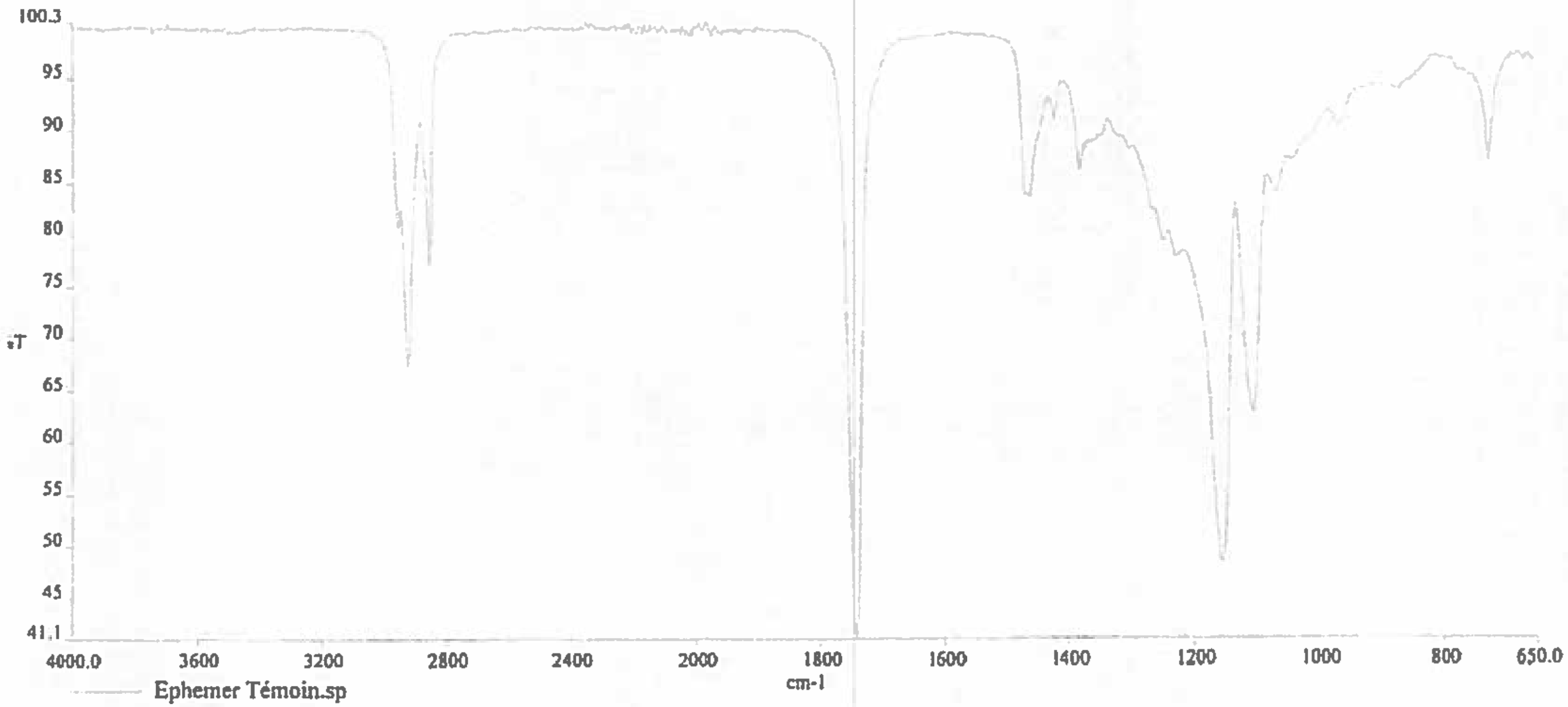
** Teneurs garanties sous contrôle statistique
** *Contents guaranteed under statistical control*

Date: vendredi 28 octobre 2016

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



EPHEMER™

INCI NAME : Caprylic/Capric Triglyceride – Undaria Pinnatifida Extract
CAS N°: 73398-61-5 – 223751-81-3
EINECS N°: 277-452-2 –
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant (Roundtable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : Standard provisoire / *Temporary standard*

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD PROVISOIRE *TEMPORARY STANDARD*
Germes totaux*** *Total germs****	MO MIC 002 / NF EN ISO 21149	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures*** *Yeasts / Moulds****	MO MIC 021 / NF EN ISO 16212	**< 100**

*** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
*** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
01 DEC 2016

CONFORME
CERTIFIED TRUE AND CORRECT
ASSURANCE QUALITE : **A. HAMON**
QUALITY ASSURANCE
01 DEC 2016

- BiotechMarine Z.I. - B.P. 72 – 22260 Pontrieux - FRANCE Tel : +33 (0) 2 96 95 31

Groupe Dermscan

EVALUATION DU POTENTIEL SENSIBILISANT D'UN PRODUIT COSMETIQUE : TEST CLINIQUE FINAL DE SECURITE SOUS CONTRÔLE DERMATOLOGIQUE

ASSESSMENT OF THE SENSITIZING POTENTIAL OF A COSMETIC PRODUCT: FINAL CLINICAL SECURITY TEST UNDER DERMATOLOGICAL CONTROL

Rapport / *Report:*	**14E0898 (version 1)**
Référence etude / *Study reference:*	**DN-1344**
Produit / *Product* :	**LCA14027 - 14P0898-1** Ephemer Undaria Pinnat. F. da Extract in Caprylic / capric Triglyceride
Promoteur / *Sponsor* :	**SEPPIC** **Biotechmarine** **Z.I.** **22260 Pontrieux** **FRANCE**
C.R.O.	**DERMSCAN** Domaine Scientifique de la Doua 56, Boulevard Niels Bohr **69623 VILLEURBANNE Cedex - FRANCE**
Moniteur de l'étude / *Study Monitor*	**LISKIN - Dr. Bogdan WICHROWSKI** IMMEUBLE FONTENAY AFFAIRES 91, rue Boucicaut **92260 FONTENAY-AUX-ROSES - FRANCE**
Investigateur / *Investigator*	**PROCOS - Dr Marlena NOWAKOWSKA**

Lyon,16/07/2014

Please note that the % of brown algae contained in each below mentioned EXTRACT ranges between 0.5 and 10%

INCI name PCPC	chemical characterization data	dermal toxicity data	dermal irritation and sensitization data -% test	description of the method of manufacture	solvent used to extract algae	presence of arsenic	presence of iodine
Water (and) **Cystoseira Baccata Extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers, was found to be no irritating.	Evaluation of the allergenic potential after repeated cutaneous applications over 50 volunteers. Results obtained under experimental conditions showed that the product was found to be no irritant with regard to the cutaneous tolerance and did not induce any significant skin reaction of contact allergy. It can be thus qualified as hypoallergenic. Concentration test : 100 %	extraction with water	water	arsenic mineral : 8,8 mg/kg (FCC V method), arsenic : 20 ppm (ICP-OES method)	/
caprylic/Capric Triglycerides (and) **Cystoseira Tamaricifolia Extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers, was found to be no irritating.	/	extraction with supercritical CO2	CO2		<1 mg/kg (colorimetry method)
Water (and) Dipropylene glycol (and) **Himanthalia elongata extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers, was found to be no irritating.	/	extraction with water and dipropylene glycol	water/dipropylene glycol		<9 mg/kg (alcaline mineralisation and potentiometric method)

Water (and) **Laminaria digitata extract** (and) Sea salt	/	Evaluation of the primary cutaneous tolerance on the rabbit, the product was found to be no irritant.	/	extraction with water	water	1,5 mg/kg (ICP-MS method)	62 mg/kg (alcaline mineralisation and potentiometric method)
Water (and) Glycerin (and) **Laminaria Digitata Extract**	see phycojuvenine						
Water (and) Dipropylene glycol (and) **Laminaria digitata extract**	/	Evaluation of the acute skin tolerance on the rabbit, the product was found to be no irritant.	/	extraction with water and dipropylene glycol	water/Dipropylene glycol	2,37 mg/kg (ICP-MS method)	87 mg/kg (alcaline mineralisation and potentiometric method), average : 110 ppm
Water (and) **Laminaria digitata extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours. The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers with sensitivie skin, was found to be non irritating.	Evaluation of the allergic potential after repeated epicutaneous applications on 50 volunteers. The results obtained in the experimental conditions retained permitted to conclude that the product was found to be very well tolerated at the cutaneous level, showing no significant reaction of a contact allergy. It can thus be qualified as hypoallergenic. Concentration test : 100 %	extraction with water	water	contains less than 10 ppm	contains approximately 550 +/- 150 ppm of iodine (mean of 12 analysis performed on 12 different industrial batches produced between 2003 and 2006). Maximum value : 700 ppm
Water (and) **Laminaria digitata extract**	/	Evaluation of the primary cutaneous tolerance on the rabbit : the product was found to be slightly irritant.	/	extraction with water	water	yes, 19,06 mg/kg (ICP-MS method)	192 mg/kg (alcaline mineralisation and potentiometric method), average : 300 ppm
water (and) **phyllacantha fibrosa extract**	/	/	/	extraction with water	water	yes, 11,35 ppm (ICP-M	yes, 97 mg/l (method ionic chromatography), average :140 ppm
Water (and) Dipropylene glycol (and) **Sphacelaria scoparia extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours. The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 11 volunteers, was found to be non irritating.	Evaluation of the allergic potential after repeated epicutaneous applications on 50 volunteers. The results obtained in the experimental conditions retained permitted to conclude that the product was found to be very well tolerated at the cutaneous level, showing no significant irritative reaction of a contact allergy reaction. It can thus be qualified as hypoallergenic. Concentration test : 100 %	extraction with water and dipropylene glycol	Water / Dipropylene glycol	yes, 0,73 mg/kg (ICP-MS method)	15 mg/kg (alcaline mineralisation and potentiometric method)

Water (and) Dipropylene glycol (and) **undaria pinnatifida extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers, was found to be no irritating.	/	extraction with water and dipropylene glycol	water/dipropylene glycol	/	<9 mg/kg (alcaline mineralisation and potentiometric method)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: October 3, 2018

SUBJECT: Undaria Pinnatifida Cell Culture Extract

Active Concepts. 2018. Product specification ACB Wakame Bioferment Advanced (Undaria Pinnatifida Cell Culture Extract).

Active Concepts. 2018. Compositional breakdown ACB Wakame Bioferment Advanced (Undaria Pinnatifida Cell Culture Extract).

Active Concepts. 2016. Study summary: *In chemico* skin sensitization ACB Wakame Bioferment Advanced (Undaria Pinnatifida Cell Culture Extract).

Active Concepts. 2016. Study summary: *In vitro* skin senstization ACB Wakame Bioferment Advanced (Undaria Pinnatifida Cell Culture Extract).

Active Concepts. 2016. Study summary: Bacterial reverse mutation test ACB Wakame Bioferment Advanced (Undaria Pinnatifida Cell Culture Extract).

