#792

February 14, 2018

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Dear Dr. Gaynor:

Re: GRAS Notices for the Use of Savory Base 100 "Corn Sauce" and Savory Base 200 "Corn Sauce" in Food Products

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, I am submitting as the notifier [Nestec S.A., Avenue Nestlé 55, CH-1800, Vevey, Switzerland], one hard copy and one electronic copy (on CD) of each Notice that Savory Base 100 "Corn Sauce" and Savory Base 200 "Corn Sauce", as defined in the enclosed documents, are GRAS, on the basis of scientific procedures, under specific conditions of use as flavoring ingredients, and therefore, are not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act*. Information setting forth the basis for the GRAS status, which includes detailed information on the notified substances and a summary of the basis for the GRAS status, as well as a consensus opinion of an independent panel of experts in support of the safety of Savory Base 100 "Corn Sauce" and Savory Base 200 "Corn Sauce", under the intended conditions of use, also are enclosed for review by the Agency.

In a meeting between the FDA's Food Labeling and Standards Staff and the notifier, which was held on February 7, 2018, a consensus was reached supporting the use of 'corn sauce' as the common or usual name for both ingredients. When the corn sauce is used as an ingredient in food, the raw materials used to create the corn sauce would be disclosed such as "corn sauce (cultured corn starch, water, salt)".

The enclosed electronic files for the Notices entitled, GRAS Notice for the Use of Savory Base 100 "Corn Sauce" in Food Products and GRAS Notice for the Use of Savory Base 200 "Corn Sauce" in Food Products were scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding these GRAS Notices, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Yours sincerely

[redacted]

Anne Petersen
Regulatory and Scientific Affairs Manager
Nestlé PTC Singen Lebensmittelforschung GmbH

GRAS NOTICE FOR THE USE OF SAVORY BASE 100 "CORN SAUCE" IN FOOD PRODUCTS

PREPARED FOR:
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD
20740

PREPARED BY:
Nestec S.A.
Avenue Nestlé 55
CH-1800 Vevey
Switzerland

DATE:
8 February 2018

GRAS Notice for the Use of Savory Base 100 “Corn Sauce” in Food Products

TABLE OF CONTENTS

List of Appendices

List of Figures and Tables

GRAS Notice for the Use of Savory Base 100 "Corn Sauce" in Food Products

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Nestec S.A. hereby informs the United States (U.S.) Food and Drug Administration (FDA) of the view that its Savory Base 100 "Corn Sauce" is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on its conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Part 1.3 below. In addition, as a responsible official of Nestec S.A., the undersigned hereby certifies that all data and information presented in this notice constitutes a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Nestec S.A. and pertinent to the evaluation of the safety and GRAS status of Savory Base 100 "Corn Sauce" as an ingredient for addition to food, as described herein.

Signed,

[redacted] 02/09/2018

Anne Petersen
Nestlé PTC Singen Lebensmittelforschung GmbH
Anne.Petersen@rdsi.nestle.com

Date

1.1 Name and Address of Notifier

Nestec S.A.
Avenue Nestlé 55
CH-1800 Vevey
Switzerland

1.2 Common Name of Notified Substance

Savory Base 100 "Corn Sauce" (Savory Base 100).

1.3 Conditions of Use

Savory Base 100 is intended for use as an ingredient in various food products, including relishes, mayonnaise, gravies and sauces, herb and spice mixes and seasonings (including mixed dishes containing these ingredients), meat and fish analogues, and soups and broths, at use levels of up to 0.76% of the final food, as consumed (Table 1.3-1). Savory Base 100 is intended to be used as an alternative to current uses of yeast extract flavoring ingredients, affirmed as GRAS under 21 CFR §184.1983 (U.S. FDA, 2017). Some of the food uses for Savory Base 100 will be in meat- and poultry-containing finished food products that are subject to the oversight by the U.S. Department of Agriculture (USDA). As such, Nestec S.A. is

simultaneously seeking a determination from the USDA Food Safety Inspection Service (FSIS) that Savory Base 100 is suitable for uses in meat-containing products that are the subject of this Notification.

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels of Savory Base 100 "Corn Sauce" (Savory Base 100) in the United States

Food Category[a]	Proposed Food Use[b]	Maximum Proposed Use Level of Savory Base 100 (g/100 g)	Maximum Proposed Use Level of Savory Base 100 (g/100 g, expressed on a dwb)[c, d]
Condiments and Relishes	Relishes	0.76	0.51
Fats and Oils	Mayonnaise[e]	0.76	0.51
Gravies and Sauces	Gravies and sauces[f]	0.38	0.25
Herbs, Seeds, Spices, Seasonings, Blends, Extracts, and Flavorings	Herb and spice mixes, and seasonings[f]	0.60	0.40
Plant Protein Products	Meat and fish analogues	0.40	0.27
Soups and Soup Mixes	Soups and broths (all types)	0.38	0.25

dwb = dry weight basis.

[a] Food categories established under 21 CFR §170.3(n) (U.S. FDA, 2017).

[b] This table lists the direct proposed food uses of Savory Base 100. The exposure assessment conducted has accounted for final products as consumed, whereby if the proposed uses are a component of a final food, *e.g.*, mixed dish containing spices, an ingredient fraction was applied to the final product as consumed.

[c] The dry weight content of Savory Base 100 is 67%, assuming typical moisture content of 33%.

[d] Values used in the exposure assessments.

[e] This food-use represents non-standardized mayonnaise. As there were a limited number of food codes identified for non-standardized mayonnaise, food codes of standardized mayonnaise were also selected as surrogate food codes in order to provide a more robust intake estimate.

[f] These food uses may fall under the USDA's jurisdiction, as some of the finished food products to which Savory Base 100 is intended to be added can contain meat/poultry products (*e.g.*, ham, sausage).

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a) and (b) of the Code of Federal Regulations (CFR), Savory Base 100 manufactured by Nestec has been concluded to have GRAS status for use as an ingredient for addition to food products defined with the specified food categories as described in Part 1.3, on the basis of scientific procedures (U.S. FDA, 2017).

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of:

Nestle PTC Singen Lebensmittelforsching GmbH
Lange Straße 21
78224 Singen
Germany

In addition, should the FDA have any questions or additional information requests regarding this Notification during or after the Agency's review of the notice, Nestec will supply these data and information.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Nestec's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

2.1.1 Common or Usual Name

FEMA Common Name: *Corynebacterium glutamicum* corn syrup fermentation product

FEMA No.: 4907

Commercial Name: Savory Base 100 "Corn Sauce" (Savory Base 100)

Historical/alternative denotations (used in supporting documentation):

- He Wei C. Essence I;
- Savory Seasoning Sauce 1 (SSS 1);
- Corn Seasoning Sauce 1; and
- Savory Corn Sauce 1 (SCS 1).

2.1.2 Chemical Name

Not applicable.

2.1.3 Chemical Abstract Service (CAS) Number

Not applicable.

2.1.4 Chemical and Physical Characteristics

Savory Base 100 is a pale brown to brownish paste with a savory taste. Some of the constituents that contribute to the characteristic savory flavor of Savory Base 100 include glutamic acid, L-alanine, succinic acid, formic acid, and an intrinsic mix of other free and bound amino acids, organic acids, Amadori and Maillard products, and minerals and their salts.

2.2 Method of Manufacturing

2.2.1 Raw Materials and Processing Aids

The raw materials (carbon and nitrogen source) and processing aids (*e.g*., salts and minerals, anti-foaming aids and pH adjustment aids) and food contact materials used during the production of Savory Base 100 are food grade quality[1] and are used in accordance with an appropriate federal regulation, or have been determined to be GRAS for their respective uses[2]. Corn glucose syrup is used as a carbon source and liquid anhydrous ammonia is used as a nitrogen source to support microbial growth and metabolism during fermentation.

2.2.2 Manufacturing Process

Savory Base 100 is manufactured by submerged fermentation of *C. glutamicum* in glucose-based media (enzymatically hydrolyzed corn starch) in compliance with requirements for risk-based preventive controls mandated by the FDA Food Safety Modernization Act (FSMA), current Good Manufacturing Practices (cGMPs) and the principles of Hazards Analysis and Critical Control Points (HACCP). Briefly, the process involves production of a fermentation broth, to which a *C. glutamicum* starter culture is added, followed by heating, filtration, and vacuum evaporation. A schematic overview of the production process is provided in Figure 2.2.2-1.

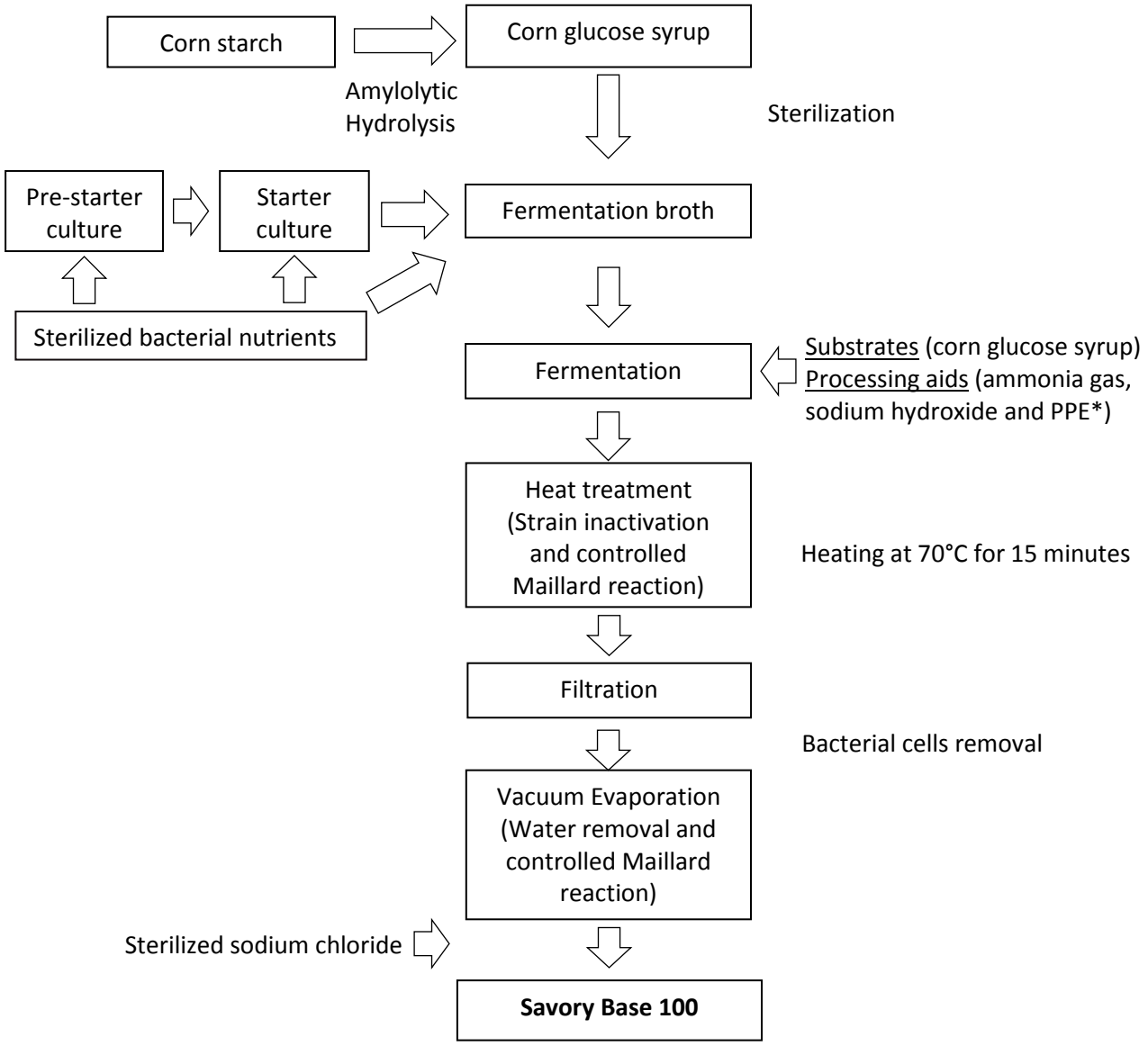
The submerged fermentation process is initiated by preparation of a fermentation broth (within a sterilized fermentation vessel), which contains sterilized nutrients for bacterial growth, substrates, and sterilized pH regulators. A small pre-starter culture is prepared separately with *C. glutamicum*, which is incubated in a medium containing the nutrients for optimum growth. This pre-starter culture is scaled up to produce the biomass, which is transferred to the primary fermentation vessel (containing the submerged fermentation broth) and then incubated. Processing aids are added during fermentation to regulate pH and reduce/prevent formation of foam. Substrates are also replenished during fermentation.

After fermentation is complete, the broth is heated to inactivate the bacteria, as well as to initiate a controlled Maillard reaction in order to achieve the desired color flavor and taste, before the broth is filtered to remove the bacterial cells (this process is monitored at Critical Control Point 3 of the HACCP plan); see Section 2.3.4 for information regarding the absence of the bacteria from the final product. The broth then undergoes vacuum evaporation, to remove water as well as initiate a second controlled Maillard reaction. At the same time sterilized sodium chloride is added to improve shelf life stability and microbial resistance against contaminants, producing the final Savory Base 100 “Corn Sauce”.

[1] Specifications compliant with U.S Food Chemicals Codex, or equivalent international standard *E.g.,* US/EU Pharmacopoeia standards.

[2] *E.g*., Antifoams or flocculants used in fermentation and recovery are used in accordance with the Enzyme Technical Association submissions to FDA.

Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for Savory Base 100 "Corn Sauce" (Savory Base 100)



*Polyoxyethylene polyoxypropylene pentaerythritol ether

2.3 Product Specifications and Batch Analysis

2.3.1 Product Specifications

The product specifications for Savory Base 100 are presented in Table 2.3.1-1.

Table 2.3.1-1 Product Specifications and Analytical Methods for Savory Base 100 "Corn Sauce" (Savory Base 100)

Specification Parameter		Specification	Method
Appearance	As is	Uniform pale brown to brownish paste	Visual test
	After preparation	Clear solution and free from visible particles or insoluble matter	
Odor ('as is' and 'after preparation')		Characteristic of Savory Base 100 flavor, free from foreign and off odors	Organoleptic test
Taste (after preparation)		Characteristic of Savory Base 100 flavor, umami, slightly salty and not bitter or burned. Free from foreign and off flavors	Organoleptic test
pH (10% dry matter solution)		5.5 to 7	APHA 4500-H+
Compositional Parameters			
Moisture content (%)		27 to 34	IDF - FIL 26A
L-Glutamic acid (%) (free)		34 to 44	AOAC 982.30
L-Alanine (%)		0.8 to 2.3	AOAC 982.30
Succinic acid (%)		0.3 to 0.7	AOAC 986.13
Formic acid (%)		0.4 to 1.2	AOAC 986.13
Total nitrogen (%)		4 to 7	ISO/FDIS 16634
Ash (%)		10 to 18	AOAC 923.03
Sodium chloride (%)		5.5 to 8	AOAC 986.26
Heavy Metals			
Arsenic (mg/kg)		≤0.5	AOAC 984.27
Lead (mg/kg)		<0.02	AOAC 984.27
Cadmium (mg/kg)		<0.01	AOAC 984.27
Mercury (mg/kg)		<0.004	AOAC 984.27
Microbiological Parameters			
Aerobic plate count (CFU/g)		≤10,000	ISO 4833:2003 AOAC method 990.12
Yeasts and molds (CFU/g)		≤100	ISO-21527-2:2008
Enterobacteriaceae (CFU/g)		≤10	ISO 21528-2:2004
Salmonella		Negative/25g	-AFNOR TRA 02/08 – 03/01 alternative method according to ISO 16140 standard:2003 -AOAC 010602

AFNOR TRA = French National Organization for Standardization; AOAC = Association of Official Agricultural Chemists; APHA = American Public Health Association; CFU = colony forming units; FDIS = Final Draft International Standard; IDF – FIL = International Dairy Federation; ISO = International Standards Organization.

2.3.2 Batch Analyses

Data from the analysis of five non-consecutive lots of Savory Base 100 demonstrating the consistency of the manufacturing process and compliance with the ingredient specifications are presented in Table 2.3.2-1.

Table 2.3.2-1 Batch Analysis Data for 5 Representative Batches of Savory Base 100 "Corn Sauce" (Savory Base 100)

Specification Parameter		Specification	Manufacturing Lot				
			G151002[a]	G160302[b]	G160304[c]	G170213[d]	G170215[e]
Appearance	As is	Uniform pale brown to brownish paste	Conforms	Conforms	Conforms	Conforms	Conforms
	After preparation	Clear solution and free from visible particles or insoluble matter	Conforms	Conforms	Conforms	Conforms	Conforms
Odor ('as is' and 'after preparation')		Characteristic of Savory Base 100 flavor, free from foreign and off odors	Conforms	Conforms	Conforms	Conforms	Conforms
Taste (after preparation)		Characteristic of Savory Base 100 flavor, umami, slightly salty and not bitter or burned. Free from foreign and off flavors	Conforms	Conforms	Conforms	Conforms	Conforms
pH (10% dry matter solution)		5.5 to 7	5.6	5.6	5.5	5.5	6.3
Compositional Parameters							
Loss on drying (%)		27 to 34	33	32	32	31	29
L-Glutamic acid (%) (free)		34 to 44	37.00	37.20	39.70	35.2	34.1
L-Alanine (%) (free)		0.8 to 2.3	1.23	0.98	0.82	2.23	1.83
Succinic acid (%)		0.3 to 0.7	0.56	0.61	0.55	0.38	0.33
Formic acid (%)		0.4 to 1.2	1	0.73	0.42	0.68	1.18
Total nitrogen (%)		4 to 7	6.3	6.4	6.2	6.2	5.8
Ash (%)		10 to 18	11	13	12	14	15
Sodium chloride (%)		5.5 to 8	5.6	7.1	6.5	6.5	7.6
Heavy Metals							
Arsenic (mg/kg)		≤0.5	<0.05	<0.02	<0.05	<0.05	<0.05
Lead (mg/kg)		<0.02	<0.02	<0.007	<0.02	0.028	<0.02
Cadmium (mg/kg)		<0.01	<0.01	<0.005	<0.01	<0.01	<0.01
Mercury (mg/kg)		<0.004	<0.003	<0.003	<0.004	<0.003	<0.003
Microbiological Parameters							
Aerobic plate count (CFU/g)		≤10,000	450	10	<10	<100	<100
Yeasts and molds (CFU/g)		≤100	<10	<10	<10	<10	<10

Table 2.3.2-1 Batch Analysis Data for 5 Representative Batches of Savory Base 100 "Corn Sauce" (Savory Base 100)

Specification Parameter	Specification	Manufacturing Lot				
		G151002[a]	G160302[b]	G160304[c]	G170213[d]	G170215[e]
Enterobacteriaceae (CFU/g)	≤10	<10	<10	<10	<10	<10
Salmonella	Negative/ 25g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g

CFU = colony forming units.
[a] Manufacturing date: October 18, 2015.
[b] Manufacturing date: March 2, 2016.
[c] Manufacturing date: March 3, 2016.
[d] Manufacturing date: February 25, 2017.
[e] Manufacturing date: February 26, 2017.

2.3.3 Additional Chemical Characterization

The mineral profile of 5 non-consecutive industrial scale lots of Savory Base 100 are presented in Table 2.3.3-1.

Table 2.3.3-1 Mineral Profile for 5 Non-Consecutive Lots of Savory Base 100 "Corn Sauce"

Parameter (values given on a dry weight basis)	Manufacturing Lot				
	G151002[a]	G160302[b]	G160304[c]	G170213[d]	G170215[e]
Mineral profile					
Sodium (%)	4.03	4.79	4.57	6.02	7.65
Potassium (%)	0.94	1.00	0.89	0.71	0.75
Magnesium (%)	0.06	0.07	0.06	0.04	0.05
Calcium (%)	0.02	0.02	0.02	0.02	0.02
Chloride (%)	3.32	3.65	4.11	3.77	4.5
Phosphate (%)	0.49	0.54	0.45	0.56	0.61
Sulfate (%)	0.15	0.20	0.14	0.16	0.15

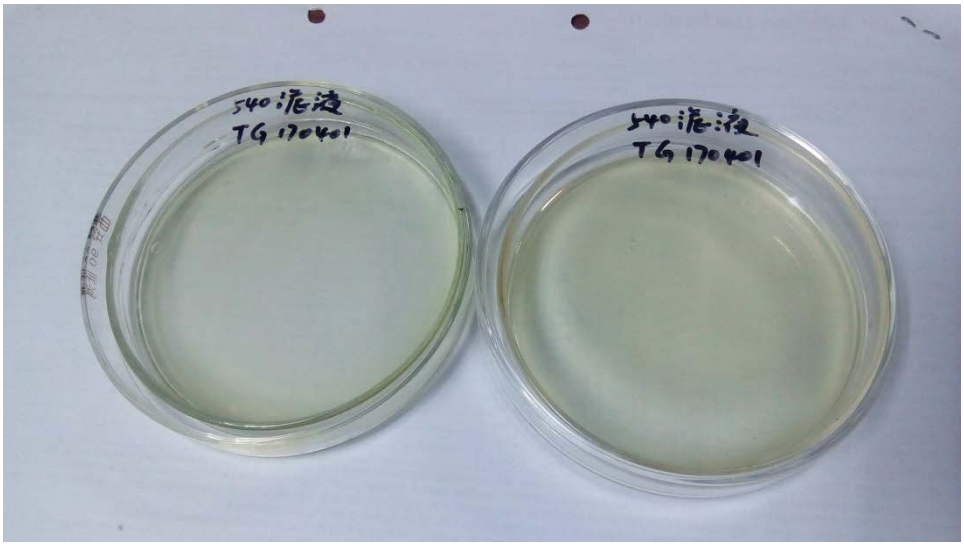
[a] Manufacturing date: October 18, 2015.
[b] Manufacturing date: March 2, 2016.
[c] Manufacturing date: March 3, 2016.
[d] Manufacturing date: February 25, 2017.
[e] Manufacturing date: February 26, 2017.

