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eurofins | Microbiology

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Wild Type, Inc.
[redacted]

ANALYTICAL REPORT

AR-24-QR-000801-02
Report Supersedes AR-24-QR-000801-01

Client Code: QR0000417
PO#: FDA RFI - Saku test 3- Nov 2023 (cells 11/16/23)
Received On: 12Dec2023
Reported On: 17Jan2024

Eurofins Sample Code:	111-2023-12120074	**Sample Registration Date:**	12Dec2023
Client Sample Code:	SAK-2023-12-08-01	**Condition Upon Receipt:**	acceptable, -25.1°C
Sample Description:	SAK-2023-12-08 made with cells harvested on 2023-11-16	**Sample Reference:**	Cells harvested on 2023-11-16

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	**Reference** AOAC 2011.19, 993.14 and 2015.01 (modified)	**Accreditation**	**Completed** 20Dec2023	**Sub** 5

Parameter	**Result**
Arsenic	<0.0100 ppm
Cadmium	<0.00500 ppm
Lead	<0.00500 ppm
Mercury	<0.00500 ppm

QD038 - Carbohydrates, Calculated	**Reference** CFR 21-calc.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Carbohydrates, Calculated	4.17 %

QD059 - Fat by Acid Hydrolysis	**Reference** AOAC 954.02	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Crude Fat By Acid Hydrolysis	14.01 %

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	**Reference** AOAC 996.06 mod.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Fatty Acid Profile	Reported as Fatty Acids
C4:0 (Butyric Acid)	<0.02 %
C6:0 (Caproic acid)	<0.02 %
C8:0 (Caprylic acid)	<0.02 %
C10:0 (Capric acid)	<0.02 %
C11:0 (Undecanoic acid)	<0.02 %

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Received On: 12Dec2023
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Client Sample Code:	SAK-2023-12-08-01	**Condition Upon Receipt:**	acceptable, -25.1°C
Sample Description:	SAK-2023-12-08 made with cells harvested on 2023-11-16	**Sample Reference:**	Cells harvested on 2023-11-16

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		28Dec2023	2

Parameter	Result
C12:0 (Lauric Acid)	<0.02 %
C14:0 (Myristic acid)	0.05 %
C14:1 (Myristoleic acid)	<0.02 %
C15:0 (Pentadecanoic acid)	<0.02 %
C15:1 (Pentadecenoic acid)	<0.02 %
C16:0 (Palmitic Acid)	0.95 %
C16:1 Omega 7	<0.04 %
C16:1 Total (Palmitoleic Acid + isomers)	<0.04 %
C16:2 (Hexadecadienoic Acid)	<0.02 %
C16:3 (Hexadecatrienoic Acid)	<0.02 %
C 16:4 (Hexadecatetraenoic Acid)	<0.02 %
C17:0 (Margaric Acid)	<0.02 %
C17:1 (Heptadecenoic Acid)	<0.02 %
C18:0 (Stearic Acid)	0.35 %
C18:1 (Vaccenic acid)	0.17 %
C18:1 Omega 9 (Oleic Acid)	7.13 %
C18:1, Total (Oleic Acid + isomers)	7.32 %
C18:2 Omega 6 (Linoleic Acid)	1.40 %
C18:2, Total (Linoleic Acid + isomers)	1.42 %
C18:3 Omega 3 (Alpha Linolenic Acid)	0.42 %
C18:3 Omega 6 (Gamma Linolenic Acid)	<0.02 %
C18:3, Total (Linolenic Acid + isomers)	0.42 %
C18:4 Omega 3 (Octadecatetraenoic Acid)	<0.02 %
C18:4 Total (Octadecatetraenoic Acid)	<0.02 %
C20:0 (Arachidic Acid)	0.05 %
C20:1 Omega 9 (Gondoic Acid)	0.06 %
C20:1 Total (Gondoic Acid + isomers)	0.08 %
C20:2 Omega 6	0.03 %
C20:2 Total (Eicosadienoic Acid)	0.03 %
C20:3 Omega 3	<0.02 %
C20:3 Omega 6	<0.02 %
C20:3, Total (Eicosatrienoic Acid)	<0.02 %
C20:4 Omega 3	<0.02 %
C20:4 Omega 6 (Arachidonic Acid)	0.03 %

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Received On: 12Dec2023
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Eurofins Sample Code:	111-2023-12120074	**Sample Registration Date:**	12Dec2023
Client Sample Code:	SAK-2023-12-08-01	**Condition Upon Receipt:**	acceptable, -25.1°C
Sample Description:	SAK-2023-12-08 made with cells harvested on 2023-11-16	**Sample Reference:**	Cells harvested on 2023-11-16

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		28Dec2023	2

Parameter	Result
C20:4, Total (Eicosatetraenoic Acid)	0.05 %
C20:5 Omega 3 (Eicosapentaenoic Acid)	0.43 %
C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02 %
C22:0 (Behenic Acid)	0.08 %
C22:1 Omega 9 (Erucic Acid)	<0.02 %
C22:1 Total (Erucic Acid + isomers)	<0.02 %
C22:2 Docosadienoic Omega 6	<0.02 %
C22:3 Docosatrienoic, Omega 3	<0.02 %
C22:4 Docosatetraenoic Omega 6	<0.02 %
C22:5 Docosapentaenoic Omega 3	0.08 %
C22:5 Docosapentaenoic Omega 6	0.05 %
C22:5 Total (Docosapentaenoic Acid)	0.13 %
C22:6 Docosahexaenoic Omega 3	0.90 %
C24:0 (Lignoceric Acid)	0.03 %
C24:1 Omega 9 (Nervonic Acid)	<0.02 %
C24:1 Total (Nervonic Acid + isomers)	<0.02 %
Total Omega 3 Isomers	1.84 %
Total Omega 5 Isomers	<0.05 %
Total Omega 6 Isomers	1.52 %
Total Omega 7 Isomers	0.19 %
Total Omega 9 Isomers	7.20 %
Total Monounsaturated Fatty Acids	7.43 %
Total Polyunsaturated Fatty Acids	3.38 %
Total Saturated Fatty Acids	1.54 %
Total Trans Fatty Acids	0.02 %
Total Fat as Triglycerides	12.92 %
Total Fatty Acids	12.37 %

QD06X - Clostridium Botulinum Toxin - Presumptive	Reference	Accreditation	Completed	Sub
	FDA-BAM, 8th ed.		11Jan2024	3

Parameter	Result
Clostridium Botulinum Toxin	Negative per 50 g

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ANALYTICAL REPORT

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Received On: 12Dec2023
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Eurofins Sample Code:	111-2023-12120074	**Sample Registration Date:**	12Dec2023
Client Sample Code:	SAK-2023-12-08-01	**Condition Upon Receipt:**	acceptable, -25.1°C
Sample Description:	SAK-2023-12-08 made with cells harvested on 2023-11-16	**Sample Reference:**	Cells harvested on 2023-11-16

QD0EK - Vitamin D (LC-MS/MS)	**Reference**	**Accreditation**	**Completed**	**Sub**
	Huang et al., Rapid Commun. Mass Spectrum 2014, 28		28Dec2023	2

Parameter	**Result**
Total Vitamin D2 and D3	<4 IU/100 g
Vitamin D2	<4 IU/100 g
Vitamin D3	<4 IU/100 g

QD148 - Moisture by Vacuum Oven	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 925.09		28Dec2023	2

Parameter	**Result**
Moisture and Volatiles - Vacuum Oven	78.6 %

QD226 - Calories, Calculated	**Reference**	**Accreditation**	**Completed**	**Sub**
	CFR - Atwater calculation		28Dec2023	2

Parameter	**Result**
Calories Calculated	150 kcal/100 g

QD250 - Ash	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 942.05		28Dec2023	2

Parameter	**Result**
Ash	<0.40 %

QD252 - Protein - Combustion	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 990.03; AOAC 992.15		28Dec2023	2

Parameter	**Result**
Protein	4.31 %
Nitrogen - Combustion	0.69 %
Protein Factor	6.25

QD493 - Clostridium Botulinum Viable Cells - Presumptive	**Reference**	**Accreditation**	**Completed**	**Sub**
	FDA-BAM, 8th ed.		11Jan2024	3

Parameter	**Result**
Clostridium botulinum (without toxin detection)	Negative per 8 g

QQ059 - Total Vitamin B9-Folate(Low Level <12.5 mg/100g)mg	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 992.05 mod.		28Dec2023	2

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Client Sample Code:	SAK-2023-12-08-01	**Condition Upon Receipt:**	acceptable, -25.1°C
Sample Description:	SAK-2023-12-08 made with cells harvested on 2023-11-16	**Sample Reference:**	Cells harvested on 2023-11-16

QQ059 - Total Vitamin B9-Folate(Low Level <12.5 mg/100g)mg	**Reference** AOAC 992.05 mod.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Total Folate as Folic Acid	0.00945 mg/100 g

QQ151 - Total Vitamin B12-Cobalamin(Low Level <3 mg/100g)	**Reference** AOAC 952.20 mod.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Vitamin B12	68.7 µg/100 g

QQ156 - Total Vitamin B5-Pan Acid(Low Level <100 mg/100g)	**Reference** AOAC 945.74 (mod.)	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Pantothenic acid	0.0768 mg/100 g

QQ182 - Total Vitamin A	**Reference** AOAC 974.29 Mod.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
ß-carotene	404 IU/100 g
Retinol	<30 IU/100 g
Total Vitamin A	404 IU/100 g

UM4BV - Yeast - FDA BAM Chapter 18 mod.	**Reference** FDA BAM Chapter 18 mod.	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 17Dec2023

Parameter	**Result**
Yeast	< 10 cfu/g
Mold	< 10 cfu/g

UM6NM - Campylobacter Species - AOAC RI #040702	**Reference** AOAC-PTM 040702	**Accreditation**	**Completed** 19Dec2023	**Sub** 4

Parameter	**Result**
Campylobacter Species	Not Detected per 25 g

UM8VD - Total Coliforms - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 13Dec2023

Parameter	**Result**

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Received On: 12Dec2023
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Eurofins Sample Code:	111-2023-12120074	**Sample Registration Date:**	12Dec2023
Client Sample Code:	SAK-2023-12-08-01	**Condition Upon Receipt:**	acceptable, -25.1°C
Sample Description:	SAK-2023-12-08 made with cells harvested on 2023-11-16	**Sample Reference:**	Cells harvested on 2023-11-16

UM8VD - Total Coliforms - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 13Dec2023

Parameter	**Result**
Total Coliforms	< 10 cfu/g
E. coli	< 10 cfu/g

UMEWE - Escherichia Coli O157:H7 - AOAC-RI 031002	**Reference** AOAC-RI 031002	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 13Dec2023

Parameter	**Result**
Escherichia coli O157:H7	Not Detected per 25 g

UMHBM - Staphylococcus aureus - BAM Chapter 12	**Reference** BAM Chapter 12	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023

Parameter	**Result**
Staphylococcus aureus	< 10 cfu/g

UMJN3 - Non-O157 Shiga toxin-Producing E.coli - AOAC-RI 091301	**Reference** AOAC-RI 091301		**Completed** 13Dec2023

Parameter	**Result**
Non-O157 Shiga toxin-Producing E.coli	Not Detected per 25 g

UMKTF - Enterobacteriaceae - CMMEF Chapter 9.62	**Reference** CMMEF Chapter 9.62	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 13Dec2023

Parameter	**Result**
Enterobacteriaceae	< 10 cfu/g

UMKXG - Staphylococcal Enterotoxin - AOAC 2007.06	**Reference** AOAC 2007.06	**Accreditation**	**Completed** 21Dec2023	**Sub** 1

Parameter	**Result**
Staphylococcal Enterotoxin	Not Detected per 25 g

UMMA7 - Bacillus cereus - BAM Chapter 14	**Reference** FDA BAM Chapter 14	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023

Parameter	**Result**

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Received On: 12Dec2023
Reported On: 17Jan2024

Eurofins Sample Code:	111-2023-12120074	**Sample Registration Date:**	12Dec2023
Client Sample Code:	SAK-2023-12-08-01	**Condition Upon Receipt:**	acceptable, -25.1°C
Sample Description:	SAK-2023-12-08 made with cells harvested on 2023-11-16	**Sample Reference:**	Cells harvested on 2023-11-16

UMMA7 - Bacillus cereus - BAM Chapter 14	**Reference** FDA BAM Chapter 14	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023

Parameter	**Result**
Bacillus cereus	< 10 cfu/g

UMQE5 - Listeria monocytogenes - AOAC-RI 061703	**Reference** AOAC-RI 061703	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 13Dec2023

Parameter	**Result**
Listeria monocytogenes	Not Detected per 25 g

UMQMM - Salmonella species - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 13Dec2023

Parameter	**Result**
Salmonella spp.	Not Detected per 25 g

UMVEP - Aerobic Plate Count - AOAC 966.23	**Reference** AOAC 966.23	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023

Parameter	**Result**
Aerobic Plate Count	< 10 cfu/g

ZM3KF - Clostridium perfringens - ISO 7937	**Reference** ISO 7937	**Completed** 14Dec2023

Parameter	**Result**
Clostridium perfringens	< 10 cfu/g

Report Comment:
Report ammended to include missing method reference for Presumptive C. botulinum toxin and Viable C. botulinum

Subcontracting partners:

1 - Eurofins Microbiology Laboratories (Des Moines), IA
2 - Eurofins Nutrition Analysis Center, Iowa
3 - Silliker, INC Food Science Center, IL
4 - Eurofins Microbiology Laboratories (Lancaster), Pennsylvania
5 - Eurofins Food Chemistry Testing US Madison, Wisconsin

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ANALYTICAL REPORT

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Report Supersedes AR-24-QR-000801-01

Client Code: QR0000417
PO#: FDA RFI - Saku test 3- Nov 2023 (cells 11/16/23)
Received On: 12Dec2023
Reported On: 17Jan2024

Respectfully Submitted,

[redacted]

Viridiana Castro
Business Unit Manager

ACCREDITED

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Eurofins Microbiology Laboratories (Los Angeles)

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Wild Type, Inc.

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ANALYTICAL REPORT

AR-24-QR-001286-02
Report Supersedes AR-24-QR-001286-01

Client Code: QR0000417
PO#: FDA RFI - Saku test 4- Nov 2023 (cells 11/09/23)
Received On: 13Dec2023
Reported On: 17Jan2024

Eurofins Sample Code:	111-2023-12130142	**Sample Registration Date:**	13Dec2023
Client Sample Code:	SAK-2023-12-12	**Condition Upon Receipt:**	acceptable, -50.3°C
Sample Description:	SAK-2023-12-12 made with cells harvested on 2023-11-09	**Sample Reference:**	Cells harvested on 2023-11-09

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	**Reference** AOAC 2011.19, 993.14 and 2015.01 (modified)	**Accreditation**	**Completed** 20Dec2023	**Sub** 5

Parameter	**Result**
Arsenic	<0.0100 ppm
Cadmium	<0.00500 ppm
Lead	<0.00500 ppm
Mercury	<0.00500 ppm

QD038 - Carbohydrates, Calculated	**Reference** CFR 21-calc.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Carbohydrates, Calculated	4.05 %

QD059 - Fat by Acid Hydrolysis	**Reference** AOAC 954.02	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Crude Fat By Acid Hydrolysis	14.11 %

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	**Reference** AOAC 996.06 mod.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Fatty Acid Profile	Reported as Fatty Acids
C4:0 (Butyric Acid)	<0.02 %
C6:0 (Caproic acid)	<0.02 %
C8:0 (Caprylic acid)	<0.02 %
C10:0 (Capric acid)	<0.02 %
C11:0 (Undecanoic acid)	<0.02 %

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ANALYTICAL REPORT

AR-24-QR-001286-02
Report Supersedes AR-24-QR-001286-01

Client Code: QR0000417
PO#: FDA RFI - Saku test 4- Nov 2023 (cells 11/09/23)
Received On: 13Dec2023
Reported On: 17Jan2024

Eurofins Sample Code:	111-2023-12130142	**Sample Registration Date:**	13Dec2023
Client Sample Code:	SAK-2023-12-12	**Condition Upon Receipt:**	acceptable, -50.3°C
Sample Description:	SAK-2023-12-12 made with cells harvested on 2023-11-09	**Sample Reference:**	Cells harvested on 2023-11-09

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		28Dec2023	2

Parameter	Result
C12:0 (Lauric Acid)	<0.02 %
C14:0 (Myristic acid)	0.05 %
C14:1 (Myristoleic acid)	<0.02 %
C15:0 (Pentadecanoic acid)	<0.02 %
C15:1 (Pentadecenoic acid)	<0.02 %
C16:0 (Palmitic Acid)	1.03 %
C16:1 Omega 7	<0.04 %
C16:1 Total (Palmitoleic Acid + isomers)	<0.04 %
C16:2 (Hexadecadienoic Acid)	<0.02 %
C16:3 (Hexadecatrienoic Acid)	<0.02 %
C 16:4 (Hexadecatetraenoic Acid)	<0.02 %
C17:0 (Margaric Acid)	<0.02 %
C17:1 (Heptadecenoic Acid)	<0.02 %
C18:0 (Stearic Acid)	0.37 %
C18:1 (Vaccenic acid)	0.20 %
C18:1 Omega 9 (Oleic Acid)	7.60 %
C18:1, Total (Oleic Acid + isomers)	7.83 %
C18:2 Omega 6 (Linoleic Acid)	1.54 %
C18:2, Total (Linoleic Acid + isomers)	1.57 %
C18:3 Omega 3 (Alpha Linolenic Acid)	0.49 %
C18:3 Omega 6 (Gamma Linolenic Acid)	<0.02 %
C18:3, Total (Linolenic Acid + isomers)	0.49 %
C18:4 Omega 3 (Octadecatetraenoic Acid)	<0.02 %
C18:4 Total (Octadecatetraenoic Acid)	<0.02 %
C20:0 (Arachidic Acid)	0.06 %
C20:1 Omega 9 (Gondoic Acid)	0.07 %
C20:1 Total (Gondoic Acid + isomers)	0.09 %
C20:2 Omega 6	0.04 %
C20:2 Total (Eicosadienoic Acid)	0.04 %
C20:3 Omega 3	<0.02 %
C20:3 Omega 6	<0.02 %
C20:3, Total (Eicosatrienoic Acid)	<0.02 %
C20:4 Omega 3	<0.02 %
C20:4 Omega 6 (Arachidonic Acid)	0.04 %

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Client Code: QR0000417
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Received On: 13Dec2023
Reported On: 17Jan2024

Eurofins Sample Code:	111-2023-12130142	**Sample Registration Date:**	13Dec2023
Client Sample Code:	SAK-2023-12-12	**Condition Upon Receipt:**	acceptable, -50.3°C
Sample Description:	SAK-2023-12-12 made with cells harvested on 2023-11-09	**Sample Reference:**	Cells harvested on 2023-11-09

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		28Dec2023	2

Parameter	Result
C20:4, Total (Eicosatetraenoic Acid)	0.05 %
C20:5 Omega 3 (Eicosapentaenoic Acid)	0.46 %
C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02 %
C22:0 (Behenic Acid)	0.08 %
C22:1 Omega 9 (Erucic Acid)	<0.02 %
C22:1 Total (Erucic Acid + isomers)	<0.02 %
C22:2 Docosadienoic Omega 6	<0.02 %
C22:3 Docosatrienoic, Omega 3	<0.02 %
C22:4 Docosatetraenoic Omega 6	<0.02 %
C22:5 Docosapentaenoic Omega 3	0.09 %
C22:5 Docosapentaenoic Omega 6	0.04 %
C22:5 Total (Docosapentaenoic Acid)	0.13 %
C22:6 Docosahexaenoic Omega 3	0.95 %
C24:0 (Lignoceric Acid)	0.03 %
C24:1 Omega 9 (Nervonic Acid)	<0.02 %
C24:1 Total (Nervonic Acid + isomers)	<0.02 %
Total Omega 3 Isomers	2.01 %
Total Omega 5 Isomers	<0.05 %
Total Omega 6 Isomers	1.67 %
Total Omega 7 Isomers	0.23 %
Total Omega 9 Isomers	7.69 %
Total Monounsaturated Fatty Acids	7.97 %
Total Polyunsaturated Fatty Acids	3.70 %
Total Saturated Fatty Acids	1.67 %
Total Trans Fatty Acids	0.03 %
Total Fat as Triglycerides	13.97 %
Total Fatty Acids	13.37 %

QD06X - Clostridium Botulinum Toxin - Presumptive	Reference	Accreditation	Completed	Sub
	FDA-BAM, 8th ed.		16Jan2024	3

Parameter	Result
Clostridium Botulinum Toxin	Negative per 50 g

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ANALYTICAL REPORT

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Client Code: QR0000417
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Received On: 13Dec2023
Reported On: 17Jan2024

Eurofins Sample Code:	111-2023-12130142	**Sample Registration Date:**	13Dec2023
Client Sample Code:	SAK-2023-12-12	**Condition Upon Receipt:**	acceptable, -50.3°C
Sample Description:	SAK-2023-12-12 made with cells harvested on 2023-11-09	**Sample Reference:**	Cells harvested on 2023-11-09

QD0EK - Vitamin D (LC-MS/MS)	**Reference**	**Accreditation**	**Completed**	**Sub**
	Huang et al., Rapid Commun. Mass Spectrum 2014, 28		28Dec2023	2

Parameter	**Result**
Total Vitamin D2 and D3	<4 IU/100 g
Vitamin D2	<4 IU/100 g
Vitamin D3	<4 IU/100 g

QD148 - Moisture by Vacuum Oven	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 925.09		28Dec2023	2

Parameter	**Result**
Moisture and Volatiles - Vacuum Oven	77.2 %

QD226 - Calories, Calculated	**Reference**	**Accreditation**	**Completed**	**Sub**
	CFR - Atwater calculation		28Dec2023	2

Parameter	**Result**
Calories Calculated	159 kcal/100 g

QD250 - Ash	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 942.05		28Dec2023	2

Parameter	**Result**
Ash	0.40 %

QD252 - Protein - Combustion	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 990.03; AOAC 992.15		28Dec2023	2

Parameter	**Result**
Protein	4.38 %
Nitrogen - Combustion	0.70 %
Protein Factor	6.25

QD493 - Clostridium Botulinum Viable Cells - Presumptive	**Reference**	**Accreditation**	**Completed**	**Sub**
	FDA-BAM, 8th ed.		16Jan2024	3

Parameter	**Result**
Clostridium botulinum (without toxin detection)	Negative per 8 g

QQ059 - Total Vitamin B9-Folate(Low Level <12.5 mg/100g)mg	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 992.05 mod.		28Dec2023	2

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ANALYTICAL REPORT

AR-24-QR-001286-02
Report Supersedes AR-24-QR-001286-01

Client Code: QR0000417
PO#: FDA RFI - Saku test 4- Nov 2023 (cells 11/09/23)
Received On: 13Dec2023
Reported On: 17Jan2024

Eurofins Sample Code:	111-2023-12130142	**Sample Registration Date:**	13Dec2023
Client Sample Code:	SAK-2023-12-12	**Condition Upon Receipt:**	acceptable, -50.3°C
Sample Description:	SAK-2023-12-12 made with cells harvested on 2023-11-09	**Sample Reference:**	Cells harvested on 2023-11-09

QQ059 - Total Vitamin B9-Folate(Low Level <12.5 mg/100g)mg	**Reference** AOAC 992.05 mod.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Total Folate as Folic Acid	0.00939 mg/100 g

QQ151 - Total Vitamin B12-Cobalamin(Low Level <3 mg/100g)	**Reference** AOAC 952.20 mod.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Vitamin B12	167 µg/100 g

QQ156 - Total Vitamin B5-Pan Acid(Low Level <100 mg/100g)	**Reference** AOAC 945.74 (mod.)	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
Pantothenic acid	0.0895 mg/100 g

QQ182 - Total Vitamin A	**Reference** AOAC 974.29 Mod.	**Accreditation**	**Completed** 28Dec2023	**Sub** 2

Parameter	**Result**
ß-carotene	419 IU/100 g
Retinol	<30 IU/100 g
Total Vitamin A	419 IU/100 g

UM4BV - Yeast - FDA BAM Chapter 18 mod.	**Reference** FDA BAM Chapter 18 mod.	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 18Dec2023

Parameter	**Result**
Yeast	< 10 cfu/g
Mold	< 10 cfu/g

UM6NM - Campylobacter Species - AOAC RI #040702	**Reference** AOAC-PTM 040702	**Accreditation**	**Completed** 19Dec2023	**Sub** 4

Parameter	**Result**
Campylobacter Species	Not Detected per 25 g

UM8VD - Total Coliforms - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023

Parameter	**Result**

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Wild Type, Inc.

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ANALYTICAL REPORT

AR-24-QR-001286-02
Report Supersedes AR-24-QR-001286-01

Client Code: QR0000417
PO#: FDA RFI - Saku test 4- Nov 2023 (cells 11/09/23)
Received On: 13Dec2023
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Eurofins Sample Code:	111-2023-12130142	**Sample Registration Date:**	13Dec2023
Client Sample Code:	SAK-2023-12-12	**Condition Upon Receipt:**	acceptable, -50.3°C
Sample Description:	SAK-2023-12-12 made with cells harvested on 2023-11-09	**Sample Reference:**	Cells harvested on 2023-11-09

UM8VD - Total Coliforms - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023	

Parameter	**Result**
Total Coliforms	< 10 cfu/g
E. coli	< 10 cfu/g

UMEWE - Escherichia Coli O157:H7 - AOAC-RI 031002	**Reference** AOAC-RI 031002	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023	

Parameter	**Result**
Escherichia coli O157:H7	Not Detected per 25 g

UMHBM - Staphylococcus aureus - BAM Chapter 12	**Reference** BAM Chapter 12	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 15Dec2023	

Parameter	**Result**
Staphylococcus aureus	< 10 cfu/g

UMJN3 - Non-O157 Shiga toxin-Producing E.coli - AOAC-RI 091301	**Reference** AOAC-RI 091301		**Completed** 14Dec2023	

Parameter	**Result**
Non-O157 Shiga toxin-Producing E.coli	Not Detected per 25 g

UMKTF - Enterobacteriaceae - CMMEF Chapter 9.62	**Reference** CMMEF Chapter 9.62	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023	

Parameter	**Result**
Enterobacteriaceae	< 10 cfu/g

UMKXG - Staphylococcal Enterotoxin - AOAC 2007.06	**Reference** AOAC 2007.06	**Accreditation**	**Completed** 21Dec2023	**Sub** 1

Parameter	**Result**
Staphylococcal Enterotoxin	Not Detected per 25 g

UMMA7 - Bacillus cereus - BAM Chapter 14	**Reference** FDA BAM Chapter 14	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 15Dec2023	

Parameter	**Result**

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ANALYTICAL REPORT

AR-24-QR-001286-02
Report Supersedes AR-24-QR-001286-01

Client Code: QR0000417
PO#: FDA RFI - Saku test 4- Nov 2023 (cells 11/09/23)
Received On: 13Dec2023
Reported On: 17Jan2024

Eurofins Sample Code:	111-2023-12130142	**Sample Registration Date:**	13Dec2023
Client Sample Code:	SAK-2023-12-12	**Condition Upon Receipt:**	acceptable, -50.3°C
Sample Description:	SAK-2023-12-12 made with cells harvested on 2023-11-09	**Sample Reference:**	Cells harvested on 2023-11-09

UMMA7 - Bacillus cereus - BAM Chapter 14	**Reference** FDA BAM Chapter 14	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 15Dec2023

Parameter	**Result**
Bacillus cereus	< 10 cfu/g

UMQE5 - Listeria monocytogenes - AOAC-RI 061703	**Reference** AOAC-RI 061703	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023

Parameter	**Result**
Listeria monocytogenes	Not Detected per 25 g

UMQMM - Salmonella species - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 14Dec2023

Parameter	**Result**
Salmonella spp.	Not Detected per 25 g

UMVEP - Aerobic Plate Count - AOAC 966.23	**Reference** AOAC 966.23	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 15Dec2023

Parameter	**Result**
Aerobic Plate Count	< 10 cfu/g

ZM3KF - Clostridium perfringens - ISO 7937	**Reference** ISO 7937	**Completed** 14Dec2023

Parameter	**Result**
Clostridium perfringens	< 10 cfu/g

Report Comment:
Report ammended to include missing method reference for Presumptive C. botulinum toxin and Viable C. botulinum

Subcontracting partners:

1 - Eurofins Microbiology Laboratories (Des Moines), IA
2 - Eurofins Nutrition Analysis Center, Iowa
3 - Silliker, INC Food Science Center, IL
4 - Eurofins Microbiology Laboratories (Lancaster), Pennsylvania
5 - Eurofins Food Chemistry Testing US Madison, Wisconsin

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Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-24-QR-001286-02
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Client Code: QR0000417
PO#: FDA RFI - Saku test 4- Nov 2023 (cells 11/09/23)
Received On: 13Dec2023
Reported On: 17Jan2024

Respectfully Submitted,

[redacted]

Viridiana Castro
Business Unit Manager

ACCREDITED

Received: 6 May 2024
Responded: 30 August 2024

Overview

This document responds to the request for additional information re. CCC 000005 transmitted by FDA to Wildtype on 6 May 2024. For ease of reference, FDA's original questions are reproduced in black text and Wildtype's responses appear below in blue text.

Substantive Information Requests

Substances used during cell culture

1. Additional analytical data are needed to support the assumptions made in the mass balance/dilution calculations. In the absence of additional analytical data, we are unable to accept the presented theoretical dilutions used for the estimated daily intakes. We typically conservatively presume that the concentration of the media component in the media is carried over to the harvested cell material when analytical data to support the theoretical dilution argument is absent. For addition to the DSN, please revise your exposure estimates (omitting CCI/TS), to presume that the concentration of the media components in the harvested cell material is the same as the level of the component in the actual media. Please include full details of the revised calculations.

Figure 1 below provides revised exposure estimates, assuming that the concentration of the media components in the harvested cell material is the same as the level of the component in the actual media (a "worst case" dilution assumption, where no subsequent rinsing occurs). Figure 1 presents those components without an applicable authorization for Wildtype's use (including components lacking an applicable authorization that have been added since we submitted CCC 000005). Note that we use the terms "component" and "input" interchangeably. Details for the revised calculations follow.

a. We start with the concentration of each input in milligrams per liter: e.g., sodium selenite is present in the starting media at a concentration of 0.043 mg/L
b. This concentration is then converted to grams / liter = 0.043 / 1,000 = 0.000043 g/L
c. The salmon cell density (specific gravity) is 1.09g/mL (cell pellet weight = 174.7g when cell volume = 0.16L). Using this cell density, we convert the concentration amount in b. to grams of media component / grams of cells by taking the concentration 0.000043 g/L and dividing by 1090 g/L = 3.94E-08 g of sodium selenite / gram of cells.
d. This concentration can be further converted to a basis of per 100 grams of cells by multiplying by 100, which will result in a mass ratio of 3.94E-06 g of sodium selenite / 100 g cells.
e. These results are then used to calculate estimated daily intake (EDI) per the assumptions in section 3.6 in CCC 000005 (pages 38-43). We used the subpopulation with the highest salmon consumption to calculate exposure. For example, the salmon consumption of children aged 2-12 at the 90th percentile was used for g/kg bw/day calculations (2.56 g/kg bw/day) and the salmon consumption of adolescents aged 13-18 at the 90th percentile for mg/day calculations (112 g/day). For sodium selenite, EDI is calculated as (3.94E-08 selenite / g cells) * (2.56 g cells /kg bw/ day) * (1000 mg / 1 g) = 1.01E-04 mg sodium selenite / kg bw / day.