Active Concepts. 2018. Study summaries: Dermal and ocular irritation tests ACB Wakame Bioferment Advanced (Undaria Pinnatifida Cell Culture Extract).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Active Concepts

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

Product Specification

Product Name: ACB Wakame Bioferment Advanced
Code Number: 20024
CAS #'s: N/A
EINECS #'s: N/A
INCI Name: Undaria Pinnatifida Cell Culture Extract
Status: Conforms

Suggested Use Levels: 0.5-2.0 %

Specification	Parameter
Appearance	Clear to Slightly Hazy Liquid
Color	Yellow to Amber
Odor	Characteristic
pH (Direct)	5.0 – 7.0
Solids (1g-1hr-105^{0}C)	24.0% Minimum Solvent: Water
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm
Microbial Content Yeast & Mold Gram Negative Bacteria	< 100 CFU/g; No pathogens < 100 CFU/g 0 CFU/g

May Sediment upon Standing; Mix Well Prior to Use

- Product Darkens over Time

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

Active Concepts

Compositional Breakdown

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

This is to certify that ACB Wakame Bioferment Advanced does not contain, neither directly nor through cross contamination, any of the 26 allergenic flavors or fragrances (Gas Chromatography-Mass Spectrometer Coupled):

ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009 amending EU Directive 2003/15/EC		
INCI NAME	CAS NUMBER	Limit (ppm)
Alpha-IsoMethyl Ionone	127-51-5	< 0.02
Amyl Cinnamal	122-40-7	< 0.10
Anise Alcohol	105-13-5	< 0.00
Benzyl Alcohol	100-51-6	< 0.01
Benzyl Benzoate	120-51-4	< 0.09
Benzyl Cinnamate	103-41-3	< 0.30
Benzyl Salicylate	118-58-1	< 0.06
Butylphenyl Methylpropional	80-54-6	< 0.50
Cinnamal	104-55-2	< 0.01
Cinnamyl Alcohol	104-54-1	< 0.30
Citral	5392-40-5	< 1.00
Citronellol	106-22-9	< 1.00
Coumarin	91-64-5	< 0.00
Eugenol	97-53-0	< 0.70
Farnesol	4602-84-0	< 0.04
Geraniol	106-24-1	< 0.08
Hexyl Cinnamal	101-86-0	< 0.40
Hydroxycitronellal	107-75-5	< 1.00
Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde	31906-04-4	< 0.00
Isoeugenol	97-54-1	< 0.06
Limonene	5989-27-5	< 0.05
Linalool	78-70-6	< 0.00
Methyl 2 Octynoate	111-12-6	< 0.20
Evernia prunastri	90028-68-5	< 0.02
Evernia furfuracea	90028-67-4	< 0.00
Amylcinnamyl Alcohol	101-85-9	< 1.00

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Compositional Breakdown

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This is to certify that ACB Wakame Bioferment Advanced does not contain pesticide levels exceeding the following (Reverse Phase High Performance Liquid Chromatography-Mass Spectrometer Coupled):

EPA Pesticide Levels	
INCI NAME	LIMIT (mg/kg)
Alachlor	< 0.02
Aldrin and Dieldrin	< 0 .05
Azinphos-methyl	< 1. 00
Bromopropylate	< 3.0 0
Chlordane(cis and trans)	< 0.05
Chlorfenvinphos	< 0.50
Chlorpyrifos	< 0.20
Chlorpyrifos-methyl	< 0.10
Cypermethrin	< 1.00
DDT	< 1.00
Deltamethrin	< 0.50
Diazinon	< 0.50
Dichlorvos	< 1.00
Dithiocarbamates	< 2.00
Endosulfan	< 3.00
Endrin	< 0.05
Ethion	< 2.00
Fenitrothion	< 0.50
Fenvalerate	< 1.50
Fonofos	< 0.05
Heptachlor	< 0.05
Hexachlorobenzene	< 0.10
Hexachlorocyclohexane	< 0.30
Lindane	< 0.60
Malathion	< 1.00
Methidathion	< 0.20
Parathion	< 0.50
Parathion-methyl	< 0.20

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Compositional Breakdown

Permethrin	< 1.00
Phosalone	< 0.10
Piperonyl butoxide	< 3.00
Pirimiphos-methyl	< 4.00
Pyrethrins	< 3.00
Quintozene(sum of 3 items)	< 1.00

Active Concepts

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OECD TG 442C: *In Chemico* Skin Sensitization

Tradename: ACB Wakame Bioferment Advanced Undaria Pinnatifida Cell Culture Extract

Code: 20024

CAS #: N/A

Test Request Form #: 2196

Lot #: 44551

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
OECD TG 442C: *In Chemico* Skin Sensitization
Direct Peptide Reactivity Assay (DPRA)

Introduction

A skin sensitizer is a substance that will lead to an allergic response following skin contact[1]. Haptenation is the covalent binding of a hapten, or low-molecular weight substance or chemical, to proteins in the skin. This is considered the prominent mechanism which defines a chemical as a sensitizer. Haptenation is described as a "molecular initiating event" in the OECD Adverse Outcome Pathway (AOP) for skin sensitization which summarizes the key events known to be involved in chemically-induced allergic contact dermatitis[2]. The direct peptide reactivity assay (DPRA) is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine. The DPRA is able to distinguish sensitizers from non-sensitizer with 82% accuracy (sensitivity of 76%; specificity of 92%)[3].

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

Assay Principle

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

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

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OECD TG 442C: *In Chemico* Skin Sensitization

Materials

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

Methods

Solution Preparation:

- 0.667mM Cysteine Peptide in 100mM Phosphate Buffer (pH 7.5)
- 0.667mM Lysine Peptide in 100mM Ammonium Acetate Buffer (pH 10.2)
- 100mM Cinnamic Aldehyde in Acetonitrile
- 100mM* **ACB Wakame Bioferment Advanced** in Acetonitrile

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

Reference Controls:

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

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

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

1:10 Ratio, Cysteine Peptide 0.5mM Peptide, 5mM Test Chemical	1:50 Ratio, Lysine Peptide 0.5mM Peptide, 25mM Test Chemical
• 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls) • 200µL Acetonitrile • 50µL Test Chemical Solution (or Acetonitrile for Reference Controls)	• 750µL Lysine Peptide Solution (or 100mM Ammonium Acetate Buffer, pH 10.2, for Co-Elution Controls) • 250µL Test Chemical Solution (or Acetonitrile for Reference Controls)

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Calibration Curve:

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

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

HPLC Analysis:

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

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

Data and Reporting

Acceptance Criteria:

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

2. The following criteria must be met for a test chemical's results to be considered valid:
   a. Maximum standard deviation should be <14.9 for percent cysteine depletion and <11.6 for percent lysine depletion.
   b. Mean peptide concentration of the three reference control C should be 0.50±0.05mM.

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Prediction Model:

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

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

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

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

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.25	Minimal Reactivity	Non-sensitizer
3.21	Minimal Reactivity	Non-sensitizer
3.23	Minimal Reactivity	Non-sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.16	Minimal Reactivity	Non-sensitizer
3.11	Minimal Reactivity	Non-sensitizer
3.14	Minimal Reactivity	Non-sensitizer

Results and Discussion

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

Percent peptide depletion is determined by the following equation:

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

Based on HPLC-UV analysis of **ACB Wakame Bioferment Advanced (20024)** we can determine this product is not classified as a sensitizer and is not predicted to cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 3.18% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

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OECD TG 442D: *In Vitro* Skin Sensitization

Tradename: ACB Wakame Bioferment Advanced Undaria Pinnatifida Cell Culture Extract

Code: 20024

CAS #: N/A

Test Request Form #: 2061

Lot #: 44551

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

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

Introduction

Skin sensitization refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals[1]. Substances are classified as skin sensitizers if there is evidence in humans that the substance can lead to sensitization by skin contact or positive results from appropriate tests, both *in vivo* and *in vitro*. Utilization of the KeratinoSens™ cell line allows for valid *in vitro* testing for skin sensitization.

This assay was conducted to determine skin sensitization potential of **ACB Wakame Bioferment Advanced** in accordance with the UN GHS.

Assay Principle

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

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

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

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OECD TG 442D: *In Vitro* Skin Sensitization

Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
B. Equipment:	Humidified incubator; Biosafety laminar flow hood; Microplate Reader; Pipettes
C. Cell Line:	KeratinoSens™ by Givaudan Schweiz AG
D. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Geneticin
E. Culture Plate:	Flat bottom 96-well tissue culture treated plates
F. Reagents:	Dimethyl Sulfoxide (DMSO); Cinnamic Aldehyde; ONE-Glo Reagent; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); sodium lauryl sulfate (SLS)
G. Other:	Sterile disposable pipette tips; wash bottles

Methods

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

24 hour post KeratinoSens™ seeding, the culture media was removed and replaced with fresh media containing 10% FBS without G418 geneticin. 50 µL of the above described test concentrations was added to the appropriate wells. The treated plates were then incubated for 48 hours at 37°C in the presence of 5% CO_2 and 95% relative humidity. After treatment incubation was complete the media was removed and the wells were washed with PBS 3 times.

One of the four plates was used for a cytotoxicity endpoint, where MTT was added to the wells and incubated for 4 hours at 37°C in the presence of 5% CO_2. SLS was then added to the wells and incubated overnight at room temperature. A spectrometer measured the absorbance at 570 nm. The absorbance values (optical density) were then used to determine the viability of each well by comparing the optical density of each test material treated well to that of the solvent control wells to determine the IC_{50} and IC_{30} values.

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

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Data and Reporting

Acceptance Criteria:

1. Gene induction obtained with the positive control, cinnamic aldehyde, should be statistically significant above the threshold of 1.5 in at least one of the tested concentrations (from 4 to 64 µM).
2. The EC1.5 value should be within two standard deviations of the historical mean and the average induction in the three replicates for cinnamic aldehyde at 64 µM should be between 2 and 8.
3. The average coefficient of variability of the luminescence reading for the negative (solvent) control DMSO should be below 20% in each experiment.

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

1. The Imax is higher than 1.5-fold and statistically significantly higher as compared to the solvent (negative) control
2. The cellular viability is higher than 70% at the lowest concentration with a gene induction above 1.5 fold (i.e., at the EC1.5 determining concentration)
3. The $EC_{1.5}$ value is less than 1000 µM (or < 200 µg/ml for test chemicals with no defined MW)
4. There is an apparent overall dose-response for luciferase induction

Results

Compound	Classification	$EC_{1.5}$ (µM)	IC_{50}	I_{max}
Cinnamic aldehyde	Sensitizer	19	289.19 µM	31.22
DMSO	Non-Sensitizer	No Induction	243.24 µM	0.16
ACB Wakame Bioferment Advanced	Non-Sensitizer	No Induction	> 1000 µM	0.34

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

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OECD TG 442D: *In Vitro* Skin Sensitization