2.3.4 Other Impurities from Fermentation Media

2.3.4.1 Production Organism

The production organism (*C. glutamicum*) is excluded from the fermentate during production of Savory Base 100 using microfiltration (0.22 µm). The effectiveness of the microfiltration system was evaluated using 1 mL of Savory Base 100 filtrate, which was mixed with 15 to 20 mL of plate count agar (PCA), cooled at 46°C and then incubated at 36±1°C for approximately 48 hours. As shown in Figure 2.3.4.1-1, no microbial growth was detectable in the media.

Figure 2.3.4.1-1 Absence of the Production Organism Following Microfiltration



Absence of the fermentation strain is also corroborated by the low residual levels of protein in Savory Base 100. Three samples of Savory Base 100 were analyzed for protein content using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue staining and the Bradford assay. As shown in Table 2.3.4.1-1, no appreciable protein levels could be detected in the ingredient. Small quantities of oligopeptides or other interfering substances likely account for the residual levels of protein that were detected.

Table 2.3.4.1-1 Protein Content of Savory Base 100 "Corn Sauce"

Sample	Bradford Assay	SDS-PAGE
	Protein Concentration (mg/mL)	Protein quantity (intact/theoretical protein content) (ppm)
1 (Lot L4K-00001)	0.005 ± 0.003	33
2 (Lot L4K-00002)	ND	92
3 (Lot L4K-00003)	0.193 ± 0.002	139

ND = not detected; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis.

2.3.4.2 Biogenic Amines

Biogenic amines are biologically active organic compounds present naturally in animals and humans. The main source of exogenous amines is through consumption of foods such as fish, fish products and fermented foodstuffs (meat, dairy, vegetables, beers, and wines) (EFSA, 2011). As detailed in Table 2.3.4.2-1 below, results of analyses for biogenic amines did not identify detectable levels of phenethylamine, cadaverine, histamine, spermidine or spermine in Savory Base 100. Only minimal levels of putrescine (1.4 mg/kg), tyramine (5.4 mg/kg) and tryptamine (3.5 mg/kg) were detected, which are far below (or within, in the case of tryptamine) reported mean values of putrescine (87.3 to 222 mg/kg), tyramine (24.7 to 235 mg/kg) and tryptamine (2.4 to 7.2 mg/kg) detected in sauerkraut (Sahu *et al.*, 2015) and also lower than maximum levels found in other commercial ready-to-eat products (Table 2.3.4.2-2).

Table 2.3.4.2-1 Biogenic Amine Levels in Savory Base 100 "Corn Sauce"

Specification Parameter	Result (mg/kg)	Quantification Limit	Method of Analysis
Phenethylamine	<LQ	1	AM-BIOGE 2014 Rev.3 - HPLC-DAD
Cadaverine	<LQ	1	
Histamine	<LQ	1	
Putrescine	1.4 ± 0.4	1	
Spermidine	<LQ	1	
Spermine	<LQ	1	
Tyramine	5.4 ± 1.3	1	
Tryptamine	3.5 ± 0.9	0.5	
Biogenic Amine Index	1.4 ± 0.43	N/A	

HPLC-DAD = high performance liquid chromatography with diode array detection; LQ = quantification limit; N/A = not applicable.

Table 2.3.4.2-2 Biogenic Amine Levels in Commercial Ready-to-Eat Products

Specification Parameter	Result (mg/kg)					
	Soy Products	Miso Products	Ketchup	Finnish Dry Sausages	Washed-Rind	Parmesan
Phenylethylamine	NR	NR	NR	<1 to 48	NR	NR
Cadaverine	nd to 128	nd to 201	1.4 to 131	NR	NR	NR
Histamine	nd to 234	nd to 221	2 to 18	<1 to 200	Nd	1.4 ± 0.04
Putrescine	nd to 360	nd to 12	2.4 to 165	NR	NR	NR
Spermidine	NR	NR	NR	NR	NR	30.7 ± 1.9
Spermine	NR	NR	NR	NR	13.6 (nd to 70.5)	NR
Tyramine	nd to 237	nd to 434	4.5 to 149	82	NR	NR
Tryptamine	NR	NR	NR	<10 to 91	NR	NR
Biogenic Amine Index	nd to 959	nd to 868	10 to 463	NR	6.6	9.8

nd = not detected; NR = not reported.
Results presented as the range (soy products, miso products and ketchup) or the mean concentration (non-irradiated blue cheese, washed-rind, and parmesan).
Sources: Eerola *et al.* (1998); Prester (2016).

2.3.5 Other Internal Quality Control Analyses

2.3.5.1 Mycotoxins

As part of Nestec's internal quality control procedures, select lots of Savory Base 100 are routinely analyzed for mycotoxin contamination. The results of analysis of 5 non-sequential batches for Savory Base 100 are summarized in Table 2.3.5.1-1.

Table 2.3.5.1-1 Analysis of Mycotoxins in 5 Batches of Savory Base 100

Parameter	Specifications	Batch Number				
		G151002[a]	G160302[b]	G160304[c]	G170213[d]	G170215[e]
Aflatoxins (Sum of B and G) (µg/kg)	≤4	<4	<4	<4	<4	<4
Ochratoxin A (µg/kg)	≤0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Fumonisins (Sum of B_1 and B_2) (µg/kg)	≤100	<100	<100	<100	<100	<100
Deoxynivalenol/Vomitoxin (µg/kg)	≤50	<50	<50	<50	<50	<50
Zearalenone (µg/kg)	≤20	<20	<20	<20	<20	<20

[a] Manufacturing date: October 18, 2015.
[b] Manufacturing date: March 2, 2016.
[c] Manufacturing date: March 3, 2016.
[d] Manufacturing date: February 25, 2017.
[e] Manufacturing date: February 26, 2017.

2.3.5.2 Heterocyclic Amines

As previously discussed in Section 2.1.4, Maillard reaction products, formed from the reaction between a reducing sugar and a food-grade nitrogen source (*e.g.*, amino acids), contribute to the distinct desirable flavor notes in Savory Base 100. However, Maillard-type reactions may also rise to undesirable substances such as heterocyclic amines (HCAs). These carcinogenic by-products are formed in the presence of creatine or creatinine (major components of muscle in meats and fish) and during heat processing of animal products at temperatures greater >130°C (Jägerstad *et al*., 1991; Skog *et al*., 1998), due to the reaction between creatine or creatinine with amino acids and sugars. Although the fermentation broth used in the manufacture of Savory Base 100 is enriched in amino acids and sugars, it does not contain creatine or creatinine, as it is not derived from animal sources. In addition, the temperature used during the manufacturing process of Savory Base 100 (*i.e.*, 70°C) does not reach a temperature at which formation of HCAs is favorable (*i.e.*, >130°C). Considering this, neither the composition nor the manufacturing process of Savory Base 100 is conducive to formation of such by-products.

2.4 Stability Data

The sensory and microbiological and chemical stability of Savory Base 100 was tested using a single lot of Savory Base 100 (lot number 363976). Each sample (100 g) was stored in a dual-layered, low-density polyethylene (LDPE) bag (enclosed within an aluminum pouch) and stored for up to 360 days (1 year). Sensory and chemical stability was evaluated at 30, 90, 150, 180, 240, 300, and 360 days, while microbiological stability was analyzed after 1 year only.

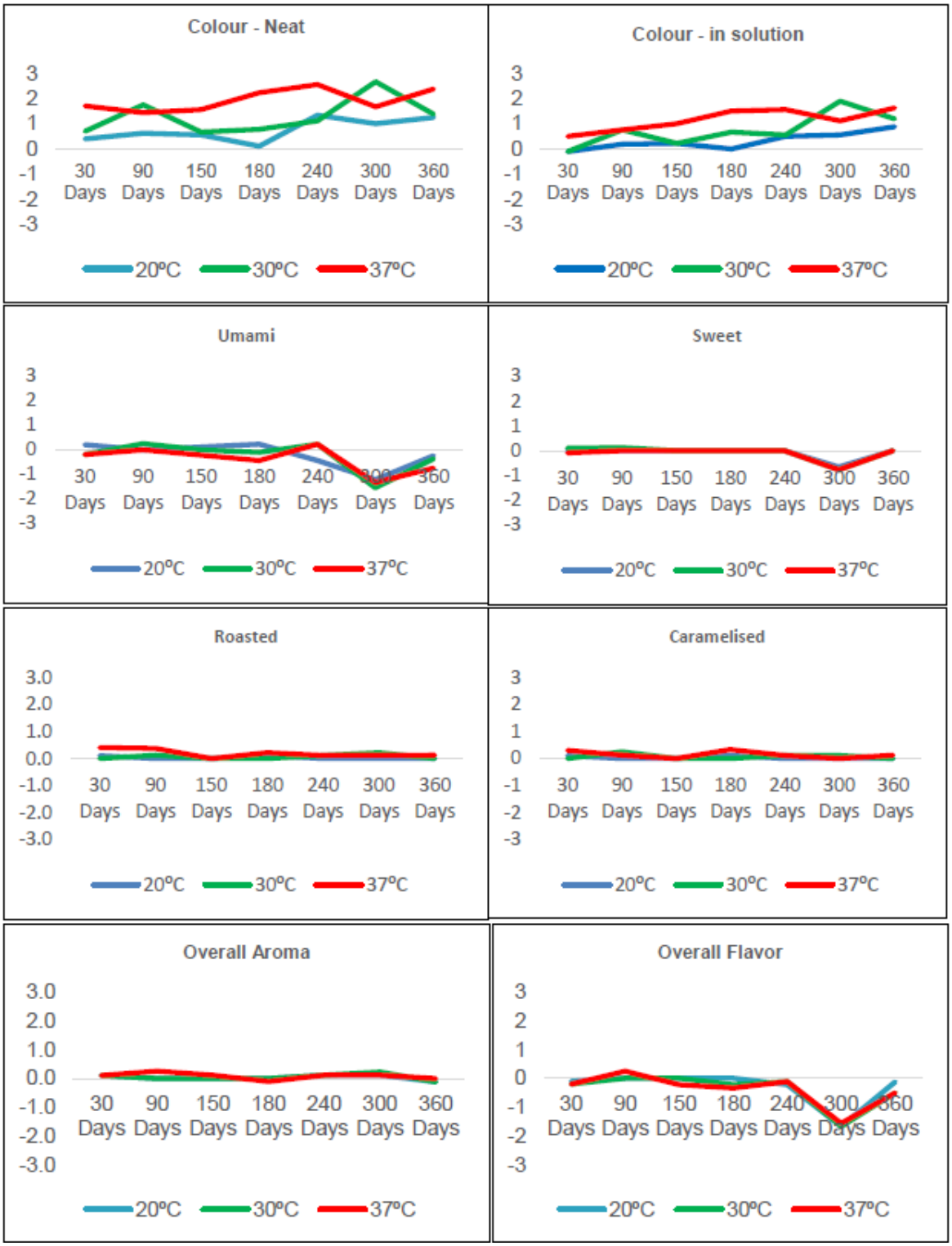
2.4.1 Sensory Stability

A panel of 8 trained internal sensory evaluators used a 7-point bipolar evaluation scale to score samples for taste (umami, sweet, roasted, caramelized and overall flavor), color [neat and in solution (as prepared for tasting)] and smell (overall aroma); the scoring scale is given as part of Figure 2.4.1-1. Tasting doses were prepared by dilution of 4 g Savory Base 100 paste in 1 liter of water (90°C) followed by stirring until visibly homogeneous; samples were served at 70°C (±5°C) for tasting. Test samples were stored (blinded and identifiable only by 3-digit code) at temperatures of 20, 30, or 37°C and at relative humidities of 50, 70, and

75%, respectively; samples stored at 4°C were assumed to be stable for the analysis period and were used as the reference (labeled as such).

As illustrated in Figure 2.4.1-1, the color of samples (whether neat or in solution) were darker with increasing temperature and humidity, and generally became darker over time. In terms of taste, there were minimal changes in roasted and caramelized flavors (regardless of temperature, humidity, or time); however, umami, sweet and overall flavor were all less detectable after 300 days (at all temperatures), then became slightly more similar to the reference after 1 year.

Figure 2.4.1-1 Sensory Stability Evaluation of Savory Base 100 "Corn Sauce"



-3 = much less; -2 = less, -1 = slightly less; 0 = same as reference; 1 = slightly more; 2 = more; 3 = much more

2.4.2 Chemical Stability

For evaluation of chemical stability, samples were homogenized before analysis of water activity, pH, and total acidity (as acetic or citric acid) when stored refrigerated (4°C) or at temperatures of 20, 30, or 37°C and at relative humidities of 50, 70, and 75%, respectively. As shown in Table 2.4.2-1 below there were no significant changes in any of the parameters measured (with all values remaining within specification, where applicable), regardless of temperature and relative humidity, when Savory Base 100 was stored for up to 1 year. Savory Base 100 is stable for at least 1 year under accelerated conditions.

Table 2.4.2-1 Accelerated Stability of Savory Base 100 "Corn Sauce" (Lot 363976)

Parameter	Specification	Analytical Data						
		Time (days)						
		30	90	150	180	240	300	360
Temperature = 4°C								
Water activity at 25°C	≤0.75	0.731	0.723	0.721	0.722	0.724	0.724	0.722
pH at 25°C	5.5-7.0	6.15	6.20	6.27	6.28	6.24	6.23	6.24
Total acidity – as acetic acid (g/100g)	N/A	2.17	2.19	2.24	2.51	2.32	2.20	2.04
Total acidity – as citric acid (g/100g)	N/A	2.53	2.55	2.61	2.93	2.57	2.50	2.38
Temperature = 20°C, RH = 50%								
Water activity at 25°C	≤0.75	0.728	0.726	0.726	0.723	0.739	0.720	0.723
pH at 25°C	5.5 to 7.0	6.16	6.27	6.19	6.29	6.22	6.22	6.24
Total acidity – as acetic acid (g/100g)	N/A	2.17	2.19	2.24	2.51	2.32	2.20	2.04
Total acidity – as citric acid (g/100g)	N/A	2.53	2.55	2.61	2.93	2.57	2.50	2.38
Temperature = 30°C, RH = 70%								
Water activity at 25°C	≤0.75	0.725	0.723	0.724	0.720	0.723	0.720	0.723
pH at 25°C	5.5 to 7.0	6.16	6.21	6.23	6.24	6.30	6.27	6.24
Total acidity – as acetic acid (g/100g)	N/A	2.19	2.20	2.17	2.47	2.22	2.11	2.06
Total acidity – as citric acid (g/100g)	N/A	2.55	2.57	2.53	2.88	2.46	2.47	2.40
Temperature = 37°C, RH = 75%								
Water activity at 25°C	≤0.75	0.723	0.720	0.720	0.717	0.716	0.725	0.725
pH at 25°C	5.5 to 7.0	6.17	6.32	6.22	6.23	6.29	6.25	6.21
Total acidity – as acetic acid (g/100g)	N/A	2.18	2.19	2.14	2.47	2.21	2.11	2.06
Total acidity – as citric acid (g/100g)	N/A	2.55	2.55	2.50	2.88	2.46	2.43	2.40

N/A = not applicable; RH = relative humidity.

2.4.3 Microbiological Stability

Savory Base 100 was also analyzed for the presence of microorganisms (Enterobacteriaceae and aerobic plate count) on Day 0 at room temperature and after 1 year when stored refrigerated (4°C) or at temperatures of 20, 30, or 37°C and at relative humidities of 50, 70, and 75%, respectively. These data are presented in Table 2.4.3-1 and show that the numbers of bacteria present in the sample after 1 year remained consistent with those on Day 0 (regardless of storage conditions), and within proposed specifications, demonstrating that Savory Base 100 is microbiologically stable for at least 1 year under accelerated conditions.

Table 2.4.3-1 Microbiological Stability of Savory Base 100 "Corn Sauce" (Lot 363976)

Time (days)	Storage Conditions	Enterobacteriaceae (CFU/g)		Aerobic Plate Count (CFU/g)	
		Specification	Analytical Data	Specification	Analytical Data
0	Room temperature	≤10	<10	≤10,000	<1,000
360	Temperature = 4°C		<10		270
	Temperature = 20°C, RH = 50%		<10		340
	Temperature = 30°C, RH = 70%		<10		200
	Temperature = 37°C, RH = 75%		<10		290

CFU = colony forming units; RH = relative humidity.

Part 3. §170.235 Dietary Exposure

3.1 Current Regulatory Status in the United States

Savory Base 100, under the substance name, "*Corynebacterium glutamicum* corn syrup fermentation product", was granted FEMA GRAS status for use as a flavoring agent in a variety of food and beverage products at use levels up to 5,100 ppm (FEMA No. 4907).

3.2 Estimated Dietary Consumption of Savory Base 100 from Intended Food Uses

3.2.1 Methodology

An assessment of the anticipated dietary exposure to Savory Base 100 as an ingredient under the intended conditions of use (see Table 3.1.2-1) was conducted using data available in the 2011-2012 cycles of the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Survey (NHANES) (CDC, 2015). A summary of the survey and methodology employed in the intake assessment of Savory Base 100 along with the pertinent results is presented herein.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2011-2012. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). In addition to collecting information on the types and quantities of foods being consumed, NHANES contain socio-economic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES data to

compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (USDA, 2014; CDC, 2015). The NHANES data were employed to assess the mean and 90th percentile intake of Savory Base 100 for each of the following population groups:

- Infants and young children, ages 0 to 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of Savory Base 100 by the U.S. population. Estimates for the daily intake of Savory Base 100 represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2011-2012 data, and these individual average amounts comprised the distribution from which mean and percentile intake estimates were generated. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of Savory Base 100 averaged over all individuals surveyed, regardless of whether they potentially consumed food products containing Savory Base 100, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing Savory Base 100 during the 2 survey days). "Consumer-only" intake refers to the estimated intake of Savory Base 100 by those individuals who reported consuming food products in which the use of Savory Base 100 is currently under consideration. Individuals were considered "consumers" if they consumed 1 or more food products in which Savory Base 100 is proposed for use on either Day 1 or Day 2 of the survey.

3.2.2 Estimated Intake of Savory Base 100 from Proposed Food-Uses

The estimates for the intake of Savory Base 100 was generated using the maximum use level indicated for each intended food-use, as presented in Table 1.3-1, together with food consumption data available from the 2011-2012 NHANES dataset. A summary of the estimated daily intake of Savory Base 100 from proposed food-uses is provided in Table 3.2.2-1 on an absolute basis (mg/person/day) and in Table 3.2.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was high among all age groups evaluated in the current intake assessment; greater than 43.4% of the population groups consisted of users of those food products in which Savory Base 100 is currently proposed for use. Female adults had the greatest percentage of users at 82.3%; infants and young children had a notably lower percent consumers than all other age groups (43.4%). The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population, the mean and 90th percentile consumer-only intakes of Savory Base 100 were determined to be 197 and 477 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of Savory Base 100 on an absolute basis, at 230 and 556 mg/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 105 and 290 mg/person/day, respectively (Table 3.2.2-1).

Table 3.2.2-1 Summary of the Estimated Daily Intake of Savory Base 100 from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	*Per capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants and Young Children	Up to 2	46	172	43.4	315	105	290
Children	3 to 11	105	291	71.7	1,138	147	359
Female Teenagers	12 to 19	138	400	76.0	391	182	443
Male Teenagers	12 to 19	170	455	75.5	384	226	537
Female Adults	20 and up	151	392	82.3	1,790	183	436
Male Adults	20 and up	186	492	80.8	1,685	230	556
Total Population	All ages	154	404	78.3	5,703	197	477

NHANES = National Health and Nutrition Examination Survey; Savory Base 100 = Savory Base 100 "Corn Sauce"; U.S. = United States.