Appendix 1 in the confidential appendices provides a full accounting of Wildtype's current media formulation with exposure estimates. Appendix 2 in the confidential appendices summarizes components that have been added and removed since CCC 000005 was submitted. Since submitting CCC 000005, we have removed 32 inputs and added five inputs; this has enabled us to remove all animal-derived components, simplify our cell feed, reduce costs, and permit larger-scale cultures.

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Figure 1 - Predicted concentration and EDI for select cell culture medium inputs

Input	Predicted pre wash concentration in cells (g/100g)	Estimated Daily Intake pre wash (mg / kg bw / day)	Estimated Daily Intake pre wash (mg / day)	Safety Reference	Safety Assessment	Safety Narrative
L-ornithine monohydrochloride	4.49E-04	1.15E-02	5.02E-01	No observed adverse effect level (NOAEL) = 3,445 mg/kg bw/day[1]	Pre-wash EDI is 0.0115 mg/kg bw/day, well below NOAEL Margin of Safety (MOS) > 290,000	Safety discussion provided below in response to question 5
Poloxamer 188	2.75E-01	7.05E+00	3.08E+02	NOAEL = 3,500 mg/kg bw/day[2]	Pre-wash EDI is 7.05 mg/kg bw/day, well below the NOAEL MOS = 496	Safety narrative provided below in Appendix 3
Salmon fibroblast growth factor-2 (also known as basic FGF or bFGF)	9.17E-07	2.35E-05	1.03E-03	Not available in the scientific literature	Salmon FGF2 is produced using conventional recombinant protein production methods and a safe source organism. FGF2 is naturally ubiquitous in all fish tissues, and is already present in the human diet without any safety concern.	Safety discussion provided below in response to question 2 and in CCC 000005 (pages 43-46)
Tween 80 (polysorbate)	5.96E-04	1.53E-02	6.68E-01	Acceptable daily intake (ADI) = 25 mg/kg bw/day[3]	Pre-wash EDI is 0.0153 mg/kg bw/day and well below the ADI	Safety narrative provided on page 8 of our January 17, 2023 Amendment (footnote 21)
D-glucuronolactone	8.62E-05	2.21E-03	9.66E-02	NOAEL= 1,000 mg/kg bw/day[4]	Pre-wash EDI is 0.00221 mg/kg bw/day, well below NOAEL, MOS > 450,000	Safety narrative provided below in Appendix 3
N-acetyl-D-glucosamine	1.88E-04	4.82E-03	2.11E-01	NOAEL = 2,323 mg/kg bw /day[5]	Pre-wash EDI is 0.00482 mg/kg bw/day, well below NOAEL, MOS > 480,000	Safety narrative provided below in Appendix 3
Sodium selenite	3.94E-06	1.01E-04	4.42E-03	NOAEL = 0.2 mg/kg bw / day[6] IOM-UL for selenium of 90-400 µg/day for various life-stages[7]	Pre-wash EDI is 0.000101 mg/kg bw/day, well below NOAEL MOS > 1,980 WT's EDI of 4.42 µg/day is well below IOM-UL of 90-400 µg/day	Safety narrative provided below in Appendix 3

[1] European Chemicals Agency (ECHA). Registration Dossier for L- Ornithine. Last modified on 29 Aug 2022. Accessed May 2024 at this link
[2] Leaf CW. (1967). Toxicology of some non-ionic surfactants. Soap Chem. Spec. 43:48 [as cited in CIR, 2008].
[3] Joint FAO/WHO Expert Committee on Food Additives. 1973. Toxicological evaluation of certain food additives with a review of general principles and of specifications. Technical report series No. 539
[4] EFSA. 2009. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on a request from the Commission on the use of taurine and D-glucurono-γ-lactone as constituents of the so called "energy" drinks. EFSA J. 935: 1-31. https://doi.org/10.2903/j.efsa.2009.935
[5] Takahashi M, Inoue K, Yoshida M, Morikawa T, Shibutani M, Nishikawa A. (2008). Lack of chronic toxicity or carcinogenicity of dietary N-acetylglucosamine in F344 rats. Food Chem Toxicol., 47(2):462-71. doi: 10.1016/j.fct.2008.12.002. Epub 2008 Dec 10. PMID: 19103248.
[6] Harr et al as cited in National Toxicology Program (NTP). 1994. NTP Technical Report on Toxicity Studies of Sodium Selenate and Sodium Selenite. NIH Publication 94-3387, July 1994.
[7] Institute of Medicine (US). 2000. Panel on Dietary Antioxidants and Related Compounds. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington (DC): National Academies Press (US); 2000. 7, Selenium.

Input (Figure 1 continues from page 2)	Predicted pre wash concentration in cells (g/100g)	Estimated Daily Intake pre wash (mg / kg bw / day)	Estimated Daily Intake pre wash (mg / day)	Safety Reference	Safety Assessment	Safety Narrative
Nicotinamide adenine dinucleotide	3.34E-04	8.55E-03	3.74E-01	NOAEL = 7.9mg/kg bw/day[8]	Pre-wash EDI is 0.00855 mg/kg bw/day; well below NOAEL MOS >900	Safety narrative provided in CCC 000005 (pages 48-49)
Taurine	1.99E-04	5.10E-03	2.23E-01	Existing dietary exposure: 29.3 mg/day (GRN 586); Observed Safe Level (OSL): 3 -6 g/day[9]	Pre-wash EDI is 0.223 mg/day and a fraction of exposure estimates in GRN 586 and well below OSL 3-6 g/day	Safety narrative provided in CCC 000005 (pages 46-48)
p-Aminobenzoic acid	3.39E-05	8.69E-04	3.80E-02	NOAEL = 100 mg/kg bw/day[10]	Pre-wash EDI is 0.000869 mg/kg bw/day, well below NOAEL MOS > 100,000	Safety discussion provided below in response to question 5
Thiamine diphosphate	4.77E-05	1.22E-03	5.34E-02	Safe intake level: up to 100 mg/day[11] US existing intake: 4.89-4.9 mg/day[12]	Pre-wash EDI is 0.0534 mg/day, well below EFSA's safe intake level and well below US background dietary intake	Safety narrative provided below in Appendix 3
Methyl-β-cyclodextrin	8.01E-02	2.05E+00	8.97E+01	ADI = 5 mg/kg bw/day for the read-across compound β-cyclodextrin[13]	Pre-wash EDI is 2.05 mg/kg bw/day and well below the ADI	Safety narrative provided below in Appendix 3
Dimethyl sulfoxide (cell cryoprotection agent)	3.5E-09	8.96E-08	3.92E-06	NOAEL = 1,000 mg/kg bw/day[14]	Pre-wash EDI is 8.96E-08 mg/kg bw/day and well below NOAEL MOS> 11.2E+09	Safety narrative provided below in Appendix 3
L2-Amino-*n*-Butyric Acid	2.64E-04	6.75E-03	2.95E-01	ADI of 30 mg/kg bw/day for the read-across compound glutamate[15]	Pre-wash EDI is 0.00673 mg/kg bw/day and well below the ADI	Safety narrative provided below in Appendix 3

[8] Birkmayer JGD and K. Nadlinger (2002). Safety of stabilized, orally absorbable, reduced nicotinamide adenine dinucleotide (NADH): a 26 weeks oral tablet administration of ENAD/NADH for chronic toxicity study in rats. Drugs Exptl. Clin. Res. XXVIII(5): 185-192.
[9] EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the safety and efficacy of taurine as a feed additive for all animal species. EFSA Journal 2012;10(6):2736. [17 pp.] doi:10.2903/j.efsa.2012.2736. Available online: www.efsa.europa.eu/efsajournal
[10] Scientific Committee On Consumer Products (SCCP). (2006). Opinion on 4-Aminobenzoic acid (PABA). European Commission, Health & Consumer Protection Directorate. Adopted by the SCCP during the 8th plenary meeting of 20 June 2006.
[11] EFSA. 2008. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS). Benfotiamine, thiamine monophosphate chloride and thiamine pyrophosphate chloride, as sources of vitamin B1 added for nutritional purposes to food supplements. EFSA J 864: 1-31.
[12] National Institutes of Health (NIH) Office of Dietary Supplements (ODS). 2021. Factsheet on Thiamine. Last updated March 2021. Accessed 19 Dec 2022.https://ods.od.nih.gov/factsheets/thiamin-healthprofessional/
[13] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2016). Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. EFSA Journal 14(12): 4628, 44 pp. doi:10.2903/j.efsa.2016.4628 & Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). (1995). beta-Cyclodextrin.
[14] ECHA (2022) Dimethyl sulfoxide CAS 67-68-5. Dossier last modified October 13, 2022 and date access December 13, 2022.
[15] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Parent-Massin D, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Boon P, Chrysafidis D, Gürtler R, Tobback P, Altieri A, Rincon AM and Lambré C, 2017. Scientific Opinion on the re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives. EFSA Journal 2017;15(7):4910, 90. Accessed August 2024. Available online:https://doi.org/10.2903/j.efsa.2017.4910

All other inputs used in Wildtype's cell feed (cell nutrient media) are widely used or present in food, are the subject of existing authorizations consistent with Wildtype's use, and fall into one of the following classes:

Amino acids: Wildtype's cell feed includes amino acids (e.g., alanine, glutamine, lysine), which are the building blocks of all proteins. Amino acids are necessary for cell growth and are found in all conventional salmon.

Fatty acids: Polyunsaturated, monounsaturated, and saturated fats (e.g., vegetable and nut oils) are common food constituents and are necessary for cell growth.

Salts: A variety of salts (e.g., sodium chloride and potassium chloride) are necessary for cell growth.

Sugars: Carbon sources such as the sugar glucose are necessary for cell growth.

Trace elements & minerals: Examples include iron and copper, which are common essential elements and necessary for cell growth.

Vitamins: A variety of vitamins are used in Wildtype's cell feed, such as vitamins A, B, and D. The levels of these vitamins are addressed further in response to question 7 below.

DNA constituents: The four DNA bases (i.e. A, T, C, G) or nucleotides are the building blocks of nucleic acids and are present in all foods. As we noted in our January 2023 amendment, DNA constituents are digested and naturally anabolized into cellular DNA or catabolized according to well-characterized physiological pathways.[16, 17]

Other substances to manage properties of the media: Examples include hydrochloric acid, which is used to control pH during cell culture and emulsifiers added to media to help mix oils and water.

2. For addition to the DSN, please provide the following information about transferrin and fibroblast growth factor (bFGF) used in the main production phase (i.e., initiation of the biomass accumulation stage through harvest).

a. **The species of origin of each recombinant protein, and information about the source organism used to produce the recombinant protein (e.g., identity, pathogenicity, toxigenicity, allergenicity).**
b. **If one, or both, of these proteins are human recombinant protein(s) (rHP), discuss the homology of each rHP used during production to orthologs from agriculturally relevant animal species (e.g., bovine, porcine). Please also include a discussion of the results of a literature search and/or in silico analyses of digestibility, glycosylation, and immunogenicity for each rHP sequence.**
c. **If the transferrin is a rHP, provide an estimated daily intake (EDI) for the substance based on analytical measurements in the harvested cellular material and state the limit of detection (LOD) of your sensitive analytical method.**
d. **For all recombinant proteins (human and other species) used during any stages of the production process, please provide information about the source organism used to produce the recombinant protein (e.g., identity, pathogenicity, toxigenicity, allergenicity) and in what stage(s) of the production process they are used**

Please be aware that FDA strongly discourages the use of recombinant human proteins at any stage of the cell culture process. We strongly encourage firms to consider replacing recombinant human

[16] Liu Y, Zhang Y, Dong P, An R, Xue C, Ge Y, Wei L, Liang X. Digestion of Nucleic Acids Starts in the Stomach. *Sci Rep.* 2015 Jul 14;5:11936. doi: 10.1038/srep11936. PMID: 26168909
[17] Hill JM, Morse PA Jr, Gentry GA. Metabolism of deoxycytidine, thymine, and deoxythymidine in the hamster. *Cancer Res.* 1975 May;35(5):1314-9. PMID: 1120315.

proteins with their counterparts from an agriculturally relevant species (e.g., bovine or porcine). When recombinant human proteins are used at any stage of the culture process, FDA requires the firm to provide analytical data to demonstrate that the recombinant human proteins are below level of detection in the harvested cellular material using a validated sensitive analytical method with a limit of detection of at least 0.1 ppb.

Transferrin is no longer used in our production process. The responses below address the sole remaining recombinant protein used in Wildtype's production process: fibroblast growth factor-2.

a. Fibroblast growth factor-2 (FGF2, basic FGF, or bFGF) from salmon is produced using conventional recombinant protein production methods. A salmon FGF gene sequence from Keta salmon (*Oncorhynchus keta*) (NCBI Reference sequence XP_035600424.1) is used. The host organism is *E. coli* BL21(DE3), a non-pathogenic, nontoxigenic, and non-allergenic strain. The safety information on the host organism is further described in point d. below.

   The relevant gene fragment, which is a truncated sequence from a whole sequence, is inserted and then transferred into *E. coli* BL21(DE3). In the host organism, the expression vector is pure, in the form of free replicating plasmid, not integrated in the host genome, and produces recombinant FGF protein through transcription and translation in the host. The host organism is grown using standard fermentation techniques. The final tag-free protein is purified using affinity chromatography and processed to remove endotoxins to meet the specification limit of <0.2 EU/µg of protein (by gel clotting endotoxin assay). The final protein (FGF2) has purity of ≥95% as analyzed by SDS-PAGE.

b. Not applicable

c. Not applicable

d. Safety information for salmon FGF2 produced in *E. coli* follows. Salmon FGF2 is used in the cell culture medium during the main production phase, comprising initiation of the biomass accumulation stage through harvest. This includes vial thaw, seed train, and cell culture in our production bioreactors.

Escherichia coli (*E. coli*) are rod-shaped (1.5 µm long and 0.5 µm wide), Gram-negative, facultative anaerobes typically found in the intestines of humans and animals.[18] *E. coli* are usually harmless, although some strains are pathogenic. Nonpathogenic *E. coli* strains are often used as hosts for gene expression and protein synthesis due to their ease of use, affordability, rapid cell proliferation, genetic simplicity, compatibility with molecular techniques and methods, and safety.[19, 20] In the past, *E. coli* has served as a platform in the production of enzymes (*i.e.* amylase[21] and protease[22]), antimicrobial peptides (*i.e.* nisin[23]), vitamins (*i.e.* B12[24]) and antioxidants and fatty acids (*i.e.* polyphenols[25] and

[18] U.S. Food and Drug Administration (FDA). (2019). *Escherichia coli*. https://www.fda.gov/food/foodborne-pathogens/escherichia-coli-e-coli (Accessed May 24, 2024).
[19] Fakruddin, M., Mohammad Mazumdar, R., Bin Mannan, K. S., Chowdhury, A., & Hossain, M. N. (2013). Critical factors affecting the success of cloning, expression, and mass production of enzymes by recombinant *E. coli*. *International Scholarly Research Notices, 2013*.
[20] Hayat, S. M., Farahani, N., Golichenari, B., & Sahebkar, A. (2018). Recombinant protein expression in *Escherichia coli* (*E. coli*): what we need to know. *Current pharmaceutical design, 24*(6), 718-725.
[21] EFSA CEP Panel. (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano V., Barat Baviera J.M., Bolognesi C., Cocconcelli P.S., Crebelli R., Gott D.M., Grob K., Lampi E., Mortensen A., Rivière G., Steffensen I-L., Tlustos C., Van Loveren H., Vernis L., Zorn H., Glandorf B., Herman L., Jany K-D, Marcon F., Penninks A., Arcella D., Gomes A., Kovalkovičová N., Liu Y., Maia J., Roncancio Peña C., Nuin I., and Chesson A. (2019). Scientific Opinion on the safety evaluation of the food enzyme maltogenic amylase from genetically modified *Escherichia coli* (strain BLASC). *EFSA Journal 2019;17*(7):5769, 16 pp. https://doi.org/10.2903/j.efsa.2019.5769
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[25] van Summeren-Wesenhagen, P. V., & Marienhagen, J. (2015). Metabolic engineering of *Escherichia coli* for the synthesis of the plant polyphenol pinosylvin. *Applied and environmental microbiology, 81*(3), 840-849.

omega-3 fatty acids[26]). For example, bioengineers have leveraged the genus to create proteins such as chymosin, an important component in cheese production, and β-galactosidase, an enzyme utilized in lactose-free dairy.[27, 28] The expression of these various biomolecules requires careful selection of the appropriate *E. coli* strain to achieve optimal yield and efficiency. One such versatile strain is *E. coli* BL21(DE3).

E. coli BL21(DE3) is derived from *E. coli* B strain and its parent strain, *E. coli* BL21. B strains are preferred for recombinant protein expression and are conveniently deficient in lon (cytoplasm) and ompT proteases (outer membrane[29]). Unlike BL21, the microbe also features a "λDE3 lysogen containing T7 RNA polymerase gene under the control of the lacUV5 promoter."[30] BL21(DE3) is typically induced by IPTG and is a suitable bioreservoir for the expression of nontoxic genes.[31] The potential for BL21(DE3)'s leaky gene expression is mitigated through the addition of 1% glucose in the medium.[32]

E. coli BL21(DE3) is often used for recombinant protein production. It is a generally non-pathogenic strain designed for laboratory use.[33] It is not found in the American Biological Safety Association's Risk Group Database[34] and has been classified as a biosafety level 1, low risk, microorganism.[35] A previous study demonstrated the lack of genes coded for an invasive phenotype in BL21(DE3)'s parent strain, BL21, as well as the absence of long-chain LPS contributing to its susceptibility to external environmental factors.[36] This study also notes the parent strain's inability to survive in mammalian hosts. BL21(DE3) is unable to produce hydrogen gas, a product that aids in the survival of some pathogens; this deficiency is noted in modifications in the FHL complex.[37] The strain does not possess the FNR gene, which has been implicated in anaerobic respiration.[38] Lastly, it does not possess a plasmid to transfer its DNA into other host organisms.[39] Overall, BL21(DE3) is absent of certain genetic traits that would make it pathogenic.

E. coli BL21(DE3) is not recognized as toxic. While BL21(DE3) strain #1540 produces endotoxins, acute oral toxicity experiments in mice confirmed they were not toxic.[40] Additionally, it was found that the parent strain of BL21(DE3), BL21, does not have the ability to express verocytotoxins, *E. coli* LT toxins, or *E. coli* ST enterotoxins.[41] In general, BL21(DE3) does not naturally produce toxins.

E. coli BL21(DE3) does not inherently express any allergenic proteins and is generally safe for use in the production of food ingredients.[42] Growth factors are naturally occurring in fish muscle tissue. The FGF2

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[28] EFSA Panel. EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Silano, V., Barat Baviera, J.M., Bolognesi, C., Cocconcelli, P.S., Crebelli, R., Gott, D.M., Grob, K., Lampi, E., Mortensen, A. and Rivière, G., Chesson, A., Steffensen, I-L., Tlustos, C., Van Loveren, H., Vernis, L., & Zorn, H. (2020). Safety evaluation of the food enzyme β-galactosidase from the genetically modified *Escherichia coli* NCIMB 30325. *EFSA Journal, 18*(1), e05977.
[29] Hayat, S. M., Farahani, N., Golichenari, B., & Sahebkar, A. (2018). Recombinant protein expression in *Escherichia coli* (*E. coli*): what we need to know. *Current pharmaceutical design, 24*(6), 718-725.
[30] *Ibid.*
[31] Zhong, C., Wei, P., & Zhang, Y. H. P. (2017). Enhancing functional expression of codon-optimized heterologous enzymes in *Escherichia coli* BL21 (DE3) by selective introduction of synonymous rare codons. *Biotechnology and bioengineering, 114*(5), 1054-1064.
[32] Gottesman, S. (1990). [11] Minimizing proteolysis in Escherichia coli: genetic solutions. In *Methods in enzymology* (Vol. 185, pp. 119-129). Academic Press.
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[34] https://my.absa.org/tiki-index.php?page=Riskgroups
[35] New England BioLabs, Inc. Safety Data Sheet
[36] Chart, H., Smith, H. R., La Ragione, R. M., & Woodward, M. J. (2000). An investigation into the pathogenic properties of *Escherichia coli* strains BLR, BL21, DH5α and EQ1. *Journal of applied microbiology, 89*(6), 1048-1058.
[37] Pinske, C., Bönn, M., Krüger, S., Lindenstrauß, U., & Sawers, R. G. (2011). Metabolic deficiencies revealed in the biotechnologically important model bacterium *Escherichia coli* BL21 (DE3). PLoS One, 6(8), e22830.
[38] *Ibid.*
[39] Health Canada. (2020). Novel Food Information – 2'fucosyllactose (2'-FL) from *Escherichia coli* BL21(DE3) Strain #1540. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/2-fucosyllactose-escherichia-coli-bl21/technical-summary.html
[40] *Ibid.*
[41] Chart, H., Smith, H. R., La Ragione, R. M., & Woodward, M. J. (2000). An investigation into the pathogenic properties of *Escherichia coli* strains BLR, BL21, DH5α and EQ1. *Journal of applied microbiology, 89*(6), 1048-1058.
[42] Health Canada. (2020). Novel Food Information – 2'fucosyllactose (2'-FL) from *Escherichia coli* BL21(DE3) Strain #1540. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/2-fucosyllactose-escherichia-coli-bl21/technical-summary.html

protein from salmon that is used in the culture of cell-cultivated salmon is already present in the human diet without any safety concern. Potential risk from increased exposure may occur if the protein is allergenic towards human consumers. Wildtype has conducted *in silico* assessments to examine the potential allergenicity risk and the findings are summarized below.

The NCBI peptide reference sequence for salmon [*Oncorhynchus keta*] FGF2 is XP_035600424.1. The peptide sequence is:
MATGEITTLPATPEDGGSGGFPPGNFKEPKRLYCKNGGYFLRINSNGSVDGIREKNDPHIKLQLQATSVG
EVVIKGVSANRYLAMNGDGRLFGTRRTTDECYFMERLESNNYNTYRSRKYPDMYVALKRTGQHKSGSKTG
PGQKAILFLPMSARR

An allergenic assessment of salmon FGF2 was conducted using the Allergen Online database system based on a scanning 80mer window of the FASTA amino acid query. The evaluation is based on recommendations by FAO/WHO that used criteria of >35% identity over any segment of 80 or more amino acids.[43,44] The salmon fibroblast growth factor, FGF2, returned zero positive hits using this algorithm, indicating they are not likely allergens. The salmon FGF2 amino acid sequence was also screened against the Allergen Online Celiac Database to determine if it would represent a potential risk of eliciting celiac disease (CD) related proteins or peptides. The FGF2 sequence returned no hits that meet the criteria of concern, which is an alignment against seven CD eliciting proteins with a score >45% overall identity.

Several factors influence the potential bioactivity of the protein, including the ability to interact with human receptors as well as the stability during digestion in the gastrointestinal tract. Protein digestion occurs throughout the digestive tract including the stomach and intestine through enzymatic activity and acid hydrolysis.[45, 46, 47] Orally administered recombinant proteins, in particular, are unstable and degrade in the digestive tract. Less than 1% of orally administered recombinant protein therapeutics arrive at the target site due to protein instability and degradation in the digestive tract.[48, 49, 50]

The breakdown of proteins can be mimicked *in silico* and based on the known enzymatic reactions from key stomach and intestinal enzymes, such as pepsin and trypsin, respectively.[51, 52] The PeptideCutter tool[53] was used to model pepsin and trypsin breakdown of salmon FGF2 to determine if sufficient protein was remaining to interact with the receptor.

In salmon (*Oncorhynchus keta*) FGF2, there are 17 amino acid residues (AA30, 33, 65, 67, 69, 97, 105, 108-113, 115, 147, 149, 151) that act as general receptor interaction sites and 5 amino acid residues (AA128, 129, 134, 138, 144) that act as heparin binding sites distributed throughout the peptide sequence (Paysan-Lafosse et al., 2023). Pepsin at pH 1.3 and pH >2 is likely to cleave the peptide at up to 25 total

[43] Codex Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant – DNA Microorganisms. CAC/GL 46-2003 Guideline For The Conduct Of Food Safety Assessment Of Foods Produced Using Recombinant-DNA Microorganisms (fao.org).
[44] Goodman RE. 2006. Practical and predictive bioinformatics methods for the identification of potentially cross-reactive protein matches. Mol Nutr Food Res 50:655-660.
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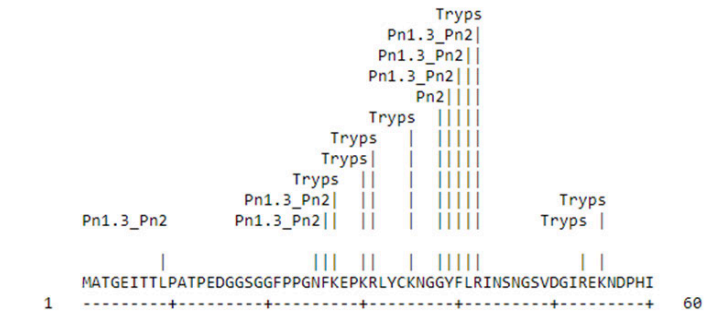
[51] Anekthanakul, K, Hongsthong, A, Senachak, J, Ruengijtchatchawalya. 2018. SpirPep: an in silico digestion-based platform to assist bioactive peptides discovery from a genome-wide database.
[52] Gasteiger, E, Hoogland, C, Gattiker, A, Duvaud, S, Wilkins, MR, Appel, RD, Bairoch, A. 2005. Protein identification and analysis tools on the ExPASy server. The Proteomics Protocols Handbook. Humana Press.
[53] *Ibid.*

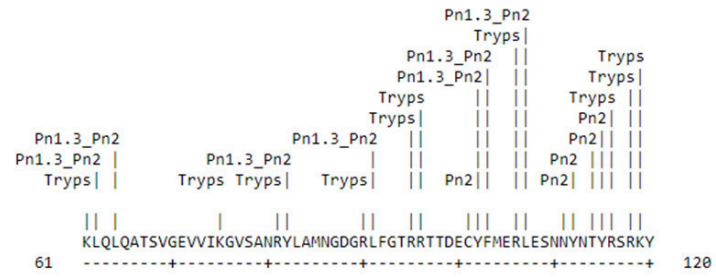
sites, including at AA115, 147 and 149 associated with general receptor interaction sites, thereby reducing potential interactions when consumed. Further, trypsin is likely to cleave the peptide at AA30 associated with general receptor interaction sites, and at AA128, 129, 134, 138, and 144 to fully hydrolyze the heparin binding sites. It is expected that gastrointestinal enzymes would substantially degrade FGF2, thereby inhibiting any bioactivity of the salmon FGF2. Please refer to Figure 2 below for further information.

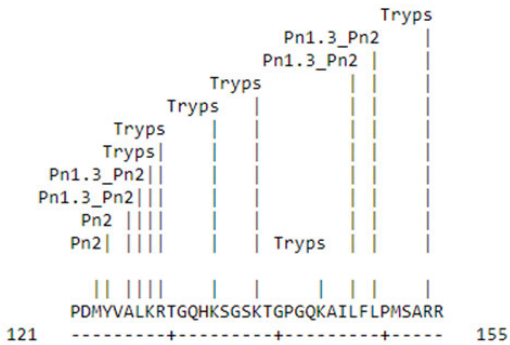
Figure 2 - *in silico* digestion of salmon FGF2 by trypsin and pepsin (pH 1.3 and pH >2)

A. The conserved protein domain family cd00058 identified in salmon FGF2 at amino acid residues AA30-152, and associated protein family and binding domain regions.

B. *In silico* digestion by trypsin (Tryps) and pepsin (Pn1.3, Pn2) results in multiple cut sites within the peptide.







3. A non-applicable GRAS notice was listed as the regulatory reference for glutathione in Table 1b of the June 3, 2022, SCM. The same table lists a food contact substance notification as the regulatory reference for the use of D-galactose. FDA notes that these are not applicable authorizations for the use of glutathione and D-galactose during the cell culture production process. Further, mass balance calculations and safety assessments are not provided for these substances.

For addition to the SCM, please demonstrate that the intake of these substances in the harvested cell material is safe by either i) showing that the EDI of these substances would not exceed the EDI from the conventional comparator; ii) that the EDI of these substances from the final food product would not substantially increase the current background intake of these substances, and/or iii) using traditional safety data (e.g., a no observed adverse effect level (NOAEL) from a subchronic animal study) or by providing an acceptable daily intake (ADI), upper limit (UL), daily value (DV), recommended dose (RD), or reference dose (RfD) (or any other safe level) derived from the comprehensive safety evaluation of these substances. Please provide a margin of safety (MOS) value between the EDIs and the safe intake level.

The use of glutathione in a wide range of foods, including highly consumed foods such as beverages, baked goods, and milk products, at levels ranging from 5 to 743 mg/serving, resulting in the 90th percentile estimated daily intake (EDI) of 961 mg/day had been concluded to be safe and generally recognized as safe (GRAS) (GRN 293). Cumulative consumption of glutathione, based on the background dietary intake and the uses in GRN 293 was approximately one gram per person per day (GRN 293). The EDI of Wildtype's harvested cell mass (pre-wash) is 0.531 mg/day—trivially small in comparison to the existing cumulative consumption in the diet. Therefore, there is no safety concern under these conditions of use.

D-galactose is no longer used in our production process.

4. Figure 7b of the January 17, 2023, amendment indicates that you no longer use glutathione-Na in your production process. Please confirm, for addition to SCM, whether this is indeed the case. If glutathione-Na is still used during the production process, along with glutathione, please note that glutathione-Na will dissociate into glutathione and sodium ion. FDA notes that the total amount of glutathione in the solution will come from both glutathione and glutathione sodium, therefore a cumulative safety assessment for glutathione is warranted. Hence, for addition to the SCM, please provide an updated mass balance calculation based on this information. When discussing the safety of glutathione, please make sure that you show that the intake of glutathione is safe from the combined source.

Glutathione-Na is no longer used in our production process.

5. The regulatory references provided for L-ornithine HCl, sodium pyruvate, potassium phosphate monobasic (anhydrous), menadione sodium bisulfite, and p-aminobenzoic acid in Table 1b of the June 3, 2022, SCM are links to FDA's Substances Added to Food (formerly EAFUS) Database. Further, no mass balance calculations or safety assessments were performed for any of these substances.

For addition to the DSN, please clearly state whether these substances, or a closely related substance (e.g., their non-salt form or another closely related salt form), have either i) an applicable authorization for use in human food in the U.S., ii) any authorization for use in human food in the U.S., and/or iii) whether these substances are naturally present in the conventional comparator or any other human food. If these substances, or their related forms, do not have applicable authorizations for use in human food in the U.S. and are not present naturally in food, please provide a safety discussion for their use in the cell culture production process.