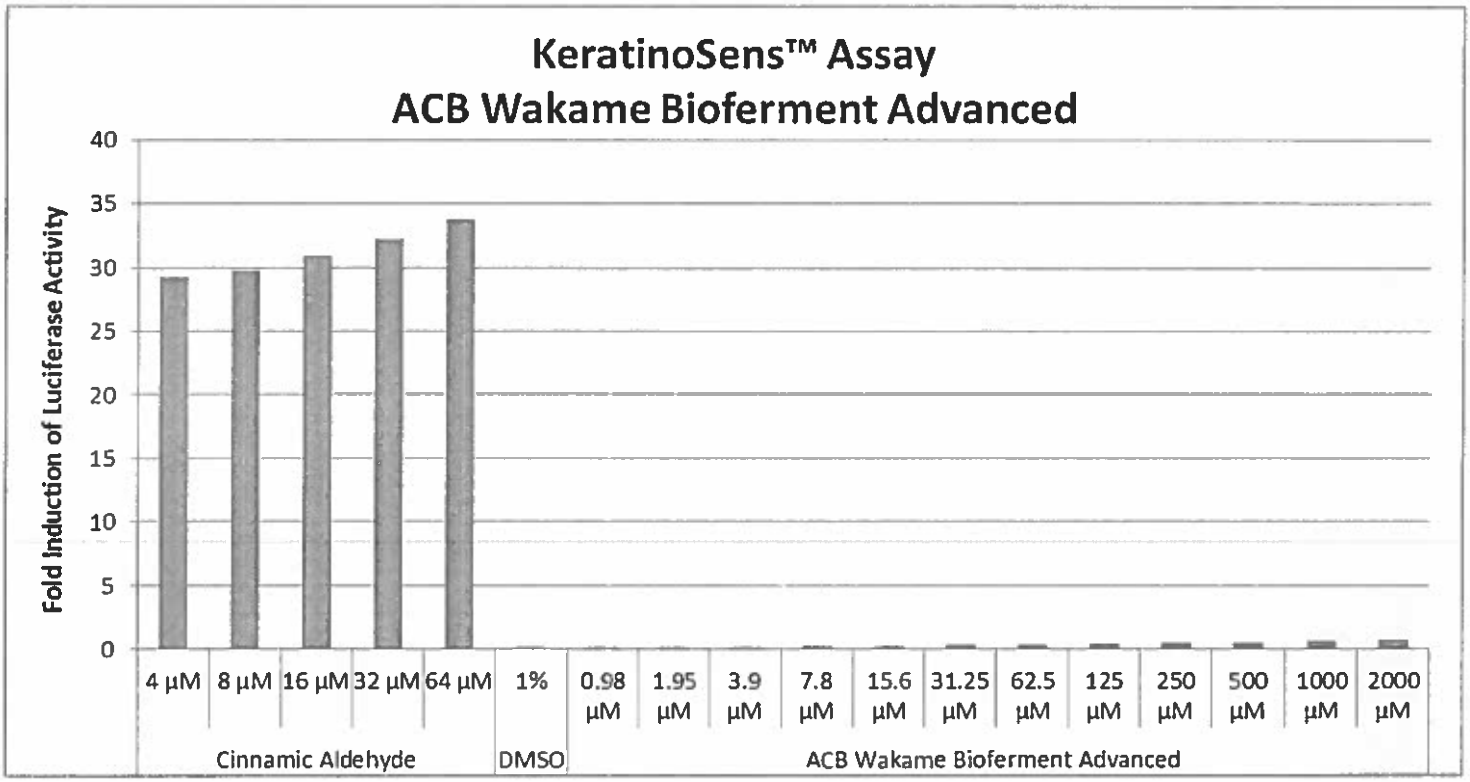

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **ACB Wakame Bioferment Advanced (20024)** was not predicted to be a skin sensitizer based on the KeratinoSens™ ARE-Nrf2 Luciferase Test Method as there was not a significant increase in luciferase expression. It can be concluded that **ACB Wakame Bioferment Advanced** can be safely used in cosmetics and personal care products at typical use levels.

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Bacterial Reverse Mutation Test

Test Article: ACB Wakame Bioferment Advanced
Code Number: 20024
CAS #: N/A

Undaria Pinnatifida Cell Culture Extract

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

Study Director: *Maureen Danaher*
Principle Investigator: *Monica Beltran*

Test Performed:
Genotoxicity: Bacterial Reverse Mutation Test

Reference:
OECD471/ISO10993.Part 3

Test Request Number: 2013

SUMMARY

A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study described by Ames *et al.* (1975) was conducted to evaluate whether a test article solution **ACB Wakame Bioferment Advanced** would cause mutagenic changes in the average number of reveratants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2*uvr*A in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The stock test article was tested at eight doses levels along with appropriate vehicle control and positive controls with overnight cultures of tester strains. The test article solution was found to be noninhibitory to growth of tester strain TA98, TA100, TA1537, TA1535 and WP2*uvr*A after Sport Inhibition Screen.

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

Under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* tester strain WP2*uvr*A. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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Bacterial Reverse Mutation Test

I. Introduction

A. **Purpose**

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

II. Materials

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

III. Test System

A. Test System

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

Tester strain	Mutations/Genotypic Relevance
TA98	hisD3052, Dgal chlD bio *uvr*B *rfa* pKM101
TA100	hisG46, Dgal chlD BIO *uvr*B *rfa* pKM101
TA1537	hisC3076, *rfa*, Dgal chlD bio *uvrB*
TA 1535	hisG46, Dgal chlD bio *uvr*B *rfa*
WP2*uvr*A	trpE, *uvr*A

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

B. Metabolic Activation

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

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Bacterial Reverse Mutation Test

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

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

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

F. Titer of the Strain Cultures:
Fresh cultures of bacteria were grown up to the late exponential or early stationary phase of growth; to confirm this, serial dilutions from each strain were conducted, indicating that the initial population was in the range of 1 to $2x10^9$/ml.

IV. Method

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

V. Evaluation

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

VI. Results and Discussion

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

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

C. Titer (Organisms/ml):
5 x 10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2 x 10^9 UFC/ml.

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

VII. Conclusion

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

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Bacterial Reverse Mutation Test

Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	*TA98*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	31	38	35
	1500	18	24	21
	500	25	21	23
	150	22	24	23
	50	16	21	18
	15	20	19	20
	5.0	10	14	12
	1.5	25	40	33
Test Solution w/o S9	5000	21	42	32
	1500	15	15	15
	500	12	17	15
	150	22	29	26
	50	34	42	38
	15	22	23	23
	5.0	19	23	21
	1.5	23	22	23
DI Water w/S9		17	29	23
DI Water w/o S9		44	48	46
2-aminoanthracen w/ S9		151	171	161
2-nitrofluorene w/o S9		140	127	134
Historical Count Positive w/S9		**43-1893**		
Historical Count Positive w/o S9		**39-1871**		
Historical Count Negative w/S9		**4-69**		
Historical Count Negative w/o S9		**3-59**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*TA100*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	157	172	165
	1500	143	162	153
	500	202	215	209
	150	160	177	169
	50	197	177	187
	15	170	168	169
	5.0	140	158	149
	1.5	140	145	143
Test Solution w/o S9	5000	137	148	143
	1500	141	148	145
	500	177	212	195
	150	132	144	138
	50	215	233	224
	15	138	127	133
	5.0	116	144	130
	1.5	124	137	131
DI Water w/S9		116	129	123
DI Water w/o S9		107	119	113
2-aminoanthracen w/ S9		607	632	620
Sodium azide w/o S9		549	630	590
Historical Count Positive w/S9		**224-3206**		
Historical Count Positive w/o S9		**226-1837**		
Historical Count Negative w/S9		**55-268**		
Historical Count Negative w/o S9		**47-250**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*TA1537*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	11	16	14
	1500	17	14	16
	500	15	24	20
	150	11	9	10
	50	18	12	15
	15	7	9	8
	5.0	17	29	23
	1.5	9	13	11
Test Solution w/o S9	5000	19	17	14
	1500	5	14	10
	500	14	14	14
	150	12	9	11
	50	14	22	18
	15	16	12	14
	5.0	7	14	11
	1.5	12	19	16
DI Water w/S9		7	14	11
DI Water w/o S9		17	12	15
2-aminoanthracen w/ S9		370	349	360
2-aminoacridine w/o S9		110	119	115
Historical Count Positive w/S9		**13-1934**		
Historical Count Positive w/o S9		**17-4814**		
Historical Count Negative w/S9		**0-41**		
Historical Count Negative w/o S9		**0-29**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*TA1535*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	17	24	21
	1500	14	17	16
	500	21	12	17
	150	11	24	18
	50	20	24	22
	15	21	29	25
	5.0	12	14	13
	1.5	29	38	34
Test Solution w/o S9	5000	25	14	20
	1500	22	30	26
	500	4	10	7
	150	22	12	17
	50	11	9	10
	15	22	25	24
	5.0	17	30	24
	1.5	12	29	21
DI Water w/S9		9	5	7
DI Water w/o S9		19	31	25
2-aminoanthracen w/ S9		140	132	136
Sodium azide w/o S9		740	762	751
Historical Count Positive w/S9		**22-1216**		
Historical Count Positive w/o S9		**47-1409**		
Historical Count Negative w/S9		**1-50**		
Historical Count Negative w/o S9		**1-45**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*WP2uvrA*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	15	17	16
	1500	17	31	24
	500	19	12	16
	150	22	14	18
	50	17	22	20
	15	22	21	22
	5.0	24	38	31
	1.5	20	27	24
Test Solution w/o S9	5000	19	16	18
	1500	28	37	33
	500	20	17	19
	150	19	20	20
	50	12	22	17
	15	22	29	26
	5.0	33	34	34
	1.5	28	34	31
DI Water w/S9		31	31	31
DI Water w/o S9		27	31	29
2-aminoanthracen w/ S9		199	169	184
Methylmethanesulfonate w/o S9		289	302	296
Historical Count Positive w/S9		**44-1118**		
Historical Count Positive w/o S9		**42-1796**		
Historical Count Negative w/S9		**8-80**		
Historical Count Negative w/o S9		**8-84**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Dermal and Ocular Irritation Tests

Sample: ACB Wakame Bioferment Advanced

Code: 20024 Undaria Pinnatifida Cell Culture Extract

CAS #: N/A

Test Request Form/Submission #: 409

Lot #:

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)
EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **ACB Wakame Bioferment Advanced** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be **non-irritating**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

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

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Dermal and Ocular Irritation Tests

I. Introduction

A. Purpose

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

II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
C. Media/Buffers:	DMEM based medium; DPBS; sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
F. Reagents:	MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
G. Other:	Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

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

B. Negative Control

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

C. Positive Control

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

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Dermal and Ocular Irritation Tests

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D. Data Interpretation Procedure

a. EpiDerm™

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

b. EpiOcular™

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

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for an additional 18 to 21 hours.