On a body weight basis, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 8.8 and 23.1 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 2.7 and 6.3 mg/kg body weight/day, respectively (Table 3.1.2-2).

Table 3.2.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Savory Base 100 from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants and Young Children	Up to 2	3.8	14.5	43.4	314	8.8	23.1
Children	3 to 11	4.0	12.0	71.7	1,138	5.6	14.5
Female Teenagers	12 to 19	2.4	7.7	76.2	383	3.1	8.8
Male Teenagers	12 to 19	2.6	6.9	75.6	382	3.4	8.6
Female Adults	20 and up	2.2	5.6	82.3	1,774	2.7	6.3
Male Adults	20 and up	2.2	6.1	80.7	1,670	2.8	6.8
Total Population	All ages	2.5	6.7	78.3	5,661	3.2	7.8

bw = body weight; NHANES = National Health and Nutrition Examination Survey; Savory Base 100 = Savory Base 100 "Corn Sauce"; U.S. = United States.

3.2.3 Summary and Conclusions

Consumption data from the 2011-2012 NHANES dataset and information pertaining to the individual proposed food-uses of Savory Base 100 were used to estimate the "*per capita*" and consumer-only intakes for specific demographic groups and for the total U.S. population. Several conservative assumptions have been included in the present assessment, which means that resulting values may be considered 'worst case' estimates of exposure for the target population. For example, it was assumed that all food products within a food category contain the ingredients at the maximum specified level of use. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently (Anderson, 1988). It should also be noted that the FEMA

GRAS uses are the same as those proposed herein, so consideration for additive exposure form FEMA GRAS uses was not deemed to be necessary.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of Savory Base 100 by the total U.S. population from all proposed food-uses in the U.S., were estimated to be 197 mg/person/day (3.2 mg/kg body weight/day) and 477 mg/person/day (7.8 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of Savory Base 100 were determined to be 230 mg/person/day (2.8 mg/kg body weight/day) and 556 mg/person/day (6.8 mg/kg body weight/day), respectively, as identified among male adults. When intakes of Savory Base 100 were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intakes of 8.8 mg/kg body weight/day and 23.1 mg/kg body weight/day, respectively.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with Savory Base 100.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable, as Savory Base 100 was not used in food before 1958.

Part 6. §170.250 Narrative and Safety Information

The safety of Savory Base 100 is demonstrated based on the following pivotal information: 1) published toxicological studies (Tafazoli *et al*., 2017), including an acute oral toxicity study, a 90-day subchronic oral toxicity study, and a battery of *in vitro* genotoxicity and mutagenicity assay; 2) information on the compositional identity of Savory Base 100 demonstrating that they are common component of the diet with a history of safe use; 3) information establishing the safety of the fermentation organism. Each of the aforementioned points is discussed in detail in the following sections.

6.1 Metabolic Fate

The absorption, distribution, metabolism, and excretion (ADME) of Savory Base 100 has not been investigated; however, Savory Base 100 is mainly composed of amino acids, minerals, water, sugars, and organic acids that are normal components of human diet and as such, are expected to be digested and metabolized in a similar manner to other commonly consumed nutrients.

6.2 Toxicological Studies

6.2.1 Acute Toxicity

The acute oral toxicity of Savory Base 100 (identified as 'GA-NRC' in the study report) in rats has been evaluated in a study conducted in compliance with the Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) (OECD, 1998a) and according to Directive 86/609/EEC (EC, 1986), Directive 2001/83/EC (EC, 2001) and Commission Regulation (EC) No 440/2008 (EC, 2008) (Tafazoli *et al*., 2017).

Groups of 5 male and 5 female Wistar rats were administered a single dose of 0 (drinking water), 100, 500, or 2,000 mg/kg body weight Savory Base 100, by gavage, at a dose volume of 10 mL/kg body weight. Animals were observed shortly after dosing, at 6 hours after dosing and then once daily until the end of the study (14 days). Body weights were recorded on the day of dosing and 3 times a week thereafter. At the end of the observation period, animals were subjected to a macroscopic necropsy, where any abnormalities were fixed and subsequently examined microscopically.

There were no deaths and no test item-related clinical signs or effects on body weight (a statistically significant (5%) reduction in body weight for males given 500 mg/kg body weight on Day 14 was considered not toxicologically relevant, due to absence of a dose-response).

There were also no macroscopic or microscopic changes that were considered to be related to Savory Base 100. White deposits observed in the spleen of 2 females from each of the low and high-dose groups were confirmed microscopically to be slight capsular fibroses. However, these were isolated instances (only seen for 2 out of 5 females in each of the affected groups) and there was no evidence of a dose-related response. Isolated instances of unilateral pelvic dilatation (1 high-dose male and 1 control female) and red sports on the thymus (1 low dose male) were also considered to be unrelated to the test item. It was concluded, therefore, that 2,000 mg/kg body weight (the highest dose tested) was the no-observed-adverse-effect level (NOAEL).

6.2.2 Repeated-Dose Toxicity

A 90-day repeat dose oral toxicity study was conducted to investigate the subchronic toxicity of NRC Mix [a combination of Savory Base 100 and the related Savory Base 200 “Corn Sauce” (Savory Base 200) in a 2:1 ratio] in rats (Tafazoli *et al*., 2017). NRC Mix contained 37.8±0.2% glutamic acid (primarily from Savory Base 100) and 14.5±0.4% IMP (primarily from Savory Base 200). Savory Base 200 is the subject of a concurrent GRAS Notice.

The study was performed in compliance with the OECD principles of GLP (OECD, 1998a) and according to Directive 2001/83/EC (EC, 2001), OECD Test Guideline 408 (OECD, 1998b) and Commission Regulation (EC) No 440/2008 (EC, 2008). Given that Savory Base 100 will often be used in combination with Savory Base 200, the test articles were used in combination.

Groups of 10 male and 10 female Wistar rats were given 0 (basal diet), 1, 2.5, or 7% NRC Mix (equivalent to approximately 500, 1,250, or 3,500 mg/kg body weight/day NRC Mix, which equates to approximately 333, 833, or 2,333 mg/kg body weight/day Savory Base 100), in the diet for 90 days; doses were selected based on data derived from an internal palatability study. An additional 5 males and 5 females were included in the control and high-dose groups and also fed for 90 days, after which time they were kept untreated for a further 4 weeks, to assess the reversibility of any effects seen during the treatment period.

Animals were observed daily for changes in behavior and appearance, with ophthalmoscopic examinations performed once before the start of dosing and once towards the end of the treatment period. Body weights were recorded 3 times each week, food intake was recorded once weekly, and water consumption was recorded every 4 days from Week 2 onwards. Blood samples were taken from the retro-orbital sinus for clinical pathology from main study animals before dosing and at the end of the treatment period, with recovery animals sampled towards the end of both the treatment and recovery periods; urine samples were collected once before dosing and at the end of the treatment and recovery periods (where applicable).

All animals were subjected to a macroscopic necropsy, where selected organs were weighed and, for animals in the control and high-dose groups only, the following tissues were examined microscopically: liver, kidneys, adrenals, spleen, pancreas, heart, lung, aorta, thymus, larynx, thyroid gland, parathyroid glands, salivary glands, tongue, trachea, bronchus, esophagus, stomach, small and large intestines, urinary bladder, prostate gland, seminal vesicles, testes, epididymides, ovaries, vagina, uterus, lymph nodes, brain, pituitary gland, skin, mammary gland, eyes, optic nerves, lacrimal glands, skeletal muscle, sciatic nerve, spinal cord, and bone marrow.

There were no test item-related deaths or clinical signs during the study. The death of 1 male in the mid-dose group on Day 90 was considered incidental as it was an isolated incident, but no reason for the death was identified at necropsy. There were also no ocular changes that were considered to be related to administration of the test item.

Mean body weights for test item-treated males were statistically significantly higher ($p<0.05$ to $p<0.005$) than those of the controls at the end of the treatment period; however, these increases were not dose-related (increases of 10, 14, and 6% at 1, 2.5, or 7.5% NRC Mix, respectively). Female groups given NRC Mix also gained slightly more weight than controls after 89 days (6 to 7%), but, as with the males, there was no dose-response relationship. All test item-treated male and female groups were heavier than controls on Day 1, despite mean body weights being similar on arrival; therefore, these animals were already gaining more weight than controls before NRC Mix was introduced into the diet. Body weight increases may in part be due to organoleptic properties of the savory base resulting in an apparent increase in food intake by the savory base groups during the early phase of the study. Nonetheless, the body weight changes were considered to be non-adverse.

Although there were statistically significant ($p<0.05$ to $p<0.005$) increases in mean food consumption in various weeks during the treatment period for both males and females (mostly for males given 1 or 2.5% NRC Mix, correlating with the increased body weights for these groups), food consumption in Week 13 was similar between test item-treated groups and controls.

High-dose males drank statistically significantly ($p<0.05$) more (18%) than controls after 90 days, with a dose-related increase in mean water consumption observed for females (increases of 13, 17, and 40% at 1, 2.5, or 7.5% NRC Mix, respectively), which was statistically significant ($p<0.005$) at the high dose; by the end of the recovery period, water consumption for high-dose groups dropped to either less than (males) or similar to (females) that of the controls. Increased water consumption was to be expected given the salt content of Savory Base ingredients. In the absence of biologically relevant changes in the kidney or in relevant clinical chemistry or urinary parameters, these findings were considered to be non-adverse.

Various statistically significant findings were reported among hematology parameters for test item-treated males and females at the end of the treatment period. Increases in hemoglobin count [4 and 7% ($p<0.005$) for high-dose males and females, respectively] and in hematocrit (for both sexes at the high-dose) were minor and there was only a dose-response relationship for females, hence these were considered to be physiological variations, unrelated to the test item. Differences in other hematological parameters were minor, inconsistent between the sexes, and/or did not show a relationship with dose and were likely also to be due to normal biological variation rather than any effect of the test item.

There were no test item-related differences in coagulation parameters at the end of the treatment period. Where statistically significant differences were reported [shortened mean activated partial thromboplastin time (APTT) for mid- (9%, $p<0.01$) and high-dose (8%, $p<0.05$) males and shorted mean prothrombin time (PT) for low dose females (4%, $p<0.05$)], there was no dose-response relationship and the changes were in the wrong direction for biological relevance (elongation of APTT and/or PT are considered to be biologically relevant changes). The statistically significantly ($p<0.01$) shortened PT (14%) for males at the end of the recovery period was also in the wrong direction for biological relevance and considered not test item-related.

There were numerous sporadic statistically significant differences in clinical chemistry parameters between test item-treated groups and controls; however, these differences were either of low magnitude, inconsistent between the sexes or did not show a dose-response relationship and were therefore considered to be toxicologically irrelevant. There were no test item-related differences in urinalyses parameters.

There were no differences in body weight-related organ weights between test item-treated groups and controls. Brain weight-relative organ weights can be notably affected by variations in terminal body weights (which were reported in this study), therefore the statistically significant differences in brain weight-related organ weights [increased thymus and spleen weights for males given 1 (thymus only), 2.5, or 7% NRC Mix, respectively, and reduced adrenal gland weight at the high dose] were considered not biologically relevant, in the absence of any changes in body weight-relative weights or of histological changes for any of these organs. Furthermore, these statistically significant differences weren't reported for females and the changes in thymus and adrenal weights were clearly not dose-related.

There were no test item-related macroscopic changes. Histopathological findings included hepatic steatosis (primarily in the periportal region), which was reported for 7 out of 20 controls and 13 out of 20 high-dose animals; this was also reported at the end of the recovery period in all 5 control males and 1 out of 5 control females and in 4 out of 5 males and 2 out of 5 females in the high-dose group. These effects were considered by the author as not test item-related, as they were not associated with any necrosis or increases in liver enzyme activities or liver weights (neither absolute nor relative), so the low and mid dose groups were not subject to histopathological examination. The histopathology report does not specify whether the changes were micro- or macrovesicular; however, as the droplets were described as "medium" this appears to indicate that these were macrovesicular fatty changes, which are the most common form of liver fatty changes that may be seen sporadically in control animals and are considered benign changes presumably as a result of nutritional, metabolic or hormonal derangement (Greaves and Faccini, 1992; Thoolen *et al*., 2010; Greaves, 2012); therefore, these changes were considered not test item-related.

Kidney tubular mineralization (also known as nephrocalcinosis) was reported in 4 out of 10 high-dose females and 1 control female at the end of the treatment period and in 4 of the 5 high-dose females at the end of the recovery period. Nephrocalcinosis is a common spontaneous minor lesion that develops in young and adult rats, primarily females (Gad, 2016); this finding was not reported in males in this study. Increased susceptibility to nephrocalcinosis is known to occur from dietary manipulation and it has been reported that imbalances in the calcium and phosphorus content of diets, calcium:phosphorus ratio of diets, deficiency of magnesium and/or chloride and high urinary pH can all contribute to the development of nephrocalcinosis (Reeves *et al*., 1993; Rao, 2002). Considering the high mineral content of Savory Base ingredients, the likely unbalanced provision of minerals in the test diet relative to the control diet could be responsible for the observed effects in the kidneys; however, no single mechanism that explains the association between the dietary factors contributing to the incidence of nephrocalcinosis has been identified. In general, these mineral deposits are of no pathological significance (Seely and Brix, 2014) and

in the absence of correlating markers of kidney impairment, were considered not to be toxicologically relevant.

At the end of the treatment period, non-specific and incidental findings included chronic focal myocarditis (4 out of 10 high-dose males and 1 of the 10 female controls) and hyperplasia of lymph follicles in both the small intestine (4 males and 2 females from the high-dose group, compared with 3 males and 1 female in the control group) and large intestine (2 and 1 high-dose males and females, respectively, compared with 4 male and 4 female controls) were reported. At the end of the recovery period, focal myocarditis was reported in only 1 high-dose male, hyperplasia of the lymph follicles in the small intestine was reported in 1 male and 2 females from the high-dose group, compared with 2 and 4 control males and females, respectively and hyperplasia of lymph follicles in the large intestine was reported in 2 males and 3 females from the high-dose group, in comparison to 3 male and 3 female controls.

The incidence of chronic focal myocarditis reported in high-dose males was considered to be toxicologically irrelevant, as these histological observations were similar to the spontaneous lesions commonly reported in test and control rats, with a higher occurrence in males (Gaunt *et al*., 1967; Jokinen *et al*., 2011). Instances of hyperplasia of lymph follicles in the small and large intestine were small in magnitude and occurred at a similar frequency in test item-treated and control groups, and were therefore also considered biologically irrelevant.

The NOAEL was reported to be 7% NRC Mix (the highest dose tested, equivalent to approximately 3,500 mg/kg body weight/day NRC Mix, which corresponds to a NOAEL of approximately 2,333 mg/kg body weight/day for Savory Base 100 (based on a the 2:1 ratio of Savory Base 100 and Savory Base 200).

6.2.3 Mutagenicity and Genotoxicity

6.2.3.1 Bacterial Reverse Mutation Test

The potential mutagenicity of Savory Base 100 (identified as 'GA-NRC' in the study report) was evaluated in a bacterial reverse mutation test (Ames test), which was performed in compliance with the OECD principles of GLP (OECD, 1998a) and according to OECD Test Guideline 471 (OECD, 1997), Commission Regulation (EC) No 2000/32/EC (EC, 2000), US EPA Health Effects Test Guidelines OPPTS 870.5100 (U.S. EPA, 1998), ICH Guidance S2A (ICH, 1995) and ICH Guidance S2B (ICH, 1997) (Tafazoli *et al*., 2017).

An initial preliminary range-finding test was conducted using the plate incorporation method at Savory Base 100 concentrations of 5 to 5,000 µg/plate, using *Salmonella typhimurium* (*S. typhimurium*) strains TA98 and TA100, in the absence and presence of S9 metabolic activation. Since the results of this test were negative, 2 separate tests (plate incorporation assay and pre-incubation assay) were conducted using *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA, which were treated with Savory Base 100 at concentrations of 51.2, 128, 320, 800, 2,000, and 5,000 µg/plate in the absence and presence of S9 mix.

Three negative control groups [untreated, vehicle (distilled water) and dimethyl sulfoxide] were used, and positive controls were also included in the absence (4-nitro-1,2-phenylene-diamine, sodium azide, 9-aminoacridine and methyl-methanesulfonate) and presence (2-aminoanthracene) of metabolic activation. A positive result for mutagenicity was defined as a dose-dependent, reproducible, and biologically relevant 2- (in *S. typhimurium* T100) or 3-fold (in the other tested strains) increase in the number of revertant colonies, compared to that of the vehicle control group.

Savory Base 100 showed no evidence of mutagenicity in any of the tests, in the absence or presence of metabolic activation. In contrast, the positive controls induced biologically relevant increases in revertant colony counts (with metabolic activation where required), which demonstrated the sensitivity of the assay and metabolic activity of the S9 preparations. It was concluded, therefore, that Savory Base 100 is non-mutagenic at concentrations up to 5,000 µg/plate, in the absence or presence of metabolic activation.

6.2.3.2 In Vitro Mammalian Cell Gene Mutation Test

The mutagenic potential of Savory Base 100 was investigated in an *in vitro* mammalian cell gene mutation test conducted in compliance with the OECD principles of GLP (OECD, 1998a) and according to OECD Test No. 476 (OECD, 2015) and Commission Directive (EC) No 2000/32/EC (EC, 2000) (Tafazoli *et al*., 2017).

A preliminary dose range-finding study (where Savory Base 100 was not cytotoxic at concentrations up to 5,000 µg/mL) was followed by 2 independent experiments (each conducted in duplicate) using V79 Chinese hamster lung (CHL) cells. For both of these experiments, the vehicle [Dulbecco's Modified Eagle's (DME) medium] and dimethyl sulfoxide (DMSO) served as the negative controls and positive controls were included in the absence (ethylmethane sulfonate) and presence (7,12-dimethyl benzanthracene) of S9 metabolic activation.

In the first experiment, CHL cells were exposed to Savory Base 100 for 3 hours at concentrations of 312.50, 625, 1,250, 2,500, or 5,000 µg/mL in the absence or presence of S9 metabolic activation. In the second, CHL cells were exposed to Savory Base 100 for 20 hours (in the absence of S9) or 3 hours (in the presence of S9) at concentrations of 156.25 (presence of S9 only), 312.50, 625, 1,250, 2,500, or 5,000 µg/mL.

After the incubation period, for both experiments, the cells were washed with DME, detached with trypsin-ethylenediaminetetraacetic acid (EDTA) solution, and cultured to determine survival and to allow for expression of the mutant phenotype. Once mutant colonies had been selected, they were fixed, stained with Giemsa, and counted for either mutant selection or cloning efficiency. Mutant frequency was calculated by division of the total number of mutant colonies by the number of cells selected, corrected for cloning efficient of cells before mutant selection. Positive mutagenic responses were defined as dose-related, reproducible, and statistically significant increases in mutant frequency.

For both experiments, in the absence or presence of S9, no statistically significant increases in mutation frequency were reported for Savory Base 100 treated cells, compared with that of the negative controls. Sensitivity of the assay and efficacy of the S9 preparations was confirmed by the significant increases in mutation frequency for the positive controls. It was concluded that Savory Base 100 is not mutagenic at concentrations up to 5,000 µg/mL, in the absence and presence of metabolic activation.

6.2.3.3 In Vitro Mammalian Cell Micronucleus Test

The clastogenic and aneugenic potential of Savory Base 100 (identified as He Wei C. Essence I in the study report) was evaluated in an unpublished corroborative *in vitro* mammalian cell micronucleus test, conducted using human lymphocytes, in compliance with the OECD principles of GLP (OECD, 1998a) and according to OECD Test No. 487 (OECD, 2014) (Chevallier, 2017). A copy of the full study report is provided in Appendix A.

An initial preliminary cytotoxicity test was conducted using Savory Base 100 at concentrations of 0 to 5,000 µg/mL, in the presence (3-hour treatment) and absence (3 and 24-hour treatments) of S9 metabolic activation; there was no evidence of cytotoxicity reported at any concentration. Cytotoxicity was assessed again in the main experiment. In the absence of S9 (at the same dose levels and under similar conditions to

those used in the preliminary test), there was no evidence of cytotoxicity after a 3-hour treatment, but slight to moderate cytotoxicity was reported at concentrations ≥2,500 µg/mL after 24 hours continuous treatment. However, there was no evidence of cytotoxicity in the presence of S9 after a 3-hour treatment under similar conditions to those described above.

In the main experiment for micronucleus analysis, 5,000 µg/mL was considered to produce extreme culture conditions, therefore, human lymphocytes were treated with Savory Base 100 at 312.5, 625, 1,250, 2,500, or 3,750 µg/mL with S9 (3 hours) and without S9 (3 and 24-hour treatments). The vehicle (water for injection) was used as a negative control and positive controls were included in the absence (colchicine and mitomycin C) and presence (cyclophosphamide) of metabolic activation. A positive result for clastogenicity/aneugenicity was defined as a dose-dependent, statistically significant increase in the frequency of micronucleated binucleated cells (MNBC), with the frequency of MNBC also being above the vehicle background range for at least 1 dose level.