Applicable authorizations for use in human food, when available, in the United States are discussed below for each input. L-ornithine HCl and *p*-aminobenzoic acid are included in Figure 1 above as they lack a clear regulatory precedent.

L-ornithine HCl

To evaluate the safety of L-ornithine HCl, a literature search was conducted in May 2024 to identify information pertinent to the toxicological potential of this substance. The searches were conducted in PubMed as well as publicly available databases, including US Food and Drug Administration (FDA), US Environmental Protection Agency (EPA), National Toxicology Program (NTP), the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO), Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA) and European Chemicals Agency (ECA). We also provide a summary of the available preclinical toxicological data in the peer reviewed literature.

There are no toxicokinetic studies for L-ornithine in the published literature. L-ornithine is a free amino acid that is not incorporated into proteins.[54] It plays a key role in mitigating the toxicity of ammonia through the urea cycle, by converting ammonia to urea; it facilitates 80% of the nitrogen excretion from the body.[55]

L-ornithine is absorbed from the ileum and distal jejunum.[56] L-ornithine is absorbed as a free amino acid into the liver where a portion is used, and the remainder passes into systemic circulation into peripheral tissue, and excreted via urine.[57]

An acute oral toxicity study conducted in accordance with OECD Guideline 420 in rats reported an LD_{50} > 2,000 mg/kg bw.[58]

In a 13-week oral repeated-dose study, Crj:CD rats (n=12/sex/group) were fed a diet containing 1.25%, 2.5%, or 5.0% L-ornithine daily, equivalent to approximately 12,500, 25,000, or 50,000 mg/kg diet. No mortality, clinical signs or other significant treatment related effects were observed at any dose. A NOAEL of 3,445 and 3,986 mg/kg bw/day was reported for male and female rats respectively.[59]

No reproductive or developmental toxicity studies were available for L-ornithine. There was no evidence of reproductive or teratogenic effects in the 13-week repeated oral dose toxicity study in rats.

There are no chronic toxicity or carcinogenicity studies for L-ornithine in the published literature. No adverse or proliferative lesions were produced in the repeated dose oral toxicity study conducted in rats.[60]

In an *in vitro* test conducted to examine genotoxicity using chromosome aberration using Chinese hamster lung fibroblast cells, L-ornithine produced no mutations at doses of up to 1,686 mg/mL, both with and without metabolic activation.[61]

Another *in vitro* test conducted to examine genotoxicity using a bacterial reverse mutation assay. Tester strains consisted of *S. typhimurium* TA98, TA100, TA1535, and TA1537 as well as *E. coli* strain WP2. The target compound was tested in doses of 313, 625, 1,250, 2,500, or 5,000 µg/plate, with and without (+/-)

[54] Joint FAO/WHO Expert Committee on Food Additives (JECFA). (2012). Safety evaluation of certain food additives. Seventy-sixth meeting of the JECFA.
[55] European Chemicals Agency (ECHA). Accessed 2024. Registration Dossier for L- Ornithine. Last modified on 29 Aug 2022. Accessed May 2024 at https://echa.europa.eu/de/registration-dossier/-/registered-dossier/21518/7/2/1
[56] *Ibid.*
[57] *Ibid.*: Toxicokinetics, metabolism and distribution
[58] Unnamed study report, 2006 as cited in ECHA, accessed 2024; Acute Toxicity: oral
[59] Unnamed report, 2013 as cited in ECHA, accessed 2024; Repeated dose toxicity: oral
[60] *Ibid.*
[61] European Chemicals Agency (ECHA). Accessed 2024. Registration Dossier for L- Ornithine. Last modified on 29 Aug 2022. Accessed May 2024 at https://echa.europa.eu/de/registration-dossier/-/registered-dossier/21518/7/2/1

S9 metabolic activation for 48 hours.[62] Based on the overall results, L-ornithine was negative for mutagenicity. No data is available for *in vivo* genotoxicity.

A 4-week clinical trial evaluated the safety and tolerability of ornithine hydrochloride. Healthy male adults (n=60/dose) completed graded doses of ornithine hydrochloride at 3.2, 6, 9.2, or 12 g/day as supplements. Outcomes measured included a broad spectrum of circulating biochemical analytes, body weight, sleep quality, and mental self-assessment. While the high dosage group of ornithine hydrochloride supplementation group showed a marginal increase in plasma aspartic acid and glutamic acid concentrations, no other parameters were altered. The study subjects tolerated the 4-week long oral supplementation of ornithine hydrochloride and a clinical NOAEL of 12 g/day was determined in this study.[63]

L-ornithine has been reported to occur in protein-rich foods, fish sauce, soya sauce, shrimp paste, and scallops. JECFA evaluated L-ornithine as a flavoring agent and concluded that there were no safety concerns associated with this compound at current estimated dietary exposures. The maximum survey-derived intake of L-ornithine (as the monohydrochloride) is 30 mg/day.[64] Lastly, the report noted that L-ornithine is an endogenous compound that is part of the urea cycle.

Safety assessment: based on the calculations in Figure 1 above, the pre-wash EDI is 0.0115 mg/kg bw/day, which is well below the lowest NOAEL of 3,445 mg/kg bw/day male rats (ECHA, 2024), with MOS >290,000. Further, the worst case EDI (0.5 mg/day) is well below the maximum survey-derived intake of 30 mg/day from approved flavoring uses (JECFA, 2012), and well below tolerable intake (12 g/day) observed in a clinical trial (Miura et al. 2022). Therefore, there is no safety concern under our intended conditions of use.

Sodium pyruvate

Sodium pyruvate is no longer used in our production process.

Potassium phosphate monobasic (anhydrous)

There is not a direct 21 CFR reference for potassium phosphate monobasic; however, its safety from dietary exposure can be established based on the GRAS status of dipotassium phosphate, which is GRAS when used in accordance with good manufacturing practice (21 CFR 182.6285). This is because in both cases, in solution, they establish an equilibrium by dissociating into their constituent potassium, hydrogen and phosphate ions. The SCOGS opinion on phosphates also concluded that "[t]here is no evidence in the available information on[…] potassium phosphate, monobasic […] that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or might reasonably be expected in the future." Furthermore, monopotassium phosphate is allowed to be added either directly to frozen eggs or in a water carrier if the amount does not exceed 0.5 percent of the weight of the frozen eggs (21 CFR 160.110). Therefore, there is no safety concern under our intended conditions of use.

Menadione sodium bisulfite

Menadione sodium bisulfite (vitamin K3) is a precursor of vitamin K2.[65] One reference shows levels in conventional salmon (raw, Alaska wild Coho, Sockeye, Chum, and King) of total vitamin K2 are ~0.3

[62] *Ibid.*
[63] Miura N, Morishita K, Yasuda T, Akiduki S, Matsumoto H. Subchronic tolerance trials of graded oral supplementation with ornithine hydrochloride or citrulline in healthy adults. Amino Acids. 2023 Mar;55(3):299-311. doi: 10.1007/s00726-022-03227-4. Epub 2022 Dec 26. PMID: 36571619; PMCID: PMC9791970.
[64] Joint FAO/WHO Expert Committee on Food Additives (JECFA). (2012). Safety evaluation of certain food additives. Seventy-sixth meeting of the JECFA.
[65] Pubchem accessed using this link on 5/23/2024

µg/100 g.[66] A broader set of vitamin K levels in foods[67] ranges from 0.3 to 600 µg per serving. Under the "worst case" assumptions described in our response to question 1 above, and assuming all menadione sodium bisulfite was converted into vitamin K2, estimated daily intake of vitamin K2 from the harvested cell material would be approximately 2 µg, roughly in the range of many conventional seafoods.[68] Therefore, there is no safety concern under our intended conditions of use.

P-aminobenzoic acid

To evaluate the safety of 4-aminobenzoic acid (*p*-aminobenzoic acid) a literature search was conducted in May 2024 to identify information pertinent to the toxicological potential of this input. The searches were conducted in PubMed as well as publicly available databases, including FDA, EPA, NTP, FAO/WHO, JECFA, ECA, and EFSA.

Oral administration of 4-aminobenzoic acid in adults resulted in 93% recovered in the urine after 5 hours of a single 80 mg dose. 4-aminobenzoic acid is rapidly absorbed from the gastrointestinal tract of humans and is quickly and nearly completely eliminated in urine within 24 hours.[69]

The reported oral LD_{50} values are >6,000 mg/kg bw in rats and 2,850 mg/kg bw in mice.[70]

In a 28-day non-GLP compliant study, rats (strain not stated) were administered at doses of 0, 600, or 1,400 mg/kg bw/day by oral gavage. All rats survived and gained body weight, with unremarkable findings at necropsy. Rats were noted to be resistant to orally administered 4-aminobenzoic acid and a NOAEL of >1,400 mg/kg bw/day was established.[71]

In a 4-week non-GLP compliant study, Sprague-Dawley rats were administered 0, 0.1, 0.5, or 1% 4-aminobenzoic acid in drinking water. No significant effects were reported on liver, kidney, or spleen weights. Plasma aspartate aminotransferase in rats administered 0.5% and 1% 4-aminobenzoic acid were statistically significantly lower than control at week 2, but not at week 1 or 4. Lipid peroxidation in the liver induced by *tert*-butyl hydroperoxide was decreased with statistical significance in rats administered 1% 4-aminobenzoic acid. Based on the decreased plasma aspartate aminotransferase, a NOAEL of 0.1% 4-aminobenzoic acid in drinking water was established.[72]

In a 108-day oral toxicity (non GLP-compliant) study, Wistar rats were administered 1,200 mg/kg bw/day 4-aminobenzoic acid to assess the influence of 4-aminobenzoic acid on porphyria induced by hexachlorobenzene. No toxicity was reported after 108 days of 4-aminobenzoic acid administered as the potassium salt and so a NOAEL of ≥1200 mg/kg bw/day was established.[73]

It was concluded that repeated oral administration of 4-aminobenzoic acid does not produce any adverse effects. However, a NOAEL of 100 mg/kg bw/day was based on the transient effect on plasma aspartate aminotransferase activities after 2 weeks, as reported in a case report of a 64-year old woman. Signs of hepatic injury were observed in humans administered 4-aminobenzoic acid at 12

[66] Elder SJ, Haytowitz DB, Howe J, Peterson JW, Booth SL. Vitamin k contents of meat, dairy, and fast food in the u.s. Diet. J Agric Food Chem. 2006 Jan 25;54(2):463-7. doi: 10.1021/jf052400h. PMID: 16417305.
[67] National Institutes of Health, Office of Dietary Supplements: Vitamin K Health Sheet for Professionals accessed via this link on 6/25/2024.
[68] USDA FoodData Central, canned tuna accessed via this link on 6/25/2024
[69] Bingham S, Cummings JH. (1982). The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. Clinical Science, 64:629-35 [as cited in SCCP, 2006] and Jakobsen J, Pedersen AN, Ovesen L. (2003). Para-aminobenzoic acid (PABA) used as a marker for completeness of 24 hour urine: effects of age and dosage scheduling. European Journal of Clinical Nutrition, 57(1):138-42 [as cited in SCCP, 2006].
[70] Scott CC, Robbins EB. (1942). Toxicity of p-aminobenzoic acid. Proceedings of the Society for Experimental Biology and Medicine, 49:184-9 [as cited in SCCP, 2006].
[71] *Ibid.*
[72] Chang T-Y, Hu M-L. (1996). Concentrations and lipid peroxidation in tissues and toxicity of paraaminobenzoic acid fed to rats in drinking water. Journal of Nutritional Biochemistry, 7(7):408-13 and Scientific Committee On Consumer Products (SCCP). (2006). Opinion on 4-Aminobenzoic acid (PABA). European Commission, Health & Consumer Protection Directorate. Adopted by the SCCP during the 8th plenary meeting of 20 June 2006.
[73] Scientific Committee On Consumer Products (SCCP). (2006). Opinion on 4-Aminobenzoic acid (PABA). European Commission, Health & Consumer Protection Directorate. Adopted by the SCCP during the 8th plenary meeting of 20 June 2006.

g/day for about 4 weeks, corresponding to 200 mg/kg bw/day for a 60 kg human. A LOAEL of 200 mg/kg bw/day was established.[74]

In a one generation reproduction toxicity study (non-GLP compliant), 4-aminobenzoic acid (0, 1, and 2%) did not affect reproduction in virgin Long-Evans rats fed orally in the diet. Oral administration of 0, 5, 15, or 50 mg/kg bw/day of 4-aminobenzoic acid dissolved in salt solution to white female rats (strain not specified) did not result in adverse effects on reproduction.[75]

4-Aminobenzoic acid was not mutagenic in vitro in *E. coli* without metabolic activation and in *Salmonella typhimurium* TA97, TA98, TA100, TA1535, and TA1537 with or without metabolic activation.[76] It did significantly increase the incidence of chromosomal aberrations in vitro in CHO cells at the highest dose.[77] No data is available for *in vivo* genotoxicity.

The Scientific Committee on Consumer Products (SCCP) evaluated 4-aminobenzoic acid in 2006. The agency based the overall safety on preclinical toxicity studies conducted in multiple animal species (i.e., rats, dogs, and rabbits) that demonstrated very low acute oral toxicity, no evidence of reproductive toxicity or carcinogenicity, but was minimally genotoxic. From the few repeated dose toxicity studies for 4-aminobenzoic acid in humans, a NOEL of 100 mg/kg bw/day and LOAEL of 200 mg/kg bw/day was established based on liver effects.[78]

Safety assessment: based on the assumptions described in our response to question 1 above, the pre-wash EDI is 0.000869 mg/kg bw/day, which is well below the lowest NOEL of 100 mg/kg bw/day in male rats, with MOS > 100,000. Therefore, there is no safety concern under our intended conditions of use.

6. For thymidine, Table 1b of the June 3, 2022, SCM states, "no precedent found" under "Evidence of use in food supply" and neither a mass balance calculation nor an EDI are provided for this substance. The safety assessment for thymidine in Table 1b is a single statement that the substance is a "DNA constituent." For addition to the DSN, please provide (omitting CCI/TS), either a theoretical EDI or an EDI based on analytical data for this substance and either i) compare the EDI for thymidine from the harvested cell material to the EDI of thymidine from the conventional comparator and show that the EDI from the harvested cell material does not exceed the EDI from the conventional comparator, ii) show that the EDI from the harvested cell material does not significantly increase the current background intake level, and/or iii) provide a safe intake level for this substance and calculate the MOS.

A theoretical EDI for 2'-deoxythymidine or thymidine is provided in Appendix 1. Thymidine (also known as deoxythymidine, deoxyribosylthymine, or thymine deoxyriboside) is a pyrimidine deoxynucleoside. Deoxythymidine is the naturally-occurring form of the DNA nucleoside T, which pairs with deoxyadenosine (A) in double-stranded DNA. DNA constituents such as thymidine are building blocks for DNA and would be used up during DNA synthesis as the cells divide.

DNA bases are digested and naturally anabolized into cellular nucleic acids (e.g. DNA) or catabolized

[74] Goerz G, Sick N, Vizethum W, Lissner R, Krieg T. (1980). Einfluss von p-Aminobenzoesaeure auf die Hexachlorbenzol-induzierte Porphyrie der Ratte [Influence of p-amino-benzoic acid on the hexachlorobenzene induced porphyria in the rat]. Arzneimittel-Forschung [Drug Research], 30(5):817-21 [Article in German translated and as cited in SCCP, 2006].
[75] Scientific Committee On Consumer Products (SCCP). (2006). Opinion on 4-Aminobenzoic acid (PABA). European Commission, Health & Consumer Protection Directorate. Adopted by the SCCP during the 8th plenary meeting of 20 June 2006.
[76] Gichner T, Baburek I, Velemínský J, Kappas A. (1991). UV-irradiation potentiates the antimutagenicity of p-aminobenzoic and p-aminosalicylic acids in Salmonella typhimurium. Mutation Research, 249(1):119-23 and Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E. (1986). Salmonella mutagenicity tests. - 2: Results from the testing of 270 chemicals. Environmental Mutagenesis, 8(Supplement; 7):1-119 [as cited in SCCP, 2006].
[77] Dean SW, Lane M, Dunmore RH, Ruddock SP, Martin CN, Kirkland DJ, et al. (1991). Development of assays for the detection of photomutagenicity of chemicals during exposure to UV light-I: assay development. Mutagenesis, 6(5):335-41 [as cited in SCCP, 2006].
[78] Scientific Committee On Consumer Products (SCCP). (2006). Opinion on 4-Aminobenzoic acid (PABA). European Commission, Health & Consumer Protection Directorate. Adopted by the SCCP during the 8th plenary meeting of 20 June 2006.

according to described physiological pathways.[79, 80] FDA has recognized nucleic acids as GRAS: "Nucleic acids are present in the cells of every living organism, including every plant and animal used for food by humans or animals, and do not raise a safety concern as a component of food. In regulatory terms, such material is presumed to be GRAS."[81] Therefore, there is no safety concern under our intended conditions of use.

[79] Liu Y, Zhang Y, Dong P, An R, Xue C, Ge Y, Wei L, Liang X. Digestion of Nucleic Acids Starts in the Stomach. *Sci Rep.* 2015 Jul 14;5:11936. doi: 10.1038/srep11936. PMID: 26168909
[80] Hill JM, Morse PA Jr, Gentry GA. Metabolism of deoxycytidine, thymine, and deoxythymidine in the hamster. *Cancer Res.* 1975 May;35(5):1314-9. PMID: 1120315.
[81] Guidance Document: Statement of Policy - Foods Derived from New Plant Varieties, FDA Federal Register, Volume 57 - 1992, May 29, 1992

Product characterization

7. A variety of vitamins are added to the cell culture media during the main production phase. The levels of these vitamins in the harvested cell material and final food product are provided in the DSN. Please discuss how the levels of these vitamins compare to the levels found in the conventional comparator. For any vitamins that are present at higher levels (EDI) than what is found in the conventional comparator, please discuss why you think that the higher intake levels of these vitamins are still safe. Please note, for certain key nutrients, additional regulatory concerns may also apply, e.g., FDA's nutrient fortification policy, "Nutritional Quality of Foods; Addition of Nutrients" which is found in 21 CFR 104.20, and/or the need for separate authorization of the nutrient in foods. These issues are of particular concern for nutrients not found in the conventional comparator or where levels are significantly higher than those in the conventional comparator and comparable to or higher than those approved for fortified food products.

As illustrated in Figure 3 below, vitamin levels in the cells at the point of harvest are lower than or similar to levels of vitamins found in salmon and other fish. COAs are provided in Appendix 7.

Figure 3: Vitamin levels in harvested cell material versus conventional comparator

Parameter	Method[82]	Specification	Cells at point of harvest			Conventional salmon / other foods
			Lot 1: 202405081	Lot 2: 202406061	Lot 3:202406062	
Vitamin A (per 100g cells)	AOAC 974.29 (eurofins) Analyst(1984)109:489 (Mérieux NutriSciences)	<50 IU	<13.32 IU (<4 µg[83])	<13.32 IU (<4 µg)	<13.32 IU (<4 µg)	50-3,150 IU[84]
Vitamin B5 (per 100g cells)	AOAC 945.74 (eurofins) AOAC 960.46 & Kit (Mérieux NutriSciences)	<5 mg	0.74 mg	0.4 mg	0.33 mg	1.14 mg[85]
Folate (per 100g cells)	AOAC 992.05 (eurofins) AOAC 960.46 & Kit (Mérieux NutriSciences)	<1 mg	0.073 mg	0.0182 mg	0.0187 mg	0.013 mg[86]
Vitamin B12 (per 100g cells)	AOAC 952.20 (eurofins) AOAC 960.46 & Kit (Mérieux NutriSciences)	<200 µg	139 µg	90 µg	101 µg	1.4-85µg[87]
Vitamin D2 & D3 (per 100g cells)	Huang et al. Rapid Commun, *Mass Spectrum* 2014, 28 (eurofins) AOAC 2016.05 Mod. (Mérieux NutriSciences)	<1,500 IU	544 IU (13.6 µg)	248 IU (6.2 µg)	291 IU (7.28 µg)	988 ± 524 IU[88]

[82] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieuex).
[83] The limit of detection for this test is 4 micrograms
[84] FoodData Central: range provided from Chum, Chinook, Atlantic, Coho, Sockeye, and Chinook liver Assumes 3.33 IU per microgram, accessed via this link on 5/23/2024
[85] FoodData Central, "Fish, salmon, coho, farmed, raw," accessed via this link on 5/23/2024
[86] FoodData Central, "Fish, salmon, coho, farmed, raw," accessed via this link on 5/23/2024
[87] Linus Pauling Institute, Oregon State: Table 2: summary of Vitamin B12 content from FoodData Central, accessed via this link on 6/25/2024
[88] A peer reviewed study estimates vitamin D levels in wild salmon at 988 ± 524 IU (mean ± standard error) per 100g. Reference: Lu Z, Chen TC, Zhang A, Persons KS, Kohn N, Berkowitz R, Martinello S, Holick MF. An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? J Steroid Biochem Mol Biol. 2007 Mar;103(3-5):642-4. doi: 10.1016/j.jsbmb.2006.12.010. Epub 2007 Jan 30. PMID: 17267210; PMCID: PMC2698592.

8. Figure 1 on page 2 of the January 24, 2024, amendment reports very high levels of vitamin D in the harvested cell material (an average 8,600 IU per 100 grams of cell material). In Appendix 5 of the same amendment, the level of vitamin D in the finished product has been significantly reduced to < 4 IU/100 g. FDA notes that the levels found in the harvested cell material are much higher than other foods that have been purposely fortified with vitamin D (see, for example, 21 CFR 172.379). For addition to the DSN, please discuss how the levels of vitamin D are reduced so significantly between the harvested cell material and the final food product. Please also explain why such high levels of vitamin D are needed in the harvested cell material. As a reminder, the consultation process considers the safety of the harvested cell material produced by your defined culture production process.

Vitamin D is critical to numerous cellular processes and is therefore included in the cell feed formulation. This concentration has been determined by Wildtype to be optimal for the normal proliferation of our salmon cell lines. As a fat-soluble molecule,[89] vitamin D may concentrate within lipid-rich components of cells.

Several previously-disclosed processing steps contribute to the significant reduction in vitamin D concentration from the harvested cell material to the finished food product. The first of these is the dilution of the harvested cell material that occurs when it is mixed with other food inputs. Second, the thermal processing steps (in particular, heating steps) are likely contributive to vitamin D degradation.[90] Much of the subsequent processing exposes the inputs to light, oxygen, and pH shifts, which have also been described as independent causes of vitamin D degradation.[91]

As illustrated in Figure 3 above, vitamin D levels in three batches of Wildtype cultivated salmon cells are within range typically found in the conventional comparator. Vitamin D levels are lower than those disclosed in Figure 1 of our January 24, 2024 amendment because we have since updated our rinsing step. This change is described in additional detail in response to question 12 below.

9. Figure 2 of the January 24, 2024, amendment reports higher than expected levels of arsenic in the harvested cell material (56.5 ppb, 81.0 ppb, and 97.5 ppb). In Appendix 4 of the same amendment, the level of arsenic found in the final food product has been reduced to below limit of detection (< 10ppb). For addition to the DSN, please comment on how the levels of arsenic are so greatly reduced between the harvested cell material and the final food product. In addition, please comment on the expected source of arsenic in the harvested cell material.

Arsenic is reduced below the limit of detection in the finished product due to the concentration of harvested cell material in the finished product. Additional detail may be found in Appendix 4 in the confidential appendices. Note that arsenic levels in both the harvested cell material as well as the finished product are lower than the conventional comparator.

Figure 7 in response to question 12 below shows that arsenic levels in the harvested cell material are substantially lower than the data presented in Figure 2 of our January 24, 2024 amendment because we have since updated our rinsing step.

[89] Kutner A, Brown G. Vitamins D: Relationship between Structure and Biological Activity. *Int J Mol Sci.* 2018 Jul 20;19(7):2119. doi: 10.3390/ijms19072119. PMID: 30037036; PMCID: PMC6073235.
[90] Mahmoodani F, Perera CO, Fedrizzi B, Abernethy G, Chen H. Degradation studies of cholecalciferol (vitamin D3) using HPLC-DAD, UHPLC-MS/MS and chemical derivatization. *Food Chem.* 2017 Mar 15;219:373-381. doi: 10.1016/j.foodchem.2016.09.146. Epub 2016 Sep 23. PMID: 27765240.
[91] Temova Rakuša Ž, Pišlar M, Kristl A, Roškar R. Comprehensive Stability Study of Vitamin D3 in Aqueous Solutions and Liquid Commercial Products. Pharmaceutics. 2021 Apr 25;13(5):617. doi: 10.3390/pharmaceutics13050617. PMID: 33922975; PMCID: PMC8147103.

10. Figure 2 of the January 24, 2024, amendment lists specifications for the harvested cell material, while Figure 4 of the same amendment lists specifications for both the harvested cell material and the final food product. On page 6 of the same amendment, you state, "For the harvested cell material, we will follow the same testing frequency for the first six months of commercial production. After six months, if there is no material discrepancy between test results for the harvested cell material and test results for finished food products, then we would consider testing of the finished food products to be sufficient to detect contamination events that were present at the point of harvest. Following the six-month period, Wildtype will routinely test the harvested cell material for all of the potential adventitious agents listed below at least quarterly to validate efficacy of controls. If this frequency is changed, we will submit a supplement to FDA." Three specifications that were included in Figure 2 are not included in Figure 4 (i.e., Escherichia coli O157:H7, Listeria monocytogenes, and Staphylococcus enterotoxin). For addition to the DSN, please describe why these three specifications were not included in Table 4.

Escherichia coli O157:H7 is included in routine microbial testing. Figure 4 from the January 24, 2024 amendment is updated as follows:

Figure 4 - testing frequency for harvested cell material and finished products

Potential Hazard	Frequency	Method[92]	Specification
Aerobic plate count	Every batch	AOAC OMA 990.12 (Eurofins) AOAC 966.23 (Merieux)	<100 cfu/g
Yeast/mold	Every batch	AOAC OMA 2014.05 (Eurofins) FDA-BAM, 7th ed. (Merieux)	<20 cfu/g
Enterobacteriaceae	Every batch	AOAC OMA 2003.01 / USP 37 <61>	<20 cfu/g
Total coliforms	Every batch	AOAC OMA 991.14	<100 cfu/g
E. coli	Every batch	AOAC OMA 991.14	<20 cfu/g
***E. coli* O157**	Every batch	AOAC - RI 031002	Negative/25g
***Campylobacter* species screen**	Every batch	AOAC RI 051201	Negative/25g
Salmonella	Every batch	AOAC OMA 2011.03 (Eurofins) AOAC 2004.03 (Merieux)	Negative/25g
***Listeria* genus**	Every batch	AOAC OMA 2013.10 (Eurofins) AOAC 2019.10 (Merieux)	Negative/25g
Staphylococcus aureus	Every batch	AOAC OMA 2003.07(Eurofins) AOAC 975.55 (Merieux)	<20 cfu/g
***Bacillus cereus* organism**	Every batch	FDA BAM(Eurofins) AOAC 980.31 (Merieux)	<100 cfu/g
***C. perfringens* organism**	Every batch	ISO 7937; AOAC 976.30	<10 CFU/g
***C. botulinum* organism**	Every batch	FDA-BAM, 8th ed.	Negative/8g
Arsenic	Every batch	AOAC 2011.19, 993.14 and 2015.01	<100 / <50 ppb[93]
Cadmium	Every batch	AOAC 2011.19, 993.14 and 2015.01	<20 ppb
Mercury	Every batch	AOAC 2011.19, 993.14 and 2015.01	<20 ppb
Lead	Every batch	AOAC 2011.19, 993.14 and 2015.01	<20 ppb

Listeria monocytogenes, and *Staphylococcus* enterotoxin were not included in Figure 4 of the January 24, 2024 amendment for the following reasons.

According to page 45 of Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry, dated January 2017, it was recommended to conduct tests for *Listeria spp.* "We recommend that you test for *Listeria spp.* because doing so will detect both *L. monocytogenes* as well as species of

[92] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieuex).
[93] We have set the lowest possible arsenic specification for cells at the point of harvest to 100 ppb and have maintained our specification for finished products at 50 ppb. We have included adventitious agent testing data for three lots of harvested cell material in Figure 6 below.

Listeria that are more common than *L. monocytogenes* and allow you to correct situations that could potentially lead to contamination with *L. monocytogenes*." Consequently, beginning in December 2023, our testing protocol shifted to *Listeria spp.* from *Listeria monocytogenes*.

Microorganism testing for *Staphylococcus aureus* was selected because the organism is a necessary precursor to the associated toxins. As stated in BAM Chapter 12: Staphylococcus aureus. "The presence of a large number of *S. aureus* organisms in a food may indicate poor handling or sanitation; however, it is not sufficient evidence to incriminate a food as the cause of food poisoning. The isolated *S. aureus* must be shown to produce enterotoxins. Conversely, small staphylococcal populations at the time of testing may be remnants of large populations that produced enterotoxins in sufficient quantity to cause food poisoning." Therefore, *Staphylococcus aureus* is needed to produce *Staphylococcus* enterotoxin and we have subsequently ceased testing for the enterotoxin.

Food safety management system

11. Page 8 of the January 24, 2024, amendment states that the thermal process is currently conducted at 70°C for a total of 110 minutes (including come-up time). FDA notes that the parameters (e.g., time, temperature) for the thermal process have been updated several times since CCC 000005 was filed in June 2022. Specifically, the original June 26, 2022, submission stated that the thermal process is conducted for one hour at 60 °C in a water bath, while the January 17, 2023, amendment, listed 65–70°C as the temperature range for the thermal process and ensures that the internal temperature reaches at least 70°C and remains at this temperature for at least 25 minutes. It is still not clear which thermal process with a validated study is used in the current production and if, or why, the process time is different. For addition to the DSN, please confirm and describe the current thermal process in detail (e.g., breakdown the come-up time, process time/temperature, and holding time) and provide an updated thermal validation study, if applicable.

We note that the thermal process occurs after the harvested cell material is rendered non-viable following the harvest process, and therefore falls outside the scope of this safety consultation per question 1 in our January 2024 amendment. We nonetheless provide additional details of the thermal process below, as requested.

Our current thermal process starts by setting an oven to 80 °C. The oven remains set to 80 °C for come-up time as well as processing / cook time. The product come-up-time is approximately 50 minutes. Subsequently, the process cooking time is 70 minutes, allowing the internal temperature of all parts of the finished product to reach at least 63 °C (145 °F) for 15 seconds. The duration of the entire thermal process is 120 minutes at 80 °C.