B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

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

b. EpiOcular™

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

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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B. Positive Control

a. EpiDerm™

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

b. EpiOcular™

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

C. Standard Deviation

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

VI. Results

A. Tissue Characteristics

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

B. Tissue Viability Assay

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

C. Test Validity

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

VII. Conclusion

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

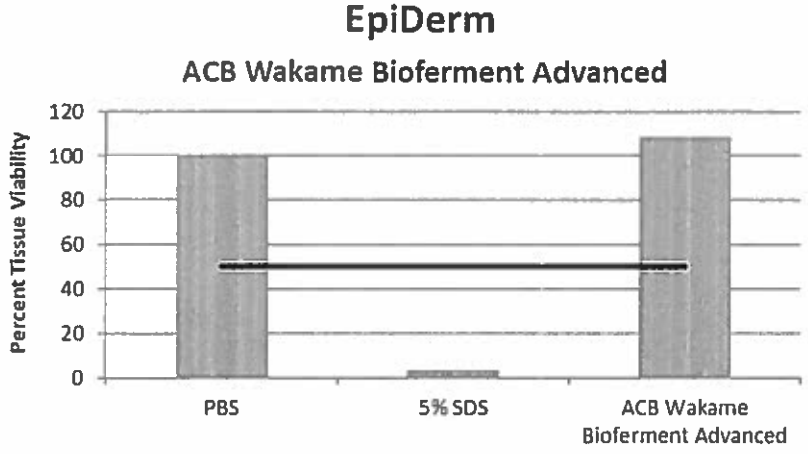

Figure 1: EpiDerm tissue viability

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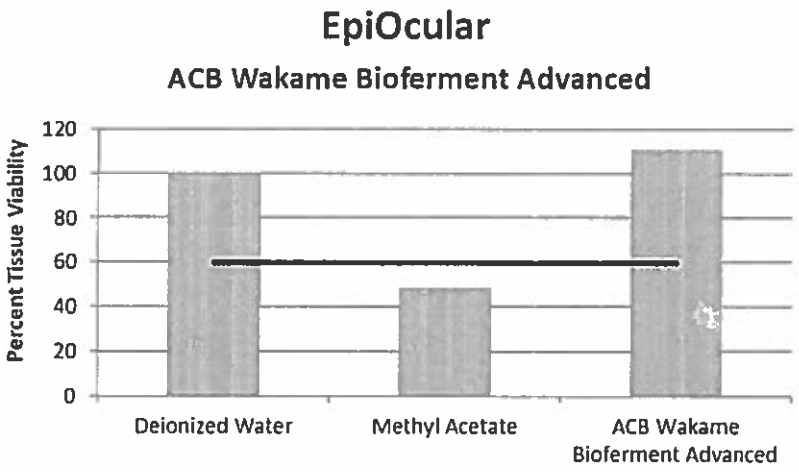

Figure 2: EpiOcular tissue viability

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: October 25, 2018

SUBJECT: Sargassum Filipendula Extract

Active Concepts. 2018. Compositional breakdown AC Algae Blend Sorb (contains 1.3% Sargassum Filipendula Extract).

Active Concepts. 2018. Dermal and ocular irritation tests (AC Algae Blend Sorb [contains 1.3% Sargassum Filipendula Extract]).

Active Concepts. 2018. OECD TG 442D: *In vitro* skin sensitization (AC Algae Blend Sorb [contains 1.3% Sargassum Filipendula Extract]).

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Compositional Breakdown

AC Algae Blend Sorb
Code: 11037MK

Compositional Breakdown:

Ingredient	%
Water	81.775
Sorbitol	14.00
Hypnea Musciformis Extract	1.40
Gellidiela Acerosa Extract	1.30
Sargassum Filipendula Extract	1.30
Methylparaben	0.20
Propylparaben	0.025

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Compositional Breakdown

This is to certify that AC Algae Blend Sorb does not contain, neither directly nor through cross contamination, any of the 26 allergenic flavors or fragrances (Gas Chromatography-Mass Spectrometer Coupled):

ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009 amending EU Directive 2003/15/EC		
INCI NAME	CAS NUMBER	Limit (ppm)
Alpha-IsoMethyl Ionone	127-51-5	< 0.02
Amyl Cinnamal	122-40-7	< 0.10
Anise Alcohol	105-13-5	< 0.00
Benzyl Alcohol	100-51-6	< 0.01
Benzyl Benzoate	120-51-4	< 0.09
Benzyl Cinnamate	103-41-3	< 0.30
Benzyl Salicylate	118-58-1	< 0.06
Butylphenyl Methylpropional	80-54-6	< 0.50
Cinnamal	104-55-2	< 0.01
Cinnamyl Alcohol	104-54-1	< 0.30
Citral	5392-40-5	< 1.00
Citronellol	106-22-9	< 1.00
Coumarin	91-64-5	< 0.00
Eugenol	97-53-0	< 0.70
Farnesol	4602-84-0	< 0.04
Geraniol	106-24-1	< 0.08
Hexyl Cinnamal	101-86-0	< 0.40
Hydroxycitronellal	107-75-5	< 1.00
Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde	31906-04-4	< 0.00
Isoeugenol	97-54-1	< 0.06
Limonene	5989-27-5	< 0.05
Linalool	78-70-6	< 0.00
Methyl 2 Octynoate	111-12-6	< 0.20
Evernia prunastri	90028-68-5	< 0.02
Evernia furfuracea	90028-67-4	< 0.00
Amylcinnamyl Alcohol	101-85-9	< 1.00

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Compositional Breakdown

This is to certify that AC Algae Blend Sorb does not contain pesticide levels exceeding the following (Reverse Phase High Performance Liquid Chromatography-Mass Spectrometer Coupled):

EPA Pesticide Levels	
INCI NAME	LIMIT (mg/kg)
Alachlor	< 0.02
Aldrin and Dieldrin	< 0 .05
Azinphos-methyl	< 1. 00
Bromopropylate	< 3.0 0
Chlordane(cis and trans)	< 0.05
Chlorfenvinphos	< 0.50
Chlorpyrifos	< 0.20
Chlorpyrifos-methyl	< 0.10
Cypermethrin	< 1.00
DDT	< 1.00
Deltamethrin	< 0.50
Diazinon	< 0.50
Dichlorvos	< 1.00
Dithiocarbamates	< 2.00
Endosulfan	< 3.00
Endrin	< 0.05
Ethion	< 2.00
Fenitrothion	< 0.50
Fenvalerate	< 1.50
Fonofos	< 0.05
Heptachlor	< 0.05
Hexachlorobenzene	< 0.10
Hexachlorocyclohexane	< 0.30
Lindane	< 0.60
Malathion	< 1.00
Methidathion	< 0.20
Parathion	< 0.50
Parathion-methyl	< 0.20

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Compositional Breakdown

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Permethrin	< 1.00
Phosalone	< 0.10
Piperonyl butoxide	< 3.00
Pirimiphos-methyl	< 4.00
Pyrethrins	< 3.00
Quintozene(sum of 3 items)	< 1.00

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Dermal and Ocular Irritation Tests

Sample: AC Algae Blend Sorb contains 1.3% Sargassum Filipendula Extract

Code: 11037MK

CAS #: 92128-82-0 & 92128-82-0 & 92128-82-0

Test Request Form/Submission #: 3952

Lot #: 55383P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)
EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **AC Algae Blend Sorb** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be **non-irritating**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2- yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

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

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Dermal and Ocular Irritation Tests

I. Introduction

A. Purpose

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

II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
C. Media/Buffers:	DMEM based medium; DPBS; sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
F. Reagents:	MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
G. Other:	Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

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

B. Negative Control

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

C. Positive Control

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

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Dermal and Ocular Irritation Tests

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D. Data Interpretation Procedure

a. EpiDerm™

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

b. EpiOcular™

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

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for an additional 18 to 21 hours.

B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

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

b. EpiOcular™

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

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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B. Positive Control

a. EpiDerm™

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

b. EpiOcular™

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

C. Standard Deviation

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

VI. Results

A. Tissue Characteristics

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

B. Tissue Viability Assay

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

C. Test Validity

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

VII. Conclusion

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

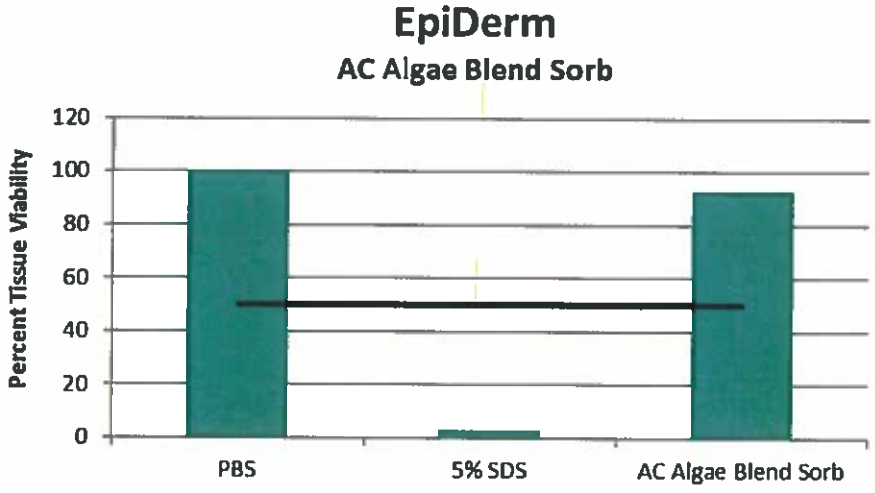

Figure 1: EpiDerm tissue viability

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Dermal and Ocular Irritation Tests

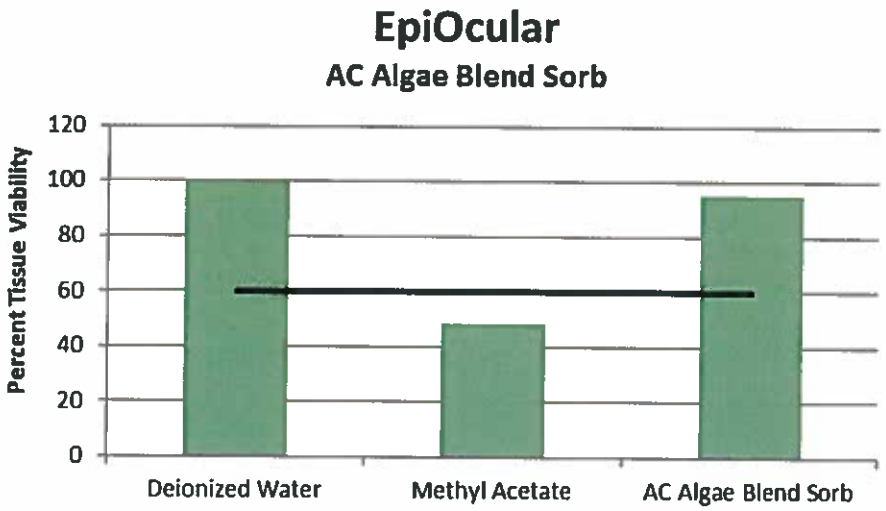

Figure 2: EpiOcular tissue viability

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OECD TG 442D: *In Vitro* Skin Sensitization

Tradename: AC Algae Blend Sorb

Code: 11037MK

CAS #: 92128-82-0 & 92128-82-0 & 92128-82-0

Test Request Form #: 3955

Lot #: 46737P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

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

Introduction

Skin sensitization refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals[1]. Substances are classified as skin sensitizers if there is evidence in humans that the substance can lead to sensitization by skin contact or positive results from appropriate tests, both *in vivo* and *in vitro*. Utilization of the KeratinoSens™ cell line allows for valid *in vitro* testing for skin sensitization.

This assay was conducted to determine skin sensitization potential of **AC Algae Blend Sorb** in accordance with the UN GHS.

Assay Principle

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

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

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

Active Concepts

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

OECD TG 442D: *In Vitro* Skin Sensitization

Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
B. Equipment:	Humidified incubator; Biosafety laminar flow hood; Microplate Reader; Pipettes
C. Cell Line:	KeratinoSens™ by Givaudan Schweiz AG
D. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Geneticin
E. Culture Plate:	Flat bottom 96-well tissue culture treated plates
F. Reagents:	Dimethyl Sulfoxide (DMSO); Cinnamic Aldehyde; ONE-Glo Reagent; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); sodium lauryl sulfate (SLS)
G. Other:	Sterile disposable pipette tips; wash bottles

Methods

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

24 hour post KeratinoSens™ seeding, the culture media was removed and replaced with fresh media containing 10% FBS without G418 geneticin. 50 µL of the above described test concentrations was added to the appropriate wells. The treated plates were then incubated for 48 hours at 37°C in the presence of 5% CO_2 and 95% relative humidity. After treatment incubation was complete the media was removed and the wells were washed with PBS 3 times.