Savory Base 100 showed no evidence of clastogenicity or aneugenicity in any of the tests, in the absence or presence of metabolic activation. In contrast, the positive controls induced biologically relevant increases in MNBC (with metabolic activation where required), which demonstrated the sensitivity of the assay and metabolic activity of the S9 preparations. It was concluded that Savory Base 100 is neither clastogenic nor aneugenic at concentrations up to 3,750 µg/mL, in the absence and presence of metabolic activation.

6.3 Additional Safety Information on Major Constituents of Savory Base 100

The constituents of Savory Base 100 have a long history of consumption as part of existing food stuffs and the characteristic savory taste of the ingredient results from a specific intrinsic mix of these compounds (including free and bound amino acids, organic acids, Amadori and Maillard products, minerals and their salts), all of which individually contribute to the overall taste. Dietary intakes of the flavoring compounds are consistent with levels commonly used in foods, and/or are well below acceptable daily intake (ADI) values that have been derived.

6.3.1 Glutamic Acid

A major constituent of Savory Base 100 is the amino acid glutamic acid. Glutamic acid is a non-essential amino acid and as a constituent of protein is consumed from a host of protein containing food sources, including meat, eggs, fish, milk, and vegetables. The safety of glutamic acid in particular has been well characterized and reported in safety evaluations of an extensive collection of animal and human studies, conducted firstly by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the 14th and 17th JECFA meetings in 1970 and 1974, respectively (JECFA, 1970, 1974). A further evaluation of additional data at the 31st JECFA (1988) resulted in the allocation of a group ADI 'not specified' for glutamic acid and its ammonium, calcium, potassium, magnesium and sodium salts, which is applicable to substances with very low toxicity and indicates that the total dietary intake of glutamic acid, arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of JECFA, represent a hazard to health. This conclusion was reiterated by the Scientific Committee on Food (SCF) in 1991 (JECFA, 1988; SCF, 1991). Furthermore, glutamic acid is approved as a food additive (E 620) in the European Union (EU), under Commission Regulation (EU) No 1129/2011. Glutamic acid (E 620) is a Group I additive, authorized at levels up to 10 g/kg in numerous food categories; additionally, it is authorized for use in salt substitutes, seasonings, and condiments at *quantum satis* (European Union, 2011).

Recently, the European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS) re-evaluated the safety of glutamic acid and its salts for use as food additives (EFSA, 2017). Following its re-evaluation of the technical, safety, and exposure data available for glutamic acid and related glutamates, the Panel derived a group ADI of 30 mg/kg body weight/day, expressed as glutamic acid, for glutamic acid and glutamates. This ADI was based on the NOAEL of 3,200 mg monosodium glutamate/kg body weight/day from the neurodevelopmental toxicity study (Vorhees *et al*., 1979), and applying the default uncertainty factor of 100.

Dietary intakes of glutamic acid from protein in the typical diet have been estimated to be *ca*. 15 g/person per day (Stamler *et al.,* 2009). Only free glutamic acid imparts flavor enhancing properties to foods, and free glutamic acid is present in a number of natural and fermented foods (Table 6.3.1-1).

Table 6.3.1-1 Foods Rich in Free Glutamic Acid

Food Product	Free Glutamic Acid (mg)	Serving Size
Human milk	300	1000 g
Cantaloupe	50	100 g
Grapes	40	100 g
Vegemite	143	10 g
Marmite	196	10 g
Tomato paste	62 to 64	10 g
Parmesan cheese	36 to 127	10 g
Soy sauce	5 to 126	10 g
Fish sauce	73 to 138	10 g
Oyster sauce	90	10 g
Condensed soups	0 to 480	100 g
Sauces, mixes, seasonings	2 to 190	10 g
Chinese restaurant meals	<10 to 1500	100 g
Italian restaurant meals	10 to 230	100 g
Western restaurant meals	<10 to 710	100 g

Sources: JECFA (1988); Yoshida (1988); Nichols and Jones (1991); Daniels *et al.* (1995).

In the U.S., L-glutamic acid and its glutamate salts are GRAS when used as a salt substitute when used in accordance with good manufacturing practice (§182.1045; §182.1047; §182.1500; §182.1516; §182.1). The GRAS use of L-glutamic acid and L-glutamates as flavoring enhancers was evaluated by the Select Committee on GRAS Substances (SCOGS) (FASEB, 1980). The committee commented on the reported cases of "Chinese Restaurant Syndrome" in certain individuals, and that the use of Monosodium Glutamate in restaurant and/or home prepared foods was not under the purview of the Select Committee since its evaluation was limited to processed foods. The committee concluded that

> "*There is no evidence in the available information on L-glutamic acid, L-glutamic acid hydrochloride, monosodium L-glutamate, monoammonium L-glutamate, and monopotassium L-glutamate that demonstrates, or suggests reasonable grounds to suspects, a hazard to the public when they are used at levels that are now current and in the manner now practices. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard*".

Another source of glutamic acid is from yeast extracts, which are commonly consumed ingredients that are GRAS under Title 21 Food and Drug of the Code of Federal Regulations (CFR) §184.1983 (U.S. FDA, 2017). Savory Base 100 is compositionally similar to yeast extracts and will be used as a replacement for them in foods. A comparison of yeast extracts [as defined in the Food Chemicals Codex (FCC, 2016)] with Savory Base 100 is presented in Table 6.3.1-2 below.

Table 6.3.1-2 Comparison of Savory Base 100 "Corn Sauce" (Savory Base 100) with Yeast Extracts (as Defined in the Food Chemicals Codex)

Parameter	Yeast Extract	Savory Base 100
Description	Yeast extract occurs as a liquid, paste, powder, or granular substance.	Savory Base 100 occurs as a pale brown to brownish paste.
	It comprises the water-soluble components of the yeast cell, the composition of which is primarily amino acids, peptides, carbohydrates, and salts.	Savory Base 100 is composed of glutamic acid (34 to 44%), water (27 to 34%), ash (10 to 18%), total nitrogen (4 to 7%), sodium chloride (5.5 to 8%) and other free amino acids (1 to 3%).
	Yeast extract is produced through the hydrolysis of peptide bonds by the naturally occurring enzymes present in edible yeasts or by the addition of food-grade enzymes.	Corn syrup serves as the substrate and *C. glutamicum* is the source of the enzymes.
	Food-grade salts may be added during processing.	Sodium chloride is added during manufacture.
Function	Flavoring agent, flavor enhancer.	Savory flavoring ingredient.
Assay		
Protein	≥42% protein	-
Total Nitrogen	-	4 to 7%.
α-Amino Nitrogen/ Total Nitrogen Percent Ratio	15 to 55%	N/A
Ammonia Nitrogen	≤2%	<2% (Analytical results)
Insoluble Matter	≤2%	Not provided
Lead	≤2 mg/kg	<0.02 mg/kg
Mercury	≤3 mg/kg	<0.003 mg/kg
Potassium	≤13%	0.94% (Analytical results)
Sodium Chloride	≤50%	5 to 7%
Microbial Limits		
Aerobic plate count	≤50,000 CFU/g	≤10,000 CFU/g
Coliforms	≤10 CFU/g	No specification
Salmonella	Negative in 25 g	Negative in 25 g
Yeast and Molds	≤50 CFU/g	≤100 CFU/g

CFU = colony forming units; N/A = not applicable.

Savory Base 100 is intended for use as an alternative to yeast extracts for general food use, and therefore, will not increase dietary intakes of glutamic acid above levels currently occurring by way of existing regulations for glutamic acid and its salts discussed above.

Based on the results of analysis of 3 batches of Savory Base 100, the glutamic acid content of the product averages about 38% (see Table 2.3.3-1). As previously indicated, EFSA has recently established an ADI of 30 mg/kg body weight/day. The 90th percentile intakes of Savory Base 100 were estimated to be 477 mg/person/day (see Table 3.1.2-1; the daily intakes of glutamic acid, as a major component of Savory Base 100, is calculated to be 174.77 mg/day (equivalent to 2.49 mg/kg body weight/day for a 70 kg individual). This intake is well below the ADI of 30 mg/kg body weight/day for glutamic acid as established by EFSA and is not expected to raise a safety concern.

6.3.2 L-Alanine

L-alanine is a non-essential amino acid, which is a natural constituent of proteins in plants and animals (Burdock, 2009). L-alanine is permitted for direct addition to foods for nutritive purposes at levels up to 6.1% by weight of total protein (21 CFR §172.320 - U.S. FDA, 2017). L-Alanine has been allocated an ADI of 'acceptable' by JECFA (2004). Based on the results of analysis of 3 batches of Savory Base 100, the L-alanine content of the product averages about 1.43% (see Table 2.3.3-1). Since the 90th percentile intakes of Savory Base 100 were estimated to be 477 mg/person/day (see Table 3.1.2-1), the daily intakes of L-alanine, as a component of Savory Base 100, was calculated to be 6.82 mg/day, and this is not expected to raise a safety concern.

6.3.3 Formic Acid

Formic acid is a natural constituent of many foods consumed by humans, such as apple, papaya, pear, raspberry, strawberry, cheeses, breads, yogurt, milk, cream, and fish (Burdock, 2009). It is also a metabolite in intermediary metabolism and a precursor in the biosynthesis of several body constituents (FASEB, 1976). Formic acid is permitted for direct addition to food intended for human consumption with no limitations other than good manufacturing practice (GMP) (21 CFR §186.1316 - U.S. FDA, 2017). Formic acid has been allocated an ADI of '0 to 3 mg/kg body weight/day' by JECFA (1997). Based on the results of the product averages about 0.80% (see Table 2.3.2-1). Since the 90th percentile consumer-only intakes of Savory Base 100 were estimated to be 477 mg/person/day (see Table 3.1.2-1), the daily intakes of formic acid, as a component of Savory Base 100, was calculated to be 3.82 mg/day (equivalent to 0.054 mg/kg body weight/day for a 70 kg individual). This intake is well below the ADI of 3 mg formic acid/kg body weight/day established by JECFA.

6.3.4 Succinic Acid

Succinic acid, an intermediate metabolite of the tricarboxylic acid cycle and an end-product of aerobic and anaerobic metabolism (Song and Lee, 2006), can be produced from yeast fermentation in the processing of sake and wine (Arikawa *et al.,* 1999; Song and Lee, 2006). In the U.S., succinic acid produced by chemical synthesis or fermentation is GRAS for use as a flavor enhancer, and pH control agent in food at levels consistent with 21 CFR §184.1091 and not to exceed cGMP (U.S. FDA, 2017). In a 13-week subchronic oral toxicity study by Maekawa *et al*. (1990), the toxicity of monosodium succinate was evaluated in groups of 10 male and 10 female F344 rats *via* the drinking water at concentrations of 0 (control), 0.3, 0.6, 1.25, 2.5, 5, or 10%. No dose-related adverse effects were reported in hematological, biochemical, or histopathological parameters at any dose. The authors concluded that the NOAEL was 1.25% (equivalent to 1,250 mg/kg body weight/day or 1,050 mg/kg body weight/day as succinic acid), based on decreased body weight gain noted at higher doses (Maekawa *et al*., 1990). The food intakes were not measured in this study. In a follow-up 2-year carcinogenicity study, no statistically significant differences were reported between the control and treated animals in overall tumor incidence, or mean survival times in either sex, when groups of 50 male and 50 female F344 rats were administered monosodium succinate through the drinking water at

doses up to 2% for 104 weeks, corresponding to daily intakes of up to 1,093 mg/kg body weight/day for males and 773 mg/kg body weight/day for females (Maekawa *et al*., 1990). The results of an *in vitro* reverse mutation assay and a chromosomal aberration test demonstrated that succinic acid was neither mutagenic nor clastogenic (Ishidate *et al*., 1984). Based on the results of analysis of 3 batches of Savory Base 100, the succinic acid content of the product averages about 0.53%. Considering that the 90th percentile consumer-only intakes of Savory Base 100 was estimated to be 477 mg/person/day, and the daily intakes of succinic acid, as a component of Savory Base 100, was calculated to be 2.53 mg/day (equivalent to intakes of 0.036 mg succinic acid/kg body weight/day for a 70-kg individual), which provides a large margin of safety when compared to the NOAEL of 1,050 mg succinic acid/kg body weight/day, as determined in the 13-week oral toxicity study by Maekawa *et al*. (1990).

6.4 Safety of the Source Organism

6.4.1 Identity

The *C. glutamicum* strain used by Nestec in the production of Savory Base 100 is deposited in several international culture collections. Initially deposited as *Micrococcus glutamicus* strain 13032 by Kyowa Ferm. Ind. Co., Ltd., the production organism currently has the strain designation *C. glutamicum* 534 [ATCC 13032] and represents the type strain for the species (ATCC, 2016; Ikeda and Nakagawa, 2003).

The complete genome of *C. glutamicum* ATCC 13032 was sequenced in 1998, which was further characterized and annotated in 2001 and 2002 (reviewed in Ikeda and Nakagawa, 2003) and is also publicly available (NCBI, 2016). The central carbon pathway, physiology, and regulation of main and specific metabolic pathways for this strain have been well characterized, as it has significant industrial applications and much interest has been focused on optimizing production performance from this microorganism (Wieschalka *et al*., 2013).

6.4.2 Pathogenicity and Toxicogenicity

There are no documented case-reports of *C. glutamicum* being pathogenic or toxic to humans or animals. *C. glutamicum* fulfils the requirements for Qualified Presumption of Safety (QPS) when it is used for amino acid production (EFSA, 2013); Savory Base 100 being enriched in amino acids. *C. glutamicum* ATCC 13032 is classified as a Biosafety Level 1 by the American Type Culture Collection (ATCC), meaning the microorganism is not known to consistently cause disease in healthy adult humans and is of minimal potential hazard to laboratory personnel and the environment.

C. glutamicum has a long history of use in the food production industry. First isolated in 1956, *C. glutamicum* was initially characterized by its unique natural ability to produce large amounts of glutamic acid (the predominant amino acid in Savory Base 100) from sugar and ammonia (Vertès *et al.*, 2013). Moreover, *C. glutamicum* has been used for the production of glutamic acid in the U.S. since 1961 (Kinoshita *et al*., 1961a,b; Kalinowski *et al*., 2003); in 2005 alone, 1.5 million tons of glutamate were produced using fermentation by *C. glutamicum*, in addition to several thousand tons of threonine, lysine, isoleucine and tryptophan (Smith *et al*., 2010). *C. glutamicum* has also been identified as a surface microflora in cheese during ripening, indicating that this organism has a history of consumption as a species in cheese (Dolci *et al.*, 2009).

A number of *Corynebacterium* spp. (*C. ammoniagenes, C. casei, C. flavescens,* and *C. variabile*) have been listed in the International Dairy Federation (IDF) 2012 inventory of microbial species with technological beneficial role in fermented food products (IDF, 2012).

Corynebacterium spp. have also been used globally for number of years in the production of a variety of foods including cereals, bread, alcoholic beverages, and native dishes. *Corynebacterium* are responsible for the hydrolysis of starch to organic acids in the production of cassava and the West African maize porridge ogi (which can be cooked and then cooled to produce agidi, a weaning food or breakfast cereal) and are also involved in the fermentation of ugba (a Nigerian snack and condiment) from African oil bean seeds (Hahn, 1988; Haard *et al*., 1999; Osungbaro, 2009; Nwagu *et al*., 2011). A novel *Corynebacterium* species (termed by the authors as *C. nuruki* strain S6-4) was isolated from an alcohol fermentation starter (nuruk), which is used in the fermentation of rice to produce the Korean alcoholic beverage makgeolli (Shin *et al*., 2011); *Corynebacterium* spp. have also been detected in doenjang-meju (Korean fermented soybean paste), sufu (Chinese fermented bean curd) and sayur asin (Indonesian fermented mustard cabbage) (Puspito and Fleet, 1985; Cheng and Han, 2014; Jung *et al*., 2016).

6.5 Expert Panel Evaluation

Nestec has concluded that Savory Base 100 meeting appropriate food-grade specifications and manufactured consistent with cGMP is GRAS for use as an ingredient in various food products, as described in Part 1.3, on the basis of scientific procedures.

The GRAS determination is based on data generally available in the public domain pertaining to the safety of Savory Base 100 and based on a unanimous opinion among a panel of experts ("the Expert Panel"), who are qualified by scientific training and experience to evaluate the safety of food ingredients. The Expert Panel consisted of the following qualified scientific experts: Professor Emeritus Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Professor Eric A. Johnson (University of Wisconsin-Madison), and Professor Emeritus John A. Thomas (Indiana University School of Medicine). The Expert Panel was selected and convened prior to issuance of the FDA's guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S., FDA 2017), and therefore no formal written GRAS Panel policy was in place at the time of Expert Panel meeting. However, the notifier confirms that prior to convening the Panel all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and microbiology, and efforts were placed on identifying conflicts of interests or relevant appearance issues that would potentially bias the outcome of the Expert Panel deliberations; no such conflicts of interests or appearance conflicts were identified. The Expert Panel received a reasonable honorarium as compensation for the Expert Panel's time, and honoraria provided to the Expert Panel were not contingent upon the outcome of the Expert Panel deliberations.

The Expert Panel, convened by Nestec, independently and critically evaluated all data and information presented herein, and concluded that Savory Base 100 is GRAS for use as an ingredient in various food products, as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the Expert Panel and evaluation of such data as it pertains to the proposed GRAS uses of Savory Base 100, are presented in Appendix B.

6.6 Conclusions

Based on the data and information presented herein, Nestec has concluded that Savory Base 100, meeting appropriate food-grade specifications and manufactured according to cGMP, is safe for use in various food products as presented in Section 1.3. Nestec also has further concluded that pivotal data and information relevant to the safety of Savory Base 100 are publicly available and therefore the intended uses of Savory Base 100 can be determined to be Generally Recognized as Safe (GRAS) on the basis of scientific procedures.

Part 7. §170.255 List of Supporting Data and Information

Anderson SA, editor (1988). *Estimation of Exposure to Substances in the Food Supply*. (Contract no FDA 223-84-2059). Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Science Research Office (LSRO).

Arikawa Y, Kuroyanagi T, Shimosaka M, Muratsubaki H, Enomoto K, Kodaira R, et al. (1999). Effect of gene disruptions of the TCA cycle on production of succinic acid in *Saccharomyces cerevisiae*. J Biosci Bioeng 87(1):28-36.

ATCC (2016). *Corynebacterium glutamicum* (Kinoshita et al.) Abe et al. (ATCC® 13032™). Manassas (VA): American Type Culture Collection (ATCC). Available at: https://www.atcc.org/Products/All/13032.aspx [Last accessed: September 2, 2016].

Burdock GA, editor (2009). *l*- and *dl*-Alanine [and] Formic acid. In: *Fenaroli's Handbook of Flavor Ingredients, 6th edition*. Boca Raton (FL): CRC Press Inc., pp. 42-43, 700-701.

CDC (2015). *National Health and Nutrition Examination Survey (NHANES): 2011-2012*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://wwwn.cdc.gov/nchs/nhanes/search/nhanes11_12.aspx. [Page last reviewed: November 6, 2015].

Cheng Y, Han B (2014). Solid-state fermented soybean [Corynebacterium, sufu, bean curd]. In: Chen J, Zhu Y, editors. *Solid State Fermentation for Foods and Beverages*. Boca Raton (FL): CRC Press (Taylor & Francis Group), pp. 155-198 [See p. 191].

Chevallier K (2016) [unpublished]. *In Vitro Micronucleus Test in Cultured Human Lymphocytes [He Wei C. Essence I]*: (Laboratory Study Number: 43957 MNH). Prepared by Evreux, France: CiToxLAB, Safety and Health Research Laboratories for Singen, Germany: Nestle PTC Singen Lebensmittelforschung GmbH.

Chevallier K (2017) [unpublished]. *In Vitro Micronucleus Test in Cultured Human Lymphocytes [He Wei C. Essence II]*: (Laboratory Study Number: 43959 MNH). Prepared by Evreux, France: CiToxLAB, Safety and Health Research Laboratories for Singen, Germany: Nestle PTC Singen Lebensmittelforschung GmbH.

Daniels DH, Joe FL Jr, Diachenko GW (1995). Determination of free glutamic acid in a variety of foods by high-performance liquid chromatography. Food Addit Contam 12(1):21-29 [Erratum, 1996, 13(6):736].

Dolci P, Barmaz A, Zenato S, Pramotton R, Alessandria V, Cocolin L, et al. (2009). Maturing dynamics of surface microflora in Fontina PDO cheese studied by culture-dependent and-independent methods. J Appl Microbiol 106(1):278-287.