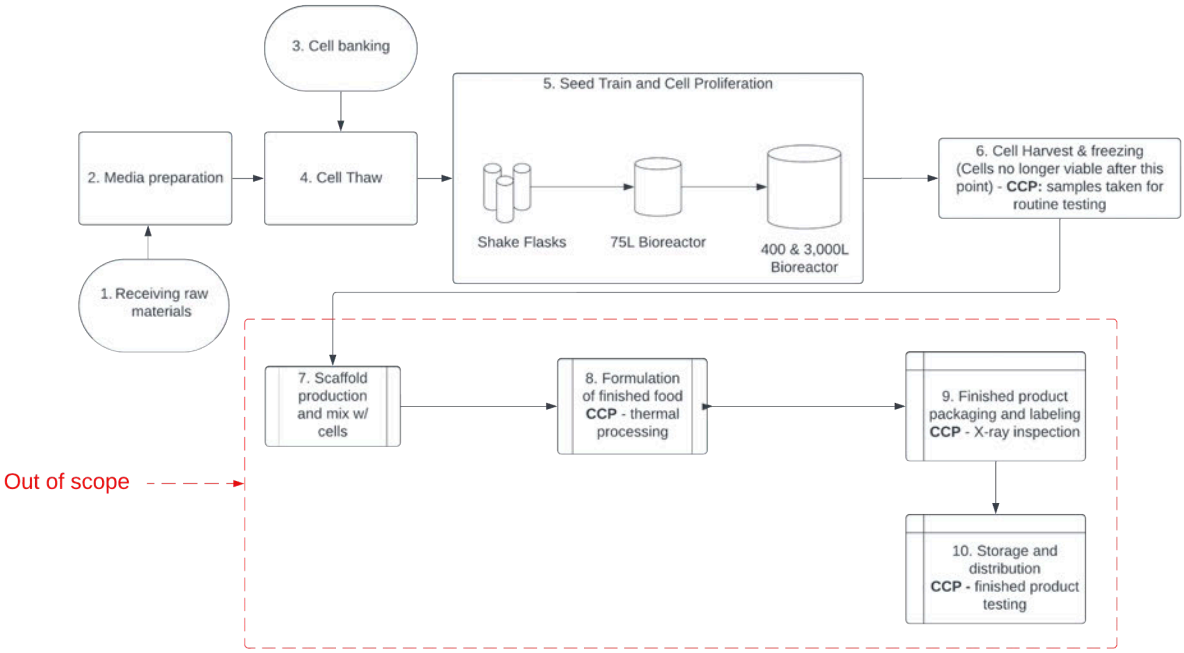
We previously had used a water-bath method for the thermal process. Starting in May 2023, we began using a dry oven for the thermal step and carried out a validation study to ensure all parts of the finished product reach at least 63 °C (145 °F) for 15 seconds. All three trials reached a temperature of at least 70 °C (158 °F) within 90 minutes and maintained at or above that temperature for the remainder of the testing period, resulting in the product being above 70 °C (158 °F) for a total of ~30 minutes and reaching a final temperature of 75 °C (167 °F). Our total cooking time of 120 minutes significantly exceeds the minimum required to achieve the target internal temperature. The oven thermal validation study is described in Appendix 5, for reference. The dry oven method is validated for its intended purpose of meeting or exceeding FDA's recommendation, which mandates cooking all parts of raw fish to an internal temperature of at least 63 °C (145 °F) for 15 seconds.[94]

[94] 2022 FDA Food Code Annex 7 -58, accessed using this link on 11/20/2023

12. For addition to the DSN, please provide an updated narrative of stepwise hazard analysis and preventive controls based on the updated hazard analysis and controls presented on pages 11-20 of the July 28, 2023, amendment. If changes in the facility setup, process, hazard analysis, and food safety plan have been implemented since the submission of the July 28, 2023, amendment, please provide updated details on the changes, including a description of any updates to the testing strategy.

Figure 5 below provides a flow chart of Wildtype's current process. The step numbers correspond to the numbering provided on pages 11-20 of our July 28, 2023 amendment. Note, steps 7 and beyond are excluded because they are out of scope for this consultation per FDA's guidance in Question 1 in the January 24, 2024 amendment. An updated narrative of each step of the process is provided below. An updated stepwise hazard analysis and preventive control table is provided in Appendix 6 with material changes since our July 28, 2024 amendment highlighted in red text.

Figure 5 - Summary flow chart of Wildtype's process



Updated narrative of stepwise hazard analysis and preventive controls

Wildtype continues to employ current good manufacturing practices (cGMP) throughout all steps of manufacturing as previously described in CCC 000005 and subsequent amendments. All process steps are subject to document and record controls, including material and product specifications, which are codified in Wildtype's master batch records (MBRs) and standard operating procedures (SOPs). A product release system requires quality assurance review of batch records, supplemented by analytical testing as described in response to question 10 above. All raw materials and finished products are tracked using an enterprise resource planning system (Netsuite), allowing traceability of raw materials and finished products.

1. Receiving raw ingredients

Upon receipt of inputs needed for the production process, such as powdered cell culture media and scaffold inputs, Wildtype's production staff validates contents and documents key attributes. Containers showing signs of tampering are rejected. Production inputs are transferred to clean, dry storage spaces until they are used for the production steps described below. There have been no material changes to this step since the July 28, 2023 amendment.

Four potential hazards were identified at this stage. First, pathogens such as *Salmonella and Listeria* could be present in the media and scaffold inputs provided by vendors. This hazard is mitigated by sterile filtration of media (described further in step 2 below), a thermal step carried out in step 8 (formulation of finished food), and ongoing testing of both the cells at the point of harvest (step 6 in Figure 5) and the final food (between steps 9 and 10 above). Our response to question 10 above and question 7 in our January 24, 2024 amendment describe our testing approach in detail.

A second biological hazard concerns the potential for our vendors to ship expired materials. This hazard is controlled with a supply chain preventive control that requires our warehouse and production staff to inspect certificates of analysis (COAs) and expiration dates on each lot of incoming materials prior to accepting.

A third hazard is the potential for undeclared allergens in inputs due to incorrect labeling or the wrong materials sent by a vendor. A supply chain preventive control mitigates this risk by requiring suppliers to pass through a supplier qualification and approval program prior to using the input. COAs are inspected for each lot and a record of the allergen statement from the vendor is reviewed prior to input acceptance. A physical inspection of the material is carried out to ensure the input matches what is disclosed on labels.

A fourth hazard is the potential for shipping materials to be damaged en route to Wildtype, thereby potentially allowing for product contamination in transit. This hazard is mitigated by a process preventive control in which a visual inspection is carried out on all packages upon receipt at Wildtype's warehouse. If damage to an input's primary package has occurred, that lot is rejected and returned to the vendor.

2. Media preparation

The media preparation stage begins by retrieving cleared raw materials that have passed the supply preventive controls described in step 1 above. Inputs are first sorted and weighed. Dry materials are measured and added to water based on exact measurements described in standard operating procedures (SOPs) and master batch records (MBRs), and mixed prior to sterile filtration. Media is sterilized by using a 0.2 µm filter and kept in a sterile vessel (stainless steel or plastic) at 4 °C until the cell proliferation stage commences. There have been no material changes to this step since the July 28, 2023 amendment.

Three potential hazards were identified at this stage. A potential biological hazard exists whereby inadequate sterilization allows the growth of potential pathogens such as *Salmonella* and *Listeria.* This hazard is controlled via strict sterilization requirements outlined in several of Wildtype's MBRs. In the event that sterilization failed, pathogens would outcompete salmon cell growth, which would be detectable in Wildtype's bioreactors (step 5 below) via real-time pH and dissolved oxygen monitoring. Visual inspection using a microscope occurs during sampling from bioreactors as an additional precautionary measure. Adventitious agent testing during steps 6 and 10 is another control.

Second, a potential exists to include the incorrect media components while making media. One of Wildtype's SOPs requires incoming material inspections for all media components, including inspection of COAs and allergen labeling. Media preparation MBRs require confirmation of corresponding lot numbers as well as expiration dates for each input.

A third potential hazard includes the introduction of materials such as metal or glass during the media mixing step. This hazard is mitigated by the sterile filtration step described above; the 0.2 µm filter would remove any potential physical contaminants. Additionally, the final product is passed through an X-ray at the conclusion of step 9.

3. Cell banking

Wildtype's production cell line has a two-tiered cell banking strategy comprising master cell banks (MCB) and working cell banks (WCB). For both MCBs and WCBs, cell lines are stored in boxes that are color coded and labeled to minimize the opportunity for operators to thaw incorrect vials or cryobags. Cryoboxes are then stored at liquid nitrogen temperatures. Wildtype's cell banks are stored both on- and off-site and are continuously monitored for temperature variation beyond designated set points. Since submitting CCC 000005, Wildtype has also implemented an enterprise resource planning software to track all inputs, including cell lines throughout the production process. Before submitting a vial to Wildtype's cell banks, species confirmation is carried out via genetic barcoding or confirmation by cytochrome C oxidase I polymerase chain reaction (PCR) amplification performed on DNA extracted from Wildtype's cell line candidates. All cell line candidates must also clear standard pathogen testing (e.g., bacterial screening) prior to being deposited in either MCBs or WCBs. There have been no material changes to this step since the July 28, 2023 amendment.

Three potential hazards were identified at the cell banking phase. First, microorganisms such as *Salmonella* and *Listeria* may migrate from the operating environment into cell cultures. This hazard is mitigated via the use of aseptic technique and ongoing adventitious agent testing previously discussed. Additionally, prior to being deposited into MCBs and WCBs, vials are tested by a 3rd-party laboratory and confirmed to be contamination-free.

A second potential hazard is the presence of cryoprotectants / freezing agents used in cell banking and persisting into the finished food product. Wildtype's cryoprotectant is included in Figure 1 above, with a safety narrative included in Appendix 3. As noted on page 26 of CCC 000005, we periodically test for the presence of DMSO in the harvested cell material. The analytical report in Appendix 7 for one of the lots presented (pg 60) shows that DMSO in the harvested cell material was below the limit of detection (<50 parts per million).

Third, a potential exists for an operator to thaw the wrong cell line when initiating the seed train. This hazard is mitigated by SOPs requiring MCBs and WCBs to be clearly labeled, and color coded. Additionally, cryoboxes with different potential allergens are not stored in the same liquid nitrogen storage containers.

4. Cell thaw

The cell proliferation stage begins by thawing a vial of cells from the working cell bank and starting the seed train and proliferation process (step 5). There have been no material changes to this step since the July 28, 2023 amendment.

Four potential hazards were identified at this stage. First, microorganisms such as *Salmonella* and *Listeria* may migrate from the operating environment into cell cultures. This hazard is mitigated via aseptic technique, the ongoing adventitious agent testing previously discussed, a subsequent lethal

step (applied during step 8), and regular monitoring of pH and dissolved oxygen levels as indicators of potential contamination.

Second, operators may introduce non-labeled allergens (e.g., by mistakenly thawing a crustacean cell line for a salmon product) by thawing an incorrect cell bank vial. This risk is mitigated by an MBR that requires operators to affix labels from thawed vials to MBR records (which are later verified by quality assurance), as well as a secondary verifier. Additionally, cryoboxes with different allergens are not stored in the same liquid nitrogen storage tanks.

Third, operators may thaw an incorrect cell line (e.g., a Coho salmon line when the specifications call for Chinook salmon) during cell thaw. This risk is mitigated by a detailed SOP that includes step-by-step instructions and controls (including secondary verification) to prevent thawing the incorrect vial.

Fourth, the potential for the presence of cryoprotectants in the finished product have already been discussed in step 3 above.

5. Seed train and proliferation

This is the main biomass accumulation phase. Cell bank vials are thawed into agitated shake flasks and combined with the sterile media prepared in step 2. This step is carried out in a laminar flow hood using aseptic technique. Cells are cultured in a facility subject to good manufacturing practices (GMP) and an environmental monitoring program (EMP) described in CCC 000005 (pages 49-53). After 1-4 weeks of growth, cell cultures in flasks and additional media are transferred via sterile tubing to a 75 liter stainless steel bioreactor. Prior to inoculation, bioreactors are first cleaned using clean-in-place (CIP) protocols, and then sterilized using steam-in-place (SIP) protocols, both of which are described in CCC 000005. After 1-4 weeks of growth, cultures are again sterile transferred to a 400 liter and then a 3,000 liter terminal bioreactor for a final 1-4 weeks in culture. During bioreactor cell culture, real-time pH, temperature, and dissolved oxygen (DO) monitoring alerts operators to potential contamination events. All cell culture processes are tightly controlled with both SOPs and MBRs. There have been no material changes to this step since the July 28, 2023 amendment.

Three potential hazards were identified at this stage. First the potential growth of pathogens such as *Salmonella* and *Listeria* in both flasks and bioreactors is mitigated through both a sanitation preventive control as well as a process preventive control. The sanitation preventive control includes an EMP complemented with rigorous cleaning governed by a regular sanitation schedule and cGMPs (e.g., gowning and boot washing requirements, facility maintenance standards, training, etc.) employed throughout the process. Process preventive controls include the use of MBRs requiring aseptic techniques and monitoring changes to process parameters such as DO and pH as an indication for contamination. DO drops of >30% over an 8-hour period in bioreactors are determined to be at risk for contamination and subjected to further screening, including microscopy. For shake flasks, turbidity is visually inspected at least five times a week as a sign for contamination. Contaminated cultures are immediately terminated. Ongoing adventitious agent testing at both steps 6 and 10 in Figure 5 are an additional control.

Second, there is a risk that CIP agents may not be adequately rinsed and removed following CIP processes. Cleaning chemical removal is verified by collection of final rinse samples, which are tested for pH, conductivity, adenosine triphosphate (ATP), as well as a visual inspection. Cleaning verification is performed as part of each bioreactor cleaning. Passing results are required before releasing equipment for the next production run. Additionally, Wildtype limits its use of clean-in-place agents to permissible chemicals widely used in the food and beverage industry in the United States.

Third, metal-to-metal contact or broken glass probes inside a bioreactor may produce metal or glass fragments. This hazard is managed by a process preventive control requiring all finished food products to be subjected to X-ray screening at the conclusion of step 9 illustrated in Figure 5 above.

6. Cell harvest from bioreactors & freezing

The harvest process begins when cells are collected from the terminal bioreactor via bowl centrifugation and washed three times with a water and sugar solution to rinse away residual cell culture medium. Following the rinsing step, cells are subjected to the analytical testing described on pages 6-7 of our January 24, 2024 amendment and in response to question 10 above. Cells that do not meet the specifications in Figure 4 above are discarded. Cells that meet testing specifications are frozen at ≤-20 °C and kept frozen until needed for process steps 7-10 as depicted in Figure 5.

There has only been one material change to the stepwise hazard analysis and preventive controls presented on pages 11-20 of our July 28, 2023 amendment. A new potential hazard has been added to step 6, “cell harvest and freezing,” describing the potential for media inputs without relevant authorization to be present in the harvested cell material. As discussed in our response to question 1 above, calculations for the "worst case" pre-rinse scenario in Figure 1 above indicate that inputs without applicable authorization are below NOAEL levels with a MOS >100, or below ADI, OSL, or background dietary intake levels.

This potential hazard has been added to our stepwise hazard analysis in Appendix 6 (red text illustrates material changes; all else remains the same vis-à-vis the hazard analysis and preventive controls presented on pages 11-20 of our July 28, 2023 amendment). Note that the stepwise hazard analysis in Appendix 6 now stops at the point of harvest from the bioreactor consistent with the scope of this consultation. Cells are frozen at the conclusion of the cell harvest process, rendering them non-viable.

CCC 000005 (pg 37) described the finished product being rinsed three times with buffered saline. This process has been moved to step 6. Additionally, a water and sugar solution is used in lieu of buffered saline to wash cells.

Two potential hazards were identified at this stage. First, pathogens such as *Salmonella* and *Listeria* may be introduced into the harvested cell material during the harvest stage. This potential hazard is mitigated by aseptic technique, a thermal process conducted during step 8 (described above in response to question 11) as well as adventitious agent testing occurring following the rinse step, but prior to freezing the cells and again at the conclusion of step 10 as outlined in Figure 5.

Second, several inputs used in Wildtype’s cell culture medium do not have an applicable authorization in the United States. As illustrated in Figure 1, before the washing step, these inputs are below NOAEL levels with a MOS >100, or below ADI, OSL, or background dietary intake levels.

As a result of this change, we collected analytical data (nutritional and microbiological) to ensure product quality was not impacted. Those data are provided in Figures 6 and 7 below. COAs are provided in Appendix 7.

No changes have been made to the testing strategy outlined in our January 24, 2024 amendment beyond the addition of an *E. coli* O157 panel to our standard testing scope as described in our response to question 10 above. No other material changes have occurred.

Figure 6 - Proximates, fatty acids, and micronutrients for cells at the point of harvest from bioreactor

Parameter	Method[95]	Specification	Lot 1: 023 2024-05-08-01	Lot 2: 024-2024-0606-01	Lot 3: 024-2024-06-06-02
Calories (per 100g)	CFR - Atwater calculation	30 - 100 kcal	31 kcal	66 kcal	72 kcal
Total fat	AOAC 954.02 (Eurofins) AOAC 948.15 (Merieux)	0.5 - 10%	1.13%	1.41%	1.75%
Protein	AOAC 990.03(Eurofins) AOAC 991.20 (Merieux)	5 - 25%	5.54%	6.34%	7.79%
Carbohydrates	CFR 21 - Calculated	<10%	0.75%	6.98%	7.60%
Ash	AOAC 942.05 (Eurofins) AOAC 938.08 (Merieux)	<5%	0.61%	0.49%	0.52%
Moisture	AOAC 925.09 (Eurofins) AOAC 950.46A, 926.08 (Merieux)	75 - 95%	92.4%	84.8%	82.9%
Saturated fat	AOAC 996.06	<2%	0.16%	0.22%	0.28%
Monounsaturated fat	AOAC 996.06	<5%	0.36%	0.44%	0.51%
Polyunsaturated fat	AOAC 996.06	<5%	0.11%	0.16%	0.21%
Trans fat	AOAC 996.06	<1%	0.04%	0.05%	0.06%
Triglycerides	AOAC 996.06	<5%	0.69%	0.92%	1.16%
Total omega 3 isomers	AOAC 996.06	<2%	<0.01%	<0.01%	0.01%
Vitamin A (per 100g)	AOAC 974.29 (Eurofins) Analyst(1984)109:489 (Merieux)	<50 IU	<13.32 IU	<13.32 IU	<13.32 IU
Vitamin B5 (per 100g)	AOAC 945.74 (Eurofins) AOAC 960.46 & Kit (Merieux)	<5 mg	0.74 mg	0.40 mg	0.33mg
Folate (per 100g)	AOAC 992.05 (Eurofins) AOAC 960.46 & Kit (Merieux)	<1 mg	0.07 mg	0.02 mg	0.02 mg
Vitamin B_{12} (per 100g)	AOAC 952.20 (Eurofins) AOAC 960.46 & Kit (Merieux)	<200 µg	139 µg	90 µg	101 µg
Vitamin D_2 & D_3 (per 100g)	Huang et al. Rapid Commun, *Mass Spectrum* 2014, 28 (Eurofins) AOAC 2016.05 Mod. (Merieux)	<1,500 IU	544 IU (13.6 µg)	248 IU (6.2 µg)	291 IU (7.28 µg)

[95] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieuex). COAs may be found in Appendix 7.

Figure 7: Heavy metals & adventitious agents of concern for cells at the point of harvest

Parameter	Method[96]	Specification[97]	Lot 1: 023 2024-05-08-01	Lot 2: 024-2024-0606-01	Lot 3: 024-2024-06-06-02
Aerobic plate count	AOAC 966.23(Eurofins) AOAC 966.23 (Merieux)	<100 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Yeast	FDA BAM Ch. 18 (Eurofins) FDA-BAM, 7th ed. (Merieux)	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Mold	FDA BAM Ch. 18 (Eurofins) FDA-BAM, 7th ed. (Merieux)	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Coliforms	AOAC OMA 991.14	<100 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
E. coli	AOAC OMA 991.14	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
***E. coli* O157:H7**	AOAC-RI 031002	Negative/25g	Negative/25g	Negative/25g	Negative/25g
Enterobacteriaceae	AOAC OMA 2003.01 / USP 37 <61>	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Staphylococcus aureus	AOAC OMA 2003.07(Eurofins) AOAC 975.55 (Merieux)	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
***Bacillus cereus* organism**	FDA BAM Ch. 14 (Eurofins) AOAC 980.31 (Merieux)	<100 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Salmonella spp	AOAC OMA 2011.03 (Eurofins) AOAC 2004.03 (Merieux)	Negative/25g	Negative/25g	Negative/25g	Negative/25g
***Listeria* genus**	AOAC OMA 2013.10 (Eurofins) AOAC 2019.10 (Merieux)	Negative/25g	Negative/25g	Negative/25g	Negative/25g
***Campylobacter* spp screen**	AOAC RI 051201	Negative/25g	Negative/25g	Negative/25g	Negative/25g
***C. botulinum* organism**	FDA-BAM, 8th ed.	Negative /8g	Negative /8g	Negative /8g	Negative /8g
***C. perfringens* organism**	ISO 7937; AOAC 976.30	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Arsenic	AOAC 2011.19, 993.14 and 2015.01	<100 ppb	20 ppb	30 ppb	30 ppb
Cadmium	AOAC 2011.19, 993.14 and 2015.011	<20 ppb	<20 ppb	<20 ppb	<20 ppb
Lead	AOAC 2011.19, 993.14 and 2015.01	<20 ppb	<10 ppb[98]	<10 ppb	<10 ppb
Mercury	AOAC 2011.19, 993.14 and 2015.01	<20 ppb	<5 ppb	<5 ppb	<5 ppb

13. For addition to the DSN, if changes in the facility setup, process, hazard analysis, and food safety plan have been implemented since the submission of the July 28, 2023, amendment, please provide updated summaries of sanitation control program and environmental monitoring program in the facility, including but not limited to the biosafety cabinet and bioreactor. Please provide your approach of root cause analysis, corrective action, verification, and validation if contamination occurs. In addition, please explain how you ensure that no biofilm formation would occur in the equipment and facility and provide a summary of your risk mitigation strategy with emerging environmental microbial contaminants.

The change described in question 12 above does not change our approach to our sanitation control program, environmental monitoring program (including biosafety cabinets and bioreactors), root cause analysis, corrective actions, verification, and validation if contamination occurs. Our approach to these topics described in CCC 000005 and subsequent amendments has not changed. Additionally, biofilm formation continues to be mitigated with clean-in-place and steam-in-place techniques described in CCC 000005 and subsequent amendments. Our risk mitigation strategy for environmental

[96] All methods are validated for their intended purposes and are carried out by an accredited external laboratory (e.g., Aemtek, Eurofins, Mérieuex).
[97] References for methods and specifications were provided in Figure 5 of the January 17, 2023 amendment.
[98] Limit of detection for lead = 10 ppb

contaminants via our environmental monitoring program remains consistent with that described in CCC 000005 and subsequent amendments.

Points of clarification

Product characterization

1. In Figure 2 of the January 24, 2024, amendment, you list the analytical method for Clostridium perfringens organism as ISO 7937. We note that this method has been withdrawn, revised, and replaced with ISO 15213-2:2023, Microbiology of the Food Chain, Horizontal Method for the Detection and Enumeration of Clostridium spp.: Part 2: Enumeration of Clostridium perfringens by Colony-Count Technique. For the administrative record, please clarify this discrepancy.

None of the three testing laboratories we use (two of which are large, international labs) have adopted ISO 1523-2:2023 as an option for testing *C. perfringens.* Our primary testing lab is currently using AOAC 976.30.

2. Page 7 of the January 24, 2024, amendment states "Microorganism testing for B. cereus, C. perfringens, and C. botulinum was selected because the organisms are a necessary precursor to the associated toxins." The COAs provided in the same amendment include analysis of C. botulinum toxin being performed (e.g., page 46). For addition to the disclosable safety narrative, please clarify whether analytical testing for the presence of C. botulinum toxin is also performed (in addition to analytical testing for the presence of C. botulinum itself).

Analytical testing for the presence of *C. botulinum* toxin is not performed because the organism is a necessary precursor to the associated toxin.

Appendix 3: safety discussion for other media inputs without an applicable authorization

Poloxamer 188 (CASRN 9003-11-6 generic CASRN for all Poloxamers)

Poloxamer 188 is a synthetic block copolymer of ethylene oxide and propylene oxide. Poloxamers function as surfactants, emulsifying agents, cleansing agents, or solubilizing agents in cosmetic products. The safety of Poloxamer 188 was comprehensively reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel[99] and is summarized herein. Other toxicity data associated with Poloxamer 188 and the generic CASRN 9003-11-6 were also considered.

Poloxamer 188 is a biocompatible block copolymer composed of repeating units of polyethylene oxide and polypropylene oxide.[100] It is also a common component in many over the counter products including laxatives, toothpaste, and cosmetics. In a systematic review conducted by Chen et al.,[101] no data on the safety and potential toxicity of Poloxamer 188 were found in the literature.

A 60-minute loading dose of 300 mg/kg bw was administered followed by a 47-hour maintenance infusion of 30 mg/kg/h. Poloxamer 188 was well tolerated with no clinically significant differences in adverse effects or other safety measures observed between the treated group and the placebo groups.[102]

In 1999, SCF evaluated polyethylene glycol and polypropylene glycol for use in food contact materials and classified in List 2 with a tolerable daily intake (TDI) of 5 mg/kg bw and list 3 as "toxicologically acceptable," respectively.[103] The SCF did not evaluate Poloxamer 188.

Following IV injection in dogs, approximately 80% of the Poloxamer 188 dose was recovered in the urine after a 24 hour period. Labeled Poloxamer 188 was found in all tissues examined with the highest concentration found in the bile, lung, and liver.[104]

In male volunteers aged 19-35, single IV infusions of Poloxamer 188 or placebo were administered for up to 72 hours. The most frequently reported adverse reactions were pain, injection site redness or swelling, and nausea. Mild to moderate and reversible elevations in alanine aminotransferase and aspartate transaminase were more common in Poloxamer-treated individuals compared to placebo. Poloxamer 188 was eliminated 72-94% via the urine.[105]

Acute oral toxicity of Poloxamer 188 was demonstrated to be low in rats with an LD_{50} of 9,380 mg/kg bw.[106] In humans, Poloxamer 188 was demonstrated to be safe when given for up to 72 hours and well tolerated upon repeated exposure in over the counter products.[107]

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[99] Cosmetic Ingredients Review[CIR]. Sigh-Joy S and McLain VC (2008). Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. Int J Toxicol.;27 Suppl 2:93-128.
[100] Chen et al. (2022). Poloxamer 188 (P188), A Potential Polymeric Protective Agent for Central Nervous System Disorders: A Systematic Review. Current Neuropharmacology, 20: 799-808. DOI: 10.2174/1570159X19666210528155801
[101] ibid
[102] Adams-Graves P, Kedar A, Koshy M, Steinberg M, Veith R, Ward D, Crawford R, Edwards S, Bustrack J, Emanuele M. (1997). RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. Blood, 1;90(5):2041-6. PMID: 9292541.
[103] European Food Safety Authority (EFSA) (2006). Opinion of the Scientific Panel on food additives, flavourings, processing aids, and material in contact with food (AFC) on a request related to an 11th list of substances for food contact materials. EFSA Journal (2006) 316 to 318; 1-10.
[104] Willcox ML, Newman MM, and Paton BC. (1978). A study of labeled pluronic F68 after intravenous injection into the dog. J. Surg. Res. 25:349–356.
[105] Jewell RC, Khor SP, Kisor DF, LaCroix KA, and Wargin WA. (1997). Pharmacokinetics of RheothRx injection in healthy male volunteers. J. Pharm. Sci. 86:808–812.
[106] Drugbank (2021). Poloxamer 188. DB11333. Date accessed July 18, 2023 at https://go.drugbank.com/drugs/DB11333
[107] Moloughney JG and Weisleder N (2012). Poloxamer 188 (P188) as a Membrane Resealing Reagent in Biomedical Applications. Recent Pat Biotechnol, 6(3):200-211.

Leaf[108] administered Poloxamer 188 dissolved in water or corn oil at a dose range of 2 to 15 g/kg bw to albino rats. The oral LD_{50} for Poloxamer 188 was >15 g/kg.

Leaf also conducted 6-month feeding studies in rats and dogs, in which groups of 45 rats were administered 0, 3, or 5% Poloxamer 188 by weight in the diet, and four dogs per group received 0, 50, or 100 mg/kg bw/day of Poloxamer 188 in capsule form prior to feeding. Overall results from both species did not reveal any toxicologically significant effects from dietary exposure to Poloxamer 188.[109] No further details of these studies were available.[110]

In a drinking water study with high molecular weight polyethylene propylene glycol (CASRN 9003-11-6) (11500 D; 70% ethylene glycol), no adverse effects were reported in rats. The NOAEL was 15,000 ppm, corresponding to approximately 1,140 mg/kg bw/day in male and 1,560 mg/kg bw/day in female rats.[111] Toxicokinetic data in dogs indicate that there is substantial absorption from the gastrointestinal tract.[112]

In two-year feeding studies of Poloxamer 188 administered in rats at doses of 0, 3, 5, and 7.5%, moderate diarrhea was observed at the two highest doses and minimally decreased growth at the highest tested dose. No other adverse treatment-related effects or effects on survival were reported.[113] No further details of this study were available. Assuming the dietary concentrations in the two-year feeding study in rats are equivalent to 30,000, 50,000, and 75,000 ppm, the calculated equivalent doses based on dose conversion for older adult rats (0.05 mg/kg bw/day per 1 ppm diet), are 1,500, 2,500, and 3,750 mg/kg bw/day Poloxamer 188 based on WHO methods.[114]

No genotoxicity data was found for Poloxamer 188.

Polyethylene propylene glycols (CASRN 9003-11-6 and 106392-12-5) with MW 3,000-5,000 Da demonstrated an equivocal potential for gene mutation induction in bacteria and mammalian cells *in vitro*, but no clastogenicity was observed. In vivo sister chromatid exchange assays in mammalian cells (Chinese hamster bone marrow cells) were negative. Based on the available *in vitro* and *in vivo* genotoxicity test data, including a poloxamer considered to be a worst case substance for toxicity, poloxamers demonstrate no genotoxic potential.[115]

The CIR Panel concluded that poloxamers including Poloxamer 188 have a low order of toxicity. Studies on carcinogenicity and reproductive and developmental toxicity were not identified for Poloxamer 188. The available toxicological data do not suggest any concerns for carcinogenesis or significant exposure to reproductive organs or to the developing fetus. A NOAEL was not established for any of the studies reported in the CIR safety assessment.[116]

Safety Assessment: the level at which no adverse effects were observed in a two-year feeding study in rats, 3,750 mg/kg bw/day, can be used in risk assessment of Poloxamer 188. Wildtype's pre-wash EDI for Poloxamer 188 is 7.05 mg/kg bw/day and well below the chronic NOAEL, resulting in a MOS of 496.