One of the four plates was used for a cytotoxicity endpoint, where MTT was added to the wells and incubated for 4 hours at 37°C in the presence of 5% CO_2. SLS was then added to the wells and incubated overnight at room temperature. A spectrometer measured the absorbance at 570 nm. The absorbance values (optical density) were then used to determine the viability of each well by comparing the optical density of each test material treated well to that of the solvent control wells to determine the IC_{50} and IC_{30} values.

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

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Data and Reporting

Acceptance Criteria:

1. Gene induction obtained with the positive control, cinnamic aldehyde, should be statistically significant above the threshold of 1.5 in at least one of the tested concentrations (from 4 to 64 µM).
2. The EC1.5 value should be within two standard deviations of the historical mean and the average induction in the three replicates for cinnamic aldehyde at 64 µM should be between 2 and 8.
3. The average coefficient of variability of the luminescence reading for the negative (solvent) control DMSO should be below 20% in each experiment.

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

1. The Imax is higher than 1.5-fold and statistically significantly higher as compared to the solvent (negative) control
2. The cellular viability is higher than 70% at the lowest concentration with a gene induction above 1.5 fold (i.e., at the EC1.5 determining concentration)
3. The $EC_{1.5}$ value is less than 1000 µM (or < 200 µg/ml for test chemicals with no defined MW)
4. There is an apparent overall dose-response for luciferase induction

Results

Compound	Classification	$EC_{1.5}$ (µM)	IC_{50}	I_{max}
Cinnamic aldehyde	Sensitizer	19	289.19 µM	32.3
DMSO	Non-Sensitizer	No Induction	243.24 µM	0.17
AC Algae Blend Sorb	Non-Sensitizer	No Induction	> 1000 µM	0.31

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

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OECD TG 442D: *In Vitro* Skin Sensitization

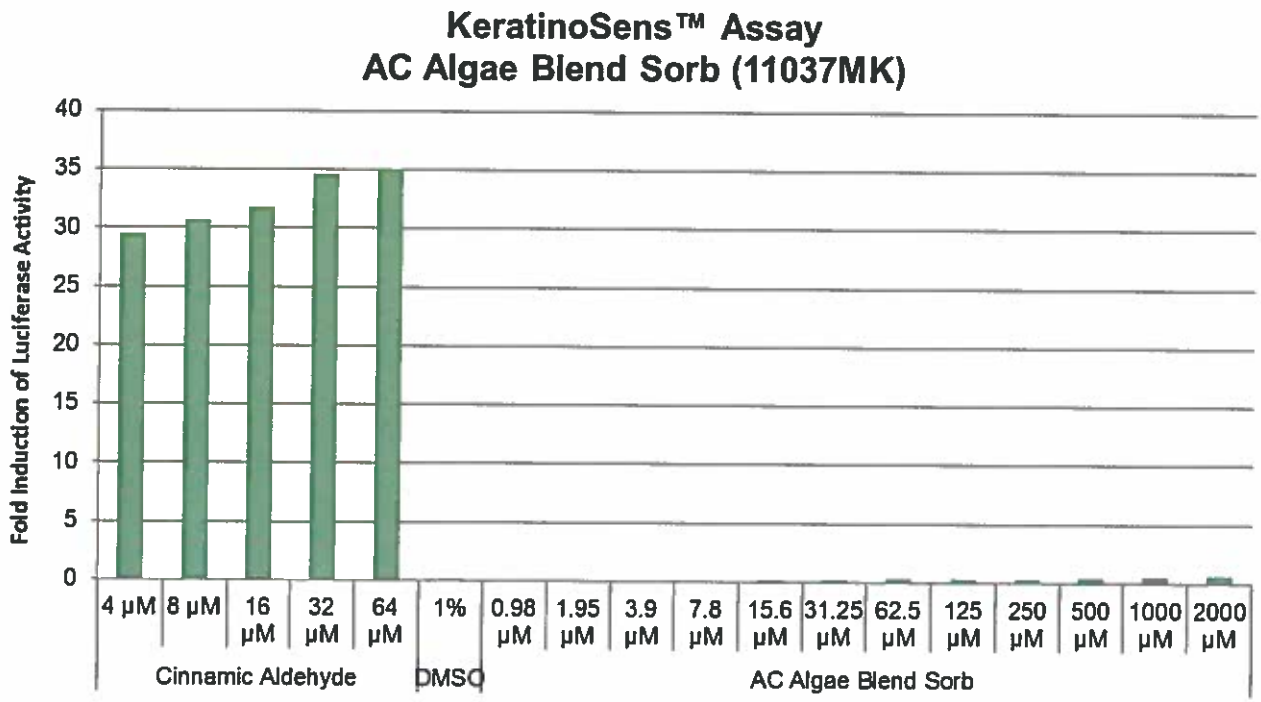


Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **AC Algae Blend Sorb (11037MK)** was not predicted to be a skin sensitizer based on the KeratinoSens™ ARE-Nrf2 Luciferase Test Method as there was not a significant increase in luciferase expression. It can be concluded that **AC Algae Blend Sorb** can be safely used in cosmetics and personal care products at typical use levels.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: October 23, 2018

SUBJECT: Macrocystis Pyrifera (Kelp) Extract

Anonymous. 2018. Summary information Macrocystis Pyrifera (Kelp) Extract (water extract).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Summary Information Macrocystis Pyrifera (Kelp) Extract

INCI name	**Macrocystis Pyrifera (Kelp) Extract**
INCI Monograph ID	8853
Use	Macrocystis Pyrifera (Kelp) Extract (water extract) is used in our products (cosmetic ingredient) at approx. 4% (two delisted products, one currently on the market).
Composition	Mainly composed of Alginate
Safety data	Studies completed on a material (Ref TEN99121) that contains approx. 4% of Macrocystis Pyrifera (Kelp) Extract: - Genotoxicity: Completed on 02/04/2001 Salmonella typhimurium Reverse Mutation Study (soluble material - 0,9% Sodium Chloride) AMES methodology employed using a 0.9% Sodium Chloride solution (1ml portion of the test article to 10 ml with 0.9% sodium chloride) with and without S-9. **The 0.9% sodium chloride test article solution was considered to be nonmutagenic to Sabnonella ryphimurium tester strains TA98, TA 100, TA 1535, TA 1537, and TA1538.** - Ocular Tolerance: Completed on 31/01/2000 HET CAM: in vitro study on Hen's egg chorion-allantoic membrane **Moderately irritant at the ocular level. May be considered as "FAIRLY WELL TOLERATED", as regards to its in vitro ocular primary tolerance.** - Primary Cutaneous Tolerance: Completed on 31/01/2000 48h occlusive single Patch test in 10 healthy adult volunteers **The index of Primary Cutaneous Irritation (P.C.I.) was equal to 0. May be considered as "VERY WELL TOLERATED" as regards to its cutaneous primary tolerance.** - Cutaneous Tolerance: Completed on 04/05/2000 Repeated Insult Patch test in 53 healthy adult volunteers **Did not indicate a potential for dermal irritation of allergic contact sensitization.**
Other safety information	Macrocystis Pyrifera (Kelp) Extract is used at approx. 4% in our products, cosmetic ingredients recommended at 10% in finished cosmetic product (≈ 0,4% Macrocystis Pyrifera (Kelp) Extract in finished cosmetics). These products are widely supplied since 2000 in the EU, the US, Canada, Korea, Japan, Australia without any complaint concerning their innocuity.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: November 5, 2018

SUBJECT: Cystoseira Amentacea/Caespitosa/Brachycarpa Extract

Anonymous. 2018. Specifications, source and safety information Cystoseira Amentacea/Caespitosa/Brachycarpa (aqueous extract).

1620 I. Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

2018

Specifications

on a control batch

- appearance	: limpid liquid brown coloured	aqueous extract
- odour	: typical	
- pH	: 5.0 ± 1.	
- density	: 1.015 ± 0.010	
- dry residue (%)	: 2.7 ± 0.4	
- solubility	: soluble in ethanol, propylene glycol, butylene glycol	
	: insoluble in oils	
- microbiology	: bacteria : < 100 germs / ml.	
	: yeasts, moulds : < 100 germs / ml.	
	: pathogens : free.	

Heavy Metal content
< 10 ppm
Arsenic 7.303 ppm
Cadmium < 0.010 ppm
Mercury < 0.010 ppm
Lead
< 0.010 ppm

Composition

Ingredients		Amounts %
Solvent	water	52
Brown alga	*Cystoseira amentacea / caespitosa / brachycarpa* extract	48
Preservative	*as required*	
Others (antioxidants ...)	none	

INCI names water CAS n° 7232-18-5 EINECS n° 231-791-2
Cystoseira amentacea / caespitosa / brachycarpa extract

Storage

should be stored in the original sealed drums, under clean conditions between 15 to 25°C. In order to avoid microbial secondary contamination, it is recommended to use the whole content of the drum once opened.

If stored under the recommended conditions, remains stable for at least 18 months.

Pack size: 1kg - 5kg -10 kg.

Safety

No animal experimentation.

Standard safety testing proves that is safe for cosmetic use.

exhibits a slightly irritant potential for ocular irritation and a non irritant potential for dermal irritation at the recommended use levels.

No direct genotoxic effect is detected (3D assay).

cf. Annex pp. 21-23.

THE BROWN ALGAE CAESPITOSE *Cystoseira*

The genus *Cystoseira* was established by C.A. Agardh in 1820. It belongs to the phylum *Heterokontophyta*, the class *Phaeophyceae*, the order Fucales and the family *Cystoseiraceae*.

The most comprehensible accounts of the morphology and biology of these brown algae are those of Sauvageau (1912 – Bull.Stn biol. Arcachon 14: 1-424), Ercegovic (1952 – Fauna Flora Adriatica 2 :1-212) and Roberts (1967- Br. Phycol. Bull. 3: 345-366).

Phycologists consider that the genus *Cystoseira* is still imperfectly known. So difficulties are inevitable in selection of suitable criteria for species speciation in field recognition (*cf.* Roberts – 1978 – In Modern Approaches to the taxonomy of red and brown algae, pp. 399-422, Academic Press).

Generally, specific separation is based on various criteria *e.g.* details of morphology, features of reproductive morphology and ecological criteria.

➤ Chemical composition and Utilizations

The chemical composition of *Cystoseira* species was studied by Pellegrini & Pellegrini (1971- Bot. mar. 14:6-16; 1972- Soc.Phycol.Fr Bull, 17:46-61).

As the other brown algae, they contain minerals, mannitol, laminaran and alginic acid. Seasonal variations in their chemical composition are present.

None industrial utilization is known for these seaweeds.