EC (1986). Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Off J Eur Union 29(L358):1-28. Available at: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:31986L0609 [Consolidated version: 10/05/2013, Repealed by Directive 2010/63/EU].

EC (2000). Commission Directive 2000/32/EC of 19 May 2000 adapting to technical progress for the 26th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Off J Eur Union 43(L136):1-89. Available at: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32000L0032.

EC (2001). Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Off J Eur Union 44(L311):67-128. Available at: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32001L0083 [Consolidated version: 16/11/2012].

EC (2008). Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Off J Eur Union 51(L142):1-739. Available at: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32008R0440 [Consolidated version: 04/03/2016].

Eerola HS, Roig Sagués AX, Hirvi TK (1998). Biogenic amines in Finnish dry sausages. J Food Safety 18(2):127-138.

EFSA (2011). Scientific Opinion on risk based control of biogenic amine formation in fermented foods (EFSA Panel on Biological Hazards/BIOHAZ) (Question no EFSA-Q-2009-00829, adopted: 21 September 2011 by European Food Safety Authority). EFSA J 9(10):2393 [93 pp.]. DOI:10.2903/j.efsa.2011.2393. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/2393.

EFSA (2013). Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update) (EFSA Panel on Biological Hazards/BIOHAZ) (Question no EFSA-Q-2013-00019, adopted: 24 October 2013 by European Food Safety Authority). EFSA J 11(11):3449 [107 pp]. DOI:10.2903/j.efsa.2013.3449. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/3449.

EFSA (2017). Scientific Opinion on the re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E622), calcium glutamate (E 623), ammonium glutamate (E 624), and magnesium glutamate (E 625) as food additives (EFSA Panel on Food Additives and Nutrient Sources added to Food/ANS) (Question no EFSA-Q-2011-00577, EFSA-Q-2011-00578, EFSA-Q-2011-00579, EFSA-Q-2011-00580, EFSA-Q-2011-00581, EFSA-Q-2011-00582, adopted: 21 June 2017 by European Food Safety Authority). EFSA J 15(7):4910 [90pp]. DOI:10.2903/j.efsa.2017.4910. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/4910.

EU (2011). Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Off J Eur Union 54(L295):1-177. Available at: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32011R1129&qid=1437072669094 [Consolidated version: 21/11/2013].

FASEB (1976). *Evaluation of the Health Aspects of Formic Acid, Sodium Formate, and Ethyl Formate as Food Ingredients*. (PB-266-282; SCOGS-71; FDA/BF-77/83). Prepared by Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office (LSRO), Select Committee on GRAS Substances (SCOGS) for Washington (DC): Food and Drug Administration, U.S. (FDA), Bureau of Foods.

FASEB (1980). *Evaluation of the Health Aspects of Certain Glutamates as Food Ingredients: Supplemental Review*. (PB80-178635; SCOGS-37a-Suppl.; Contract No. FDA 223-75-2004). Prepared by Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office (LSRO), Select Committee on GRAS Substances (SCOGS) for Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Bureau of Foods.

FCC (2016). Yeast extract. In: *Food Chemicals Codex, 10th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 1360-1361.

Gad SC, editor (2016). Pathology of old rats. In: *Animal Models in Toxicology, 3rd edition*. Boca Raton (FL): CRC Press (Taylor & Francis Group), pp. 212-220.

Gaunt IF, Grasso P, Gangolli SD (1967). Short-term toxicity study of emulsifier YN* in rats. Food Cosmet Toxicol 5(5):623-629.

Greaves P (2012). Liver (chapter 9). In: *Histopathology of Preclinical Toxicity Studies, 4th edition*. New York (NY): Elsevier B.V., pp. 432-544 [see pages 432-481].

Greaves P, Faccini JM (1992). Digestive system. In: *Rat Histopathology: A Glossary for Use in Toxicity and Carcinogenicity Studies, 2nd edition*. Amsterdam, The Netherlands / New York (NY): Elsevier, pp. 105-158 [see pages 107-116].

Haard NF, Odunfa SA, Lee C-H, Quintero-Ramírez R, Lorence-Quiñones A, Wacher-Radarte C (1999). Cereal fermentations in African countries (chapter 2). In: *Fermented Cereals a Global Perspective*. (FAO Agricultural Services Bulletin no 138). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO). Available at: http://www.fao.org/docrep/x2184e/x2184e07.htm.

Hahn SK (1988). An overview of traditional processing and utilization of cassava in Africa. In: Hahn SK, Reynolds L, Egbunike GN, editors. *Cassava as Livestock Feed in Africa*: Proceedings of the IITA/ILCA/University of Ibadan Workshop on the Potential Utilization of Cassava as Ibadan, Nigeria: International Institute of Tropical Agriculture (IITA) / Addis Ababa, Ethiopia: International Livestock Centre for Africa (ILCA). Available at: http://www.fao.org/wairdocs/ilri/x5458e/x5458e05.htm.

ICH Steering Committee (1995). *Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals: S2A*. (ICH Harmonised Tripartite Guideline - Recommended for adoption at Step 4 of the ICH Process on 19 July 1995 by the ICH Steering Committee). Geneva, Switz.: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human Use (ICH).

ICH Steering Committee (1997). *Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals: S2B*. (ICH Harmonised Tripartite Guideline - Current Step 4 Version Dated July 16, 1997). Geneva, Switz. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human Use (ICH).

IDF (2012). *Safety Demonstration of Microbial Food Cultures (MFC) in Fermented Food Products*. (Bulletin (IDF) No. 455). Brussels, Belgium: International Dairy Federation (IDF). Available at: http://jdta.or.jp/JIDF/wp-content/uploads/2015/03/455-2012-Safety-demonstrations-of-MFC-in-fermented-food-products-1-1.pdf.

Ikeda M, Nakagawa S (2003). The *Corynebacterium glutamicum* genome: features and impacts on biotechnological processes. Appl Microbiol Biotechnol 62(2/3):99-109.

Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 22(8):623-636.

Jägerstad M, Skog K, Grivas S, Olsson K (1991). Formation of heterocyclic amines using model systems. Mutat Res 259(3/4):219-233.

JECFA (1970). Monosodium glutamate. In: *Toxicological Evaluation of Some Extraction Solvents and Certain Other Substances*. Fourteenth Report of the Joint FAO/WHO Expert Committee on Food Additives, June 24-July 2, 1970, Geneva, Switz. (FAO Nutrition Meetings Report Series no 48A; WHO/FOOD ADD/70.39). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v48aje09.htm.

JECFA (1974). L-Glutamic acid and its ammonium, calcium, monosodium and potassium salts. In: *Toxicological Evaluation of Some Food Additives including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents.* Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switz. (WHO Technical Report Series, no 539; Nutrition Meetings Report Series, no 53a). Geneva, Switz.: World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je85.htm.

JECFA (1988). L-Gluta[m]ic acid and its ammonium, calcium, monosodium and potassium salts. In: *Toxicological Evaluation of Certain Food Additives*. 31st Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Feb. 16-25, 1987, Geneva, Switz. (WHO Food Additives Series no 22). New York (NY) / Geneva, Switz.: Cambridge University Press / World Health Organization (WHO), pp. 97-161. Available at: http://www.inchem.org/documents/jecfa/jecmono/v22je12.htm.

JECFA (1997). Formic acid. In: *Summary of Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 1956-2014 (1st through 79th Meetings) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA) / Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO) / International Programme on Chemical Safety (IPCS). Available at: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1561.

JECFA (2004). DL-Alanine. In: *Summary of Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 1956-2014 (1st through 79th Meetings) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA) / Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO) / International Programme on Chemical Safety (IPCS). Available at: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=5314.

Jokinen MP, Lieuallen WG, Boyle MC, Johnson CL, Malarkey DE, Nyska A (2011). Morphologic aspects of rodent cardiotoxicity in a retrospective evaluation of National Toxicology Program studies. Toxicol Pathol 39(5):850-860.

Jung WY, Jung JY, Lee HJ, Jeon CO (2016). Functional characterization of bacterial communities responsible for fermentation of doenjang: A traditional Korean fermented soybean paste. Front Microbiol 7:Article 827 [10pp]. DOI:10.3389/fmicb.2016.00827.

Kalinowski J, Bathe B, Bartels D, Bischoff N, Bott M, Burkovski A, et al. (2003). The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. J Biotechnol 104(1/3):5-25.

Kinoshita S, Tanaka T and Akita S, inventors; Chiyoda-ku, Tokyo, Japan: Kyowa Hakko Kogyo Co., Ltd., assignee (1961a). *Method of Producing L-Glutamic Acid.* US Patent 3,002,889 [Oct. 3, 1961]. Available at: http://www.freepatentsonline.com/3002889.html.

Kinoshita S, Udaka S, Akita S, inventors; Tokyo, Japan: Kyowa Hakko Kogyo Co., Ltd., assignee (1961b). *Method of Producing L-Glutamic Acid by Fermentation.* US Patent 3,003,925 [Oct. 10, 1961]. Available at: http://www.freepatentsonline.com/3003925.html.

Maekawa A, Todate A, Onodera H, Matsushima Y, Nagaoka T, Shibutani M, et al. (1990). Lack of toxicity/carcinogenicity of monosodium succinate in F344 rats. Food Chem Toxicol 28(4):235-241.

NCBI (2016). *Corynebacterium glutamicum ATCC 13032 DNA, complete genome.* (GenBank BA000036.3). In: *NCBI Protein Database*. Bethesda (MD): National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine (NLM). Available at: http://www.ncbi.nlm.nih.gov/nuccore/BA000036 [Last accessed: September 2, 2016].

Nichols PG, Jones SM (1991). Monosodium glutamate in Western Australian foods. Chem Aust 58:556-558.

Nwagu TNT, Orji MU, Nwobodo I, Nwobodo HA (2011). Mixed microbial flora as starter culture for production of ugba from African oil bean seed. Asian J Biol Sci 4(1):62-69.

OECD (1997). Bacterial reverse mutation test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 471) [Updated & Adopted: 21 July 1997]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=1ijgh8o34k0qd.delta.

OECD (1998a). *OECD Principles of Good Laboratory Practice*. (Series on Principles of Good Laboratory Practice and Compliance Monitoring, no. 1 [ENV/MC/CHEM(98)17]). Paris, France: Organisation for Economic Co-Operation & Development (OECD), Environment Directorate, Chemicals Group and Management Committee, OECD Environmental Health and Safety Publications. Available at: http://www.oecd-ilibrary.org/environment/oecd-principles-on-good-laboratory-practice_9789264078536-en [As revised in 1997].

OECD (1998b). Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 408) [Updated & Adopted: 21 September 1998]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en;jsessionid=1m6bvwspup322.delta.

OECD (2014). In Vitro Mammalian Cell Micronucleus Test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 487) [Updated & Adopted: 26 September 2014]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264224438-en.

OECD (2015). In Vitro Mammalian Cell Gene Mutation Tests Using the Hprt and Xprt Genes. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 476) [Updated & Adopted: 28 July 2015]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-476-in-vitro-mammalian-cell-gene-mutation-tests-using-the-hprt-and-xprt-genes_9789264243088-en [replaced by OECD Guideline no 476, adopted: 29 July 2016].

Osungbaro TO (2009). Physical and nutritive properties of fermented cereal foods. Afr J Food Sci 3(2):023-027.

Prester L (2016). Biogenic amines in ready-to-eat foods (chapter 22). In: Kotzekidou P, editor. *Food Hygiene and Toxicology in Ready-to-Eat Foods*. San Diego (CA) / Amsterdam, The Netherlands: Academic Press, Elsevier Inc., pp. 397-416.

Puspito H, Fleet GH (1985). Microbiology of sayur asin fermentation. Appl Microbiol Biotechnol 22(6):442-445.

Rao GN (2002). Diet and kidney diseases in rats. Toxicol Pathol 30(6):651-656.

Reeves PG, Rossow KL, Lindlauf J (1993). Development and testing of the AIN-93 purified diets for rodents: Results on growth, kidney calcification and bone mineralization in rats and mice. J Nutr 123(11):1923-1931.

Sahu L, Panda SK, Paramithiotis S, Zdolec N, Ray RC (2015). Biogenic amines in fermented foods: overview (chapter 16). In: Montet D, Ray RC, editors. *Fermented Foods. Part I: Biochemistry and Biotechnology*. (Food Biology Series). Boca Raton (FL): CRC Press (Taylor & Francis Group), pp. 303-317.

SCF (1991). *Reports of the Scientific Committee for Food First Series of Food Additives of Various Technological Functions (Opinion expressed on 18 May 1990)*. (Reports of the Scientific Committee for Food, 25th series). Brussels, Belgium: Commission of the European Communities (EEC), Scientific Committee for Food (SCF), pp. 21-25. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_reports_25.pdf.

Seely JC, Brix A (2014). Kidney – mineralization. In: Cesta MF, Herbert RA, Brix AL, Malarkey DE and Sills RC, editors. *Nonneoplastic Lesion Atlas: A Guide for Standardizing Terminology in Toxicologic Pathology for Rodents*. Research Triangle Park (NC): National Toxicology Program (NTP). Available at: https://ntp.niehs.nih.gov/nnl/urinary/kidney/mineral/index.htm [Last updated: Jan. 14, 2014].

Shin N-R, Jung M-J, Kim M-S, Roh SW, Nam Y-D, Bae J-W (2011). *Corynebacterium nuruki* sp. nov., isolated from an alcohol fermentation starter. Int J Syst Evol Microbiol 61(10):2430-2434.

Skog KI, Johansson MAE, Jägerstad MI (1998). Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. Food Chem Toxicol 36(9/10):879-896.

Smith KM, Cho KM, Liao JC (2010). Engineering *Corynebacterium glutamicum* for isobutanol production. Appl Microbiol Biotechnol 87(3):1045-1055.

Song H, Lee SY (2006). Production of succinic acid by bacterial fermentation. Enzyme Microb Technol 39(3):352-361.

Stamler J, Brown IJ, Daviglus ML, Chan Q, Kesteloot H, Ueshima H, et al. (2009). Glutamic acid, the main dietary amino acid, and blood pressure: the INTERMAP Study (International Collaborative Study of Macronutrients, Micronutrients and Blood Pressure). Circulation 120(3):221-228 [Includes supplemental material].

Tafazoli S, Vo TD, Petersen A, Constable A, Coulet M, Phothirath P, et al. (2017). Genotoxicity, acute and subchronic toxicity evaluation of savory food ingredients. Regul Toxicol Pharmacol 87:71-87.

Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, et al. (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicol Pathol 38(7, Suppl.):5S-81S.

U.S. EPA (1998). *Health Effects Test Guidelines: OPPTS 870.5100: Bacterial Reverse Mutation Test.* (EPA 712-C-98-247). Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Office of Prevention, Pesticides and Toxic Substances (OPPTS). Available at: https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0156-0022 [Last accessed: September 2, 2016].

U.S. FDA (2017). U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.ecfr.gov/cgi-bin/ECFR?SID=b3913f1005fd08fc916003094862a5e3&mc=true&page=browse [current to August 8, 2017].

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food Additives Permitted for Direct Addition to Food for Human Consumption	172.320	Amino acids
184—Direct Food Substances Affirmed as Generally Recognized as Safe	184.1091	Succinic acid
	184.1983	Bakers yeast extract
186— Indirect food substances affirmed as generally recognized as safe	186.1316	Formic acid

USDA (2014). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2011-2012*. Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release. [Last Modified: 10/2/2014].

Vertès AA, Inui M, Yukawa H (2013). The biotechnological potential of *Corynebacterium glutamicum*, from *Umami* to *Chemurgy*. In: Tatsuni N, Inui M, editors. *Corynebacterium* glutamicum*: Biology and Biotechnology*. (Microbiology Monographs, vol 23). Heidelberg, Germany / New York (NY): Springer, pp. 1-49.

Vorhees CV, Butcher RE, Brunner RL, Sobotka TJ (1979). A developmental test battery for neurobehavioral toxicity in rats: a preliminary analysis using monosodium glutamate calcium carrageenan, and hydroxyurea. Toxicol Appl Pharmacol 50(2):267-282.

Wieschalka S, Blombach B, Bott M, Eikmanns BJ (2013). Bio-based production of organic acids with *Corynebacterium glutamicum*. Microb Biotechnol 6(2):87-102.

Yoshida Y (1998). Umami taste and traditional seasonings. Food Rev Int 14(2/3):213-246.

Appendix A
Full Study Report for *In Vitro* Mammalian Cell Micronucleus Test (Chevalier, 2016)

CiToxLAB
Safety and Health Research Laboratories

SPONSOR

PTC Singen
Nestle PTC Singen Lebensmittelforschung GmbH
Lange Str. 21
78224 Singen
Germany

TEST ITEM

He Wei C.Essence I

STUDY TITLE

***IN VITRO* MICRONUCLEUS TEST**
IN CULTURED HUMAN LYMPHOCYTES

STUDY DIRECTOR

Kelly Chevallier

DATE OF ISSUE

27 December 2016

TEST FACILITY

CiToxLAB France
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER

43957 MNH

Phone +33 (0)2 32 29 26 26
Fax +33 (0)2 32 67 87 05
Email contact.france@citoxlab.com
B.P. 563 - 27005 Evreux cedex, France

CIT
SAS au capital de 2 003 329 €
788 060 465 RCS Evreux
VAT : FR 19 788 060 465
Agrément Crédit Impôt Recherche

CONTENTS

GLP COMPLIANCE STATEMENT OF THE STUDY DIRECTOR

The study was performed at CiToxLAB France, BP 563, 27005 Evreux, France, in compliance with CiToxLAB France's standard operating procedures and the following principles of Good Laboratory Practice:

- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17 and all subsequent OECD consensus documents,
  Conformance to these GLP standards satisfies the Mutual Acceptance of Data (MAD) between members of OECD including the United States and Japan,
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L50 of 20.2.2004),
- Article Annexe 2 à l'article D523-8 du code de l'environnement concernant les principes de l'OCDE des Bonnes Pratiques de Laboratoire (BPL).

Reference data used as historical data in the present study were obtained from non-GPL studies, nevertheless, they were prepared in compliance with CiToxLAB France's standard operating procedures then audited by CiToxLAB France Quality Assurance Unit. Since CiToxLAB France is a Test facility certified by the French National Authorities for Good Laboratory Practice, and the procedures undertaken are the same, the use of these non-GLP data was considered not to prejudice the overall GLP status of the study and the scientific reliability of the study conclusions.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

K. Chevallier
Study Director
MSc

Study completion date: 27 December 2016

SIGNATURE PAGE

Reviewed and approved by:

Test facility
Management Representative

P. Singh, PhD, DABT
Director of Toxicology

Date: 27 Dec. 2016

STATEMENT OF QUALITY ASSURANCE UNIT

Inspections performed at CiToxLAB France

The CiToxLAB France Quality Assurance Unit conducted the inspections detailed below:

Type of inspection	Dates		
	Inspection	Reported to the Study Director	Reported to Management
Study plan	26 May 2016	26 May 2016	26 May 2016
Study plan amendment No. 1	28 July 2016	29 July 2016	29 July 2016
Treatment	18 August 2016	18 August 2016	18 August 2016
Report	02 and 05 December 2016	05 December 2016	05 December 2016

In addition, process and facility based inspection are carried out, as detailed below:

Type of inspection	Dates		
	Inspection	Reported to the Director of Department	Reported to Management
SOP / training	17 and 18 March 2016	18 March 2016	18 March 2016
Apparatus, Material, Reagents	04 April 2016	05 April 2016	05 April 2016
Test and reference item	21 April 2016	21 April 2016	21 April 2016
Facility	20 and 24 May 2016	24 May 2016	24 May 2016
Apparatus, Material, Reagents	04 July 2016	07 July 2016	07 July 2016
Apparatus, Material, Reagents	08 and 09 September 2016	09 September 2016	09 September 2016
Dosages form analysis	05 April 2016	05 April 2016	05 April 2016
Dosages form analysis	06 September 2016	06 September 2016	06 September 2016

The inspections were performed in compliance with CiToxLAB France Quality Assurance Unit procedures and the principles of Good Laboratory Practices.

The final report is considered to constitute an accurate and complete reflection of the study raw data.

D. LEGRAND Date: 27 Dec. 2016

CiToxLAB France Quality Assurance Unit

SUMMARY

The objective of this study was to evaluate the potential of the test item, He Wei C.Essence I, to induce an increase in the frequency of micronucleated cells in cultured human lymphocytes.