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[108] Leaf CW. (1967). Toxicology of some non-ionic surfactants. Soap Chem. Spec. 43:48 [as cited in CIR, 2008].
[109] *Ibid.*
[110] Cosmetic Ingredients Review[CIR]. Sigh-Joy S and McLain VC (2008). Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. Int J Toxicol.;27 Suppl 2:93-128.
[111] European Food Safety Authority (EFSA) (2006). Opinion of the Scientific Panel on food additives, flavourings, processing aids, and material in contact with food (AFC) on a request related to an 11th list of substances for food contact materials. EFSA Journal (2006) 316 to 318; 1-10.
[112] *Ibid.*
[113] Leaf CW. (1967). Toxicology of some non-ionic surfactants. Soap Chem. Spec. 43:48 [as cited in CIR, 2008].
[114] World Health Organization [WHO] (2000). Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. Geneva, December 2000.
[115] European Food Safety Authority (EFSA) (2006). Opinion of the Scientific Panel on food additives, flavourings, processing aids, and material in contact with food (AFC) on a request related to an 11th list of substances for food contact materials. EFSA Journal (2006) 316 to 318; 1-10.
[116] Cosmetic Ingredients Review[CIR]. Sigh-Joy S and McLain VC (2008). Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. Int J Toxicol.;27 Suppl 2:93-128.

Additionally, we note that the use of Poloxamer 188 in the manufacture of a cell cultivated product was previously addressed in CCC 000001 (pages 54-58). Therefore, there is no safety concern under our intended conditions of use.

D-Glucuronolactone (CASRN 32449-92-6)

D-Glucuronolactone is a naturally-occurring metabolite of glucose that is an important structural component of connective tissues. A scientific opinion paper on the safety of the use of glucuronolactone in energy drinks in 2009 established a NOAEL from a 13-week oral gavage toxicity study of D-glucuronolactone in rats, with specific focus on the kidneys.[117] This study used the same rat strain as the previous study reported in the Scientific Committee on Food (SCF) Opinion of 2003.[118] Extensive urinalysis and histopathological examinations demonstrated no treatment-related effects. Based on the results of this study, the NOAEL for daily oral administration of D-glucuronolactone in rats was 1,000 mg/kg bw/day, the highest dose tested.[119]

Safety assessment: the calculated pre-wash EDI for D-Glucuronolactone is 0.00221 mg/kg bw/day, well below NOAEL, with a MOS >450,000, therefore, there is no safety concern under our intended conditions of use.

N-acetyl-glucosamine (CASRN 7512-17-6)

To evaluate the safety of *N*-acetyl-glucosamine a literature search was conducted in May 2024 to identify information pertinent to the toxicological potential of this substance. The searches were conducted in PubMed as well as publicly available databases, including FDA, EPA, NTP, FAO/WHO, JECFA, ECA, and EFSA. Safety assessments conducted by regulatory authorities are presented first, followed by a summary of the available preclinical toxicological data in the peer reviewed literature.

The safety of *N*-acetyl glucosamine and glucosamine are considered together due to structural similarities based on the amino monosaccharide core group. Glucosamine is an amino monosaccharide that is an essential component of mucopolysaccharides and chitin.[120] Glucosamine and its derivative *N*-acetylglucosamine are endogenously synthesized from glucose. Extensive reviews of glucosamine conclude that oral glucosamine is safe and well tolerated based on animal and human studies.[121, 122, 123] Key safety information of *N*-acetyl-glucosamine and glucosamine that have been documented in these reviews are summarized below.

No absorption, distribution, metabolism, excretion (ADME) data are available for this compound in the reviewed literature. The hydrochloride salt of glucosamine was reported to be rapidly absorbed following oral ingestion in humans and dogs.[124]

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[117] EFSA. 2009. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on a request from the Commission on the use of taurine and D-glucurono-γ-lactone as constituents of the so called "energy" drinks. EFSA J. 935: 1-31. https://doi.org/10.2903/j.efsa.2009.935
[118] Opinion of the Scientific Committee on Food on Additional information on "energy" drinks, European Commission Health & Consumer Protection Directorate-General, (expressed on 5 March 2003), accessed via this link in August 2024.
[119] EFSA. 2009. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on a request from the Commission on the use of taurine and D-glucurono-γ-lactone as constituents of the so called "energy" drinks. EFSA J. 935: 1-31. https://doi.org/10.2903/j.efsa.2009.935
[120] Anderson JW, Nicolosi RJ, and Borzelleca JF. (2005). Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, 43(2), 187–201. https://doi.org/10.1016/j.fct.2004.11.006.
[121] *Ibid.*
[122] European Food Safety Authority (EFSA) (2006). Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission on the safety of glucosamine hydrochloride from *Aspergillus niger* as food ingredient. The EFSA Journal (2009) 1099, 1-19. Available at: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.1099.
[123] Cosmetic Ingredient Review (CIR) Panel. (2022). Safety Assessment of Glucosamine Ingredients as Used in Cosmetics. CIR Expert Panel. Scientific Literature Review for Public Comment. Release Date: 11 February 2022.
[124] *Ibid.*

Low acute oral toxicity was reported with LD_{50} values for glucosamine are >5,000 mg/kg bw in mice, and >8,000 mg/kg bw in rats and rabbits.[125] An LD_{50} >15,000 mg/kg bw in mice was reported for glucosamine hydrochloride.[126]

A 13-week study of *N*-acetyl glucosamine was conducted in F344 rats (10 rats/sex/group) that were fed pelleted diets containing 0, 0.625, 1.25, 2.5, or 5% of *N*-acetyl glucosamine.[127] Measured parameters included clinical signs, food intake, hematology, serum biochemistry, and histopathology. No mortality was reported. Overall, no treatment-related adverse or toxicologically significant effects were observed. Thus, the NOAEL was determined to be >5% equivalent to 2,476 and 2,834 mg/kg bw/day for male and female rats respectively.

Echard et al.[128] administered 0.5% w/w glucosamine hydrochloride (~300 mg/kg bw/day) in the diet of 8 male spontaneously hypertensive rats and 8 male Sprague–Dawley rats for 9 weeks. Compared to a basal diet, there were no treatment-related effects on blood analyses (e.g., serum alanine aminotransferase, aspartate aminotransferase and blood urea nitrogen) or organ histology in either tested strain of rat.

In an unpublished 26-week dietary study,[123] dogs that received glucosamine sulfate at doses ranging from 159-2,149 mg/kg bw/day did not demonstrate any adverse effects up to the highest tested dose.

In a 52-week study, F344 rats (n=10/sex/group) were fed a diet with levels of 0, 1.25, 2.5, or 5% *N*-acetyl glucosamine. No mortality, clinical signs and other significant adverse effects associated with treatment were observed at any dose. A NOAEL was identified at the highest dose of 5% in both sexes, equivalent to 2,323 and 2,545 mg/kg/day in males and females, respectively.[129]

In an unpublished 1-year dietary study,[123] Sprague-Dawley rats that received glucosamine sulfate at doses ranging from 300-2,700 mg/kg bw did not result in any adverse effects up to the highest tested dose.

In an Ames assay (OECD TG 471) *S. typhimurium* strains TA 1537, TA 1535, TA 98, TA 100, and TA 102 were exposed to *N*-acetyl glucosamine at concentrations of 156.25, 312.5, 625, 1,250, 2,500, and 5,000 µg/plate, with and without metabolic activation in triplicates for 48 hours. No genotoxicity was observed at any dose with and without metabolic activation.[130]

Glucosamine hydrochloride (derived from *Aspergillus niger*) is non-mutagenic in an Ames assay at doses up to 5,000 µg per plate, with and without metabolic activation.[131] Negative results were also reported for *Aspergillus niger*-derived glucosamine hydrochloride in an in vivo micronucleus assay conducted in mice.[132]

In a 16-week safety assessment study, human subjects (n=22/group) were given 500 mg/day or 1,000 mg/day *N*-acetyl glucosamine. Adverse effects observed were mild. Routine physical and

[125] Anderson JW, Nicolosi RJ, and Borzelleca JF. (2005). Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, 43(2), 187–201. https://doi.org/10.1016/j.fct.2004.11.006.
[126] *Ibid.*
[127] Lee KY, Shibutani M, Takagi H, et al. (2004). Subchronic toxicity study of dietary N-acetylglucosamine in F344 rats. Food Chem Toxicol, 42(4):687-695.
[128] Echard BW, Talpur NA, Funk KA, Bagchi D, Preuss HG. (2001). Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats. Mol Cell Biochem, 225(1):85-91.
[129] Takahashi M, Inoue K, Yoshida M, Morikawa T, Shibutani M, Nishikawa A. (2008). Lack of chronic toxicity or carcinogenicity of dietary N-acetylglucosamine in F344 rats. Food Chem Toxicol., 47(2):462-71. doi: 10.1016/j.fct.2008.12.002. Epub 2008 Dec 10. PMID: 19103248.
[130] Cosmetic Ingredient Review (CIR) Panel. (2022). Safety Assessment of Glucosamine Ingredients as Used in Cosmetics. CIR Expert Panel. Scientific Literature Review for Public Comment. Release Date: 11 February 2022.
[131] *Ibid.*
[132] *Ibid.*

cardiovascular evaluation, hematology, and blood chemistry did not show any other significant abnormalities.[133]

Safety assessment: based on the "worst case" calculations presented in Figure 1, the pre-wash EDI is 0.00482 mg/kg bw/day, which is well below the lowest chronic NOAEL of 2,323 mg/kg bw/day in male rat for *N*-acetyl-glucosamine, with MOS >480,000. Therefore, there is no safety concern under our intended conditions of use.

Sodium selenite / selenium (CASRN 10102-18-8)

Naturally occurring selenium in foods, such as fish, Brazil nuts, eggs and cereals, is primarily incorporated as amino acids (i.e. L-selenomethionine and L-selenocysteine). Inorganic selenium is present as both selenite and selenate in the diet through supplementation. Sodium selenite has selenium present in the +4 oxidation state, which will oxidize gradually to result in selenium in the more stable +6 oxidation state to form sodium selenate.[134]

Subchronic studies[135] in rats reported a NOAEL of 0.4 mg selenium/kg bw/day (0.88 mg/kg bw/day sodium selenite)[136] based on mortality, body weight depression, decreased water consumption, and renal papillary lesions and a NOAEL of 0.9 mg selenium/kg bw/day (2 mg/kg bw/day sodium selenite[137]) in mice based on body weight depression and decreased water consumption, after dietary exposure to sodium selenite for 90 days. A NOAEL of 4 ppm (0.2 mg/kg bw/day[138]) for sodium selenite was identified in a chronic-duration study reported in the National Toxicology Program (NTP) where rats were subjected to dietary exposure.[139] Oral exposure to sodium selenite did not have an adverse effect on reproduction and development. Sodium selenite has the potential to be genotoxic at high doses, however, there is a narrow concentration range that elicits mutagenicity but not lethality. This makes it ambiguous to determine the genotoxic potential of sodium selenite, which is further bolstered by the conflicting results reported in the database for selenium genotoxicity. Additionally, no carcinogenicity was reported after oral exposure to sodium selenite for chronic duration in the NTP (1994) study.

Several clinical studies have investigated the effects of formula with selenium provided as sodium selenite compared to formula without added selenium and/or breastmilk.[140, 141, 142, 143, 144, 145] Formulas with added sodium selenite provided a total of 16 to 34 µg/L selenium (2.4 to 5.1 µg/100 kcal) from intrinsic and added sources combined. In most studies, the non-supplemented formulas were reported to provide 3 to 5 µg/L selenium (0.4 to 0.7 µg/100 kcal) from intrinsic sources, though one study reported

[133] Kubomura D, Ueno T, Yamada M, Tomonaga A, Nagaoka I. Effect of N-acetylglucosamine administration on cartilage metabolism and safety in healthy subjects without symptoms of arthritis: A case report. Exp Ther Med. 2017 Apr;13(4):1614-1621. doi: 10.3892/etm.2017.4140. Epub 2017 Feb 21. PMID: 28413518; PMCID: PMC5377572.
[134] National Toxicology Program (NTP). 1994. NTP Technical Report on Toxicity Studies of Sodium Selenate and Sodium Selenite. NIH Publication 94-3387, July 1994.
[135] *Ibid.*
[136] Weight of sodium selenite for rats = Weight of selenium (0.4 mg) * (1 g / 1000 mg) * (1 mole / 78.97g selenium) * 172.95 g selenite / 1 mole) = 0.88 mg / kg / day
[137] Weight of sodium selenite for mice = Weight of selenium (0.9mg) * (1g / 1000mg) * (1 mole / 78.97 g selenium) * (172.95g selenite / 1 mole) = 2 mg / kg / day
[138] Mg/kg bw/day estimated based on Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives (2000) (4 ppm * 0.05 mg/kg bw/day = 0.2 mg/kg bw/day)
[139] Harr et al. 1967, as cited in NTP, 1994
[140] Darlow BA, Inder TE, Sluis KB, Nuthall G, Mogridge N, Winterbourn CC. Selenium status of New Zealand infants fed either a selenium supplemented or a standard formula. J Paediatr Child Health. 1995;31(4):339-44.Dennert G, Zwahlen M, Brinkman M, Vinceti M, Zeegers MP, Horneber M. 2011. Selenium for preventing cancer. Cochrane Database Syst Rev. (5):CD005195.
[141] Johnson CE, Smith AM, Chan GM, Moyer-Mileur LJ. Selenium status of term infants fed human milk or selenite-supplemented soy formula. J Pediatr. 1993;122(5 Pt 1):739-41.
[142] Kumpulainen J, Salmenperä L, Siimes MA, Koivistoinen P, Lehto J, Perheentupa J. Formula feeding results in lower selenium status than breast-feeding or selenium supplemented formula feeding: a longitudinal study. Am J Clin Nutr. 1987;45(1):49-53.
[143] Litov RE, Sickles VS, Chan GM, Hargett IR, Cordano A. Selenium status in term infants fed human milk or infant formula with or without added selenium. Nutr Res 1989;9:585-96.
[144] Lönnerdal B, Hernell O. Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace element fortified milk-based infant formula. Acta Paediatrica. 1994;83(4):367-73.
[145] McGuire MK, Burgert SL, Milner JA, Glass L, Kummer R, Deering R, Boucek R, Picciano MF. Selenium status of infants is influenced by supplementation of formula or maternal diets. Am J Clin Nutr. 1993;58(5):643-8.

selenium content of 13 to 15 µg/L (1.9 to 2.2 µg/100 kcal) from intrinsic sources (Litov et al., 1989). The identified clinical studies were largely conducted in infants prior to mandatory inclusion of selenium in infant formula, with study durations ranging from 2 to 10 months, and consumption of the supplemented formulas commencing from birth to 6 weeks of age.

Overall, there were no reports of adverse events in infants fed formulas with added sodium selenite, and no reported untoward effects on tolerance or growth. Plasma selenium was significantly lower in infants fed formula without sodium selenite supplementation compared to breastfed and infants fed formula with supplementary sodium selenite.[146, 147] One study found significantly lower plasma and erythrocyte selenium levels in infants fed with a sodium selenite fortified formula compared to breastfed infants; however, plasma and erythrocyte glutathione peroxidase activities were comparable between groups, suggesting that the physiologic requirement for selenium was being met.[148] In other studies, selenium status as assessed by serum glutathione peroxidase activity was similar in infants fed formula fortified with selenium compared to breastfed infants, but lower in infants fed unfortified formula.[149, 150] The results from these studies suggest that supplementary selenium in the form of sodium selenite is required for formula fed infants in order to ensure comparable selenium status to breastfed infants.

Selenium is an essential mineral with the recommended dietary allowance (RDA) of 55 µg/day for males and females 14 years of age or older and in the range of 20-40 µg/day for children aged 1 to 13 years.[151] While selenium is an essential mineral, excessive intake can be toxic. The Institute of Medicine (IOM) conducted a risk assessment of dietary selenium in the course of developing DRIs.[152] Adverse effects reported from high intakes of selenium included selenosis (hair and nail brittleness and loss), gastrointestinal disturbances, skin rash, garlic-breath odor, fatigue, irritability, and nervous system abnormalities. Hair and nail brittleness and loss were selected as the critical endpoints on which to base a tolerable upper intake level (UL) as these signs and symptoms of chronic selenosis were reported more frequently than others. Specifically, the IOM chose to use the data reported by Yang and Zhou (1994)[153] to determine the dose-response of selenium toxicity from food sources.[154] Based on this study, the IOM established the NOAEL of selenium intake as 800 µg per day and applied an uncertainty factor of 2 to derive a UL of 400 µg per day for selenium from food and supplements. The IOM ULs for selenium established for children and adolescents range from 90 to 400 µg/day.

Along with the IOM, the US EPA evaluated the available health information on selenium.[155] Their assessment provides an oral reference dose (RfD) which is an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The EPA reported a NOAEL of 853 µg/day and applied an uncertainty factor of 3 to account for sensitive individuals and derived a reference dose (RfD) of 5 µg/kg bw/day. The Agency for Toxic Substances and Disease Registry (ATSDR) also derived a chronic

[146] *Ibid.*
[147] Kumpulainen J, Salmenperä L, Siimes MA, Koivistoinen P, Lehto J, Perheentupa J. Formula feeding results in lower selenium status than breast-feeding or selenium supplemented formula feeding: a longitudinal study. Am J Clin Nutr. 1987;45(1):49-53.
[148] Johnson CE, Smith AM, Chan GM, Moyer-Mileur LJ. Selenium status of term infants fed human milk or selenite-supplemented soy formula. J Pediatr. 1993;122(5 Pt 1):739-41.
[149] Lönnerdal B, Hernell O. Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace element fortified milk-based infant formula. Acta Paediatrica. 1994;83(4):367-73.
[150] McGuire MK, Burgert SL, Milner JA, Glass L, Kummer R, Deering R, Boucek R, Picciano MF. Selenium status of infants is influenced by supplementation of formula or maternal diets. Am J Clin Nutr. 1993;58(5):643-8.
[151] Institute of Medicine (US). 2000. Panel on Dietary Antioxidants and Related Compounds. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington (DC): National Academies Press (US); 2000. 7, Selenium.
[152] *Ibid.*
[153] Yang G, Zhou R. Further observations on the human maximum safe dietary selenium intake in a seleniferous area of China. J Trace Elem Electrolytes Health Dis. 1994 Dec;8(3-4):159-65. PMID: 7599506.
[154] Institute of Medicine (US). 2000. Panel on Dietary Antioxidants and Related Compounds. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington (DC): National Academies Press (US); 2000. 7, Selenium.
[155] U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS). 1992b. Chemical Assessment Summary for Selenious acid; CASRN 7783-00-8.

minimum risk level (MRL) at 5 µg/kg bw/day; both values were based on the Chinese human cohort exposed to selenium in soil and the food supply.[156]

Safety Assessment: A NOAEL of 4 ppm (0.2 mg/kg bw/day) for sodium selenite was identified in a chronic-duration study where rats were subjected to dietary exposure (Harr et al. 1967, as cited in NTP, 1994). Wildtype's estimated pre-wash daily intake (EDI) for sodium selenite is 1.01E-04 mg/kg bw/day. The margin of safety is therefore large (MOS = 0.2 mg/kg bw day/ 0.000101 mg/kg bw/day = 1,980). Further, Wildtype's EDI on a selenium basis (0.22 µg/kg bw/day) is well below the EPA RfD and ATSDR MRL of 5 µg/kg bw/day; and well below the IOM - UL range for selenium of 90 – 400 µg/day (Wildtype's EDI = 4.42 µg/day). Sodium selenite is a safe source of selenium in infant formulas. Therefore, there is no safety concern under our intended conditions of use.

Thiamine diphosphate (CASRN 154-87-0)

Thiamine diphosphate also known as cocarboxylase is the active form of vitamin B_1 and an important dietary supplement. Estimated food intake of vitamin B_1 (97.5th percentile) in some European countries varied from 1.90 mg/day to 6.35 mg/day.[157] In US adults aged 20 and older, the average daily thiamine intake from foods and supplements is 4.89 mg in men and 4.90 mg in women.[158] EFSA evaluated the use of thiamine diphosphate as a food supplement and determined that there is no safety concern when use at up to 100 mg/day corresponding to 1.7 mg/kg bw/day.[159]

Safety assessment: pre-wash EDI is 0.0534 mg/day, well below EFSA's safe intake level and well below US background dietary intake. Therefore, there is no safety concern under our intended conditions of use.

Methyl-β-cyclodextrin (CASRN 128446-36-6)

To evaluate the safety of methyl-β-cyclodextrin a literature search was conducted in May 2024 to identify information pertinent to the toxicological potential of this substance. The searches were conducted in PubMed as well as publicly available databases, including FDA, EPA, NTP, FAO/WHO, JECFA, ECA, and EFSA. Safety assessments conducted by regulatory authorities are presented first, followed by a summary of the available preclinical toxicological data in the peer reviewed literature.

Based on the structural similarity between β-cyclodextrin and methyl-β-cyclodextrin, the published safety data on β-cyclodextrin will be used to read across to methyl-β-cyclodextrin. The additional methyl group in this context is not a moiety that typically raises concern about a change in the overall safety profile of the substance. The safety data from preclinical toxicity studies conducted in multiple animal species (i.e., rats, dogs, and rabbits) demonstrated low acute oral toxicity, and there is no evidence of genotoxicity or carcinogenicity via oral administration at high doses.

Based on the results of toxicity studies in dogs, rats, and mice, β-cyclodextrin has little systemic activity.[160] β-Cyclodextrin is poorly absorbed and digested following oral administration in animals and humans. Toxicokinetic analysis in beagle dogs demonstrated the presence of unchanged β-cyclodextrin in urine and, to a lesser extent in feces.[161] Urinary excretion of this compound varies

[156] Agency for Toxic Substances and Disease Registry (ATSDR). 2003. Toxicological Profile for Selenium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
[157] EFSA. 2008. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS). Benfotiamine, thiamine monophosphate chloride and thiamine pyrophosphate chloride, as sources of vitamin B 1 added for nutritional purposes to food supplements. EFSA J 864: 1-31.
[158] National Institutes of Health (NIH) Office of Dietary Supplements (ODS). 2021. Factsheet on Thiamine. Last updated March 2021. Accessed 19 Dec 2022.
[159] EFSA. 2008. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS). Benfotiamine, thiamine monophosphate chloride and thiamine pyrophosphate chloride, as sources of vitamin B_1 added for nutritional purposes to food supplements. EFSA J 864: 1-31.
[160] European Commission Food Science and Techniques. (1997). Reports of the Scientific Committee for Food. Opinion on β -Cyclodextrin manufactured by the action of the enzyme cycloglycosyltransferase obtained from bacillus circulans on partially hydrolyzed starch
[161] HRC (1994a). Beta-cyclodextrin: Toxicity to rats by dietary administration for 52 weeks. Unpublished report no. ROQ 4/931090 from Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, UK. Submitted to WHO by Roquette Frères, Lestrem, France [as cited in JECFA, 1995].

between species but is less than 5% of the oral dose. The proportion of undigested β-cyclodextrin excreted in the feces was dose-dependent and low, with 1–2% of the daily doses.[162]

EFSA[163] reported that this compound has low acute oral toxicity with LD_{50} values for male and female mice of >12,500 mg/kg bw and for male and female rats of 12,000 mg/kg bw.[164]

In a 28-day dietary study, Wistar rats (4 groups/5 animals/sex per group) were fed 4,856, 4,454, 4,192, or 3,297 mg/kg bw/day for males, and 4,667, 4,314, 4,048, or 3,619 mg/kg bw/day for females. No adverse effects were observed at any of the doses tested (unpublished report as cited in EFSA, 2016). Other short-term toxicity studies discussed in EFSA (2016)[165] and JECFA (1995)[166] also did not report any adverse effects to the compound.

In a 90-day sub-chronic duration study conducted by Olivier et al.,[167] Sprague–Dawley rats (6 groups/20 animals/sex per group) were fed diets supplemented with β-cyclodextrin at concentrations of 1.25, 2.5, 5, or 10% (equal to 668, 1,335, 2,676, or 5,439 mg/kg bw/day and to 738, 1,488, 3,045, or 6,074 mg/kg bw/day for males and females, respectively). The only treatment-related effect was a statistically significant increase in filled cecal weights for both sexes. The authors stated that cecal enlargement was an adaptive response to poorly digestible sugars and other carbohydrates in rats and mice. Based on these findings, the Panel considered that the NOAEL of this study was 5,439 mg/kg bw/day.[168]

Other sub-chronic duration toxicity studies discussed in the EFSA and JECFA reports also did not report any adverse effects to the compound.

In a lifetime feeding study,[169] CD-1 mice (n=50/sex/group) were fed diets of 0, 25, 75, 225, or 675 mg/kg bw/day of β-cyclodextrin for 104 weeks. No treatment related effects on survival, body weight, good consumption, or hematological parameters were observed up to the highest dose. Treatment related lesions were reported only in decedent animals.

At the highest dose group, histopathological examination was conducted on all organs. At 25, 75, or 225 mg/kg bw/day, only abnormalities and major organs were examined. At the highest dose, one male showed treatment-related lesions in the cecum, colon, and/or rectum. These lesions were observed in rats that had a treatment related death (1 male at 75 mg/kg bw/day, 1 male at 225 mg/kg bw/day, 4 males and 4 females at 225 mg/kg bw/day). No other treatment related non-neoplastic lesions were observed. A NOEL was determined based on the inflammatory changes seen in the lower gastrointestinal tract at 25 mg/kg bw/day. Neoplastic findings were reported in the uterus at all doses but there were no dose-related effects. Pheochromocytoma of the adrenal gland was also reported at 75 and 225 mg/kg bw /day, however the study authors concluded that these were not treatment related.[170] JECFA (1995) reported a NOAEL of 25 mg/kg bw/day based on inflammatory effects in the lower gastrointestinal tract. However, the EFSA (2016) panel noted that, unlike JECFA (1995), the SCF (1997) study considered the inflammatory effects observed in mice were species specific and not

[162] Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). (1995). beta-Cyclodextrin.
[163] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2016). Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. EFSA Journal 14(12): 4628, 44 pp. doi:10.2903/j.efsa.2016.4628
[164] Mifune A and Shima A. (1977). Cyclodextrins and their application. Journal of Synthetic Organic Chemistry Japan, 35, 116–130 [in Japanese, as cited in EFSA, 2016].
[165] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2016). Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. EFSA Journal 14(12): 4628, 44 pp. doi:10.2903/j.efsa.2016.4628
[166] Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). (1995). beta-Cyclodextrin.
[167] Olivier P, Verwaerde F, and Hedges AR. (1991). Subchronic toxicity of orally administered beta-cyclodextrin in rats. Journal of American College of Toxicology, 10, 407–419.
[168] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2016). Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. EFSA Journal 14(12): 4628, 44 pp. doi:10.2903/j.efsa.2016.4628
[169] Gur E, Nyska A, and Waner T. (1993a). ß-cyclodextrin: Oncogenicity study in the mouse by dietary administration. LSRI project no. CHS/066/BCD. Unpublished report from Life Science Research Israel Ltd, Ness Ziona 70 451, Israel. Submitted to WHO by Roquette Frères, Lestrem, France [as cited in JECFA, 1995 and EFSA, 2016].
[170] *Ibid.* and Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). (1995). beta-Cyclodextrin.

observed in studies in rats and dogs therefore, the NOAEL of 25 mg/kg bw/day was not relevant to humans and a NOAEL of 225 mg/kg bw/day was determined.[171]

In a chronic-duration toxicity study, F344 rats were fed diets of 0, 25, 75, 225, or 675 mg/kg bw/day of β-cyclodextrin for 104 weeks. No treatment related effects were observed and no carcinogenic effects were observed up to the highest dose. Gur et al.[172] concluded that the neoplastic lesions observed were not treatment related and lacked a dose-response relationship. EFSA reported the NOAEL was 675 mg/kg bw/day for this study.[173]

In a 52-week study in SD rats (20 animals/sex/group) fed diets containing β-cyclodextrin at concentrations of 12,500, 25,000, or 50,000 mg/kg diet (equal to 654, 1,313 or 2,655 mg/kg bw/day for males and 864, 1,743, or 3,614 mg/kg bw/day for females), no statistically significant treatment related effects on body weight, food consumption, organ weights, hematological, or urinalysis parameters were observed. Alanine aminotransferase and aspartate aminotransferase were statistically significantly increased compared to controls in both males and females of the mid and high dose groups. Treatment related changes in the liver and kidney were observed on histological examination. Significantly increased incidences of single cell necrosis, centrilobular hepatocyte enlargement, and inflammatory cell infiltration were observed in male and female rats at the high dose group compared to controls. Males fed 2.5% β-cyclodextrin had increased incidence of portal inflammatory cell infiltration, while females showed an increased incidence of single cell necrosis and focal basophilic hepatocytes. Females fed 2.5 or 5% β-cyclodextrin showed an increased incidence of pigment in the epithelium of the cortical tubules of the kidneys, but no treatment related changes were observed in males. The authors concluded that the centrilobular hepatocellular hypertrophy is a common adaptive response in female rats due to exacerbated aging in the liver caused by β-cyclodextrin. Further, the necrosis and inflammatory responses are considered to be mild and consistent with elevated liver enzyme concentrations. The changes in liver enzyme concentrations were not associated with microscopic changes, and therefore not considered to be toxicologically significant. The NOEL in this study was 1.25% β-cyclodextrin in the diet, equivalent to 650 mg/kg bw/day, based on the treatment-related hepatotoxicity.[174]

In a 52-week study, beagle dogs (n=4/sex/group) were fed a diet of 0, 0.62, 1.25, or 5% β-cyclodextrin. No mortality was observed at any dose. Male dogs in the highest treatment group had increased protein concentration. There were no changes in clinical signs or other significant treatment related adverse effects were observed at other doses. A NOEL of 1.25% corresponding to 470 mg/kg bw/day was reported in males based on the urinary effects in male dogs.[175]

Overall, there was no evidence of carcinogenic potential for β-cyclodextrin based on the safety assessments conducted by EFSA (2016) and JECFA (1995).

In a 2-generation reproductive toxicity study, Sprague-Dawley rats (n= 32 /sex/ group) were fed β-cyclodextrin at concentrations of 10,000, 25,000, or 50,000 mg/kg diet (equal to 1,108, 2,713, or 5,444 mg/kg bw/day for males and 655, 1,584, or 3,164 mg/kg bw/day for females of the F0 generation in the first week of treatment and for the F1a generation equal to 1,531, 3,882, or 7,996 mg/kg bw/day for males

[171] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2016). Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. EFSA Journal 14(12): 4628, 44 pp. doi:10.2903/j.efsa.2016.4628
[172] Gur E, Nyska A, and Waner T. (1993a). ß-cyclodextrin: Oncogenicity study in the mouse by dietary administration. LSRI project no. CHS/066/BCD. Unpublished report from Life Science Research Israel Ltd, Ness Ziona 70 451, Israel. Submitted to WHO by Roquette Frères, Lestrem, France [as cited in JECFA, 1995 and EFSA, 2016].
[173] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2016). Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. EFSA Journal 14(12): 4628, 44 pp. doi:10.2903/j.efsa.2016.4628
[174] HRC (1994a). Beta-cyclodextrin: Toxicity to rats by dietary administration for 52 weeks. Unpublished report no. ROQ 4/931090 from Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, UK. Submitted to WHO by Roquette Frères, Lestrem, France [as cited in JECFA, 1995].
[175] HRC (1994b). Beta-cyclodextrin: Toxicity to dogs by repeated dietary administration for 52 weeks. Unpublished report no. ROQ 3/931848 from Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, UK. Submitted to WHO by Roquette Frères, Lestrem, France [as cited in JECFA, 1995].

and 1,525, 3,815, or 7,819 mg/kg bw/day for females. The study observed evidence of some adverse effects in both parents (decreased in body weight) and offspring (litter losses, lower fetal weights, and pup body weights) at the higher doses of 25,000, and 50,000 mg/kg bw/day. These effects were minimal but consistent across generations. Therefore, a NOAEL of 10,000 mg/kg bw/day was determined for parent-treated animals and/or their offspring based on growth development, fertility, and general performance from this study.[176]

In a 3-generation reproductive toxicity study, SD rats (n= 30/sex/dose) were fed β-cyclodextrin at dose levels of 0, 1.25, 2.5, or 5%. The parental generation males and females were maintained on these diets for 10 and 2 weeks, respectively, before pairing and during the gestation and lactation periods of three successive mating periods. Two subsequent generations, comprising 25 males and 25 females which were randomly selected from the F1b and F2b litters, and were treated with concentrations of 0, 0.31, 0.62, or 1.25% β-cyclodextrin.