ANNEX

Evaluation of ocular irritation

STUDY SUMMARY

EVALUATION OF THE POTENTIAL IRRITANCY OF A PRODUCT THROUGH ITS APPLICATION ON THE CHORIOALLANTOIC MEMBRANE OF A CHICKEN EGGSHELL: *Het Cam Method*

- **Tested product :** water 50 % Cystoseira Amentacea / Caespitosa / Brachycarpa Extract 48 %
- **Promoter :**
- **Objective:** To assess the irritant potential of the tested product
- **Methodology:** The principle of this study is based on the visual observation, by a trained person, of the possible irritations (hyperaemia, haemorrhaging, coagulation / thrombosis) that may appear during the five minutes that follow the application of the said product on the chorioallantoic membrane of an embryonic chicken egg after eleven days of incubation.
- **Dates of study :** 12/12/2006
- **Place of study:** EUROFINS ATS, Pôle d'activité d'Aix en Provence
  Actimart, 1140, rue Ampère,
  13851 AIX EN PROVENCE cedex 3
- **Results :**

Denomination	ATS Reference	Initial concentration	Results	
			Score	Classification
SEA HEATHER	167111	100%	2.5	Slightly Irritant

- **Conclusion :**

According to the performed experimental conditions, the product SEA HEATHER tested by the HET CAM method, at 100 %, can be considered as slightly irritant regarding its ocular primary tolerance.

Evaluation of cutaneous irritation

eurofins | ATS

STUDY SUMMARY

EVALUATION OF THE CUTANEOUS TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION UNDER OCCLUSIVE PATCH DURING 48 HOURS:
Patch test method

- **Product tested :** water 50%
- **Promoter :** Cystoseira Amentacea / Caespitosa / Brachycarpa Extr
- **Monitor :** 48%
- **Objective :** Assessment of the cutaneous local tolerance of the studied product after an epicutaneous test performed in occlusive conditions, during 48 hours.
- **Place of the study:** EUROFINS SCIENTIFIC TEST CENTER,
  3 allée des Ingénieurs
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Investigator :** Doctor Mary CREST
- **Date of study:** from 28/11/06 to 30/11/06 and from 12/12/06 to 14/12/06
- **Methodology:**
  - ✓ ***Application modes:***

Area of application : on the back
Quantity of product : 0.02 ml
Frequency and duration : only one application during 48 hours
Conditions of application : product applied pure under occlusive patch.

- ✓ ***Assessment method:***

A dermatologist makes the clinical observation, after the removal of the patches. The quantification of the cutaneous irritation is given according to a numeric scale (rash, oedema, dryness, blister). The average irritant score of the product to be tested is measured with the average of the quotations obtained for the whole volunteers, allowing ranking the product from "not irritant to very irritant". The assessment is always made by comparison with the "negative" control: patch alone.

- **Population:** 11 healthy adult volunteers.
- **Results:** The average irritant score of the product is 0,0.
- **Conclusion:**

According to the experimental conditions taken into account, after only one application of 0.02 ml of product, under occlusive patch and during 48 hours, on 11 healthy adult volunteers, and according to the scale used for the interpretation of the results, the raw material **I, can be considered as not irritant regarding its primary cutaneous tolerance.**

Eurofins Scientific Test Center -Pôle d'activité d'Aix-en-Provence - Actimart - 1140, Rue Ampère - 13851 Aix-en-Provence Cedex 3 - France
TEL +33 (0)4 42 39 78.08 - FAX +33 (0)4 42 39.77.81
N° SIRET : 33761796300067 – Code APE : 743 B

Evaluation of genotoxic effects

Method

This chemiluminescent 3D Assay is an ELISA-like assay, realized by the well known company S.F.R.I.(St Jean d'Illac, France), by using plasmid DNA adsorbed on sensitized microplates as the substrate.

This method is based on a repair reaction of DNA (Salles & *al.*, 1995 – Analytical Biochemistry 232:37-42; Patent FR n° 95003230).

DNA lesions are repaired by the excision repair pathway which implies an incision-excision reaction followed by DNA repair synthesis.

In the present experiment, these lesions were performed by singlet oxygen generated by methylene blue (10 µg/ml in extrapure water).

is added according to 4 concentrations: 10 – 1 – 0.1 and 0.01%. The positive standard is MMS.

Test material: Water 50 %; Cystoseira Amentacea / Caespitosa / Brachycarpa Extract 48 %

Results

The ability of a molecule to alter DNA is measured by the reparation ratio R .

R = RLU sample at a known dilution / RLU solvent alone
RLU: Relative Light Units

When R is inferior to 2, there is no genotoxicity,

When R is superior to 2, there is a significant genotoxicity.

Results represent the mean of two independent experimentations. They are expressed comparatively to control (irradiated or no-irradiated solvent).

Product	Concentrations (%)	Ratio of genotoxicity
Test material	10	0.29
	1	0.51
	0.1	0.87
	0.01	0.84
Positive standard (MMS)	5mM	2.71
	2mM	2.02

➤ No direct genotoxicity *in vitro* with the used conditions.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: November 5, 2018

SUBJECT: Himanthalia Elongata Extract and Undaria Pinnatifida Extract

Anonymous. 2018. Specifications, source and safety information Himanthalia Elongata Extract and Undaria Pinnatifida Extract (aqueous extracts).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

2018

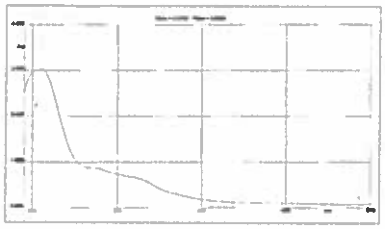
Specifications

on a control batch

Aqueous extract of Himanthalia elongata and Aqueous extract of Undaria pinnatifida

- Appearance : limpid liquid orange-amber coloured
- odour : typical
- pH : 6.0 ± 1.0
- density : 1.02 ± 0.02
- dry residual (%) : 2.9 ± 0.5

-UV spectrum (5% in water)



Heavy metals < 10ppm
Arsenic 0.510ppm
Cadmium < 0.010ppm
Mercury < 0.010pp
Lead 0.030ppm

- solubility : soluble in ethanol, propylene glycol, butylene glycol
  : insoluble in oils.
- microbiology : bacteria < 100 germs / ml.
  : yeasts, moulds < 100 germs / ml.
  : pathogens free.

INCI nomenclature

INCI names	CAS n°	EINECS n°	Amounts (%)
water	7732-18-5	231-791-2	43
Himanthalia elongata extract	223751-70-0	-	20
Undaria pinnatifida extract	-	-	37
Preservative	as required	-	-

Addition of preservative by selection: Phenoxyethanol or Microcare SB.

ECOCERT/COSMOS compliant

CHINA compliant (list 2015)

06260	水	WATER
06011	伸长海条藻（HIMANTHALIA ELONGATA）提取物	HIMANTHALIA ELONGATA EXTRACT
05477	裙带菜（UNDARIA PINNATIFIDA）提取物	UNDARIA PINNATIFIDA EXTRACT

ALGAL SOURCE

combines two extracts prepared from the brown seaweeds
-*Himanthalia elongata*
-*Undaria pinnatifida.*

Himanthalia elongata

➤ Classification

Empire	Eukaryota
Kingdom	Chromista
Phylum	Ochrophyta
Class	Phaeophyceae
Subclass	Fucophycidae
Order	Fucales
Family	*Himanthaliaceae*
Genus	*Himanthalia* Lyngbye 1819
Species	*elongata* (Linnaeus) S.F. Gray 1821

Origin of genus name from Greek "himas ": a thong and "hals" : the sea

Homotypic Synonym(s)

Fucus elongatus Linnaeus 1753

Heterotypic Synonym(s)

Spongia dichotoma Hudson 1762
Fucus loreus Linnaeus 1767
Fucus pruniformis Gunnerus 1772
Fucus tomentosus Hudson 1778
Ulva tomentosa (Hudson) De Candolle 1805
Funicularius tuberculatus Roussel 1806
Himanthalia lorea (Linnaeus) Lyngbye 1819
Himanthalia elongata var. β *inequalis* S.F. Gray 1821

Common names

- as *Himanthalia lorea* (Linnaeus) Lyngbye

  Gaeilge: Ruadhálach, Ruadhánach, Ruabhánach, Ríseach, Raif, Sreanga

- as *Himanthalia elongata* (Linnaeus) S.F.Gray

  English: Sea thong (Duddington 1966), Thongweed, Buttonweed, Sea Haricots, Sea Spaghetti) Thong Weed

  French: Spaghetti de Mer

  Gaeilge: Ríseach, Ruálach, Ruánach, Imleacán cloch, Raif

  German: Riementang

  Portuguese: Esparguete-do-mar, Cintas, Cordas, Corriolas.

➤ Chemical composition

The chemical composition of *Himanthalia elongata* has been often studied due to the fact of the food value of this alga (Lahaye, 1991- J. Sci. Food Agr. 54:587-594; Rouxel & Crouan 1995 – Acta Bot. Gall. 142: 109-118, Bobin-Dubigeon *et al.* 1997- Sciences des aliments 17:619-639; Cofrades *et al.* 2010- Food Science and Technol. Doi 10.1177/1082013210367049 ; S. COX, 2012 – Thesis Dublin; CEVA Fiche 2015).

Major data are regrouped here after (*cf.* CEVA nutritional sheet 2015)

in g/100g dehydrated

Proteins	5.0 - 23.9
Minerals	4.7 – 42.3
Dietary fibre	20.2 - 49.0
Lipids	0.8 - 8.2
Polyphenols	0.64 - 1.99 (eq. phloroglucinol)

In µg/100g dehydrated

Vitamin D	0.3
Vitamin B8	34.0
Vitamin B9	24.8 - 91.0

In mg/100g dehydrated

Potassium	3676 - 8180
Sodium	3006 - 4136
Magnesium	515 - 7077
Calcium	460 - 919
Phosphorus	74 - 129
Manganese	1.4 - 4.8
Iron	1.4 – 3.5
Copper	0.1 - 0.4
Zinc	2.5 - 8.1
Iodine	7.4 - 24.8
vitamin A	0.10 - 0.41
vitamin E	3.6 - 6.6
vitamin C	27.5 - 133.3
Vitamin B1	0.3
Vitamin B2	0.5
beta carotene	7

Himanthalia elongata is characterized by a high amount of alginic acid and laminarin in button (respectively 215.40 and 21.18 mg per gm DW).

Cellulose has been identified in cell walls and equals 8% (Cronshaw *et al.* 1958 – Biochim. et Biophys. Acta 27: 89-103).

This alga also contains sulphated polysaccharides isolated firstly by Percival & Ross, 1950 – J. Chem. Soc. 717) as fucoidan. This study revealed in the most highly purified samples the presence of half ester sulphate (38%), only 56.7% fucose beside galactose (4%), xylose (1.5%), uronic acid (3%) and metals (8%).

In addition to mannitol, *Himanthalia elongata* contains hexitol D-altritol (Chudek *et al.* 1984 – Phytochemistry 23:1081-1082). The presence of the carbohydrate altritol has been well proved by several studies [Gray *et al.* 1985- J. Exp. Mar. Biol. Ecol. 93 (1-2): 183-190; Wright *et al.* 1987- Phycologia 26 (4): 429-434; Reed *et al.* 1995- J. Mycol. 30(3):169-178). It may be involved in osmotic adjustment (Chudek *et al.*1984- Phytohemistry 23: 1081-1082].

The presence of phorotannin has been shown [Groβe- Damhues & Glombitza 1983 – Phytochemistry 22 (9): 2043-2046: Glombitza & Groβe-Damhues , 198S – Planta Med. 51:42-46]. Their amount equals 0.2% DW. They include fucols, diphloroethol, tetraphloethol and several fucophloroethols.