Methods

After two preliminary cytotoxicity tests, the test item He Wei C.Essence I, diluted in water for injections, was tested in a single experiment, with and without a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction (S9 fraction) of rats induced with Aroclor 1254, as follows:

Without S9 mix	3 h treatment + 24 h recovery
	24 h continuous treatment (no recovery)
With S9 mix	3 h treatment + 24 h recovery

At harvest, cells were collected by centrifugation and submitted to a hypotonic treatment. The cells were then fixed in a methanol/acetic acid mixture (3/1; v/v).
Following fixation, cells were kept at +4°C for at least an overnight period, then, they were centrifuged at 1250*g* for 3 minutes, supernatant was removed and cells were re-suspended in a methanol/acetic acid mixture (7/1; v/v). After a second centrifugation (3 minutes at 1250*g*) and removal of the supernatant, two drops were spread on glass slides and stained with 5% Giemsa in Evian water.
Slides were coded, so that the scorer was unaware of the treatment group of the slide under evaluation ("blind" scoring) for the micronucleus analysis.

Each treatment was coupled to an assessment of cytotoxicity at the same dose-levels. Cytotoxicity was evaluated by determining the RI (Replication Index).
For the main experiment (with or without S9 mix), micronuclei were analyzed for three dose-levels of the test item, for the vehicle and the positive controls, in 1000 Binucleated Cells per culture (total of 2000 Binucleated Cells per treatment level), except for the positive control following the short treatment without S9 mix (total of 1592 Binucleated Cells analyzed).

Results

Since the test item was found to be non-cytotoxic and freely soluble in the second preliminary test, the highest dose-level selected for the main experiment was 5000 µg/mL, according to the criteria specified in the international regulations.

The mean frequency of cells that have undergone mitosis (binucleated + multinucleated cells), as well as the mean background frequency of MNBC for the vehicle control were as specified in the acceptance criteria. Also, positive control cultures showed clear statistically significant increases in the frequency of MNBC. The study was therefore considered to be valid.

With a treatment volume of 3% (v/v) in culture medium, the selected dose-levels were 312.5, 625, 1250, 2500, 3750 and 5000 µg/mL for the 3-hour treatments with and without S9 mix, as well as for the continuous 24-hour treatment without S9 mix.

At the end of the treatment periods, no precipitate was observed in the culture medium at any of the tested dose-levels.

Cytotoxicity
Following the 3-hour treatment without S9 mix, no cytotoxicity was induced at any of the tested dose-levels, as shown by the absence of any noteworthy decrease in the RI.
Following the 24-hour continuous treatment without S9 mix, a slight to moderate cytotoxicity was noted at dose-levels ≥ 2500 µg/mL, as shown by a 30 to 43% decrease in the RI.
Following the 3-hour treatment with S9 mix, no noteworthy cytotoxicity was induced at any of the tested dose-levels, as shown by the absence of any noteworthy decrease in the RI.

Micronucleus analysis
The dose-levels selected for micronucleus analysis were 1250, 2500 and 3750 µg/mL for the 3-hour treatments with and without S9 mix, as well as for the continuous 24-hour treatment without S9 mix, the highest dose-level of 5000 µg/mL being considered to produce extreme culture conditions (osmolality increase of more than 50 mOsm/kg H_2O at 5000 µg/mL when compared to the vehicle control medium).

Following the 3-hour treatments with and without S9 mix, as well as for the continuous 24-hour treatment without S9 mix, neither statistically significant nor dose-related increase in the frequency of MNBC was noted at any of the analyzed dose-levels in comparison to the corresponding vehicle controls. Moreover, none of the analyzed dose-levels showed frequency of MNBC of both replicate cultures above the corresponding vehicle control historical range. These results met the criteria of a negative response.

Conclusion

Under the experimental conditions of the study, the test item He Wei C.Essence I did not induce any chromosome damage, or damage to the cell division apparatus, in cultured mammalian somatic cells, using human lymphocytes, either in the presence or absence of a rat liver metabolizing system.

1. INTRODUCTION

1.1 OBJECTIVE

The objective of this study was to evaluate the potential of the test item, He Wei C.Essence I, to induce an increase in the frequency of micronucleated cells in cultured human lymphocytes.

The micronuclei observed in the cytoplasm of interphase cells may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes that are unable to migrate with the rest of the chromosomes during the anaphase of cell division. The assay thus has the potential to detect the activity of both clastogenic and aneugenic chemicals [(a, b)].

In order to ensure that the cells scored for micronuclei have undergone mitosis during the treatment or the recovery period, Cytochalasine B (CytoB) was used to block cytokinesis. This treatment leads to the formation of binucleated cells, by preventing separation of daughter cells after mitosis. The micronucleus analysis was then only performed in binucleated cells.

This test was performed in the presence and absence of a rat liver metabolizing system (S9 mix).

1.2 REGULATORY COMPLIANCE

The study design was based on the OECD guideline No. 487, adopted 26 September 2014.

2. MATERIALS AND METHODS

2.1 TEST ITEM, VEHICLE AND POSITIVE CONTROL ITEMS

2.1.1 Identification

2.1.1.1 Test item

Name:	.	He Wei C.Essence I
Synonyms:	.	HeWei C.Essence I, SSS1, NMF 1, Athos Gold, He Wei C.Essence I paste
Batch No.:	.	G160304
Description:	.	Brown paste
Containers:	.	Two transparent plastic flasks
Storage condition:	.	At room temperature
Specific test item requirements (handling conditions):	.	The test item was homogenized by vigourous mixing using a Polytron® before any sampling
Molecular Weight:	.	Not applicable
Composition:	.	See test item analysis certificate
Manufacturing process:	.	93.5% fermented cereal paste and 6.5% salt
	.	The test item received 11 July 2016 was sterilized through heat treatment (autoclaved at 121°C for 15 minutes) (*information provided by the Sponsor*)
Correction factor:	.	1.471 (*taking into account the water content*) for the sample received 14 April 2016
	.	1.458 (*taking into account the water content*) for the sample received 11 July 2016
Dates of receipt:	.	14 April 2016 (first preliminary test)
	.	11 July 2016 (second preliminary test and main test)
Expiry date:	.	02 December 2016
Disposal of the test item:	.	Destruction (except the archived test item sample) (any remaining test item is kept for at least 6 months after last use in the project and then disposed of according to instructions described in CiToxLAB France in-house procedures)

Data relating to the characterization of the test item are documented in a test item information sheet (archived with study files) and a test item analysis certificate (presented in Appendix 1) provided by the Sponsor.
As confirmed by the Sponsor in an e-mail dated 13 May 2016 (archived with study files), the test item had to be homogenized by vigourous mixing using a Polytron® before any sampling.
Confirmation of identity of the test item is the responsibility of the Sponsor.

The sample received 14 April 2016 was found to be contaminated during the first preliminary cytotoxicity test (see § Study plan adherence).
The sample received 11 July 2016 was exempt of microbiological contamination.

2.1.1.2 Vehicle

According to available solubility data, the vehicle was water for injections.

2.1.1.3 Positive control items

The positive controls were dissolved in water and were used at the final concentrations described in the following table:

	Short treatment (3 hours)	Continuous treatment (24 hours)
Without S9 mix	Aneugen Colchicine (COL): 0.1 µg/mL	Clastogen Mitomycin C (MMC): 0.1 µg/mL
With S9 mix	Clastogen Cyclophosphamide (CPA): 6 µg/mL	-

2.1.1.4 Calculation of correction factors

To obtain the amount of test item to be weighed for preparation of dose formulations, the amount of test item expressed in active moiety was multiplied by the following correction factor for water content:

For the sample received 14 April 2016:

100/(100-**(W1)**) = 1.471

Water content (W1): 32%

For the sample received 11 July 2016:

100/(100-**(W2)**) = 1.458

Water content (W2): 31.42%

2.1.2 Dose formulation preparation

All the test item concentrations and dose-levels were expressed as dry matter, taking into account the water content (32% or 31.42%). Thus, a correction factor of 1.471 for the first preliminary cytotoxicity test or 1.458 for the second preliminary cytotoxicity test and the main cytogenetic experiment was applied.

The test item was homogenized by vigourous mixing using a Polytron® before any sampling. It was then dissolved in the vehicle at a concentration of 166.67 mg/mL for both preliminary cytotoxicity tests and for the main cytogenetic experiment.

The stock solutions and their dilutions were prepared within 4 hours of use, and then kept at room temperature and protected from light until use.

2.1.3 Chemical analysis of the dose formulations

Analytical technique:
. High Performance Liquid Chromatography with UV detection (HPLC/UV)

Principle and validation of the method:
. Analytical method provided by the Sponsor and validated at CiToxLAB France *(CiToxLAB France/Study No. 43956 VAA (Study not yet finalized))* prior to dose formulation analysis

. Checked parameters, acceptance criteria and obtained results are described in the validation report

Determination of test item concentrations in dose formulations
. The concentration of the test item was determined according to the validated method in samples of each vehicle control and test item stock formulation prepared for the main cytogenetic experiment

Acceptance criterion:
. Measured concentration = nominal concentration ± 10%

2.2 TEST SYSTEM

2.2.1 Cells

Cultures of human lymphocytes are primary cell cultures recommended by international regulations for the *in vitro* mammalian cell micronucleus test. They have a stable karyotype with 46 chromosomes and an average cell cycle time of 12-14 hours.
Cultures of human lymphocytes were prepared from whole blood samples (supplied by ImmuneHealth, Belgium) obtained from young (*i.e.* 18 to 35 years old), healthy, non-smoking donors and collected into heparinized sterile tubes.

2.2.2 Culture conditions

The culture medium was RPMI 1640 medium (HEPES-buffered) containing 20% fetal calf serum, L-glutamine (2 mM), antibiotic and antimycotic.
Phytohemagglutinin (PHA, a mitogen to stimulate the lymphocytes to divide) was added at a final concentration of 0.216 mg/mL for the 44-48 hours culture period.

2.2.3 Metabolic activation system

The S9 mix consists of induced enzymatic systems contained in rat liver post-mitochondrial fraction (S9 fraction) and the cofactors necessary for their function [(d)]. S9 fraction was purchased from Moltox (Molecular Toxicology, INC, Boone, NC 28607, USA) and obtained from the liver of rats treated with Aroclor 1254 (500 mg/kg) by intraperitoneal route.
The S9 fraction was preserved in sterile tubes at -80°C, until use.

The S9 mix was prepared at +4°C immediately before use and maintained at this temperature until added to culture medium.

The composition of S9 mix was as follows:

Ingredient	Volume(s)
Glucose-6-phosphate (180 mg/mL)	1
NADP (25 mg/mL)	1
KCl (150 mM)	1
S9 fraction (final concentration in S9 mix: 40% (v/v)): batch No. 3556, protein concentration: 42.5 mg/mL	2

In the assay with metabolic activation, the culture medium was supplemented with 5.28% of this S9 mix (see § Study plan adherence) so that the final concentration of S9 in the treatment medium was 2%.

2.2.4 Culture conditions

For each experiment, cell cultures were prepared from the blood of one donor.
To prepare each culture, 0.4 mL of heparinized human whole blood was added to 8 mL of culture medium containing PHA. The cultures were then placed at 37°C for 44 to 48 hours.

2.2.5 Treatment, rinsing and recovery period

Following the 44- to 48-hour culture period, lymphocyte cultures were centrifuged at 300*g* for 10 minutes. Then, supernatants were discarded and cells were re-suspended in fresh culture medium (supplemented with S9 mix for the treatment with metabolic activation).
For the 24-hour treatment, CytoB dissolved in dimethylsulfoxide (at 3 mg/mL) was added in each culture (20 µL/culture) to reach the final concentration of 6 µg/mL.
Cells suspensions were then exposed to the test item or to the control items (vehicle or positive controls) and the final volume was set to 10 mL with culture medium.

The cultures were placed at 37°C for the treatment duration (see § Preliminary cytotoxicity test and § Main cytogenetic experiment).

At the end of treatment, cell cultures were centrifuged (300*g* for 10 minutes) and rinsed twice with 10 mL of 0.9% NaCl pre-warmed at 37°C. Then, following the 3-hour treatment, the cultures were incubated at 37°C for a recovery period (corresponding to 1.5 - 2 normal cell cycles) in fresh culture medium, in which CytoB (dissolved in dimethylsulfoxide at 3 mg/mL) was added (20 µL/culture) to reach the final concentration of 6 µg/mL.

2.2.6 Cell harvesting and slides preparation

At harvest, the cells were collected by centrifugation (300*g* for 10 min) and submitted to a hypotonic treatment to induce cells swelling (*i.e.* incubation of 3 minutes in 4 mL of KCl 0.075 M pre-warmed at 37°C). The cells were then fixed in a methanol/acetic acid mixture (3/1; v/v).
Following fixation, the cells were kept at +4°C for at least an overnight period, then, they were centrifuged at 1250*g* for 3 minutes, the supernatant was removed and cells were resuspended in a methanol/acetic acid mixture (7/1; v/v). After a second centrifugation (3 minutes at 1250*g*) and removal of the supernatant, two drops were spread on glass slides and stained for 7 minutes with 5% Giemsa in Evian water.
The slides were coded, so that the scorer was unaware of the treatment group of the slide under evaluation ("blind" scoring) for the micronucleus analysis.

2.2.7 Analysis of the slides

2.2.7.1 Assessment of cytotoxicity

The assessment of cytotoxicity was performed without blinding.
Using a microscope, the numbers of binucleated and multinucleated cells were scored on 500 cells per culture (*i.e.* 1000 cells per treatment-level).

For each culture, the Replication Index (RI) was calculated and used relative to that of the vehicle control.

$$RI = \frac{\text{number of Binucleated Cells + 2 (number of multinucleated cells)}}{\text{total number of cells}}$$

Cytotoxicity (or cytostasis) was shown by the decrease in the RI when compared to the vehicle control culture.

2.2.7.2 Micronucleus analysis

Three appropriate test item dose-levels for the scoring of micronuclei were selected mainly on the basis of the cytotoxicity (*i.e.* achieved reduction of the RI) and on the presence of precipitate.

The micronucleus analysis was performed "blind", under a microscope.
Micronuclei (MN) were analyzed in 1000 Binucleated Cells (BC) per culture (total of 2000 Binucleated Cells per treatment-level), except for the positive control following the short treatment without S9 mix (total of 1592 binucleated cells analyzed; see § Study plan adherence).

The Binucleated Cells selected for micronucleus analysis must meet the following criteria:
- cells should have two nuclei situated within the same cytoplasmic boundary,
- the two nuclei of Binucleated Cells should be approximately equal in size and staining, Binucleated Cells should have intact and distinguishable nuclear and cytoplasmic membranes.

Among the Binucleated Cells, Micronucleated Binucleated Cells (MNBC) were scored according to the following criteria [e, f]:
- micronuclei should be within the same cytoplasmic boundary as the two main nuclei and clearly surrounded by a nuclear membrane,
- micronuclei should be round or oval in shape,
- the micronucleus diameter should be less than one-third of the diameter of the main nuclei (*i.e.* the micronucleus area should be less than $1/9^{th}$ of the area of one of the main nuclei),
- micronuclei should be non-refractile (can be distinguished from artefacts such as staining particles),
- micronuclei should have similar staining intensity to that of the main nuclei (or occasionally more intense),
- micronuclei should not be linked to the main nuclei *via* nucleoplasmic bridges,
- micronuclei may touch but not overlap the main nuclei and the micronuclear boundary should be distinguishable from the nuclear boundary,
- only Binucleated Cells with a number of micronuclei ≤ 5 were scored to exclude apoptosis and nuclear fragmentation.

Number of Micronucleated Binucleated Cells and number of micronuclei per Binucleated Cell were given separately for each treated and control culture.

2.3 EXPERIMENTAL DESIGN

2.3.1 Preliminary cytotoxicity tests

To assess the cytotoxicity of the test item received 14 April 2016, a single culture (one culture/dose-level) was tested, in presence and absence of S9 mix, with:

. six dose-levels of the test item,
. the vehicle control.

The treatment durations were as follows:

Without S9 mix	3 h treatment + 24 h recovery
	24 h continuous treatment (no recovery)
With S9 mix	3 h treatment + 24 h recovery

Since the test item received 14 April 2016 was found contaminated during the first preliminary cytotoxicity test, a second preliminary cytotoxicity test was performed under the same experimental conditions using the sterile test item received 11 July 2016.

Assessment of cytotoxicity was performed by evaluation of Replication Index (RI; see § Assessment of cytotoxicity).
No micronucleus analysis was undertaken on the slides of the preliminary cytotoxicity test.

2.3.2 Main cytogenetic experiment

In a single experiment using duplicate culture (*i.e.* two cultures/dose-level), each culture was tested, in presence and absence of S9 mix, with:

. six dose-levels of the test item received 11 July 2016,
. the vehicle control,
. the appropriate positive control.

The treatment durations were as follows:

Without S9 mix	3 h treatment + 24 h recovery
	24 h continuous treatment (no recovery)
With S9 mix	3 h treatment + 24 h recovery

2.4 COMPUTER SYSTEMS

The CiToxLAB France's computer systems used in the study are detailed in the following table:

Software	Version number	Application function
CITAC-CITMaster	2	CIT Application Center: Web business portal Master schedule sheet (including Study note) Master schedule sheet - Study event
Empower 2	Build 2154	Acquisition and management of chromatographic data
CIT Pharma (CITAC)	2	Test item receipt and inventories, reagent, matrix
Panorama E^2	2.60.0000	Acquisition of temperature and humidity in study rooms (study and laboratory rooms, cold chambers)
SAS	9.2	Statistical analysis (Server)

2.5 EVALUATION OF THE RESULTS

2.5.1 Acceptance criteria

The main experiment was considered valid if the following criteria were met:

. the mean frequency of cells that have undergone mitosis (Binucleated + Multinucleated Cells) in the vehicle control cultures is at least 50%,
. the mean background frequency of Micronucleated Binucleated Cells in the vehicle control cultures should be consistent with the historical vehicle control range for the Laboratory (Appendix 2),
. a statistically significant increase in the frequency of MNBC has to be obtained in the positive controls over the background frequency of the vehicle control cultures.

2.5.2 Statistical analysis

Treated cell cultures were compared to that of the vehicle control cell cultures. Unless treated culture data were lower than or equal to the vehicle control data, the statistical comparison was performed using the χ^2 test, in which p = 0.05 was used as the lowest level of significance.

To assess the dose-response trend, a linear regression was performed between the mean frequencies of Micronucleated Binucleated Cells and the dose-levels. This statistical analysis was performed using SAS Enterprise Guide software.

2.5.3 Evaluation criteria

The biological relevance of the results was considered first.

Evaluation of a positive response: a test item is considered to have clastogenic and/or aneugenic potential if, in any of the experimental conditions examined, all the following criteria are met:

. a statistically significant increase in the frequency of MNBC, in comparison to the corresponding vehicle control, is obtained at one or more dose-levels,
. a dose-response relationship (dose-related increase in the frequency of MNBC) is demonstrated by a statistically significant trend test,
. for at least one dose-level, the frequency of MNBC of each replicate culture is above the corresponding vehicle historical range.

Evaluation of a negative response: a test item is considered clearly negative if none of the criteria for a positive response are met.

2.6 DISTRIBUTION OF THE FINAL REPORT

Sponsor: one electronic copy + second original paper.
CiToxLAB France: first original paper.

2.7 ARCHIVING

The following study materials are retained in the archives of CiToxLAB France (BP 563, 27005 Evreux, France) for 3 years after the signature of the study report by the Study Director:

. study plan and amendment,
. raw data,
. slides,
. correspondence,
. final report and any amendments,
. a sample of the test item.

According to French GLP regulation (see § Regulatory compliance), the total duration of archiving must be 10 years. After the archiving period at CiToxLAB France, archiving responsibility for the remaining of the 10-year period will be transferred to the Sponsor.

The total duration of archiving (depending on regulations) is the responsibility of the Sponsor.

2.8 CHRONOLOGY OF THE STUDY

The chronology of the study is summarized as follows:

Procedure	Date
Study plan approved by:	
. Study Director	26 May 2016
. Sponsor Representative	30 May 2016
Experimental starting date (day of the first generated data)	16 June 2016
Day of treatment of the first preliminary test	23 June 2016
Day of treatment of the second preliminary test	18 August 2016
Day of treatment of the cytogenetic experiment	31 August 2016
Last day of the last incubation	01 September 2016
Experimental completion date (end of microscopic slide analysis)	30 September 2016

2.9 STUDY PLAN ADHERENCE

The study was performed in accordance with study plan No. 43957 MNH and subsequent amendment, with the following deviations from the agreed study plan:

. § 2.1.1.1: during the evaluation of the absence or presence of precipitate in the first preliminary cytotoxicity test, a turbidity of the culture medium was noted at the end of the 24-hour treatment period at the dose-levels ≥ 1000 µg/mL. After a microscopic inspection, this turbidity was attributed to a bacterial contamination. Since the test item must be exempt of microbiological contamination, data obtained in the first preliminary cytotoxicity test were not taken into account for the determination of test item cytotoxicity. Since the Sponsor has sent a new sterilized sample (received 11 July 2016) and since a second preliminary cytotoxicity test was performed with this sterilized sample, this deviation was considered not to have compromised the validity or integrity of the study,

. § 2.2.3: in order to allow the use of a treatment volume of 3% (v/v), for the treatment with metabolic activation, cells were re-suspended in 9 mL of fresh culture medium supplemented with 5.28% of S9 mix, instead of in 9.5 mL of fresh culture medium supplemented with 5% of S9 mix; typing error in the study plan. Since this step was performed in compliance with a CiToxLAB France's standard operating procedure, this deviation was considered not to have compromised the validity or integrity of the study,

. § 2.2.7.2: following the short treatment without S9 mix, a total of 1592 Binucleated Cells instead of 2000 were analyzed for the positive control. Indeed, no enough cells were available despite the spreading of a third slide of the Culture No. 2, and the slides of the Culture No. 1 were read completely thus it was impossible to complete on this culture. However, based on these 1592 Binucleated Cells analyzed, a statistically significant increase in the frequency of MNBC has been obtained in this positive control over the background frequency of the vehicle control cultures ($p < 0.001$). Since the sensibility of the test system was demonstrated, this deviation was considered not to have compromised the validity or integrity of the study.