In the parental generation, body weight gain in the high dose female group was statistically significantly higher than in controls during the premating period but statistically significantly less during the first lactation period. There were no treatment related effects on mating performance or gestation at any of the three mating. Pup viability did not show a treatment related effect but pup weights were significantly reduced at the highest dose group. The females in the F1 generation had lower body weight than controls on day 1 and 14 of lactation only. No treatment related effects were observed on reproductive performance, litter parameters, pup viability, body weight gain, or development. In the F2 generation. No treatment related effect was observed on paternal or maternal body-weight. No adverse effects were seen on reproductive performance, pup viability, body weight gain or development. A NOEL of 1.25% β-cyclodextrin in diet was identified, equivalent to 560 – 2,900 mg/kg bw/day over the different phases of the study.[177]

Based on other multigeneration reproductive studies in animals from various species, EFSA (2016) panel concluded that doses of up to 10,000 mg/kg bw/day in the diet (equal to 1,108 –1,531 mg/kg bw/day for males and 655–1,525 mg/kg bw/day for females) did not affect reproductive parameters and parental toxicity. Additionally, there were no adverse effects on developmental parameters at doses up to 2,500 and 5,000 mg/kg bw/day.[178]

β-cyclodextrin was tested in a bacterial reverse mutation assay with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with and without metabolic activation at concentrations of 0.1, 0.5, 1.0, 2.0, or 4.0 mg/plate, in triplicate. β-Cyclodextrin was negative for genotoxicity with and without metabolic activation. β-Cyclodextrin produced negative results in an HPRT assay using V79 Chinese hamster cells, an in vitro chromosomal aberration assay and in an in vivo micronucleus test which were of limited reliability. Based on these data, the EFSA panel considered that there was no indication for genotoxicity associated with this compound (unpublished reports as cited in EFSA, 2016).

The Scientific Committee for Food (SCF, 1997), JECFA (1995)[179], and EFSA (2016)[180] evaluated the safety of β-cyclodextrin. SCF (1997) established an ADI of 5 mg/kg bw/day based on a NOAEL of 466 mg/kg bw/day in the 1-year dog study and a safety factor of 100. JECFA (1995) revised the previous temporary ADI of 6 mg/kg bw/day and allocated an ADI of 5 mg/kg bw/day for β-cyclodextrin based on a NOEL of

[176] HRC (Huntingdon Research Centre Ltd), 1992b. Beta-cyclodextrin (RP64237) A study of the effect on reproductive function of two generations in the rat. Report No. RNP 363/911058 of Huntingdon Research Centre Ltd. Submitted by Société Roquette Frères, Lestrem, France, 2012. [as cited in EFSA 2016]
[177] Pharmakon Europe (1994). Beta-cyclodextrin: Three generation oral (dietary administration) reproduction toxicity study in the rat. Study no. 430/006. Unpublished report from Pharmakon Europe, L'Arbresle, France. Submitted to WHO by Roquette Frères, Lestrem, France [as cited in JECFA, 1995].
[178] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2016). Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. EFSA Journal 14(12): 4628, 44 pp. doi:10.2903/j.efsa.2016.4628
[179] Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). (1995). beta-Cyclodextrin.
[180] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2016). Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. EFSA Journal 14(12): 4628, 44 pp. doi:10.2903/j.efsa.2016.4628

1.25% in the diet (equal to 470 mg/kg bw/day) in a 1-year study in dogs (HRC, 1994)[181] and a safety factor of 100. EFSA (2016) concluded that there was no reason to revise the current ADI of 5 mg/kg bw/day for β-cyclodextrin based on the available toxicological database.

Safety assessment: based the "worst case" exposure estimates in Figure 1, pre-wash EDI is 2.05 mg/kg bw/day, which is below the ADI of 5 mg/kg bw/day for the read-across compound β-cyclodextrin (JECFA, 1995; EFSA, 2016). Therefore, there is no safety concern under our intended conditions of use.

Dimethyl sulfoxide (CASRN 67-68-5)

Dimethyl sulfoxide (DMSO) is listed in 21 CFR 172.859, 177.1655 and 177.244 as a flavoring agent or adjuvant.

DMSO is rapidly absorbed following oral administration in rhesus monkeys.[182] DMSO is metabolized to either dimethyl sulfone or dimethyl sulfide. Approximately 85% of DMSO and its metabolites are excreted in both urine and feces.[183]

Dogs were given oral doses of 2.5, 5, 10, 20, or 40 g/kg bw/day, 5 days/week for 23 weeks. High doses of 20 and 40 g/kg bw/day were not well tolerated and were reduced. Changes in lens refractiveness were observed and persisted after withdrawal of treatment.[184] The established LOEL was 2,500 mg/kg bw/day.[185]

In an 18-month study, SD rats were administered DMSO by oral gavage 1,100, 3,300, or 9,900 mg/kg bw/day, a NOAEL of 3,300 mg/kg bw/day was established based on slight body weight reduction (<10%). A LOAEL of 9,900 mg/kg bw/day was established based on ophthalmology and hematology effects.[186]

In a 45-day oral study in Wistar rats administered 2,000 or 5,000 mg/kg bw/day of a 50% DMSO solution, 5,000 mg/kg bw/day caused reduced weight gain and some liver damage. The NOAEL was determined to be 1,000 mg/kg bw/day (2,000 mg/kg bw/day of a 50% solution).[187]

No adverse effects were observed in the mother or offspring of Wistar rats administered 5,000 mg/kg bw/day orally for 4 days pre-mating and throughout pregnancy.[188] In Swiss mice administered 5-12 g/kg bw/day orally on days 6-12 of gestation, no fetal deaths, reduction in fetal weight, or abnormalities were observed. Maternal toxicity was observed at all doses except the low dose.[189]

In an OECD 421 guideline reproductive/developmental toxicity study, SD rats were administered 100, 300, or 1,000 mg/kg bw/day by oral gavage 15 days before mating, during mating, and throughout pregnancy and lactation until day 21 post-partum (females) or until sacrifice, at least 4 weeks in total (males). No treatment-related effects were observed on male or female reproductive performance. No fetotoxicity was noted. A reproductive, fetotoxic, and maternal NOAEL of 1,000 mg/kg bw/day was established based on the study findings.[190]

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[181] HRC (1994a). Beta-cyclodextrin: Toxicity to rats by dietary administration for 52 weeks. Unpublished report no. ROQ 4/931090 from Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, UK. Submitted to WHO by Roquette Frères, Lestrem, France [as cited in JECFA, 1995].
[182] Layman DL and Jacob SW. 1985. The absorption, metabolism, and excretion of dimethyl sulfoxide by rhesus monkeys. Life Sci, 37(25), 2431-2437
[183] IUCLID Data Set for DMSO. 2003. Submitted to the US EPA's HPV Challenge Program by the Dimethyl Sulfoxide Producers Association. Atofina Chemicals, Inc. Last revised August 12, 2003
[184] Rubin LF and Barnett KC. 1967. Ann NY Acad Sci, 141(1), 333-345
[185] Rubin LF and Mattis PA Science 1966 153 83-4
[186] Noel PRB, et al. 1975. The toxicity of dimethyl sulphoxide (DMSO) for the dog, pig, rat and rabbit. Toxicology, 3(2), 143-69
[187] Caujolle FM, Caujolle DH, Cros SB, Calvet MM. 1967. Limits of toxic and teratogenic tolerance of dimethyl sulfoxide. *Ann NY Acad Sci.* 1967;141(1):110-126. doi:10.1111/j.1749-6632.1967.tb34871.x
[188] *Ibid.*
[189] *Ibid.*
[190] European Chemicals Agency (ECHA). 2020. Registration Dossier - Dimethyl sulfoxide. Last modified October 2022. Accessed December 2022, available at https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15007/7/1.

In a developmental toxicity study in SD rats administered 0, 200, 1,000, or 5,000 mg/kg bw/day by oral gavage on gestation days 6 to 15, maternal toxicity was observed at 5,000 mg/kg bw/day (decreased body weight gain and food consumption). Fetal body weights were also decreased at the maternally toxic dose in addition to increased incidence of dilated renal pelvis and dilated ureter. These fetotoxicity findings were not accompanied by microscopic changes in the kidneys and were not considered to be adverse effects, but rather related to the diuretic properties of DMSO. Delayed ossification observed at 5,000 mg/kg bw/day were considered to be related to the decreased fetal body weight. No treatment-related malformations or skeletal variations were observed. The maternal and developmental toxicity NOAELs were both 1000 mg/kg bw/day, and the LOAELs 5,000 mg/kg bw/day.[191]

DMSO was negative in vitro in Ames assay and other bacterial tests, Chinese hamster ovary cells, and in host mediated assay.[192] However, it was positive in *Salmonella typhimurium* strains TA 1537 and TA2637 and in *E.coli* WP2uvrA at high concentrations, with and without metabolic activation (cytotoxic concentrations). There was a dose-related increase in the frequencies of cytogenetic aberrations in an in vivo study.[193] DMSO did not induce micronuclei in the polychromatic erythrocytes of bone marrow of male and female Han Wistar rats treated in vivo at doses up to 5,000 mg/kg bw/day for 5 consecutive days.[194]

The lowest NOAEL for oral exposure is 1,000 mg/kg bw/day from reproductive/developmental toxicity studies in SD rats.[195, 196] Based on the lowest NOAEL of 1,000 mg/kg bw/day and an overall safety factor of 600, an oral derived no observed effect level (DNEL) of 1.67 mg/kg bw/day was reported.[197] Also based on the NOAEL of 1,000 mg/kg bw/day from a 45-day study in Wistar rats, FDA previously calculated the permitted daily exposure (PDE) of 50 mg/day for DMSO assuming a 50 kg body weight and applying 1000-fold safety factors.[198]

Safety assessment: Wildtype's calculated pre-wash EDI for DMSO (8.96E-08 mg/kg bw/day) is well below the DNEL of 1.67 mg/kg bw/day. Also based on the NOAEL of 1,000 mg/kg bw/day, a very large MOS = 11.2E+09 can be calculated. Therefore, there is no safety concern under our conditions of use.

L-2-amino-*n*-butyric acid (CASRN 1492-24-6)

Aminobutyric acids are non-proteinogenic amino acids, which include alpha (α), beta (β), and gamma (γ) isomers; the α and β isomers each have *L* and *D* enantiomers. These three isomers have identical physical and chemical properties but can have different biological activities.[199] α-Aminobutyric acid is a metabolite in isoleucine biosynthesis, and is found exogenously in the diet.[200, 201] β-Aminobutyric acid is a natural product in plants' immune system, but is not commonly found in humans.[202, 203] γ-Aminobutyric

[191] Regnier JF and Richard J. 1998. Toxicologist, 42(1-s), 256-257
[192] US Food and Drug Administration. 1998. Appendix 6. Toxicological Data for Class 3 Solvents. Guidance Document, Q3C: Appendix 6. March 1998. Accessed December 2022 at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q3c-appendix-6
[193] US Environmental Protection Agency. 2006. Inert Reassessments: One Exemption from the Requirement of a Tolerance for Dimethyl sulfoxide (CAS Reg. No. 67-68-5). June 16, 2006. Accessed December 2022 at: https://www.epa.gov/sites/default/files/2015-04/documents/dimethyl.pdf
[194] European Chemicals Agency (ECHA). 2020. Registration Dossier - Dimethyl sulfoxide. Last modified October 2022. Accessed December 2022, available at https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15007/7/1
[195] Regnier JF and Richard J. 1998. Toxicologist, 42(1-s), 256-257
[196] European Chemicals Agency (ECHA). 2020. Registration Dossier - Dimethyl sulfoxide. Last modified October 2022. Accessed December 2022, available at https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15007/7/1
[197] *Ibid.*
[198] US Food and Drug Administration. 1998. Appendix 6. Toxicological Data for Class 3 Solvents. Guidance Document, Q3C: Appendix 6. March 1998. Accessed December 2022 at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q3c-appendix-6
[199] Wang, Z., Bian, L., Mo, C. *et al.* Quantification of aminobutyric acids and their clinical applications as biomarkers for osteoporosis. *Commun Biol* 3, 39 (2020). https://doi.org/10.1038/s42003-020-0766-y
[200] PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 80283, L-2-Aminobutyric acid; [reported 2024 Aug. 20]. Accessed August 2024. Available online:: https://pubchem.ncbi.nlm.nih.gov/compound/L-2-Aminobutyric-acid
[201] Human Metabolome Database (HMDB). 2024. Metabocard for L-alpha-Aminobutyric acid (HMDB0000452). Accessed August 2024. Available online: https://hmdb.ca/metabolites/HMDB0000452
[202] Parker ET, Chan QHS, Glavin DP and Dworkin JP. 2022., Non-protein amino acids identified in carbon-rich Hayabusa particles. Meteorit Planet Sci. 57: 776-793.
[203] Baccelli I, Glauser G, & Mauch-Mani B. 2017. The accumulation of β-aminobutyric acid is controlled by the plant's immune system. Planta 246: 791–796

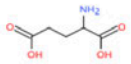
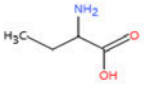
acid, also known as GABA, is a well characterized, endogenous major inhibitory neurotransmitter in the mammalian central nervous system.[204]

L-2-aminobutyric acid is an optically active form of α-aminobutyric acid that is produced endogenously and can be chemically synthesized using fermentation with genetically engineered microbes and enzymatic processes .[205] Wang et al. (2020) investigated the presence of aminobutyric acids in biological fluids including serum, plasma, and cerebrospinal fluid and found that only the *L*-α-aminobutyric acid enantiomer is naturally occurring in the biological fluids analyzed. The normal concentration range for α-aminobutyric acid is reported to be <41 µM in human plasma, increasing up to 151 µM in patients with pathological conditions such as sepsis, though the concentration of each enantiomer was not quantified (Chiarla et al., 2011).[206] There are no data in the public literature informing the extent to which exogenous or dietary α-aminobutyric acid is absorbed into systemic circulation or tissues, or potential toxicity of L-2-aminobutyric acid.

Glutamate (CASRN 56-86-0), an α amino acid that is central to amino acid metabolism, including aminobutyric acids, is endogenous to mammalian systems and naturally present in foods in free form or bound to proteins.[207] Glutamic acid is structurally similar to L-2-aminobutyric acid with the same key functional groups, and differs by one carboxylic acid. The additional carboxylic acid is not a moiety that typically raises concern about a change in the overall safety profile of the substance. The structure of L-2-aminobutyric acid also differs from L-alanine (an essential proteinogenic amino acid) by one carbon atom[208], further diminishing the likelihood of a difference in its overall safety profile. *In silico* quantitative structure-activity relationship analysis using OECD Toolbox (v4.6) shows that glutamic acid and L-2-aminobutyric acid have the same structural alert profile (see Figure 8 below). Neither chemical has structural alerts for acute oral toxicity, carcinogenicity, genotoxicity, or developmental and reproductive toxicity. Both chemicals have a structural alert for repeated dose toxicity for hepatoxicity however, these alerts are based on the presence of an ethionine functional group. Neither of these chemicals have a sulfur atom or ethionine group thus is not a concern for hepatoxicity. Based on the structural similarity and structural profilers for toxicity, glutamic acid is a suitable surrogate for read across to L-2-aminobutyric acid.

[204] Jewett BE, Sharma S. 2024. Physiology, GABA. [Updated 2023 Jul 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513311/
[205] Xu JM, Li JQ, Zhang B, Liu ZQ, Zheng YG. 2019. Fermentative production of the unnatural amino acid L-2-aminobutyric acid based on metabolic engineering. Microb Cell Fact.18(1):43.
[206] Chiarla C, Giovannini I. & Siegel J H. 2011. Characterization of alpha-amino-n-butyric acid correlations in sepsis. Transl. Res. 158:328–333.
[207] Loï C, Cynober L. 2022. Glutamate: A Safe Nutrient, Not Just a Simple Additive. Ann Nutr Metab. 78 (3): 133–146.
[208] PubChem reference: https://pubchem.ncbi.nlm.nih.gov/compound/alanine

Figure 8: *in silico* quantitative structure-activity relationship analysis (OECD toolbox)

	Glutamic acid	L-2-aminobutyric acid
Substance identity		
Structure		
CAS number	56-86-0	1492-24-6
Chemical name	glutamic acid	2-aminobutanoic acid
Other identifier		
SMILES	NC(CCC(O)=O)C(O)=O	CCC(N)C(O)=O
Profilers		
Predefined		
US-EPA New Chemical Categories	Aliphatic Amines	Aliphatic Amines
OECD HPV Chemical Categories	Not categorized	Not categorized
General Mechanistic		
DNA binding by OECD	No alert found	No alert found
Estrogen Receptor Binding	Non binder, non cyclic structure	Non binder, non cyclic structure
Toxic hazard classification by Cramer	Low (Class I)	Low (Class I)
Protein binding by OECD	No alert found	No alert found
DNA binding by OASIS	No alert found	No alert found
Protein binding by OASIS	No alert found	No alert found
Endpoint Specific		
Carcinogenicity (genotox and nongenotox) alerts by	No alert found	No alert found
Protein binding alerts for skin sensitization by OASIS	No alert found	No alert found
rtER Expert System - USEPA	No alert found	No alert found
Oncologic Primary Classification	Not classified	Not classified
Acute Oral Toxicity	Not categorized	Not categorized
in vitro mutagenicity (Ames test) alerts by ISS	No alert found	No alert found
DNA alerts for AMES, CA and MNT by OASIS	No alert found	No alert found
Respiratory sensitisation	No alert found	No alert found
in vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
DART scheme	Not known precedent reproductive and developmental toxic potential	Not known precedent reproductive and developmental toxic potential
Protein binding alerts for Chromosomal aberration by OASIS	No alert found	No alert found
Empiric		
Organic functional groups (US EPA)	Acid, aliphatic attach [-COOH] Alcohol, olefinic attach [-OH] Aliphatic Carbon [-CH2-] Aliphatic Carbon [CH] Alpha Amino acid Amino acid, non-alpha carbon type Amino, aliphatic attach [-N<] Amino, aliphatic attach [-NH2] Carbonyl, aliphatic attach [-C(=O)-] Miscellaneous sulfide (=S) or oxide (=O) Olefinic carbon [=CH- or =C<]	Acid, aliphatic attach [-COOH] Alcohol, olefinic attach [-OH] Aliphatic Carbon [-CH2-] Aliphatic Carbon [-CH3] Aliphatic Carbon [CH] Alpha Amino acid Amino, aliphatic attach [-N<] Amino, aliphatic attach [-NH2] Carbonyl, aliphatic attach [-C(=O)-] Miscellaneous sulfide (=S) or oxide (=O) Olefinic carbon [=CH- or =C<]
Organic functional groups	Aliphatic amine, primary alpha-Aminocarboxylic acid Amine, primary Carboxylic acid	Aliphatic amine, primary alpha-Aminocarboxylic acid Amine, primary Carboxylic acid
Structure similarity	Target not set	Target not set
Toxicological		
Repeated dose (HESS)	Ethionine (Hepatotoxicity) Alert	Ethionine (Hepatotoxicity) Alert

Measured and predicted data

Physical Chemical Properties#Dissociation Constant

sublevel	*endpoint*	*value*	*unit*	*species, duration, test type, type of method, assay, strain, test guideline, year, reference, database*	*value*	*unit*	*species, duration, test type, type of method, assay, strain, test guideline, year, reference, database*
Dissociation Constant (pKa)	Acidic pKa	2.13		Endpoint: Acidic pKa Reference source: Dissociation Constants Of Organic Acids And Bases, https://www.zirchrom.com/organic.htm Database: pKa OASIS			

The safety profiles of glutamate, or L-glutamic acid, and its salts, including monosodium glutamate (MSG), are well characterized, therefore the pertinent toxicological data will be used for read across to L-2-aminobutyric acid, as summarized below.

Glutamate is readily absorbed and metabolized to a significant extent in the gastrointestinal tract following oral exposure. Studies in animals and humans suggest that the majority of glutamate is eliminated through first pass metabolism, leaving <20% available for systemic availability.[209, 210, 211] Systemically available glutamate is metabolized in several organs, including in the liver, skeletal tissue, and brain, and is subjected to urinary excretion in humans via the kidneys.[212, 213]

The oral LD_{50} values for glutamate include >2,300 mg/kg bw in rabbits, 12,961 and 19,200 mg/kg bw in mice, >5,110 mg/kg bw in rats.[214, 215]

In two GLP and OECD test guideline compliant 28-day studies in Sprague-Dawley (SD) rats (10/sex/group), MSG was administered in the diet at 4,800 and 4,900mg/kg bw/day in females and 5,100 and 5,300 mg/kg bw/day in males. No adverse effects were observed in either study.[216, 217]

One 13-week study in dogs and two 13-week studies in rats were provided to EFSA and are summarized below. The studies summarized below were provided to EFSA and not available in the public literature.

In a GLP and OECD guideline compliant 13-week study provided to EFSA, beagle dogs (5/sex/group) were administered 0, 150, 500, or 1,500 mg MSG monohydrate/kg bw/day. Transient and non-treatment related clinical signs (vomiting, loose stools, and diarrhea) and changes in clinical chemistry and hematological parameters (not specified) were reported. Statistically significant increases in absolute and relative weight of the thymus (+100%) compared to controls were reported, however no correlated histopathological findings were observed thus considered non adverse. The study authors stated that no adverse effects were seen at any dose level and concluded that the NOAEL was 1,500 mg/kg bw/day. The EFSA Panel agreed with the authors' conclusions (BRC 2007b as reported in EFSA, 2017).[218]

In a GLP and OECD guideline compliant 13-week study provided to EFSA, SD rats (20/sex/group) were administered MSG in the diet at concentrations equivalent to 0, 308, 931, or 3,170 mg/kg bw/day in males and 0, 354, 1,066, or 3,620 mg/kg bw/day in females. Increased blood urea nitrogen was noted in the high dose males, though the authors did not consider this to be toxicologically significant because the increase in urea was derived from the metabolite of glutamate through urea cycle. Increased urine sodium concentration was attributed to the sodium in the glutamate salt. No test related effects were

[209] Reeds PJ, Burrin DG, Jahoor F, Wykes L, Henry J and Frazer EM. 1996. Enteral glutamate is almost completely metabolized in first pass by the gastrointestinal tract of infant pigs. American Journal of Physiology-Endocrinology and Metabolism. 270:E413–E418.
[210] Burrin DG and Stoll B. 2009. Metabolic fate and function of dietary glutamate in the gut. American Journal of Clinical Nutrition, 90:850S–856S.
[211] Hays SP, Ordonez JM, Burrin DG and Sunehag AL. 2007. Dietary glutamate is almost entirely removed in its first pass through the splanchnic bed in premature infants. Pediatric Research. 62:353–356.
[212] Ragginer C, Lechner A, Bernecker C, Horejsi R, Möller R, Wallner-Blazek M, Weiss S, Fazekas F, Schmidt R, Truschnig-Wilders M and Gruber HJ. 2012. Reduced urinary glutamate levels are associated with the frequency of migraine attacks in females. European Journal of Neurology, 19, 1146–1150.
[213] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Parent-Massin D, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Boon P, Chrysafidis D, Gürtler R, Tobback P, Altieri A, Rincon AM and Lambré C, 2017. Scientific Opinion on the re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives. EFSA Journal 2017;15(7):4910, 90. Accessed August 2024. Available online:https://doi.org/10.2903/j.efsa.2017.4910
[214] ibid
[215] Takasaki Y, Narui K and Shioya S. 1990. Toxicity of salts of L-glutamate. Acute toxicity of four salts of L-glutamate in mice and rats, and mutagenicity test. Iyakuhin Kenkyu. 21: 257–264 [as reported in EFSA, 2017].
[216] Center International de Toxicologie (CIT), 1997a. 4-week toxicity study by oral administration (dietary admixture) in rats. CIT/Study No 14458 TSR/MSG (MSG)/Société Orsan. Submitted by Ajinomoto, 22 August 2016.
[217] Center International de Toxicologie (CIT), 1997b. Complementary 4-week toxicity study by oral administration (dietary admixture) in rats. CIT/Study No 14716 TSR/MSG (RC035/01)/Société Orsan. Submitted by Ajinomoto, 22 August 2016.
[218] Biosafety Research Center, 2007b. Monosodium L-glutamate monohydrate produced by a new method: 90-day repeated oral dose toxicity study in dogs. Experiment No. 9959 (258-059). Submitted by Ajinomoto, 22 August 2016.

reported in any other parameter evaluated. The authors identified a NOAEL of 3,170 mg/kg bw/day for males and 3,620 mg/kg bw/day for females.[219]

In a GLP and OECD guideline compliant 13-week study provided to EFSA, rats (species not specified) were administered MSG monohydrate at 0, 700, 1,300, or 2,700 mg/kg bw/day for males and 0, 700, 1,500, or 2,900 mg/kg bw/day for females by gavage. No findings were reported in any parameter evaluated including clinical signs, mortality, clinical chemistry, hematology, or macroscopic and microscopic examinations. Dose related increases in sodium excretion noted in all groups at week 12 was attributed to the high sodium intake from MSG monohydrate. EFSA established NOAELs of 2,700 mg/kg bw/day in males and 2,900 mg/kg bw/day in females (TNO, 2014 as reported in EFSA, 2017).[220]

In a 2-year chronic toxicity study,[221] beagle dogs (5/sex/group) were administered diets containing MSG concentrations equivalent to 0, 625, 1,250, or 2,500 mg/kg bw/day. Hematology, blood chemistry, urinalysis, and ophthalmoscopic and electrocardiographic examinations were conducted at intervals of 13 weeks. Two animals/sex/group were subjected to gross and histopathological examination and organ weight analysis at 13 weeks. No significant differences in clinical signs, food consumption, body weight, ophthalmoscopy, electrocardiography, hematology, blood chemistry, organ weights, mortality, or histopathology were observed between controls and treated animals. The EFSA Panel established a NOAEL of 2,500 mg/kg bw/day, the highest dose tested. EFSA also noted that the increase in thymus weight reported in the 13-week study provided by BRC[222] and summarized above was not observed in the study by Owen et al.[223]

Owen et al.[224] reported on a similar 2-year study in CD rats (40/sex/group) fed diets containing MSG concentrations equivalent to 0, 450, 900, or 1,800 mg/kg bw/day and 0, 580, 1,160, or 2,320 mg/kg bw/day in males and females, respectively. Evaluations of hematology, blood chemistry, and urinalysis were conducted before treatment and at 13, 26, 52, 78, and 104 weeks. Ten animals/sex/group were sacrificed at 12 weeks and subjected to gross and histopathological examinations and organ weight analysis. No statistically significant or adverse effects on food consumption, ophthalmoscopy, hematology, blood chemistry, organ weight, or mortality were reported between treated animals and controls. The 2017 EFSA Panel concluded that the NOAELs in this study were 1,800 mg/kg bw/day in males and 2,320 mg/kg bw/day in females, the highest doses tested.

Ebert[225] conducted a 2-year chronic toxicity study in SD rats (35-40/sex in treatment groups and 61-89/sex in controls) fed diets containing MSG concentrations equivalent to 0, 59, or 133 mg/kg bw/day for males and 0, 33, or 73 mg/kg bw/day for females. Six animals of each sex from the control group and three animals of each sex from the treatment groups were sacrificed at study day 63 and subjected to gross and histopathological examinations. No statistically significant changes in clinical signs, food consumption, mortality, hematology, organ weights, or histopathology were observed between treated and control rats. Tumor incidences were similar in controls and treated animals. The EFSA Panel concluded that the NOAEL was 133 and 73 mg/kg bw/day in males and females, respectively (EFSA, 2017).

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[219] *Ibid.*
[220] TNO (Netherlands Organisation for Applied Scientific Research), 2014. Repeated-dose (13-week) oral toxicity study in rats with Monosodium L-Glutamate monohydrate produced by a GMM production strain. TNO project number 093.25059. Submitted by Ajinomoto, 4 November 2016.
[221] Owen G, Cherry CP, Prentice DE and Worden AN. 1978a. The feeding of diets containing up to 4% MSG to rats for 2 years. Toxicology Letters. 1:221–226.
[222] Biosafety Research Center, 2007b. Monosodium L-glutamate monohydrate produced by a new method: 90-day repeated oral dose toxicity study in dogs. Experiment No. 9959 (258-059). Submitted by Ajinomoto, 22 August 2016.
[223] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Parent-Massin D, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Boon P, Chrysafidis D, Gürtler R, Tobback P, Altieri A, Rincon AM and Lambré C, 2017. Scientific Opinion on the re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives. EFSA Journal 2017;15(7):4910, 90. Accessed August 2024. Available online:https://doi.org/10.2903/j.efsa.2017.4910
[224] Owen G, Cherry CP, Prentice DE and Worden AN. 1978a. The feeding of diets containing up to 4% MSG to rats for 2 years. Toxicology Letters. 1:221–226.
[225] Ebert AG. 1979b. The dietary administration of MSG or glutamic acid to C-57 black mice for two years. Toxicology Letters. 3: 65–70.

In a study reported in Shibata et al.,[226] Fischer 344 rats (50/sex/group) were fed diets containing MSG concentrations equivalent to 0, 231, 481, 875, or 1,982 mg/kg bw/day in males, and 0, 268, 553, 1,121, or 2,311 mg/kg bw/day in females for 2 years. Urinalysis was performed after week 1 and months 1, 3, 6, 12, 18, and 24. Hematological evaluations were conducted at study termination, with the exception of creatinine and blood urea nitrogen parameters. Gross pathological examination was performed and the brain, heart, liver, spleen, kidneys, urinary bladder, adrenals, and gonads were weighed, followed by histopathological examination on all major organs from animals in the control and high dose groups. No significant differences were reported between treated animals and controls in clinical signs, food consumption, mortality, and hematology. Final body weight of males in the high dose group was significantly decreased compared to controls. Statistically significant increase in pH and sodium concentrations of the urine, as well as statistically significant decrease in potassium concentrations were reported in both sexes at the mid and high dose levels. Kidney weights relative to body weights were statistically significantly increased in males (12.7%) and in females (11.9%) at the high dose. Transitional cell hyperplasia of the renal pelvis associated with moderate or severe chronic nephropathy was reported in males at 553 and 2,311 mg/kg bw/day though not statistically significant compared to controls. The tumor incidences were similar in treated animals and control. EFSA (2017) concluded that the 10% increase in relative kidney weight was non-adverse since there were no histopathological correlates and identified a NOAEL of 1,982 mg/kg bw/day in males and 2,311 mg/kg bw/day in females, the highest doses tested.