The fatty acids composition has been also evaluated [Herbreteau *et al.* 1997 - Bot Mar 40 (1): 25-27; Sanchez-Machado *et al.* Food Chemistry 8S:439-444]. According to the first study, the major fatty acids present are (in $mg.g^{-1}$): C16:0 (palmitic) = 1.04 ± 0.15 – C18:2 (linoleic) = 0.55 ± 0.03 – C18:1 (oleic) 0.55 ± 0.03 - C14:0 (myristic) 0.42 ± 0.02 – C18:4 (octadecatetraenoic) 0.46 ± 0.02.

Undaria pinnatifida

➤ Classification

Empire	Eukaryota
Kingdom	Chromista
Phylum	Ochrophyta
Class	Phaeophyceae
Subclass	Fucophycidae
Order	Laminariales
Family	*Alariaceae*
Genus	*Undaria*
Species	*pinnatifida* (Harvey) Suringar 1873

Basionym

Alaria pinnatifida Harvey

Origin of species name

Adjective (Latin), pinnately cleft

Homotypic Synonym(s)

Alaria pinnatifida Harvey 1860
Ulopteryx pinnatifida (Harvey) Kjellman 1885

Heterotypic Synonym(s)

Undaria pinnatifida var. *distans* Miyabe & Okamura
Undaria pinnatifida f. *distans* (Miyabe & Okamura) Yendo
Undaria pinnatifida f. *narutensis* Yendo
Undaria pinnatifida f. *typica* Yendo
Undaria pinnatifida f. *subflabellata* Suringar
Alaria amplexicaulis Martens
Undaria pinnatifida var. *vulgaris* Suringar 1872
Undaria pinnatifida var. *elongata* Suringar 1872

Common names

Chinese	Qun dai cai
English	Sea mustard, Precious sea grass, Wakame
Japanese	Wakame
Korean	Miyok, Miyeouk

Major compounds of *Undaria pinnatifida* are regrouped here after (*cf.* CEVA nutritional data 2015).

in g/100g dehydrated

Minerals	12.6 - 41.1
Proteins	8.9 - 21.4
Dietary fibers	13.6 - 71.0
Lipids	0.4 - 7.2
Polyphenols	0.08 - 0.60

In µg/100g dehydrated

Selenium	5.4 - 274.4
Vitamin K	732
Vitamin B8	14.6 - 20.1
Vitamin B9	72.2 - 503.0
Vitamin B12	0.03 - 0.5

In *mg*/100 g dehydrated

Potassium	380 - 16 919
Sodium	1463 -10 060
Magnesium	370 - 2789
Phosphorus	155 - 979
Calcium	274 - 2744
Manganese	2.4
Iron	1.4 - 131.1
Copper	0.6
Zinc	0.3 - 6.5
Iodine	2.0 - 143.7

Vitamin A	0.02 - 1.14
Vitamin E	0.2 - 2.4
Vitamin C	2.8 - 137.2
Vitamin B1	0.3
Vitamin B2	0.1 - 1.7
Vitamin B3	0.8 - 11.0
Vitamin B5	0.15 - 0.18
Vitamin B6	0.1
Beta carotene	1 - 402

Undaria pinnatifida contains alginic acid: 34-40 and fucans: 2-3 (in % DW).

Fucoidans extracted from sporophylls of this alga show a higher sulfate and L-fucose content than others fucoidans (Kim *et al.* 2010 – Food Chem. Toxicol. 48: 1101-1104).

ANNEX

Evaluation of ocular irritation

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RAPPORT D'ETUDE

Les résultats qui suivent ne s'appliquent qu'aux échantillons soumis au laboratoire et tels qu'ils sont définis dans le présent document.
Les échantillons seront conservés dans nos locaux pendant une période de 3 mois à compter de la date figurant sur ce document.
L'échantillon et les informations concernant l'échantillon ont été fournis par le client. Toutes les informations relatives à l'échantillon sont sous la responsabilité du client et n'ont pas été vérifiées par la société Eurofins ATS.

Le 8 décembre 2015

EVALUATION DU POTENTIEL IRRITANT D'UN PRODUIT PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE :
Méthode du Het Cam

Donneur d'ordre :

N° de devis : 2015 / 43137 / v2

N° d'étude : 725082

Elément d'essai :

- **Dénomination :**
- **Référence :** -
- **N° échantillon ATS :** 531186
- **Marque :** -

water 43 %
Himanthalia Elongata Extract 20 %
Undaria pinnatifida Extact 37 %
→ the tested product

SUMMARY

The HET-CAM test is a method to detect the potential irritancy of compounds applied on the surface of the chorioallantoic membrane (CAM) of a fertilized hen's egg. The CAM is a vascular foetal membrane which represents an in vitro model to analyse the effects induced by chemicals that *in vivo* are observed on the conjunctiva.

The principle of this test is based on a visual observation, by a trained person, of the possible end-points (hyperaemia, haemorrhaging, coagulation / thrombosis) that may appear during the five minutes that follow the application of the product on this membrane.

This method is registered in the Official Journal of French Republic (JORF - Decree of 5 April 1971 modified by the decree of 29 November 1996).

In the performed experimental conditions, the **product** . tested by the HET-CAM method at 10 % and according to the JORF classification, is considered as **slightly irritant**.

Evaluation of cutaneous irritation

STUDY SUMMARY

ASSESSMENT OF THE SKIN TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION UNDER OCCLUDED PATCH DURING 48H ON 10 VOLUNTEERS:
48 hours patch tests

- **Product tested:**
- **Promotor:**
- **Objective:** Assessment of the skin local tolerance of the studied product after an epicutaneous test performed in occluded conditions, during 48 hours, on healthy adult volunteers.
- **Investigator:** Audrey PERRET-COURT, M.D. Dermatologist
- **Place of the study:** EUROFINS ATS
  Pôle d'activité Aix-Les-Milles - ACTIMART
  4 allée des Informaticiens
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Dates of study:** from 27/10/2015 to 29/10/2015
- **Method:**

the tested product:
Water 43%
Himanthalia Elongata Extract 20%
Undaria Pinnatifida Extract 37%

✓ ***Application:***
Area: on the back
Quantity of product: 0.02 mL
Frequency and duration: only one application during 48 hours
Conditions of application: product applied pure under occluded patch.

✓ ***Assessment method:***
A dermatologist performs the clinical observation, after the removal of the patches. The quantification of the skin irritation is given through a numeric scale (erythema, oedema, dryness/desquamation, vesicle). The average irritant score of the product to be tested is calculated from the average of the quotations obtained for each volunteer, allowing to rank the product from "non irritant to very irritant". The assessment is always made by comparison with the "negative" control.

- **Panel:** 10 healthy adult volunteers.
- **Result:** The average irritant score of the product is 0.10.
- **Conclusion:**

According to the experimental conditions of the study, the product, referenced 1 BATCH 15-05-220, can be considered as very slightly irritant regarding its primary skin tolerance.

Personal Care Products Council
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Memorandum

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

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

DATE: November 5, 2018

SUBJECT: Halidrys Siliquosa Extract

Anonymous. 2018. Specifications, source and safety information Halidrys Siliquosa Extract (aqueous extract).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Aqueous extract of Halidrys siliquosa

Specifications

on a control batch

- appearance	: liquid limpid brown coloured with possible brown precipitates	
- odour	: typical	
- pH	: 5 ± 1	
- density	: 1.02 ± 0.02	
- dry residual (%)	: 3.6 ± 0.4	
- solubility	: soluble in ethanol, propylene glycol, butylene glycol	
	: insoluble in oils.	
- microbiology	: bacteria	: < 100 germs / ml.
	: yeasts, moulds	: < 100 germs / ml.
	: pathogens	: free.

Composition

Ingredients		Amounts %
Solvent	water	52
Brown alga	*Halidrys siliquosa* extract	48
Preservative	as required	
Others (antioxidants ...)	none	

INCI names: water CAS n° 7232-18-5 EINECS n°: 231-791-2.
Halidrys siliquosa extract.

♦ Contents in minerals

The contents in minerals have been evaluated by the testing Company EUROFINS (France).

➢ Macrominerals (ppm)

Potassium	: 2100
Sodium	: 1600
Magnesium	: 220
Calcium	: 120

➢ Trace minerals (ppm)

Iron	: 220
Silicon	: 24
Zinc	: 0.9
Manganese	: 0.35
Selenium	: < 0.1
Copper	: < 0.1
Iodine	: 13.4

➢ Heavy metals (ppm) < 10 ppm

Arsenic	: 0.01 ppm
Cadmium	: < 0.010 ppm
Mercury	: < 0.010 ppm
Lead	: < 0.010 ppm

♦ Content in polyphenols

The total content of polyphenolic compounds has been evaluated by the Folin-Ciocalteu assay. The principle of the method is based on the oxidation of phenols in the presence of phosphomobybdic and phosphotungstic acids leading to the formation of blue compounds, the absorption maximum of which being at 760 nm. The calibration series is prepared from phloroglucinol.

contains 0.16 % total polyphenols phloroglucinol equivalent.

Storage

should be stored in the original sealed drums, under clean conditions between 15 to 25°C. In order to avoid microbial secondary contamination, it is recommended to use the whole content of the drum once opened.

If stored under the recommended conditions, remains stable for at least 18 months.

Pack size: 1 Kg – 5Kg – 10 Kg.

Safety

No animal experimentation.

Standard safety testing proves that I is safe for cosmetic use at the recommended use levels:

- slightly irritant for ocular irritation (Het Cam test)
- no irritant for dermal irritation (Human Patch test)
- no mutagenic and no promutagenic (Ames test).
- hypoallergenic (RIPT test).

cf. Annex pp.42-45.

Source and Safety Information Halidrys siliquosa Extract

2018

ALGAL SOURCE

is extracted from the brown seaweed: *Halidrys siliquosa*.

➤ Classification

The species *Halidrys siliquosa* belongs to:

Empire	*Eukaryota*
Kingdom	*Chromista*
Infrakingdom	*Heterokonta*
Phylum	*Ochrophyta*
Class	*Phaeophyceae*
Order	Fucales
Family	*Sargassaceae*
Genus	*Halidrys* Lyngbye 1819
Species	*siliquosa* (Linnaeus) Lyngbye 1819.

- Synonyms

Fucus siliquosus Linnaeus, 1753
Cystoseira siliquosa (Linnaeus) C.Agardh 1820.

- Origins of the name

From Greek "*hals*" : the sea and "*drus*" : an oak
From Latin "*siliquose*"

- Common names

Sea oak — Great Britain

Rupán
Schotentang
Pod-weed
Meereiche
Crůba préacháin
Fraoch freangach
} Ireland

➤ Chemical composition

The amount of total minerals present in *Halidrys siliquosa* reaches 11.19 % (dry mass) (Vinogradov, A.P., 1953 – The elementary chemical composition of Marine organisms, Moscow, 647p).

Cell walls contains about 16-17 % of alginic acid (dry mass) (*cf.* Chapman, V.J. & Chapman , D.J. , 1980 – Seaweeds and their uses , 334 p. Chapman & Hall, London, N-Y).

The ratio mannuronic acid / guluronic acid varies from 1.1 to 0.75 (*cf.* in Stewart, W.D.P. – 1974 - Algal Physiology & Biochemistry, Blackwell Scientific Publications, Ltd.).

Are also present cellulose about 14 % (dry mass) and a water soluble polysaccharide fraction about 62 % (Cronshaw J. *et al.*, 1958 – Biochim & Biophys. Acta , 27: 89-103).