3. RESULTS AND DISCUSSION

3.1 CHEMICAL ANALYSIS OF THE DOSE FORMULATIONS (Appendix 2)

No test item was found in the sample of the vehicle control dose formulation.

The test item concentration in the stock formulation was found satisfactory, since it remained within the acceptable range of ± 10% of the nominal concentration (bias of +4.0%).

Details of the results obtained are presented in the corresponding Appendix.

3.2 PRELIMINARY CYTOTOXICITY TESTS (Tables 1 and 2)

Using a test item concentration of 166.67 mg/mL in the vehicle (*i.e.* water for injections) and a treatment volume of 3% (v/v) in culture medium, the highest recommended dose-level of 5000 µg/mL was achievable (the test item being a UVCB). Thus, the dose-levels selected for the treatment of the preliminary cytotoxicity tests were 10, 100, 500, 1000, 2500 and 5000 µg/mL.

As the test item received on 14 April 2016 was found to be contaminated, it was decided in accordance with the Sponsor to implement a second preliminary cytotoxicity test under the same experimental conditions, but using a sterile sample of the test item received on 11 July 2016.
Data obtained in the first preliminary cytotoxicity test are presented in this report only as information (see Table 1), and are not taken into account for the determination of test item cytotoxicity.

Second preliminary cytotoxicity test
At the highest dose-level of 5000 µg/mL, the pH of the culture medium was approximately 7.4 (as for the vehicle control).

At the time of test item addition, the osmolality values in the final treatment medium were as follows:

	Dose-levels (µg/mL)						
	0	10	100	500	1000	2500	5000
Osmolality (mOsm/kg H_2O)	281	280	282	289	299	314	351

Since the highest dose-level of 5000 µg/mL resulted in an osmolality increase in the culture medium of more than 50 mOsm/kg H_2O when compared to the vehicle control, this dose-level was considered to produce extreme culture conditions.

No precipitate was observed in the culture medium at any of the tested dose-levels, at the end of the treatment periods.

No noteworthy cytotoxicity was induced at any of the tested dose-levels following either the 3-hour treatments with and without S9 mix or the 24-hour treatment without S9 mix, as shown by the absence of any noteworthy decrease in the RI (Table 2).

3.3 MAIN EXPERIMENT (Tables 3 to 8, Appendices 3 and 4)

Since the test item was found to be non-cytotoxic and freely soluble in the second preliminary test, the highest dose-level selected for the main experiment was 5000 µg/mL, according to the criteria specified in the international regulations.

The mean frequency of cells that have undergone mitosis (Binucleated + Multinucleated Cells), as well as the mean background frequency of MNBC for the vehicle control were as specified in the acceptance criteria. Also, positive control cultures showed clear statistically significant increases in the frequency of MNBC. The study was therefore considered to be valid.

With a treatment volume of 3% (v/v) in culture medium, the selected dose-levels were 312.5, 625, 1250, 2500, 3750 and 5000 µg/mL for the 3-hour treatments with and without S9 mix, as well as for the continuous 24-hour treatment without S9 mix.

At the time of test item addition, the osmolality values in the final treatment medium were as follows:

	Dose-levels (µg/mL)						
	0	312.5	625	1250	2500	3750	5000
Osmolality (mOsm/kg H_2O)	288	292	296	303	318	333	349

Since the highest dose-level of 5000 µg/mL resulted in an osmolality increase in the culture medium of more than 50 mOsm/kg H_2O when compared to the vehicle control, this dose-level was considered to produce extreme culture conditions and could not be selected for micronucleus analysis.

At the end of the treatment periods, no precipitate was observed in the culture medium at any of the tested dose-levels.

Experiments without S9 mix

Cytotoxicity

Following the 3-hour treatment followed by a 24-hour recovery period, no cytotoxicity was induced at any of the tested dose-levels, as shown by the absence of any noteworthy decrease in the RI (Table 3).

Following the 24-hour continuous treatment, a slight to moderate cytotoxicity was noted at dose-levels ≥ 2500 µg/mL, as shown by a 30 to 43% decrease in the RI (Table 5).

Micronucleus analysis

The dose-levels selected for micronucleus analysis were 1250, 2500 and 3750 µg/mL for the 3- and 24-hour treatments, the highest dose-level of 5000 µg/mL being considered to produce extreme culture conditions.

Following the 3- and 24-hour treatments without S9 mix, neither statistically significant nor dose-related increase in the frequency of MNBC was noted at any of the analyzed dose-levels in comparison to the corresponding vehicle controls (Tables 4 and 6 and Appendix 4). Moreover, none of the analyzed dose-levels showed frequency of MNBC of both replicate cultures above the corresponding vehicle control historical range (see Reference Data in Appendix 3). These results met the criteria of a negative response.

Experiment with S9 mix

Cytotoxicity

No noteworthy cytotoxicity was induced at any of the tested dose-levels, as shown by the absence of any noteworthy decrease in the RI (Table 7).

Micronucleus analysis

The dose-levels selected for micronucleus analysis were 1250, 2500 and 3750 µg/mL, the highest dose-level of 5000 µg/mL being considered to produce extreme culture conditions.

Neither statistically significant nor dose-related increase in the frequency of MNBC was noted at any of the analyzed dose-levels in comparison to the vehicle control (Table 8 and Appendix 4). Moreover, none of the analyzed dose-levels showed frequency of MNBC of both replicate cultures above the corresponding vehicle control historical range (see Reference Data in Appendix 3). These results met the criteria of a negative response.

4. CONCLUSION

Under the experimental conditions of the study, the test item He Wei C.Essence I did not induce any chromosome damage, or damage to the cell division apparatus, in cultured mammalian somatic cells, using human lymphocytes, either in the presence or absence of a rat liver metabolizing system.

5. BIBLIOGRAPHICAL REFERENCES

(a) Kirsch-Volders M. (1997) Towards a validation of the micronuclei test. Mutation Res., 392, 1-4.

(b) Parry J.M. and Sorrs A. (1993) The detection and assessment of the aneugenic potential of environmental chemicals: the European Community aneuploidy project. Mutation Res., 287, 3-15.

(c) Ames B.N., Durston W.E., Yamasaki E. and Lee F.D. (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. USA 1973, 70, 2281-2285.

(d) Kirsch-Volders M., Sofuni T., Aardema M., Albertini S., Eastmond D., Fenech M., Ishidate M. Jr, Kirchner S., Lorge E., Morita T., Norppa H., Surralles J., Vanhauwaert A. and Wakata A. (2003) Report from the *in vitro* micronucleus assay working group. Mutation Res., 540, 153-63.

(e) Miller B.M., Pujadas E. and Gocke E. (1995) Evaluation of the micronucleus test *in vitro* using chinese hamster cells: results of four chemicals weakly positive in the *in vivo* micronucleus test. Environ. Mol. Mutagen. 26, 240-247.

(f) Fenech M., Chang W.P., Kirsch-Volders M., Holland N., Bonassi S., Zeiger E. (2003) HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures, Mutation Research 534 65-75.

TABLES

Table 1. First preliminary cytotoxicity test (*for information purpose only*)

Conditions	Treatment	Number of mononucleated cells	Number of binucleated cells	Number of multinucleated cells	Total number of cells	RI	RI as % of control	Decrease in RI (%)
Without S9 mix: 3-h treatment + 24-h recovery	**Vehicle control**	32	394	74	500	1.08		
	Test item (µg/mL) [(1)]							
	10	23	399	78	500	1.11	102	none
	100	37	397	66	500	1.06	98	2
	500	23	393	84	500	1.12	104	none
	1000	32	391	77	500	1.09	101	none
	2500	23	411	66	500	1.09	100	none
	5000	16	427	57	500	1.08	100	0
Without S9 mix: 24-h continuous treatment (no recovery)	**Vehicle control**	34	371	95	500	1.12		
	Test item (µg/mL) [(1)]							
	10	26	391	83	500	1.11	99	1
	100	42	351	107	500	1.13	101	none
	500	25	387	88	500	1.13	100	none
	1000	32	367	101	500	1.14	101	none
	2500	38	415	47	500	1.02	91	9
	5000	61	408	31	500	0.94	84	16
With S9 mix: 3-h treatment + 24-h recovery	**Vehicle control**	39	354	107	500	1.14		
	Test item (µg/mL) [(1)]							
	10	29	383	88	500	1.12	98	2
	100	29	371	100	500	1.14	101	none
	500	21	364	115	500	1.19	105	none
	1000	18	389	93	500	1.15	101	none
	2500	21	361	118	500	1.19	105	none
	5000	31	307	154	492	1.25	110	none

[(1)]: expressed as active item
RI: Replication Index
Vehicle control: Water for injections

Table 2. Second preliminary cytotoxicity test

Conditions	Treatment	Number of mononucleated cells	Number of binucleated cells	Number of multinucleated cells	Total number of cells	RI	RI as % of control	Decrease in RI (%)
Without S9 mix: 3-h treatment + 24-h recovery	**Vehicle control**	23	391	86	500	1.13		
	Test item (µg/mL) [(1)]							
	10	38	365	97	500	1.12	99	1
	100	17	400	83	500	1.13	101	none
	500	41	358	101	500	1.12	99	1
	1000	19	413	68	500	1.10	98	2
	2500	25	374	101	500	1.15	102	none
	5000	30	371	99	500	1.14	101	none
Without S9 mix: 24-h continuous treatment (no recovery)	**Vehicle control**	50	345	105	500	1.11		
	Test item (µg/mL) [(1)]							
	10	44	336	120	500	1.15	104	none
	100	21	387	92	500	1.14	103	none
	500	32	322	146	500	1.23	111	none
	1000	36	367	97	500	1.12	101	none
	2500	33	412	55	500	1.04	94	6
	5000	49	400	51	500	1.00	90	10
With S9 mix: 3-h treatment + 24-h recovery	**Vehicle control**	46	366	88	500	1.08		
	Test item (µg/mL) [(1)]							
	10	29	376	95	500	1.13	104	none
	100	62	387	51	500	0.98	90	10
	500	47	384	69	500	1.04	96	4
	1000	35	388	77	500	1.08	100	0
	2500	50	375	75	500	1.05	97	3
	5000	50	373	77	500	1.05	97	3

[(1)]: expressed as active item
RI: Replication Index
Vehicle control: Water for injections

Table 3. Main experiment without S9 mix (3-h treatment + 24-h recovery), cytotoxicity

Treatment		Number of mononucleated cells	Number of binucleated cells	Number of multinucleated cells	Total number of cells	RI	RI as mean % of control	Mean Decrease in RI (%)
Vehicle control	C1	166	320	14	500	0.70		
	C2	249	248	3	500	0.51		
Test item (µg/mL) [(1)]								
312.5	C1	186	302	12	500	0.65	108	none
	C2	181	315	4	500	0.65		
625	C1	150	343	7	500	0.71	115	none
	C2	171	321	8	500	0.67		
1250	C1	172	315	13	500	0.68	110	none
	C2	183	310	7	500	0.65		
2500	C1	179	311	10	500	0.66	103	none
	C2	213	283	4	500	0.58		
3750	C1	142	353	5	500	0.73	113	none
	C2	190	302	8	500	0.64		
5000	C1	195	297	8	500	0.63	103	none
	C2	198	299	3	500	0.61		
Positive controls								
COL (0.1 µg/mL)	C1	330	158	12	500	0.36	63	37
	C2	312	177	11	500	0.40		

[(1)]: expressed as active item
RI: Replication Index
Vehicle control: Water for injections
COL: Colchicine
C1: Culture 1
C2: Culture 2

Table 4. Main experiment without S9 mix (3-h treatment + 24-h recovery), cytogenetic results

Treatment	RI as mean % of control	Culture	Number of binucleated cells analyzed	Number of binucleated cells with n micronuclei					Total micronucleated binucleated cells		Frequency of micronucleated binucleated cells (‰)	
				n = 1	n = 2	n = 3	n = 4	n = 5	per culture	per dose		
Vehicle control		**C1**	**1000**	**1**	**0**	**0**	**0**	**0**	**1**	**2**	**1.0**	
		C2	**1000**	**1**	**0**	**0**	**0**	**0**	**1**			
Test item (µg/mL) [(1)]												
1250	**110**	**C1**	**1000**	**3**	**1**	**0**	**0**	**0**	**4**	**7**	**3.5**	
		C2	**1000**	**3**	**0**	**0**	**0**	**0**	**3**			
2500	**103**	**C1**	**1000**	**2**	**0**	**0**	**0**	**0**	**2**	**4**	**2.0**	
		C2	**1000**	**1**	**1**	**0**	**0**	**0**	**2**			
3750	**113**	**C1**	**1000**	**2**	**0**	**0**	**0**	**0**	**2**	**6**	**3.0**	
		C2	**1000**	**3**	**1**	**0**	**0**	**0**	**4**			
Positive controls												
COL (0.1 µg/mL)	**63**	**C1**	**1000**	**8**	**0**	**0**	**0**	**0**	**8**	**16**	**10.1**	*******
		C2	**592**	**8**	**0**	**0**	**0**	**0**	**8**			

[(1)]: expressed as active item
RI: Replication Index
Vehicle control: Water for injections
COL: Colchicine
C1: Culture 1
C2: Culture 2

Statistics: 2 x 2 contingency table:
***: $p < 0.001$

Table 5. Main experiment without S9 mix (24-h treatment; no recovery), cytotoxicity

Treatment		Number of mononucleated cells	Number of binucleated cells	Number of multinucleated cells	Total number of cells	RI	RI as mean % of control	Mean Decrease in RI (%)
Vehicle control	C1	198	291	11	500	0.63		
	C2	174	319	7	500	0.67		
Test item (µg/mL) [(1)]								
312.5	C1	183	314	3	500	0.64	105	none
	C2	155	329	16	500	0.72		
625	C1	215	274	11	500	0.59	98	2
	C2	165	332	3	500	0.68		
1250	C1	274	220	6	500	0.46	83	17
	C2	197	301	2	500	0.61		
2500	C1	308	190	2	500	0.39	70	30
	C2	258	226	16	500	0.52		
3750	C1	346	151	3	500	0.31	57	43
	C2	291	205	4	500	0.43		
5000	C1	340	157	3	500	0.33	57	43
	C2	305	186	9	500	0.41		
Positive controls								
MMC (0.1 µg/mL)	C1	316	178	6	500	0.38	64	36
	C2	282	212	6	500	0.45		

[(1)]: expressed as active item
RI: Replication Index
Vehicle control: Water for injections
MMC: Mitomycin C
C1: Culture 1
C2: Culture 2

Table 6. Main experiment without S9 mix (24-h treatment; no recovery), cytogenetic results

Treatment	RI as mean % of control	Culture	Number of binucleated cells analyzed	Number of binucleated cells with n micronuclei					Total micronucleated binucleated cells		Frequency of micronucleated binucleated cells (‰)	
				n = 1	n = 2	n = 3	n = 4	n = 5	per culture	per dose		
Vehicle control		C1	1000	2	0	0	0	0	2	5	2.5	
		C2	1000	2	0	1	0	0	3			
Test item (µg/mL) [(1)]												
1250	83	C1	1000	3	0	0	0	0	3	5	2.5	
		C2	1000	2	0	0	0	0	2			
2500	70	C1	1000	2	1	1	0	0	4	5	2.5	
		C2	1000	1	0	0	0	0	1			
3750	57	C1	1000	1	0	0	0	0	1	5	2.5	
		C2	1000	4	0	0	0	0	4			
Positive controls												
MMC (0.1 µg/mL)	64	C1	1000	9	0	0	0	0	9	29	14.5	***
		C2	1000	20	0	0	0	0	20			

[(1)]: expressed as active item
RI: Replication Index
Vehicle control: Water for injections
MMC: Mitomycin C
C1: Culture 1
C2: Culture 2

Statistics: 2 x 2 contingency table:
***: $p < 0.001$

Table 7. Main experiment with S9 mix (3-h treatment + 24-h recovery), cytotoxicity

Treatment		Number of mononucleated cells	Number of binucleated cells	Number of multinucleated cells	Total number of cells	RI	RI as mean % of control	Mean Decrease in RI (%)
Vehicle control	C1	57	418	25	500	0.94		
	C2	127	344	29	500	0.80		
Test item (µg/mL) [(1)]								
312.5	C1	125	339	36	500	0.82	93	7
	C2	110	378	12	500	0.80		
625	C1	96	378	26	500	0.86	98	2
	C2	100	377	23	500	0.85		
1250	C1	92	383	25	500	0.87	99	1
	C2	87	397	16	500	0.86		
2500	C1	49	431	20	500	0.94	99	1
	C2	127	358	15	500	0.78		
3750	C1	106	382	12	500	0.81	94	6
	C2	114	361	25	500	0.82		
5000	C1	89	403	8	500	0.84	97	3
	C2	98	381	21	500	0.85		
Positive controls								
CPA (6 µg/mL)	C1	353	142	5	500	0.30	36	64
	C2	344	154	2	500	0.32		

[(1)]: expressed as active item
RI: Replication Index
Vehicle control: Water for injections
CPA: cyclophosphamide
C1: Culture 1
C2: Culture 2

Table 8. Main experiment with S9 mix (3-h treatment + 24-h recovery), cytogenetic results

Treatment	RI as mean % of control	Culture	Number of binucleated cells analyzed	Number of binucleated cells with n micronuclei					Total micronucleated binucleated cells		Frequency of micronucleated binucleated cells (‰)	
				n = 1	n = 2	n = 3	n = 4	n = 5	per culture	per dose		
Vehicle control		C1	1000	1	0	0	0	0	1	4	2.0	
		C2	1000	3	0	0	0	0	3			
Test item (µg/mL) [(1)]												
1250	99	C1	1000	1	0	0	0	0	1	3	1.5	
		C2	1000	2	0	0	0	0	2			
2500	99	C1	1000	0	0	0	0	0	0	0	0.0	
		C2	1000	0	0	0	0	0	0			
3750	94	C1	1000	3	0	0	0	0	3	4	2.0	
		C2	1000	0	1	0	0	0	1			
Positive controls												
CPA (6 µg/mL)	36	C1	1000	16	0	1	0	0	17	34	17.0	***
		C2	1000	16	1	0	0	0	17			

[(1)]: expressed as active item
RI: Replication Index
Vehicle control: Water for injections
CPA: cyclophosphamide
C1: Culture 1
C2: Culture 2

Statistics: 2 x 2 contingency table:
***: $p < 0.001$

APPENDICES

1. Test item analysis certificate

广东肇庆星湖生物科技股份有限公司
Star Lake Bioscience Co.,Inc.Zhaoqing Guangdong

成品检验报告书
Certificate of Analysis

产品名称/Product Name	: HE WEI C.ESSENCE I
批号/Batch No.	: G160304
批量/Batch Quantity	: 1445.3 kg
生产日期/Date of Manufacture	: Mar 3, 2016
报告日期/Date of Report	: Mar 9, 2016
有效期/Date of Expiry	: Dec 2, 2016

COA No. 540-00-CKB-16-029

项目/Test	标准/Specification	结果/Result
外观/Appearance as is	浅棕色至棕色均匀膏体 Uniform pale brown to brownish paste	棕色均匀膏体 Brownish paste
气味/Odor as is	具天然调味品 1 特征性气味，无异味 Characteristic of Natural Savory Flavor 1, free from foreign and/or off odors	符合规定 Conforms
溶液外观/Appearance after preparation	浅棕色至正棕色澄清液体，无肉眼可见颗粒物 Clear, light brown to moderate brown solution and free from foreign particles	符合规定 Conforms
溶液气味/Odor after preparation	具天然调味品 1 特征性气味，无异味 Characteristic of Natural Savory Flavor 1, free from foreign and/or off odors	符合规定 Conforms
溶液味道/Taste after preparation	具黄金风味特征性鲜味，味微咸，无苦味或焦味，无异味 Characteristic of Natural Savory Flavor 1, umami with slight saltiness. No bitter or burnt note. Free from foreign and/or off flavors	符合规定 Conforms
L-谷氨酸/Glutamic acid, L	34~44 %	438 %
干燥失重/Loss on drying	27~34 %	32 %
水活度/Water activity	≤0.75	0.68
氯化钠/Sodium chloride	5.5~8.0 %	6.5 %
pH(10% dry matter solution)	5.5~7.0	5.5
其他氨基酸/Other amino acids	1.0~3.0 %	1.8 %
总氮/Total nitrogen	4.0~7.0 %	6.2 %
灰分/Ash	10~18 %	12 %
重金属/Heavy metals(as Pb)	≤10 mg/kg	<10 mg/kg
砷/Arsenic	≤0.5 mg/kg	<0.5 mg/kg
细菌总数/Aerobic plate count	≤10000 cfu/g	<10 cfu/g
酵母菌及霉菌/Yeasts and Molds	≤100 cfu/g	<10 cfu/g
肠道杆菌/Enterobacteriaceae	≤10 cfu/g	<10 cfu/g
沙门氏菌/Salmonella	Negative/25 g	Negative/25 g

结论/Conclusion: Passes all specifications.