Based on the three 2-year studies in rats, no increase in tumor incidence was observed up to the highest doses tested, thus EFSA (2017) concluded that MSG is not carcinogenic in rats.

In a three-generation reproductive toxicity study,[227] CD-1 mice were fed diets containing MSG concentrations equivalent to approximately 0, 1,500, or 6,000 mg/kg bw/day for males and 0, 1,800, or 7,200 mg/kg bw/day for females. The parent generation (17-33 males/group and 51-99 females/group) were fed MSG diets from 8-9 weeks prior to mating until the end of lactation. Some of the F1 generation (116-370 animals/sex/group) were weaned at 4 weeks until 36 weeks of age, and some were mated at 13-14 weeks or 20-21 weeks to obtain two F2 generations (59-229 animals/sex/group). Some of the F2 mice were weaned at 4 weeks and maintained on the test diet until 27-32 weeks of age, while other F2 mice were mated at 16 or 32 weeks to obtain two F3 generations (27-110 animals/sex/group). No statistically significant differences in parental, reproductive, or developmental parameters were observed between the treated groups and control animals. EFSA (2017) concluded the NOAEL for reproductive toxicity was 6,000 and 7,200 mg/kg bw/day for males and females, respectively, the highest doses tested.

In a GLP and OECD guideline 416 compliant two-generation reproductive toxicity study reported to EFSA (2017), MSG was administered in the diet to Charles-River rats (30/sex/group) at concentrations of 0, 0.5, 1.5, or 5%. The w/w intake of F0 parental males and females at 1.5% was equivalent to 939 and 1,039 mg/kg bw/day, respectively, and 3,131 and 3,496 mg/kg bw/day, respectively, at 5%. The w/w intake for F1 parental males and females was 4404 and 4,618 mg/kg bw/day, respectively. No effects were reported on estrous cycle or sperm parameters in the parental animals, and no effects on reproductive indices or offspring viability indices. No test related effects were reported in litter observations. Increased absolute and relative kidney weights were observed in high dose males and females of the F0 generation (absolute: 10.1% in males and 9.5% in females; relative: 9% in males and 14.1% in females), and the F1 generation (absolute: 9% in males and 19.7% in females; relative: 10.9% in males and 17.8% in females). Increased absolute and relative ovary weights were observed in F1 females of the high dose

[226] Shibata MA, Tanaka H, Kawabe M, Sano M, Hagiwara A and Shirai T. 1995. Lack of carcinogenicity of monosodium L-glutamate in Fischer 344 rats. Food and Chemical Toxicology. 33:383–391.
[227] Anantharaman K, 1979. In utero and dietary administration of monosodium L-glutamate to mice: reproductive performance and development in a multigeneration study. In: LJ Filer, S Garattini, MR Kare, Reynolds WA and Wurtman RJ (eds.). Glutamic Acid: Advances in Biochemistry and Physiology. Raven Press, New York. 231–253 [as reported in EFSA, 2017].

group. Reduced absolute and relative spleen weights were observed at weaning in both sexes in the F1 generation at the high dose, but not in the F2 generation. No macroscopic or histopathological correlates in the kidneys, ovaries, or spleen were reported. The authors identified a NOAEL of 1.5% MSG (equivalent to 939 and 1,039 mg/kg bw/day for males and females, respectively) for parental toxicity, and 5% (equivalent to 3,131 and 3,496 mg/kg bw/day for males and females, respectively), the highest dose tested, for reproductive and developmental toxicity (as reported in EFSA, 2017). The EFSA Panel agreed with the author's NOAEL for reproductive and developmental toxicity; however considered the highest dose tested as the NOAEL for parental toxicity because no histopathological changes were observed in the organs in which weight was increased (EFSA, 2017).

In a GLP and FDA guideline compliant prenatal developmental toxicity study reported to EFSA,[228] pregnant SD rats (25/group) were fed diets containing MSG at 0, 302, 898, or 3,019 mg/kg bw/day from gestation day 6 to 20. The animals were monitored at regular intervals for clinical signs, body weight, and food intake, and subjected to cesarean section and necropsy on gestation day 20. Heart, lung, liver, kidney, spleen, adrenal, ovary, and uterus weights were measured. The number of corpora lutea, implantations, resorptions, fetus survival, fetal weight, and external abnormalities were examined. No maternal or developmental effects were observed therefore, the authors established a NOAEL of 3,019 mg/kg bw/day, the highest dose tested (as reported in EFSA, 2017).

In a developmental neurotoxicity study, SD rats were administered diets containing MSG at concentrations equivalent to 0, 1,900, 3,700, or 5,300 mg/kg bw/day for males and 0, 1,600, 3,200, or 5,000 mg/kg bw/day for females in the pre-breeding period, and 0, 1,800, 3,900, or 6,200 mg/kg bw/day and 0, 2,000, 4,300, or 6,600 mg/kg bw/day in male and female offspring, respectively, from 14 days prior to mating until conception for males and through gestation and lactation in females. On postnatal day 1, litters were reduced to 12 pups/litter and continued on the same diet until 90 days of age. Body weight and food intake were recorded for dams and offspring, and length of gestation, litter size, sex distribution, and number of dead pups were noted. Behavioral tests were conducted on two animals per sex per litter before weaning and after weaning. No statistically significant differences were reported in body weights or food intake. Mortality was statistically significantly increased in the offspring of mid-dose dams. Other measures of reproductive performance were unaffected. Delayed early swimming development, diminished rearing frequency in the open field, altered active avoidance acquisition and extinction, and prolonged day-2 passive avoidance retention were observed in the high dose group.[229] The EFSA Panel considered the mid dose, 3,200 mg/kg bw/day as the NOAEL based on the neurobehavioral effects observed at the high dose (EFSA, 2017).

Six reverse mutation assays with glutamate or its salts using *Salmonella typhimurium* TA92, TA98, TA100, TA1535, TA1537, and TA1538 tester strains up to a maximum concentration of 5,000 or 10,000 µg/plate,

[228] Biosafety Research Center, 2007c. Monosodium L-glutamate monohydrate produced by a new method: teratogenicity study in rats. Experiment No. 9958 (258-058). Submitted by Ajinomoto, 22 August 2016.
[229] Vorhees CV. A Test of Dietary Monosodium Glutamate Developmental Neurotoxicity in Rats: A Reappraisal. Ann Nutr Metab. 2018;73 Suppl 5:36-42. doi: 10.1159/000494781. Epub 2018 Dec 3. PMID: 30508817.

both in the absence and presence of liver S9 metabolic activation, were not mutagenic.[230, 231, 232, 233, 234, 235, 236]

In documentation provided to EFSA (2017),[237, 238] MSG monohydrate was tested for its potential to induce chromosomal aberrations in Chinese hamster lung cells both in the absence and presence of S9 metabolic activation at concentrations of 0.48, 0.95, or 1.9 mg/mL for 6 hours, and for 24 hours only in the absence of metabolic activation. EFSA evaluated two studies conducted by the same authors, using MSG monohydrate of 99% and 99.6% purity. MSG monohydrate did not induce statistically significant increases in structural chromosomal aberrations and polyploid cells in the 6-hour treatment both in the absence and presence of metabolic activation in either GLP and OECD guideline 473 compliant studies. A 4% increase in the induction of structural aberrations was observed at the highest concentration in the 24-hour treatment, however, was not considered to be biologically relevant by the study authors.

In a GLP and OECD guideline 487 compliant study reported to EFSA (2017),[239] MSG monohydrate (purity 98%) was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneugenicity in the presence and absence of rat 29-metabolic activation at concentrations of 3.7, 7.3, 14.6, 29.2, 58,5, 117, 234, 468, 936, or 1,871 µg/mL for the 4 hour treatment and 98.3, 197, 393, 492, 614, 768, 960, 1,200, 1,500, or 1,871 µg/mL for the 20-hour treatment. MSG monohydrate did not induce micronuclei both in the absence and presence of metabolic activation.

In an *in vivo* dominant lethal test reported in JECFA,[240] 12 male albino Charles River mice were administered MSG at doses of 0, 2,700, or 5,400 mg/kg bw once by gavage. Each treated male was mated with three untreated females each week for 6 weeks. No differences in the number of implantations, resorptions, or embryos were reported.

In an in vivo micronucleus test provided to EFSA,[241] ICR mice (5/sex/group) were administered 500, 1,000, or 2,000 mg/kg bw/day MSG monohydrate (purity 99%) by gavage once daily for 3 days. The presence of micronuclei were not statistically significantly increased in any treated group compared to control.

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[230] JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1988. L-Glutamic acid and its ammonium, calcium, monosodium and potassium salts. Toxicological evaluation of certain food additives. Prepared by the thirty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the meeting in Geneva 16–25 February 1987. Food Additives Series, 22. Accessed August 2024. Available online:: http://www.inchem.org/documents/jecfa/jecmono/v22je12.htm

[231] Litton-Bionetics. 1975a. Mutagenic evaluation of compound FDA 73-58, 000997-42-2, monopotassium glutamate. US Department of Commerce, National Technical Information Service PB-254.511, as referred to by JECFA, 1988.

[232] Litton-Bionetics. 1975b. Mutagenic evaluation of compound FDA 75-11, 007558-63-6, monoammonium glutamate, FCC. US Department of Commerce, National Technical Information Service PB-254.512, as referred to by JECFA, 1988.

[233] Notox, 2010. Evaluation of the mutagenicity activity of L-glutamic acid in the salmonella typhimurium reserve mutation assay and the Escherichia coli reserved mutation assay (with independent repeat). Notox Project 493744. Submitted by Ajinomoto, 22 August 2016 as reported in EFSA, 2017

[234] Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K. 1992. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environmental and Molecular Mutagenesis. 19: 2–141.

[235] De Flora S, Zanacchi P, Camoirano A, Bennicelli C and Badolati GS. 1984. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutation Research - Genetic Toxicology. 133:161–198.

[236] Ishidate Jr M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A. 1984. Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology. 22:623–636.

[237] Hatano Research Institute, 2006a. Chromosomal aberration test of monosodium L-glutamate monohydrate produced by a new method using cultured Chinese hamster lung cells. Contract No. 06-K-078. Submitted by Ajinomoto, 22 August 2016.

[238] Hatano Research Institute, 2007. Chromosomal aberration test of monosodium L-glutamate monohydrate produced by a new method (Lot No. 20061222BLD3) using cultured Chinese hamster lung cells. Contract No. G-06-090. Submitted by Ajinomoto, 22 August 2016.

[239] TNO (Netherlands Organisation for Applied Scientific Research), 2013a. Bacterial reverse mutation test with Monosodium L-Glutamate, monohydrate. TNO project number 093.25061/01.41. Submitted by Ajinomoto, 4 November 2016.

[240] Industrial-Bio-test-Laboratories. 1973. Mutagenic study with accent brand monosodium L-glutamate in albino mice. Northbrook, IL, USA. 1-12, as referred to by JECFA, 1988 as reported in JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1988. L-Glutamic acid and its ammonium, calcium, monosodium and potassium salts. Toxicological evaluation of certain food additives. Prepared by the thirty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the meeting in Geneva 16–25 February 1987. Food Additives Series, 22. Accessed August 2024. Available online: http://www.inchem.org/documents/jecfa/jecmono/v22je12.htm

[241] Hatano Research Institute, 2006b. Micronucleus test of monosodium L-glutamate monohydrate produced by a new method. Contract No. 06-K-077. Submitted by Ajinomoto, 22 August 2016 as reported to EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Parent-Massin D, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Boon P, Chrysafidis D, Gürtler R, Tobback P, Altieri A, Rincon AM and Lambré C, 2017. Scientific Opinion on the re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives. EFSA Journal 2017;15(7):4910, 90. Accessed August 2024. Available online:https://doi.org/10.2903/j.efsa.2017.4910

EFSA noted that the study was performed according to GLP and OECD test guideline 474, with the exception of the analysis of 2,000 polychromatic erythrocytes per animal rather than 4,000.

Overall, the *in vitro* and *in vivo* studies reviewed by the EFSA Panel[242] do not suggest concerns for genotoxicity of glutamate or its salts.

The European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food evaluated the safety profile of glutamate and its salts. EFSA[243] considered the findings from the available animal studies deemed to be adequate for hazard characterization and concluded that no adverse effects were observed in repeated dose oral toxicity studies. The Panel used the NOAEL from the neurodevelopmental toxicity study as reported by Vorhees (1979) of 3,200 mg/kg bw/day to derive an ADI of 32 mg MSG/kg bw/day, or 27.8 mg glutamate/kg bw/day. The derived ADI of 30 mg/kg bw/day was established by EFSA as the group ADI, expressed as glutamate.

Safety assessment: Based on the read-across to glutamate, an ADI of 30 mg/kg bw/day (EFSA 2017) can be applied to L-2-aminobutyric acid. WT's estimated daily intake (EDI) for L-2-aminobutyric acid is 0.00673 mg/kg bw/day, which is well below the ADI. Therefore, there is no safety concern under our intended conditions of use.

[242] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Parent-Massin D, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Boon P, Chrysafidis D, Gürtler R, Tobback P, Altieri A, Rincon AM and Lambré C, 2017. Scientific Opinion on the re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives. EFSA Journal 2017;15(7):4910, 90. Accessed August 2024. Available online:https://doi.org/10.2903/j.efsa.2017.4910

[243] ibid

Appendix 5: thermal validation study

This report summarizes the oven thermal validation study for the Sheldon Dry heat oven carried out on Wildtype's finished product during the kill step described in response to question 11. This occurs after the cell harvest step, at which point the harvested cell material is considered non-viable (see step 8 in Figure 5 above).

Acceptance criterion

All parts of Wildtype finished product must be cooked to an internal temperature of at least 145 °F (63 °C) for 15 seconds. (FDA Food Code 2022, 3-404.11)

Background

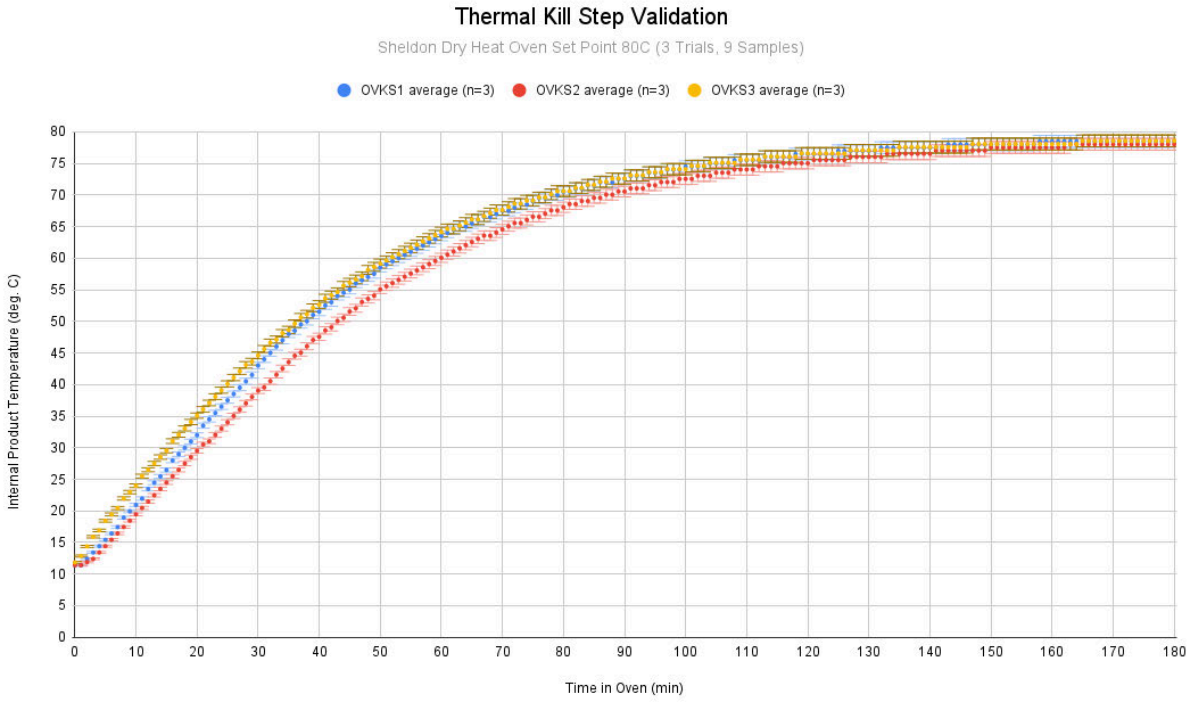
Wildtype's dry heat oven uses a forced-air circulation and reserve heating power for quick recovery after door openings. The unit is equipped with a stainless steel interior for long life operation and easy cleaning. After using the system, the system is cleaned prior to the next run.

Procedures

Temperature monitoring was conducted using calibrated data loggers to measure the internal temperature of the finished product at multiple points to ensure uniform heating. Multiple finished product samples were selected from different locations within the oven to account for potential temperature variations within the oven.

The time required to reach the target internal temperature of at least 145 °F (63 °C) for 15 seconds for each sample was monitored and documented throughout the heating process.

Results



The graph above summarizes the results of a thermal validation study for the Sheldon dry heat oven and Wildtype's finished product. The objective of this study was to verify that the oven reaches and maintains an internal temperature of at least 145 °F (63 °C) for 15 seconds for the product, which is necessary to be in compliance with FDA Food Code 2022, 3-404.11 for fish. The x-axis of the graph represents the time in minutes that the product was in the oven, and the y-axis represents the internal temperature of the product at the coldest location in degrees Celsius. The graph shows three lines, each representing the temperature data for one of the three trials (OVKS1, OVKS2, and OVKS3).

All three trials reached a temperature of at least 70 °C within 90 minutes and maintained at or above that temperature for the remainder of the testing period, resulting in the product being above 70 °C for a total of ~30 minutes and reaching a final temperature of 75 °C. The oven was set to a setpoint of 80 °C for 120 minutes to ensure that the product reaches the target temperature throughout the process. The graph above shows that all samples were cooked to an internal temperature of at least 145 °F (63 °C) for well beyond 15 seconds. (FDA Food Code 2022, 3-404.11)

There is some variation in the temperature data between the three trials. This variation is relatively small, but it does indicate that there may be some minor inconsistencies in how the oven heats products in various parts of the oven.

In all cases, however, the data above show that all products exceed FDA guidance for reaching at least 145 °F (63 °C) for 15 seconds based on Wildtype's current heating time of 120 minutes at 80 °C.

In conclusion, the thermal validation study confirms the Sheldon dry heat oven at a setpoint of 80 °C for 120 minutes is effective at achieving and maintaining the necessary internal temperature for the Wildtype Saku product to meet FDA Food Code 2022, 3-404.11 for fish.

WILDTYPE

Appendix 6: Hazard analysis and preventive controls (changes in red text)

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
1	Receiving Raw Materials	Biological	Potential pathogens (e.g., *Salmonella, L. monocytogenes)* present in media & scaffold inputs	x		Scaffold/media-borne pathogens may survive into final product	**Process Preventive Controls** Thermal process in subsequent step inactivates potential pathogens Ongoing adventitious agent testing (see Figure 4 above) as well as ongoing monitoring of heavy metals and spore formers. For brevity, this verification method is abbreviated as "ongoing adventitious testing" in subsequent steps in this hazard analysis.		x
		Biological	Expired materials may be provided by raw material suppliers	x		Expired materials may introduce pathogens into products	**Supply Chain Preventive Control** COAs and expiration dates inspected for each lot	x	
		Chemical	Potential for undeclared allergens in scaffold and media inputs; incorrect materials sent by the vendor	x		Suppliers may inadvertently include undeclared allergens by cross-contact, incorrect labeling.	**Supply Chain Preventive Control** - Scaffold suppliers pass through supplier qualification program prior to using input - COAs inspected for each lot - Record of allergen statement from the vendor, physical inspection of material along with their label	x	
		Physical	Potential packing/shipping materials in scaffold inputs	x		Damaged packaging or shipping materials	**Process Preventive Controls** - Visual inspection of all packages - If damage to primary package; lot is rejected	x	

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
2	Media Preparation	Biological	Potential for media sterilization failure allowing growth of pathogens (e.g., *Salmonella, L. monocytogenes)*		x	Media prep MBRS (e.g., 044 and 048) include strict sterilization requirements In the event that sterilization failed, pathogens would outcompete or affect cell growth (detectable via real-time monitoring), leading to the destruction of the batch. Ongoing adventitious agent testing in subsequent step			
		Chemical	Potential for inclusion of incorrect media components		x	SOP-012 requires incoming material inspection for all media components, which includes certificate and allergen confirmation Media prep MBRs (e.g., 044 and 048) require confirmation of lot # and expiration for each input			
		Physical	Introduction of foreign material such as metal or glass fragments during media mixing step		x	Sterile filtration process is included in the media preparation batch records with a 0.2 µm filter. Each final product is passed through the X-ray in step 9			

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
3	Cell banking	Biological	Potential introduction of microorganisms in cell banks (*Salmonella, L. monocytogenes, Staphylococcus aureus*) from the environment/personnel handling		x	Ongoing adventitious agent testing: Per SOP-002, cell vials tested via 3rd party laboratory required to be free from microbial contamination before releasing to cell banks			
		Chemical	Potential for foods with unclear regulatory precedence (freezing agents) in product		x	Mass balance calculations and analytical testing of finished product shows absence of freezing agents.			
		Chemical	Potential for cross contamination between different species during storage, undesirable species identified as part of the vial		x	Per SOP-002, MCBs and WCBs are clearly labeled and color coded. Cryoboxes with different allergens are not stored in same liquid nitrogen storage			
		Physical	None						

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
4	Cell Thaw	Biological	Potential introduction of microorganisms in cell culture (*Salmonella, L. monocytogenes, Staphylococcus aureus*) from the environment/personnel		x	Pathogens would outcompete cell growth - pathogens controlled by monitoring for contamination in each batch Subsequent lethal step Ongoing adventitious agent testing at subsequent step			
		Chemical	Potential introduction of non-labeled allergens		x	The cell thaw MBR requires operators to affix labels from thawed vials to MBR and a secondary verifier Cryoboxes with different allergens are not stored in same liquid nitrogen storage			
		Chemical	Potential for thawing incorrect cell line for production		x	SOP-002 (cell banking) includes step-by-step instructions and controls to prevent thawing incorrect vial			
		Chemical	Potential for inputs without applicable authorization (freezing agents) in harvested cell material		x	Mass balance calculations and analytical testing of finished product shows absence of freezing agents.			
		Physical	None						

5	Seed train and cell proliferation	Biological	Potential growth of pathogens from the environment / human contact. Utensils/tools, bioreactor, or any equipment contaminated and not cleaned.	x		Cell culture conditions in flasks and bioreactors are amenable to pathogen growth	**Sanitation Preventive Control** Environmental monitoring program, master sanitation schedule, & GMPs mitigate environmental pathogens **Process Preventive Controls** Upstream production MBRs include aseptic techniques, process parameters to record and monitor DO changes as an indicator for contamination. Dissolved oxygen drops of >30% over an 8-hour period in bioreactors are determined to be at risk for contamination and subjected to further screening including microscopy. For shake flasks, turbidity is visually inspected at least 5x / week as a sign for contamination. If contaminated then cultures are terminated. Ongoing adventitious agent testing at subsequent step	x	
		Chemical	Cleaning chemical residue may be present in bioreactor	x		Clean-in-place chemistry may not be adequately rinsed and removed following CIP process	**Process Preventive Control** Cleaning development study is performed which determines the cleaning process. Cleaning chemistry removal and cleaning effectiveness is verified by collection of final rinse samples which are tested for pH, conductivity, ATP, and a visual inspection. Cleaning verification testing is performed as part of each bioreactor cleaning. Passing results are required for releasing the equipment for the next production run.	x	
		Physical	Potential for metal or glass fragments	x		Metal-to-metal contact inside a bioreactor or broken glass probes may produce metal or glass fragments	**Process Preventive Control** X-ray (conducted in a subsequent step)		x

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
6	Cell Harvest from bioreactors	Biological	Potential growth of pathogens such as *Salmonella, L. monocytogenes, Staphylococcus aureus*	x		Pathogens, if present in the environment, have the opportunity to be introduced however, the process is short and GMPs are followed throughout the process Cells are frozen at ≤-20 °C following this step and banked for further processing using standard food inputs and manufacturing techniques.	**Process Preventive Controls** 1. Thermal process in subsequent step inactivates potential pathogens 2. **Critical control point:** Ongoing adventitious agent testing as described in Figure 4 above		x
		Chemical	Potential for inputs without applicable authorization (some media components) in product		x	Several inputs used in Wildtype's cell culture medium do not have an applicable authorization. Calculations for the "worst case" exposure scenarios in Figure 1 above indicate that inputs without applicable authorization are below NOAEL levels with a MOS >100, or below ADI, OSL, or background dietary intake levels.			
		Physical	None						

Appendix 7: certificates of analysis (COAs) for analytical testing (address omitted for privacy)

Mérieux NutriSciences

SILLIKER, Inc.

Salida, CA Laboratory
5262 Pirrone Court, Salida, CA 95368
Tel. 1-844-277-1680 Fax. 209-545-0245
Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48276902-0
Supersedes:	None
COA Date	6/7/24
Page 1 of 7	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	5/17/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 435399216 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1
Sample Name: 023-2024-05-08-01

Additional Field 1: T302

Analyte	Result	Units	Method Reference	Test Date	Loc.
Amino Acids Complete			USDA MSS2 (1993)	6/3/24	CHG
Aspartic Acid	0.45	% (w/w)			
Threonine	0.21	% (w/w)			
Serine	0.24	% (w/w)			
Glutamic Acid	0.68	% (w/w)			
Glycine	0.28	% (w/w)			
Alanine	0.27	% (w/w)			
Valine	0.27	% (w/w)			
Methionine	0.16	% (w/w)			
Isoleucine	0.22	% (w/w)			
Leucine	0.39	% (w/w)			
Tyrosine	0.17	% (w/w)			
Phenylalanine	0.23	% (w/w)			
Lysine	0.38	% (w/w)			
Histidine	0.11	% (w/w)			
Arginine	0.29	% (w/w)			
Proline	0.26	% (w/w)			
Hydroxyproline	<0.01	% (w/w)			
Cysteine	0.08	% (w/w)			
Tryptophan	0.05	% (w/w)			
* Ash	0.61	% (w/w)	AOAC 938.08	5/28/24	CHG
Calories by Calculation	31	Cal/100g	Atwater Factors	6/4/24	CHG
Carbohydrates - Calculation	0.75	% (w/w)	Calculation	6/4/24	CHG
Fat - Mojo, Acid Hydrolysis	1.13	% (w/w)	AOAC 948.15	5/23/24	CHG
* Fat by Fatty Acid Profile			AOAC 996.06 (mod)	5/28/24	CHG

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Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48276902-0
Supersedes:	None
COA Date	6/7/24
Page 2 of 7	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	5/17/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Sample Name: 023-2024-05-08-01

Additional Field 1: T302

Fat Analysis by GC - Summary

Fat by Fatty Acid Profile	0.69	g/100g
Total Saturated Fatty Acids	0.16	g/100g
Total Monounsaturated Fatty Acids	0.36	g/100g
Total Polyunsaturated Fatty Acids	0.11	g/100g
Total Trans Fatty Acids	0.04	g/100g
Total Conjugated Fatty Acids	0.00	g/100g

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48276902-0
Supersedes:	None
COA Date	6/7/24
Page 3 of 7	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	5/17/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Sample Name: 023-2024-05-08-01

Additional Field 1: T302

Fat Analysis by GC — **Laboratory ID:** 435399216

Fatty Acids	% Fatty Acid in Product (Weight/Weight Basis)					% as Triglyceride	% FA of Total FA
	Saturated	Cis MUFA	Cis PUFA	Trans	Conjugated		
4:0 Butanoic (Butyric)						0.000	0.000
5:0 Pentanoic (Valeric)						0.000	0.000
6:0 Hexanoic (Caproic)						0.000	0.000
7:0 Heptanoic (Enanthic)						0.000	0.000
8:0 Octanoic (Caprylic)						0.000	0.000
9:0 Nonanoic (Pelargonic)						0.000	0.000
10:0 Decanoic (Capric)						0.000	0.000
11:0 Undecanoic						0.000	0.000
12:0 Dodecanoic (Lauric)						0.000	0.000
12:1 Dodecenoic						0.000	0.000
14:0 Tetradecanoic (Myristic)	0.010					0.011	1.542
14:1 trans-Tetradecenoic						0.000	0.000
14:1 Tetradecenoic (Myristoleic)						0.000	0.000
15:0 Pentadecanoic	0.002					0.002	0.301
15:1 Pentadecenoic						0.000	0.000
16:0 Hexadecanoic (Palmitic)	0.089					0.094	13.433
16:1 trans-Hexadecenoic						0.000	0.000
16:1 Hexadecenoic (Palmitoleic)		0.013				0.013	1.899
17:0 Heptadecanoic (Margaric)						0.000	0.000
17:1 Heptadecenoic (Margaroleic)		0.043				0.045	6.496
18:0 Octadecanoic (Stearic)	0.058					0.060	8.673
18:1 trans-Octadecenoic (incl. Elaidic)				0.005		0.005	0.704
18:1 Octadecenoic (incl. Oleic)		0.284				0.297	42.790
18:2 trans-Octadecadienoic				0.032		0.033	4.787
18:2 Octadecadienoic (Linoleic)			0.071			0.074	10.723
20:0 Eicosanoic (Arachidic)						0.000	0.000
18:3 trans-Octadecatrienoic						0.000	0.000
18:3 g-Linolenic			0.005			0.006	0.816
20:1 trans-Eicosenoic						0.000	0.000

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Mérieux NutriSciences

SILLIKER, Inc.