The total proteins content equals 9.6 % (dry mass), the most abundant free amino acids being aspartic acid, threonine and alanine (Citharel, J. 1971 – Thesis, Rennes).

The total lipids content reaches 3.61 % (dry mass), glycolipids being the most common. The total fatty acids content has been evaluated to 4.0 $mg.g^{-1}$ (dry mass) (Fleurence, J. *& al.,* 1994 – J. Applied Phycol. 6:527-532).

Biotin has been detected (0.21 μg/g in May) by Larsen, B. (1961 – Norsk. Inst.for tan-og taref, Report 26).

The amount of phenols varies from 5.6 to 15.4 % mg-g^{-1} (dry mass) according to the season, the period of harvest ect... (*cf.* Haug, A. & Larsen, B. 1958 – Norsk. Inst.for tan-og taref, Report 22, 18p); Glombitza, K.W. & Sattler, E. 1973 – Tetrahedron Letters. 43: 4277-4280).

Free phloroglucinol has been detected (Glombitza, K.W. & *al.*, 1973 – Planta Med. 24: 301-303).

Numerous oligomers of phloroglucinol can be identified as phlorotannins : fucols (difucol) and fuhalols (bi-, tri-, tetra-, penta- and heptafuhalols) (*cf.* Glombitza ,K.W., 1979 in Marine Algae in Pharmaceutical Science, eds H.A. Hoppe & *al.*, : 303-342 , Walter de Gruyter, Berlin, N.Y.).

The polyphenolics compounds of brown algae differ from the tannins derived from terrestrial plants by their biosynthesis pathway.

Other secondary metabolites have been isolated too *e.g.* meroditerpenoids (tetraprenyltoluquinol-related metabolites) and several hydroquinols (Culioli G. & *al.*, 2008 – J. Natural Products, 71(7) : 1121-1126); Higgs , M.D. & Mulheiron, L.J., 1981 – Tetrahedron, 37 (18) : 3209-3213).

ANNEX

Evaluation of ocular irritation

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RAPPORT D'ETUDE

Le 18/12/2008

EVALUATION DU POTENTIEL IRRITANT D'UN PRODUIT PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE :
Méthode du Het Cam

SUMMARY

The Het Cam test is performed on the chorioallantoic membrane of an embryonic chicken egg after eleven days of incubation. This membrane has the characteristic to be vascularized.

The principle of this study is based on the visual observation, by a trained person, of the possible irritations (hyperaemia, haemorrhaging, coagulation / thrombosis) that may appear during the five minutes that follow the application of the said product on this chorioallantoic membrane.

This study has been carried out following the official method of the Official Journal of French Republic, according to the order of april 5th, 1971, modified in the last by the order of november 29th, 1996.

Haldrys Siliquosa Extract without preservativ

According to the performed experimental conditions, **the product** , tested by the HET CAM method, at 5 %, can be considered as **slightly irritant** regarding its ocular primary tolerance.

Eurofins ATS -Pôle d'activité d'Aix-Les Milles - Actimart - 1140, Rue Ampere - 13851 Aix-en-Provence Cedex 3 - France
TEL +33 (0)4 42.39 78 08 - FAX +33 (0)4 42.39.77 81
N° SIRET : 33761796300083 – Code APE : 7120 B

Evaluation of cutaneous irritation

eurofins | ATS

N° Etude : 301720F01
Version : N° 1
Page : 13/15 + annexe 2
P05.0 DOC.00017.02

STUDY SUMMARY

EVALUATION OF SKIN TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION UNDER OCCLUDED PATCH DURING 48H ON 13 VOLUNTEERS:
48 hours occluded patch tests

Halidrys Siliquosa Extract without preservatives

- **Product tested:**
- **Promotor:**
- **Objective:** Assessment of the cutaneous local tolerance of the studied product after an epicutaneous test performed in occluded conditions, during 48 hours.
- **Place of the study:** EUROFINS ATS
  Pôle d'activité Aix-Les-Milles - ACTIMART
  3 allée des Ingénieurs
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Investigator:** Doctor Mary Crest
- **Dates of study:** from 02/12/08 to 04/12/08
- **Method:**

✓ ***Application:***

Area: on the back
Quantity of product: 0.02 ml
Frequency and duration: only one application during 48 hours
Conditions of application: product applied diluted at 5% under occluded patch.

✓ ***Assessment method:***

A dermatologist performs the clinical observation, after the removal of the patches. The quantification of the cutaneous irritation is given through a numeric scale (erythema, oedema, dryness/desquamation, vesicles). The average irritant score of the product to be tested is calculated from the average of the quotations obtained for each volunteer, allowing to rank the product from "non irritant to very Irritant". The assessment is always made by comparison with the "negative" control.

- **Panel:** 13 healthy adult volunteers.
- **Result:** The average irritant score of the product is 0,00.
- **Conclusion:**

According to the experimental conditions of the study, ' ' can be considered as **not Irritant regarding its primary cutaneous tolerance.**

Evaluation of mutagenicity

vivotecnia

Final Report B-00746
Rapport final B-00746

BACTERIAL REVERSE MUTATION TEST

B-00746 FINAL REPORT Halidrys siliquosa Extract

The present bacterial reverse mutation test (Ames test) was performed in order to evaluate the mutagenic potential of the test item.

The test was performed in accordance with OECD Guideline 471 for the Testing of Chemicals (Bacterial Reverse Mutation Test. Adopted 21st July 1997) and the test Method B13/B14 of Commission Directive 2000/32/EC.

Doses ranging from 5µL to 0,06µL per plate were tested. No cytotoxicity was observed at any dose.

Suspensions of 5 amino-acid requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537) were exposed by the direct plate incorporation method to five doses of the test item in the presence and in the absence of an exogenous metabolic activation system. Both tests were repeated with the pre-incubation method.

Revertant bacteria due to point or frameshift-mutations at specific locus are able to grow, forming colonies. These colonies were counted and compared to the number of spontaneous revertant colonies on solvent control plate (negative control). Similarly, specific standard mutagens were tested and used as positive controls.

Based on the results obtained in this study, the test item was found to be NON MUTAGENIC and NON-PROMUTAGENIC under the test conditions.

Test facility
VIVOTECNIA Research S.L.
Parque Cientifico de Madrid
C/Santiago Grisolia, 2
28760 Tres Cantos (Madrid)
Spain

Evaluation of sensitizing potential

ROBEN PRODUCTION GRUP SRL

CENTRUL DE CERCETARE A PLANTELOR
STRADA LUGOJ NR. 63 SECTOR 1, BUCURESTI, ROMANIA

EVALUATION DU POUVOIR SENSIBILISANT CHEZ LE VOLONTAIRE ADULTE SELON LA METHODE DE MARZULLI-MAIBACH

ASSESSMENT OF SENSITIZING POTENTIAL IN THE ADULT VOLUNTEER FOLLOWING THE METHOD OF MARZULLI-MAIBACH

Etude clinique sur 107 volontaires, tout type de peau
Clinical study on 107 volunteers, with all skin type

- Etude/ *Study*: 3.04
- Produit/ *Product*: RB11/0006

PRODUIT / *Product*	: Halidrys Siliquosa Extract without preservatives
CODE PRODUIT / *Code product*	
DILUTION / *Dilution*	: PUR PURE
INVESTIGATEUR / *Investigator*	: DR. ANNE-MARIE MARINESCU

CONCLUSIONS/ *CONCLUSIONS*

Dans les conditions d'une application répétée de la procédure de patch-test conduite auprès d'un panel de 107 volontaires présentant tout type de peau, le produit [ ] **RB11/0006** a été «Testé dermatologiquement» et n'a pas présenté de risque d'irritation de la peau cliniquement significative ni montrer de réaction de type allergique au contact de la peau humaine.

Under the conditions of a repeated insult (occlusive) patch test procedure conducted in a panel of 105 subjects, with all skin type, the product [ ] was "Dermatologist-Tested" and did not induce clinically significant skin irritation nor show any evidence of induced allergic contact dermatitis in human subjects.

Le produit [ ] peut être considéré comme «hypoallergénique».

The product [ ] can be considered as "hypoallergenic".

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: September 18, 2018

SUBJECT: Draft Report: Safety Assessment of Brown Algae-Derived Ingredients as Used in Cosmetics (draft prepared for the September 24-25, 2018 CIR Expert Panel meeting)

The Council respectfully submits the following comments on the draft report, Safety Assessment of Brown Algae-Derived Ingredients as Used in Cosmetics.

Key Issues

Unless there is an additional ingredient in this report that is not in Table 1, there are 82 ingredients in this report, not 83 as stated in the Introduction and Summary.

As this report is about brown algae-derived ingredients, it is not necessary to describe the taxonomy for all types of algae. As some types of algae, such as brown algae are in a separate class (brown algae are in the phylum Ochrophyta which includes many other classes including the class Bacillariophyceae, the diatoms), the Introduction is not correct when it says "The different algal phyla...". Table 3 is also not correct. Some of the names in the Class column, such as Chlorophyta, Cyanophyta and Rhodophyta are actually phylum names not class names.

Impurities, Phthalates - The statement that DBP and DEHP were found in "*Laminaria japonica* at concentrations of 60 and 70%" is misleading. This study was trying to determine whether or not the phthalate compounds were naturally produced by measuring how much ^{14}C was incorporated into the phthalate compounds. The abstract states: "The natural abundance ^{14}C content of di-(2-ethylhexyl) phthalate (DEHP) obtained from the same algae was about 50–80% of the standard sample and the ^{14}C content of the petrochemical (industrial) products of DBP and DEHP were below the detection limit."

Toxicological Studies - In each section, it would be helpful if the information on one species would be presented together.

Rather than a separate subsection, the studies on hydrolyzed fucoidan should be presented in the appropriate endpoint section.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Additional Considerations

Composition - As some information has now been provided by cosmetic ingredient suppliers, it is not correct to state that: "There have been no data found or submitted on the composition of any of the ingredients in this report as used in cosmetics specifically."

Please clarify the composition of Undaria Pinnatifida Extract. What do the percentages for monosaccharides represent, the percent of total composition, or the percent of total monosaccharides?

Impurities - Heavy Metals should be a subheading (needs to be underlined and bolded, followed by a hard return)

Short-Term, Subchronic, Chronic, Oral, Summary - Was there a dose of Ecklonia Cava Extract given to female rats that did not cause any effects (reference 9)?

What dose/drinking water concentration of Undaria Pinnatifida Extract was given to rats for 32 weeks?

Other Relevant Studies, Estrogen Effects and Progesterone Receptor Binding - As the aromatase activity was also studied, the subheading should be changed to Endocrine Effects (which would be consistent with the outline of CIR reports at https://www.cir-safety.org/sites/default/files/CIR%20Report%20Format%20Outline.pdf).

Please correct: "7-methoxy-4-rifluoromethyl..."

Photoprotection - Please state the type of *Sargassum muticum* extract used (the title says ethyl acetate fraction).

Use Studies - The use study of the eye cream should be moved to the ocular irritation section.

Table 5 - Why are some species not included in Table 5? e.g., *Cladosiphon novae-caledoniae* and *Cladosiphon okamuranus*.

Reference 2 - As there have been recent changes in the taxonomy of brown algae, reference 2, published in 1985 is not an appropriate reference for the "systematics" of brown algae. This reference is used in the Introduction to indicate that kelp are in the order Laminariales. The Algae data base would be a more up to date reference.