Salida, CA Laboratory

5262 Pirrone Court, Salida, CA 95368

Tel. 1-844-277-1680 Fax. 209-545-0245

Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48276902-0
Supersedes:	None
COA Date	6/7/24
Page 4 of 7	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	5/17/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Sample Name: 023-2024-05-08-01

Additional Field 1: T302

Fat Analysis by GC

Laboratory ID: 435399216

Fatty Acids	% Fatty Acid in Product (Weight/Weight Basis)					% as Triglyceride	% FA of Total FA
	Saturated	Cis MUFA	Cis PUFA	Trans	Conjugated		
20:1 Eicosenoic (incl. Gadoleic)		0.014				0.014	2.085
18:3 Octadecatrienoic (Linolenic)			0.001			0.001	0.177
21:0 Heneicosanoic						0.000	0.000
18:2 conj Linoleic						0.000	0.000
18:4 Octadecatetraenoic (Moroctic)						0.000	0.000
20:2 Eicosadienoic			0.007			0.007	1.081
20:3 5,8,11-Eicosatrienoic						0.000	0.000
22:0 Docosanoic (Behenic)						0.000	0.000
20:3 8,11,14-Eicosatrienoic (gamma)			0.024			0.025	3.602
22:1 trans-Docosaenoic (Brassidic)						0.000	0.000
22:1 Cetoleic						0.000	0.000
22:1 Docosaenoic (Erucic)		0.001				0.001	0.216
20:3 11,14,17-Eicosatrienoic						0.000	0.000
20:4 Eicosatetraenoic (Arachidonic)			0.004			0.005	0.673
23:0 Tricosanoic						0.000	0.000
22:2 Docosadienoic						0.000	0.000
24:0 Tetracosanoic (Lignoceric)						0.000	0.000
20:5 Eicosapentaenoic						0.000	0.000
24:1 Tetracosaenoic (Nervonic)						0.000	0.000
22:3 Docosatrienoic						0.000	0.000
22:4 Docosatetraenoic						0.000	0.000
22:5 Docosapentaenoic						0.000	0.000
22:6 Docosahexaenoic						0.000	0.000
Total (g per 100g)	0.16	0.36	0.11	0.04	0.00	0.69	100.00
% of Total Fatty Acid Concentration	23.95	53.49	17.07	5.49	0.00		

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48276902-0
Supersedes:	None
COA Date	6/7/24
Page 5 of 7	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	5/17/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 435399216 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1
Sample Name: 023-2024-05-08-01

Additional Field 1: T302

Analyte	Result	Units	Method Reference	Test Date	Loc.
Folic Acid (Microbiological Assay)	7.30	mcg/100g	AOAC 960.46 & Kit	6/4/24	CHG
Glutathione	0.0454	% (w/w)	Internal HPLC H068	5/24/24	BRN
* ICP MS Heavy Metals (4 analytes)			AOAC2015.01Mod<2232>	5/31/24	CHG
Arsenic	0.02	ppm (w/w)			
Cadmium	0.005	ppm (w/w)			
Lead	<0.01	ppm (w/w)			
Mercury	<0.005	ppm (w/w)			
ICP Sample Prep - Microwave	Microwave	-	AOAC 2011.14	5/24/24	CHG
* Iron	<0.25	mg/100g	AOAC 984.27 (mod.)	5/28/24	CHG
* Magnesium	7.97	mg/100g	AOAC 984.27 (mod.)	5/28/24	CHG
* Moisture - Vacuum Oven	92.41	% (w/w)	AOAC 926.08	5/30/24	CHG
Omega 3 Fatty Acids	<0.01	g/100g	Calculation	5/28/24	CHG
Omega 6 Fatty Acids	0.10	g/100g	Calculation	5/28/24	CHG
Omega 9 Fatty Acids	0.29	g/100g	Calculation	5/28/24	CHG
Pantothenic Acid (Microbiological Assay)	0.74	mg/100g	AOAC 960.46 & Kit	6/4/24	CHG
Phosphate			AOAC 984.27	5/28/24	CHG
as	PO4	-			
Phosphate	475	mg/100g			
* Phosphorus	155	mg/100g	AOAC 984.27 (mod.)	5/28/24	CHG
* Potassium	145	mg/100g	AOAC 984.27 (mod.)	5/28/24	CHG
* Protein - Kjeldahl			AOAC 991.20	5/24/24	CHG
Protein Factor	6.25	-			
As Received	5.54	% (w/w)			
* Salt (calculated from Chloride)			AOAC 983.14	5/24/24	CHG

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48276902-0
Supersedes:	None
COA Date	6/7/24
Page 6 of 7	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	5/17/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 435399216 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1
Sample Name: 023-2024-05-08-01

Additional Field 1: T302

Analyte	Result	Units	Method Reference	Test Date	Loc.
Salt	0.23	% (w/w)			
Chloride	0.14	% (w/w)			
* Selenium	0.1	ppm (w/w)	AOAC2015.01Mod<2232>	5/31/24	CHG
* Sodium	77.1	mg/100g	AOAC 984.27 (mod.)	5/28/24	CHG
Solvent Analysis			USP/NF Current Ver.	5/31/24	BRN
-	-	-			
Dimethyl Sulfoxide	<50	ppm (w/w)			
Thiamine	0.34	mg/100g	AOAC 942.23	6/5/24	CHG
* Total Vitamin A			Analyst(1984)109:489	5/30/24	CHG
Retinol (mcg RAE)	<3	mcg RAE/100 g			
Beta Carotene (mcg RAE)	<1	mcg RAE/100 g			
Total Vitamin A (mcg RAE)	<4	mcg RAE/100 g			
Total Vitamin B12	139.00	mcg/100g	AOAC 960.46 & Kit	6/7/24	CHG
Trehalose	0.33	% (w/w)	Internal HPLC	5/29/24	CHG
Vitamin D			AOAC 2016.05 Mod.	5/24/24	CHG
Vitamin D2	13.6	mcg/100g			
Vitamin D3	<0.13	mcg/100g			
Total Vitamin D (mcg/100g)	13.6	mcg/100g			
Vitamin E	<0.25	mg a-tocoph/100g	AOAC 992.03	5/24/24	CHG

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48276902-0
Supersedes:	None
COA Date	6/7/24
Page 7 of 7	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	5/17/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

[redacted]
Julienne Mørtensen Laboratory Director

Noted Test Locations: CHG-Silliker, Inc. Crete, IL Laboratory, 3600 Eagle Nest Drive, North Building, Crete, IL 60417
Noted Test Locations: BRN-Silliker Canada Co., Burnaby Laboratory, 106-8255 North Fraser Way, Burnaby, BC V3N 0B9

I Customer supplied information * ISO17025 Accredited Analysis † Indicates reason for COA amendent when applicable

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Mérieux NutriSciences

SILLIKER, Inc.

Salida, CA Laboratory
5262 Pirrone Court, Salida, CA 95368
Tel. 1-844-277-1680 Fax. 209-545-0245
Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48402777-0
Supersedes:	None
COA Date	7/18/24
Page 1 of 2	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/24/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 436173977 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 2.1
Sample Name: 023-2024-05-08-01

Analyte	Result	Units	Method Reference	Test Date	Loc.
* ICP-MS Sample Prep	Acid Digest	-	AOAC2015.01Mod<2232>	7/16/24	BRN
* Speciated Arsenic			FDA EAMS HPLC-ICPMS	7/17/24	BRN
Monomethyl arsonic acid	4.1	ppb (w/w)			
Dimethylarsinic acid	8.4	ppb (w/w)			
Arseno-betaine	5.4	ppb (w/w)			
Inorganic Arsenic	7.8	ppb (w/w)			
Organic Arsenic	17.9	ppb (w/w)			
Total Arsenic	20	ppb (w/w)			
* Aerobic Plate Count	<10	/g	AOAC 966.23	6/27/24	
* Bacillus cereus	<10	/g	AOAC 980.31	6/26/24	
C. perfringens - Presumptive	<10	/g	AOAC 976.30	6/26/24	
Campylobacter - ELFA	Negative	/25g	AOAC-RI 051201	6/28/24	ATL
* E. coli / Coliform - Petrifilm			AOAC 991.14	6/27/24	
Coliform-Petrifilm	<10	/g			
E. coli-Petrifilm	<10	/g			
* E. coli O157:H7 PCR	Negative	/25g	AOAC RI 031002	6/27/24	
* Enterobacteriaceae - Petrifilm	<10	/g	AOAC 2003.01	6/26/24	
Genus Listeria - PCR	Negative	/25g	AOAC 2019.10	6/27/24	
Presumptive Viable C.botulinum	Negative	/8g	FDA-BAM, 8th ed.	7/18/24	RES
Salmonella - ELFA	Negative	/25g	AOAC 2004.03	6/27/24	
* Staphylococci - coag. positive	<10	/g	AOAC 975.55	6/27/24	
* Yeast and Mold			FDA-BAM, 7th ed.	6/30/24	
Yeast	<10	/g			
Mold	<10	/g			

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48402777-0
Supersedes:	None
COA Date	7/18/24
Page 2 of 2	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/24/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

[redacted]
Julienne Mørtensen Laboratory Director

Noted Test Locations: BRN-Silliker Canada Co., Burnaby Laboratory, 106-8255 North Fraser Way, Burnaby, BC V3N 0B9
Noted Test Locations: ATL-Silliker, Inc. Stone Mountain, GA Laboratory, 2169 West Park Court, Suite G, Stone Mountain, GA 30087
Noted Test Locations: RES-Silliker, Inc. Food Science Center Laboratory, 3600 Eagle Nest Drive, South Building, Crete, IL 60417

I Customer supplied information * ISO17025 Accredited Analysis † Indicates reason for COA amendent when applicable

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Salida, CA Laboratory
5262 Pirrone Court, Salida, CA 95368
Tel. 1-844-277-1680 Fax. 209-545-0245
Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48371528-0
Supersedes:	CCA-48300839-4
COA Date	7/8/24
Page 1 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/7/24
P.O.# / ID:	OPs
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 435831080 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1
Sample Name: Lot: 024-2024-06-06-01 Nutritional

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Ash	0.49	% (w/w)	AOAC 938.08	6/14/24	CHG
Calories by Calculation	66	Cal/100g	Atwater Factors	6/18/24	CHG
Carbohydrates - Calculation	6.98	% (w/w)	Calculation	6/18/24	CHG
Fat - Mojo, Acid Hydrolysis	1.41	% (w/w)	AOAC 948.15	6/13/24	CHG
* Fat by Fatty Acid Profile			AOAC 996.06 (mod)	6/19/24	CHG
Fat Analysis by GC - Summary					
Fat by Fatty Acid Profile	0.92	g/100g			
Total Saturated Fatty Acids	0.22	g/100g			
Total Monounsaturated Fatty Acids	0.44	g/100g			
Total Polyunsaturated Fatty Acids	0.16	g/100g			
Total Trans Fatty Acids	0.05	g/100g			
Total Conjugated Fatty Acids	0.00	g/100g			

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Mérieux NutriSciences

SILLIKER, Inc.

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5262 Pirrone Court, Salida, CA 95368
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Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48371528-0
Supersedes:	CCA-48300839-4
COA Date	7/8/24
Page 2 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/7/24
P.O.# / ID:	OPs

Location of Test: (except where noted)
Salida, CA

Analytical Results

Sample Name: Lot: 024-2024-06-06-01 Nutritional

Fat Analysis by GC

Laboratory ID: 435831080

Fatty Acids	% Fatty Acid in Product (Weight/Weight Basis)						
	Saturated	Cis MUFA	Cis PUFA	Trans	Conjugated	% as Triglyceride	% FA of Total FA
4:0 Butanoic (Butyric)	0.001					0.001	0.072
5:0 Pentanoic (Valeric)						0.000	0.000
6:0 Hexanoic (Caproic)						0.000	0.000
7:0 Heptanoic (Enanthic)						0.000	0.000
8:0 Octanoic (Caprylic)						0.000	0.000
9:0 Nonanoic (Pelargonic)						0.000	0.000
10:0 Decanoic (Capric)						0.000	0.000
11:0 Undecanoic						0.000	0.000
12:0 Dodecanoic (Lauric)						0.000	0.032
12:1 Dodecenoic						0.000	0.000
14:0 Tetradecanoic (Myristic)	0.011					0.012	1.294
14:1 trans-Tetradecenoic						0.000	0.000
14:1 Tetradecenoic (Myristoleic)						0.000	0.000
15:0 Pentadecanoic	0.003					0.003	0.294
15:1 Pentadecenoic						0.000	0.000
16:0 Hexadecanoic (Palmitic)	0.122					0.128	13.969
16:1 trans-Hexadecenoic						0.000	0.000
16:1 Hexadecenoic (Palmitoleic)		0.014				0.015	1.622
17:0 Heptadecanoic (Margaric)						0.000	0.039
17:1 Heptadecenoic (Margaroleic)		0.034				0.035	3.828
18:0 Octadecanoic (Stearic)	0.083					0.087	9.491
18:1 trans-Octadecenoic (incl. Elaidic)				0.008		0.008	0.890
18:1 Octadecenoic (incl. Oleic)		0.380				0.398	43.437
18:2 trans-Octadecadienoic				0.042		0.044	4.778
18:2 Octadecadienoic (Linoleic)			0.099			0.103	11.299
20:0 Eicosanoic (Arachidic)						0.000	0.000
18:3 trans-Octadecatrienoic						0.000	0.000
18:3 g-Linolenic			0.009			0.010	1.052
20:1 trans-Eicosenoic						0.000	0.000
20:1 Eicosenoic (incl. Gadoleic)		0.014				0.014	1.585

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SILLIKER, Inc.

Salida, CA Laboratory
5262 Pirrone Court, Salida, CA 95368
Tel. 1-844-277-1680 Fax. 209-545-0245
Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-48371528-0
Supersedes:	CCA-48300839-4
COA Date	7/8/24
Page 3 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/7/24
P.O.# / ID:	OPs
Location of Test: (except where noted)	Salida, CA

Analytical Results

Sample Name: Lot: 024-2024-06-06-01 Nutritional

Fat Analysis by GC — **Laboratory ID:** 435831080

Fatty Acids	% Fatty Acid in Product (Weight/Weight Basis) Saturated	Cis MUFA	Cis PUFA	Trans	Conjugated	% as Triglyceride	% FA of Total FA
18:3 Octadecatrienoic (Linolenic)			0.001			0.001	0.106
21:0 Heneicosanoic						0.000	0.000
18:2 conj Linoleic						0.000	0.000
18:4 Octadecatetraenoic (Moroctic)						0.000	0.000
20:2 Eicosadienoic			0.011			0.012	1.305
20:3 5,8,11-Eicosatrienoic						0.000	0.000
22:0 Docosanoic (Behenic)						0.000	0.000
20:3 8,11,14-Eicosatrienoic (gamma)			0.037			0.038	4.189
22:1 trans-Docosaenoic (Brassidic)						0.000	0.000
22:1 Cetoleic						0.000	0.000
22:1 Docosaenoic (Erucic)		0.002				0.002	0.196
20:3 11,14,17-Eicosatrienoic						0.000	0.000
20:4 Eicosatetraenoic (Arachidonic)			0.005			0.005	0.521
23:0 Tricosanoic						0.000	0.000
22:2 Docosadienoic						0.000	0.000
24:0 Tetracosanoic (Lignoceric)						0.000	0.000
20:5 Eicosapentaenoic						0.000	0.000
24:1 Tetracosaenoic (Nervonic)						0.000	0.000
22:3 Docosatrienoic						0.000	0.000
22:4 Docosatetraenoic						0.000	0.000
22:5 Docosapentaenoic						0.000	0.000
22:6 Docosahexaenoic						0.000	0.000
Total (g per 100g)	0.22	0.44	0.16	0.05	0.00	0.92	100.00
% of Total Fatty Acid Concentration	25.19	50.67	18.47	5.67	0.00		

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48371528-0
Supersedes:	CCA-48300839-4
COA Date	7/8/24
Page 4 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/7/24
P.O.# / ID:	OPs
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 435831080 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1
Sample Name: Lot: 024-2024-06-06-01 Nutritional

Analyte	Result	Units	Method Reference	Test Date	Loc.
Folic Acid (Microbiological Assay)	18.20	mcg/100g	AOAC 960.46 & Kit	6/19/24	CHG
ICP MS Heavy Metals (4 analytes)			AOAC2015.01Mod<2232>	6/14/24	BRN
Arsenic	0.03	ppm (w/w)			
Cadmium	0.004	ppm (w/w)			
Lead	<0.01	ppm (w/w)			
Mercury	<0.005	ppm (w/w)			
Moisture - Vacuum Oven	84.78	% (w/w)	AOAC 950.46A	6/13/24	CHG
Omega 3 Fatty Acids	<0.01	g/100g	Calculation	6/19/24	CHG
Omega 6 Fatty Acids	0.16	g/100g	Calculation	6/19/24	CHG
Omega 9 Fatty Acids	0.40	g/100g	Calculation	6/19/24	CHG
Pantothenic Acid (Microbiological Assay)	0.40	mg/100g	AOAC 960.46 & Kit	7/2/24	CHG
* Protein - Kjeldahl			AOAC 991.20	6/18/24	CHG
Protein Factor	6.25	-			
As Received	6.34	% (w/w)			
* Speciated Arsenic			FDA EAMS HPLC-ICPMS	6/20/24	BRN
Monomethyl arsonic acid	<3.4	ppb (w/w)			
Dimethylarsinic acid	22.9	ppb (w/w)			
Arseno-betaine	<3.4	ppb (w/w)			
Total Arsenic	31	ppb (w/w)			
Inorganic Arsenic	4.7	ppb (w/w)			
Organic Arsenic	26.3	ppb (w/w)			
* Total Vitamin A			Analyst(1984)109:489	6/18/24	CHG
Retinol (mcg RAE)	<3	mcg RAE/100 g			
Beta Carotene (mcg RAE)	<1	mcg RAE/100 g			
Total Vitamin A (mcg RAE)	<4	mcg RAE/100 g			
Total Vitamin B12	90.00	mcg/100g	AOAC 960.46 & Kit	6/19/24	CHG

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48371528-0
Supersedes:	CCA-48300839-4
COA Date	7/8/24
Page 5 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/7/24
P.O.# / ID:	OPs
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 435831080 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 1
Sample Name: Lot: 024-2024-06-06-01 Nutritional

Analyte	Result	Units	Method Reference	Test Date	Loc.
Vitamin D			AOAC 2016.05 Mod.	6/21/24	CHG
Vitamin D2	6.20	mcg/100g			
Vitamin D3	<0.13	mcg/100g			
Total Vitamin D (mcg/100g)	6.20	mcg/100g			

[redacted]
Julienne Mørtensen Laboratory Director

Noted Test Locations: CHG-Silliker, Inc. Crete, IL Laboratory, 3600 Eagle Nest Drive, North Building, Crete, IL 60417
Noted Test Locations: BRN-Silliker Canada Co., Burnaby Laboratory, 106-8255 North Fraser Way, Burnaby, BC V3N 0B9

I Customer supplied information * ISO17025 Accredited Analysis † Indicates reason for COA amendent when applicable

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48300838-2
Supersedes:	CCA-48300838-1
COA Date	6/28/24
Page 1 of 1	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/7/24
P.O.# / ID:	OPs
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 435831066 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** -1.3
Sample Name: Lot: 024-2024-06-06-01 Microbiological

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Aerobic Plate Count	<10	/g	AOAC 966.23	6/10/24	
* Bacillus cereus	<10	/g	AOAC 980.31	6/9/24	
C. perfringens - Presumptive	<10	/g	AOAC 976.30	6/9/24	
Campylobacter - ELFA	Negative	/25g	AOAC-RI 051201	6/13/24	ATL
* E. coli / Coliform - Petrifilm			AOAC 991.14	6/10/24	
Coliform-Petrifilm	<10	/g			
E. coli-Petrifilm	<10	/g			
* E. coli O157:H7 PCR	Negative	/25g	AOAC RI 031002	6/9/24	
* Enterobacteriaceae - Petrifilm	<10	/g	AOAC 2003.01	6/9/24	
Genus Listeria - PCR	Negative	/25g	AOAC 2019.10	6/9/24	
Presumptive Viable C.botulinum	Negative	/8g	FDA-BAM, 8th ed.	6/28/24	RES
Salmonella - ELFA	Negative	/25g	AOAC 2004.03	6/10/24	
* Staphylococci - coag. positive	<10	/g	AOAC 975.55	6/10/24	
* Yeast and Mold			FDA-BAM, 7th ed.	6/13/24	
Yeast	<10	/g			
Mold	<10	/g			

[redacted]
Julienne Mørtensen Laboratory Director

Noted Test Locations: ATL-Silliker, Inc. Stone Mountain, GA Laboratory, 2169 West Park Court, Suite G, Stone Mountain, GA 30087
Noted Test Locations: RES-Silliker, Inc. Food Science Center Laboratory, 3600 Eagle Nest Drive, South Building, Crete, IL 60417

[redacted]

I Customer supplied information * ISO17025 Accredited Analysis † Indicates reason for COA amendent when applicable

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48371529-0
Supersedes:	CCA-48300821-3
COA Date	7/8/24
Page 1 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/10/24
P.O.# / ID:	OPs

Location of Test: (except where noted)
Salida, CA

Analytical Results

Laboratory ID: 435842482 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.8
Sample Name: Lot: 024-2024-06-06-02 Nutritional

Additional Field 1: 3rd wash

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Ash	0.52	% (w/w)	AOAC 938.08	7/1/24	CHG
Calories by Calculation	72	Cal/100g	Atwater Factors	7/1/24	CHG
Carbohydrates - Calculation	7.63	% (w/w)	Calculation	7/1/24	CHG
Fat - Mojo, Acid Hydrolysis	1.75	% (w/w)	AOAC 948.15	7/1/24	CHG
* Fat by Fatty Acid Profile			AOAC 996.06 (mod)	7/1/24	CHG
Fat Analysis by GC - Summary					
Fat by Fatty Acid Profile	1.16	g/100g			
Total Saturated Fatty Acids	0.28	g/100g			
Total Monounsaturated Fatty Acids	0.51	g/100g			
Total Polyunsaturated Fatty Acids	0.21	g/100g			
Total Trans Fatty Acids	0.06	g/100g			
Total Conjugated Fatty Acids	0.05	g/100g			

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48371529-0
Supersedes:	CCA-48300821-3
COA Date	7/8/24
Page 2 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/10/24
P.O.# / ID:	OPs
Location of Test: (except where noted)	Salida, CA

Analytical Results

Sample Name: Lot: 024-2024-06-06-02 Nutritional

Additional Field 1: 3rd wash

Fat Analysis by GC

Laboratory ID: 435842482

Fatty Acids	% Fatty Acid in Product (Weight/Weight Basis) Saturated	Cis MUFA	Cis PUFA	Trans	Conjugated	% as Triglyceride	% FA of Total FA
4:0 Butanoic (Butyric)	0.002					0.002	0.191
5:0 Pentanoic (Valeric)						0.000	0.000
6:0 Hexanoic (Caproic)						0.000	0.000
7:0 Heptanoic (Enanthic)						0.000	0.000
8:0 Octanoic (Caprylic)						0.000	0.000
9:0 Nonanoic (Pelargonic)						0.000	0.000
10:0 Decanoic (Capric)						0.000	0.000
11:0 Undecanoic						0.000	0.000
12:0 Dodecanoic (Lauric)						0.000	0.000
12:1 Dodecenoic						0.000	0.000
14:0 Tetradecanoic (Myristic)	0.013					0.014	1.196
14:1 trans-Tetradecenoic						0.000	0.000
14:1 Tetradecenoic (Myristoleic)						0.000	0.000
15:0 Pentadecanoic	0.002					0.002	0.179
15:1 Pentadecenoic						0.000	0.000
16:0 Hexadecanoic (Palmitic)	0.154					0.161	13.820
16:1 trans-Hexadecenoic						0.000	0.000
16:1 Hexadecenoic (Palmitoleic)		0.017				0.018	1.563
17:0 Heptadecanoic (Margaric)						0.000	0.000
17:1 Heptadecenoic (Margaroleic)						0.000	0.000
18:0 Octadecanoic (Stearic)	0.103					0.107	9.262
18:1 trans-Octadecenoic (incl. Elaidic)				0.013		0.014	1.178
18:1 Octadecenoic (incl. Oleic)		0.472				0.493	42.490
18:2 trans-Octadecadienoic				0.048		0.050	4.349
18:2 Octadecadienoic (Linoleic)			0.128			0.133	11.486
20:0 Eicosanoic (Arachidic)						0.000	0.000
18:3 trans-Octadecatrienoic						0.000	0.000
18:3 g-Linolenic			0.014			0.015	1.259
20:1 trans-Eicosenoic						0.000	0.000

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48371529-0
Supersedes:	CCA-48300821-3
COA Date	7/8/24
Page 3 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/10/24
P.O.# / ID:	OPs
Location of Test: (except where noted)	Salida, CA

Analytical Results

Sample Name: Lot: 024-2024-06-06-02 Nutritional

Additional Field 1: 3rd wash

Fat Analysis by GC — **Laboratory ID:** 435842482

Fatty Acids	% Fatty Acid in Product (Weight/Weight Basis) Saturated	Cis MUFA	Cis PUFA	Trans	Conjugated	% as Triglyceride	% FA of Total FA
20:1 Eicosenoic (incl. Gadoleic)		0.017				0.017	1.488
18:3 Octadecatrienoic (Linolenic)						0.000	0.000
21:0 Heneicosanoic						0.000	0.000
18:2 conj Linoleic					0.052	0.055	4.697
18:4 Octadecatetraenoic (Moroctic)						0.000	0.000
20:2 Eicosadienoic			0.014			0.014	1.234
20:3 5,8,11-Eicosatrienoic						0.000	0.000
22:0 Docosanoic (Behenic)						0.000	0.000
20:3 8,11,14-Eicosatrienoic (gamma)			0.049			0.051	4.404
22:1 trans-Docosaenoic (Brassidic)						0.000	0.000
22:1 Cetoleic						0.000	0.000
22:1 Docosaenoic (Erucic)		0.002				0.002	0.184
20:3 11,14,17-Eicosatrienoic			0.007			0.007	0.619
20:4 Eicosatetraenoic (Arachidonic)						0.000	0.000
23:0 Tricosanoic						0.000	0.000
22:2 Docosadienoic						0.000	0.000
24:0 Tetracosanoic (Lignoceric)	0.003					0.003	0.233
20:5 Eicosapentaenoic						0.000	0.000
24:1 Tetracosaenoic (Nervonic)		0.002				0.002	0.167
22:3 Docosatrienoic						0.000	0.000
22:4 Docosatetraenoic						0.000	0.000
22:5 Docosapentaenoic						0.000	0.000
22:6 Docosahexaenoic						0.000	0.000
Total (g per 100g)	0.28	0.51	0.21	0.06	0.05	1.16	100.00
% of Total Fatty Acid Concentration	24.88	45.89	19.00	5.53	4.70		

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Mérieux NutriSciences

SILLIKER, Inc.

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48371529-0
Supersedes:	CCA-48300821-3
COA Date	7/8/24
Page 4 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/10/24
P.O.# / ID:	OPs
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 435842482 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.8
Sample Name: Lot: 024-2024-06-06-02 Nutritional

Additional Field 1: 3rd wash

Analyte	Result	Units	Method Reference	Test Date	Loc.
Folic Acid (Microbiological Assay)	18.65	mcg/100g	AOAC 960.46 & Kit	7/1/24	CHG
ICP MS Heavy Metals (4 analytes)			AOAC2015.01Mod<2232>	7/1/24	BRN
Arsenic	0.03	ppm (w/w)			
Cadmium	0.005	ppm (w/w)			
Lead	<0.01	ppm (w/w)			
Mercury	<0.005	ppm (w/w)			
Moisture - Vacuum Oven	82.90	% (w/w)	AOAC 950.46A	7/1/24	CHG
Omega 3 Fatty Acids	0.01	g/100g	Calculation	7/1/24	CHG
Omega 6 Fatty Acids	0.20	g/100g	Calculation	7/1/24	CHG
Omega 9 Fatty Acids	0.50	g/100g	Calculation	7/1/24	CHG
Pantothenic Acid (Microbiological Assay)	0.33	mg/100g	AOAC 960.46 & Kit	7/1/24	CHG
* Protein - Kjeldahl			AOAC 991.20	7/1/24	CHG
Protein Factor	6.25	-			
As Received	7.79	% (w/w)			
* Speciated Arsenic			FDA EAMS HPLC-ICPMS	7/1/24	BRN
Monomethyl arsonic acid	<3.4	ppb (w/w)			
Dimethylarsinic acid	22.8	ppb (w/w)			
Arseno-betaine	<3.4	ppb (w/w)			
Total Arsenic	30	ppb (w/w)			
Inorganic Arsenic	<2	ppb (w/w)			
Organic Arsenic	30	ppb (w/w)			
* Total Vitamin A			Analyst(1984)109:489	7/1/24	CHG
Retinol (mcg RAE)	<3	mcg RAE/100 g			
Beta Carotene (mcg RAE)	<1	mcg RAE/100 g			
Total Vitamin A (mcg RAE)	<4	mcg RAE/100 g			

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SILLIKER, Inc.

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48371529-0
Supersedes:	CCA-48300821-3
COA Date	7/8/24
Page 5 of 5	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/10/24
P.O.# / ID:	OPs

Location of Test: (except where noted)
Salida, CA

Analytical Results

Laboratory ID: 435842482 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.8
Sample Name: Lot: 024-2024-06-06-02 Nutritional

Additional Field 1: 3rd wash

Analyte	Result	Units	Method Reference	Test Date	Loc.
Total Vitamin B12	101.00	mcg/100g	AOAC 960.46 & Kit	7/1/24	CHG
Vitamin D			AOAC 2016.05 Mod.	7/1/24	CHG
Vitamin D2	7.28	mcg/100g			
Vitamin D3	<0.13	mcg/100g			
Total Vitamin D (mcg/100g)	7.28	mcg/100g			

[redacted]
Julienne Mørtensen Laboratory Director

Noted Test Locations: CHG-Silliker, Inc. Crete, IL Laboratory, 3600 Eagle Nest Drive, North Building, Crete, IL 60417
Noted Test Locations: BRN-Silliker Canada Co., Burnaby Laboratory, 106-8255 North Fraser Way, Burnaby, BC V3N 0B9

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CERTIFICATE OF ANALYSIS

COA No:	CCA-48402778-0
Supersedes:	None
COA Date	7/18/24
Page 1 of 1	

TO:
Ms. [redacted]
Quality and Food Safety Manager
Wildtype
[redacted]

Received From:	San Francisco, CA
Received Date:	6/24/24
P.O.# / ID:	PF4852
Location of Test: (except where noted)	Salida, CA

Analytical Results

Laboratory ID: 436174005 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 2.1
Sample Name: 024-2024-06-06-02

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Aerobic Plate Count	<10	/g	AOAC 966.23	6/27/24	
* Bacillus cereus	<10	/g	AOAC 980.31	6/26/24	
C. perfringens - Presumptive	<10	/g	AOAC 976.30	6/26/24	
Campylobacter - ELFA	Negative	/25g	AOAC-RI 051201	6/28/24	ATL
* E. coli / Coliform - Petrifilm			AOAC 991.14	6/27/24	
Coliform-Petrifilm	<10	/g			
E. coli-Petrifilm	<10	/g			
* E. coli O157:H7 PCR	Negative	/25g	AOAC RI 031002	6/27/24	
* Enterobacteriaceae - Petrifilm	<10	/g	AOAC 2003.01	6/26/24	
Genus Listeria - PCR	Negative	/25g	AOAC 2019.10	6/27/24	
Presumptive Viable C.botulinum	Negative	/8g	FDA-BAM, 8th ed.	7/18/24	RES
Salmonella - ELFA	Negative	/25g	AOAC 2004.03	6/27/24	
* Staphylococci - coag. positive	<10	/g	AOAC 975.55	6/27/24	
* Yeast and Mold			FDA-BAM, 7th ed.	6/30/24	
Yeast	<10	/g			
Mold	<10	/g			

[redacted]
Julienne Mørtensen Laboratory Director

Noted Test Locations: ATL-Silliker, Inc. Stone Mountain, GA Laboratory, 2169 West Park Court, Suite G, Stone Mountain, GA 30087
Noted Test Locations: RES-Silliker, Inc. Food Science Center Laboratory, 3600 Eagle Nest Drive, South Building, Crete, IL 60417

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