QC 主管/QC Manager [redacted]

Lianhua Rd., Dinghu District, Zhaoqing City, Guangdong P.R.China 526070
Website: www.starlake.com.cn

Version SLK-QC-1-21.01-5

Nestlé R&D Center (Pte) Ltd
UEN 197903876E
TELEPHONE (65) 6890 0100
TELEFAX (65) 6265 5633

Nestlé Research
Research & Development Centre
Singapore

CIT SAS- Pharmacy department
CiToxLAB
RN13 / Route de Pacy
27930 Miserey
FRANCE
Tel: +33 2 32 29 26 56 / 97

Date: 14/07/2016

CERTIFICATE OF ANALYSIS

Product: He Wei C.Essence I paste

This product was sterilized through heat treatment
1) 300g of paste was weighed into glass bottle
2) Autoclaved at 121°C for 15 minutes

This is to certify that the above shipment of sterilized sample had been analyzed and the results are as follows:

Batch:	G160304
Manufacturing date:	Mar 3, 2016
Expiry date:	Dec 2, 2016
Colour:	Brown
Apperance:	Paste
*Aerobic Mesophilic Microorganisms (cfu/ g):	<10
**Total solid: (g/100g)	68.58

*Refer result at Nestlé NesTMS AR: 167307.0004
**Refer result at Nestlé NesTMS AR: 167313.0002

Product is not suitable for food allergics.

Yours faithfully,
NESTLÉ R&D CENTER (PTE) LTD SINGAPORE

Suresh Damodaran
Group manager, RDSG-QM

Validated Analysis Results

Origin	Operator	Analysis request	Analysis type	Parameter
Sample: 167307.0003 / Trial: 18293.034 - Natural Savory Flavor I & II paste mixing	Renukambal Narayanan	167307 - Micro- HeWei C.Essence I (autoclave)	201 - AEROBIC MESOPHILIC MICROORGANISMS	201.001 - AMC < 10.00 CFU/g
Sample: 167307.0004 / Trial: 18293.034 - Natural Savory Flavor I & II paste mixing	Renukambal Narayanan	167307 - Micro- HeWei C.Essence I (autoclave)	201 - AEROBIC MESOPHILIC MICROORGANISMS	201.001 - AMC < 10.00 CFU/g

2. Determination of He Wei C.Essence I in the dose formulations

2.1 Chemical analysis of the dose formulations

Chemical analysis of the dose formulations

1. PRINCIPLE OF THE ANALYTICAL METHOD

The analytical method was developed at CiToxLAB France. It consisted of sampling 0.2 mL of dose formulation and diluting it appropriately with diluents to reach the nominal concentration of injection (0.1 mg/mL). The diluted samples were analyzed by Ultra High Performance Liquid Chromatography with Ultra-Violet detection (UHPLC/UV), bracketed by standard solutions and quantified by the mean response factors calculated for the standard solutions.

The validation data demonstrating the suitability of the method for analysis of the dose formulations (from 0.8 to 200 mg/mL in water for injection) are presented in the *CiToxLAB France/Study No. 43956 VAA*.
Complete details of the analysis process are described in the analytical procedure which is presented in Appendix 2.2.

2. SOLVENTS, REAGENTS AND LABORATORY EQUIPMENT
2.1SOLVENT AND REAGENT

Solvents and reagents	Reference	Suppliers
Water for injection	Not applicable	CDM Lavoisier
Methanol	525102	Carlo Erba
Acetonitrile	412392000	Carlo Erba
Potassium phosphate monobasic (KH_2PO_4)	P5379-500 g	Sigma-Aldrich
Orthophosphoric acid	79606-100 mL	Fluka / Sigma Aldrich

Diluent 1: Water for injection.
Diluent 2: Methanol.
Diluent 3: a solution of water for injection / methanol (10v/90v).

2.2 EQUIPMENT

Equipments	Suppliers
Ultra Performance Liquid Chromatography (UPLC) Systems: . Acquity UPLC	Waters
Micro-balance; Balance	Mettler-Toledo
pH meter model Seven Compact	Mettler-Toledo
Automatic pipettes	Biohit
PT 2500 E	Polytron

. equipments for agitating which could be used (magnetic stirrers),
. class A volumetric flasks,
. filters Millipore 0.22 µm GVWP.

2.3 SOFTWARE
Empower 2 (Waters).

2.4 ANALYTICAL STANDARD
The analytical standard used to prepare the standard solutions was the test item described in the study plan (sample received 11 July 2016).

3. SAMPLES PREPARATION
Dose formulations were sampled and diluted as follows:

Dose formulations concentration (mg/mL)	1st Dilution		2nd Dilution		3nd Dilution		Theoretical injection concentration (mg/mL)
	Volume v1 of dose formulation taken (mL)	Total volume V1 of diluent 1 (mL)	Volume of solution taken v2 (mL)	Total volume V2 of diluent 1 (mL)	Volume v3 of solution taken (mL)	Total volume V3 of diluent 2 (mL)	
0	0.2	-	-	-	-	2 (volumetric flask)	0
166.67	0.2	5 (volumetric flask)	0.75	5 (volumetric flask)	1	10 (volumetric flask)	0.1

Note: for the determination of nominal concentrations, the dilution volumes used were calculated by measuring the weight of each sampling and assuming the density of the dose formulation sampled (d = 1.00 to 1.03).

Dilution factor = (V2 x V1 x V3) / (v1 x v2 x v3)

With:
V1 = total volume for the first dilution
V2 = total volume for the second dilution
V3 = total volume for the third dilution
v1 = volume taken for each sample (with v1 = weight of sample / density of the formulation)
v2 = volume taken for the second dilution
v3 = volume taken for the third dilution

4. ANALYTICAL SEQUENCES RULES
The samples were assayed according to the following principles.

4.1 COMPOSITION OF AN ANALYTICAL SEQUENCE
Analytical sequences are composed of at least:
. blank samples (diluent 1, 2 and 3) were checked for the absence of chromatographic interferences,
. vehicle sample (when requested),
. a LOQ solution,
. at least 10 standard samples at nominal concentration prepared from two independent solutions (standard 1 (STD 1) and standard 2 (STD 2)),
. study samples prepared from the aliquots of the dose formulations bracketed by the standard samples.

4.2 QUANTIFICATION

Peak areas were determined for standard solutions prepared at 0.1 mg/mL of He Wei C.Essence I (STD 1 and STD 2). The response factor of each standard solution and the mean response factor for all the standard solutions were calculated.

Samples were quantified using the mean response factor obtained from the standard samples according to the following equation:

$$[\text{Concentration}_{\text{sample test item}}] = (\text{Area}_{\text{sample test item}} / \text{Standard mean response factors}) \times \text{dilution factor}$$

Where:
$\text{Area}_{\text{sample test item}}$ = peak area of He Wei C.Essence I in the sample
Standard mean response factors = Mean response factor of standard solutions 1 and 2 (n = 10)
Dilution factor (also including conversion between units if required)
Response factor = $\text{Area}_{\text{standard solution}} / \text{Concentration}_{\text{standard solution}}$

4.3 ACCEPTANCE CRITERIA OF AN ANALYTICAL SEQUENCE

Acceptance of the analytical sequence depends on the precision of the standard samples and on the agreement of the standard sample results. Acceptance criteria are defined in CiToxLAB France Standard Operating Procedures (SOPs).

Criteria for the acceptance of an analytical sequence are:

- precision of response factor for standard solution STD 1: % RSD ≤ 3.0%,
- precision of response factor for standard solution STD 2: % RSD ≤ 3.0%,
- precision of response factor for standard solutions STD 1 and STD 2: % RSD ≤ 3.0%,
- accuracy of the response factor of the standards (ratio of mean response factor of standard solution 1 with mean response factor of standard solution 2): 95.0%-105.0%.

5. DOSE FORMULATION ANALYSIS

5.1 ASSAY

Diluted samples of dose formulations were analyzed in single by Ultra High Performance Liquid Chromatography with Ultra-Violet detection.

The test item peak area was determined for each sample and the corresponding concentration was calculated using the equation obtained from the calibration data.
All the results are expressed as mg/mL of He Wei C.Essence I.

5.2 ACCEPTANCE CRITERION

Deviation calculated between measured concentration and theoretical concentration should be within ± 10%.

$$\%\ \text{DEV} = \frac{\text{Cm} - \text{Ct}}{\text{Ct}} \times 100$$

With: Cm: measured concentration
Ct: theoretical concentration

6. SUMMARY OF ANALYTICAL SEQUENCES

Date	Analytical sequence	Valid (Yes/No)	Comments	System suitability results		Raw data register
				Criterion description	Results (%)	
31 August 2016	Determination of content in administered dose formulations (first experiment)	Yes	No comment	STD 1 response factor % RSD (n = 5)	2.1	2
				STD 2 response factor % RSD (n = 5)	1.0	
				STD 1 & STD 2 response factor % RSD (n = 10)	2.2	
				Standard agreement	96.9	

RSD: standard deviation.
%: percentage.
STD: standard solution.

7. RESULTS

The results are presented in Table 1.

Table 1: Concentration of He Wei C.Essence I in administered dose formulations

Nominal concentrations (mg/mL)	Measured concentrations	
	First Experiment - Assay 1	
	(mg/mL)	Bias (%)
0	nd	nc
166.67	173	4.0

%: Percentage.
nc: not calculated.
nd: no peak detected.

2.2 Analytical method

CiToxLAB	Pharmaceutical analysis Laboratory
METHOD FOR THE ANALYSIS OF HeWei C.Essence I BY HPLC-UV IN WATER FOR INJECTION (from 0.8 to 200 mg/mL)	
Version : 43956VAA.MET.V02	

MODIFICATIONS FOLLOW-UP:

DATE	VERSION	REASONS
18/08/2016	01	Creation
30/08/2016	02	§6 : Add information for correction factor from the date of receipt

Written by : ROVEYAZ L.	Checked and approved by : J. Decorde
Visa : [redacted]	Visa : [redacted]
Date : 30/08/2016	Date : 30 August 2016

1 GENERAL RULES

. Each mixture prepared should be agitated before use (manually or magnetically).
. Each analytical sample prepared (diluted dosage formulations, standard solutions...) should be agitated before injection (vortex or manually).
. Diluent or mobile phases should eventually be sonicated when necessary.

Note: Any other specific requirements for the preparation should be clearly indicated in the present method and documented in the raw data.

2 SOLVENTS AND REAGENTS

Solvents and Reagents	Reference	Suppliers
milli-Q water	Not applicable	CiToxLAB
acetonitrile for HPLC	412392000	Carlo Erba
Methanol for HPLC	525102	Carlo Erba
potassium phosphate monobasic (KH_2PO_4)	P5379-500G	Sigma-Aldrich
water for injection	/	C.D.M. Lavoisier
orthophosphoric acid	79606-100ML	Fluka

Note : Equivalent solvents and reagents may be used with clear documentation.

3 EQUIPMENT / APPLIANCES

Equipments	Suppliers
Ultra Performance Liquid Chromatography (UPLC) Systems:	
. Acquity UPLC	Waters
Micro-balance; Balance	Mettler-Toledo
pH meter model Seven Compact	Mettler-Toledo
Automatic pipettes	Biohit
PT 2500 E	Polytron
Ultrasonic bath model Elmasonic S300	Grosseron

Others:
- Equipments for agitating which could be used (magnetic stirrers)
- Class A volumetric flasks
- Filters Millipore 0.22µm GVWP

Note : Equivalent equipment can be used (but clear documentation should be provided).

4 SOFTWARE

Empower 2 (Waters)

5 VEHICLE AND CONCENTRATION RANGE

Vehicle : water for injection
Range of concentrations tested: from 0.8 mg/mL to 200 mg/mL.

6 REFERENCE SUBSTANCE

Name : HeWei C.Essence I
Batch No. : G160304
Purity : Not applicable
Correction factor : 1.471
Expiry : 02 dec. 2016
The sample received : 14 April 2016

Name : HeWei C.Essence I
Batch No. : G160304
Purity : Not applicable
Correction factor : 1.458
Expiry : 02 dec. 2016
The sample received : 11 July 2016

Note: If possible, the batch of the reference substance will be the same batch of the active substance used in test dosage form. When they are different, a particular attention will be pay on the purity of the two batches.

7 PREPARATION OF DILUENT AND MOBILE PHASE

7.1Diluent

7.1.1 Diluent 1

Use water for injection as diluent 1

Stability period/storage conditions: *7 days* at room temperature

7.1.2 Diluent 2

Use methanol as diluent 2

Stability period/storage conditions: *3 months* at room temperature

7.1.3 Diluent 3

Prepare a solution of water for injection / methanol (10v/90v)
100 mL of water for injection and 900 mL of methanol are mixed together.

Stability period/storage conditions: *3 months* at room temperature

7.2Mobile phase

Mobile phase A: 20 mM phosphate buffer at pH2.85
Prepare a solution of 20 mM phosphate buffer at pH2.85
About 2.72g (± 0.05g) of potassium dihydrogen phosphate are mixed with approximately 900 mL of milli-Q water in a 1L volumetric flask. The pH is adjusted to 2.85 ± 0.1 with orthophosphoric acid. The volumetric flask is then completed to final volume with milli-Q water and filtrated with 0.22 µm GVWP filters.

Mobile phase B: ACN
Use acetonitrile as mobile phase B

Stability period/storage conditions: phase A: 7 days at room temperature
phase B: 3 months at room temperature

Note: Based on these ratios, the total volumes prepared could be adapted regarding the volumes required for sample analysis.

8 PREPARATION OF STANDARD SOLUTIONS

THE TEST ITEM WILL BE HOMOGENIZED BY VIGOUROUS MIXING USING AN ULTRA TURRAX® OR POLYTRON® BEFORE ANY SAMPLING.

8.1Stock standard solutions (SM1 and SM2) of HeWei C.Essence I at 1 mg/mL

Stock standard solutions (SM1 and SM2) are prepared by:
. precisely weighing about 25 mg **(x correction factor)** of HeWei C.Essence I into a 25 mL volumetric flask,
. add 2.5 mL (P5000) of diluent1, shake until complete dissolution
.making up to final volume with diluent 2.

Stability period/storage conditions: use on the day of preparation at room temperature

8.2Standard solutions (STD1 et STD2) of HeWei C.Essence I at 0.1 mg/mL in diluent 3

Standard solutions (STD1 and STD2) are prepared by:
. diluting 2.5 mL of stock standard solutions (SM1 or SM2) into a 25-mL volumetric flask,
. making up to the final volume with diluent 3.

Stability period/storage conditions: 6 days, +5°C, protect from the light

8.3Preparation of LOQ solution at 10 µg/mL in diluent 3 to check control group

LOQ solution is prepared by:
. diluting 0.5 mL of STD1 taken with a 1-mL pipette into a 5-mL volumetric flask,
. making up to the final volume with diluent 3.

Stability period/storage conditions: use on the day of preparation at room temperature

9 PREPARATION OF WORKING SOLUTIONS

THE TEST ITEM WILL BE HOMOGENIZED BY VIGOUROUS MIXING USING AN ULTRA TURRAX® OR POLYTRON® BEFORE ANY SAMPLING.

The working solutions are prepared following the table below:

Water for injection :

Dose formulations concentration (mg/mL)	1st Dilution		2nd Dilution		3nd Dilution		Theoretical injection concentration (mg/mL)
	Volume of dose formulation taken (mL)	Total volume of diluent 1 (mL)	Volume of solution taken (mL)	Total volume of diluent 1 (mL)	Volume of solution taken (mL)	Total volume of diluent 2 (mL)	
0	0.2 (P1000)	-	-	-	-	2 (volumetric flask)	0
1	0.2 (P1000)	-	-	-	-	2 (volumetric flask)	0.1
166.67	0.2 (P1000)	5 (volumetric flask)	0.75 (P1000)	5 (volumetric flask)	1 (P1000)	10 (volumetric flask)	0.1

Stability period/storage conditions: use on the day of preparation, protect from the light

10 CHROMATOGRAPHIC CONDITIONS

Column : Kinetex HILIC 100A (Phenomenex), particle size = 2.6 µm
length = 150 mm, inner diameter = 2.1 mm

Mobile phase : Mobile phase A: *20 mM phosphate buffer at pH2.85*
Mobile phase B: acetonitrile

Elution mode : Isocratic

Time (min)	A (%)	B (%)
0	18	82
3	18	82

Flow rate : 0.7 mL/min

Software : Empower 2 (Waters)

Column temperature : +40°C

Injector temperature : +5°C

Injected volume	: 10 µL
Needle wash	: ACN / milli-Q water (80v/20v)
Column wash	: ACN / milli-Q water (80v/20v)
Time of column wash	: at least 30 minutes
Wavelength	: 200 nm
Retention time	: HeWei C.Essence I: 1.6 min
Analysis time	: 3 min

11 QUANTIFICATION

The concentration of the test item is determined from the mean response factor of HeWei C.Essence I in standard solutions.

The sample concentrations are determined using the following equation:

$$[\text{Concentration}_{\text{dosage form}}] = (\text{Area}_{\text{sample}} / \text{Standard mean response factors}) \times \text{dilution factor}$$

Where:

$\text{Area}_{\text{sample}}$ = Area of sample

Standard mean response factors = Mean response factor of standard solutions 1 and 2 (n=10)

Dilution factor (also including conversion between units if required)

Response factor = Area_{Std} / $\text{Concentration}_{\text{Std}}$

12 RESULTS

Results are expressed in: mg/mL

13 ACCEPTANCE CRITERIA

Criteria applied for the acceptance of the analytical sequences are defined in CiToxLAB France Standard Operating Procedures (SOPs) as:

Criteria for the acceptance of the analytical sequence are:

- precision of response factor for standard solution STD1: Coefficient of Variation CV% ≤ 3.0 %,
- precision of response factor for standard solution STD2: Coefficient of Variation CV% ≤ 3.0 %,
- precision of response factor for standard solutions STD1 and STD2: Coefficient of Variation CV% ≤ 3.0 %,
- accuracy of the response factor of the standards (ratio of mean response factor of standard solution 1 with mean response factor of standard solution 2) should be between 95.0% and 105.0%

14 TYPICAL CHROMATOGRAMS

Figure 1. Chromatogram of diluent 1

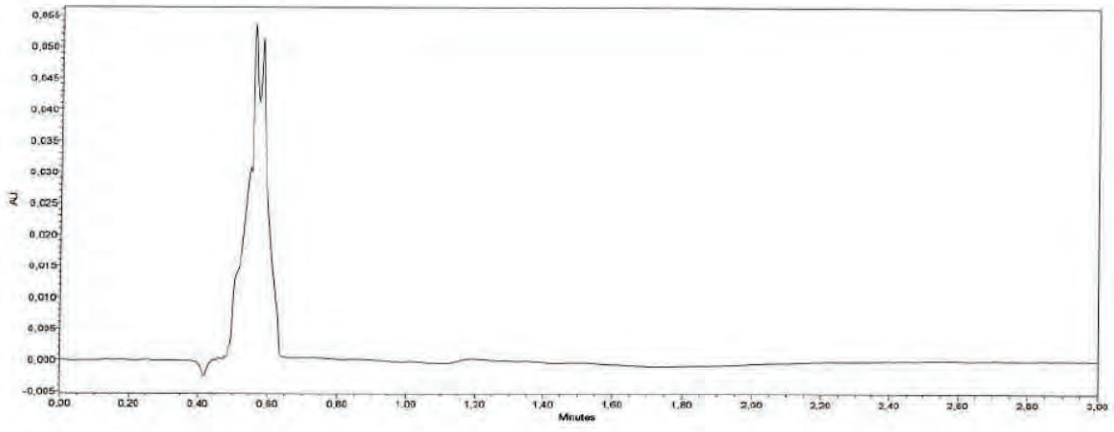


Figure 2. Chromatogram of diluent 2

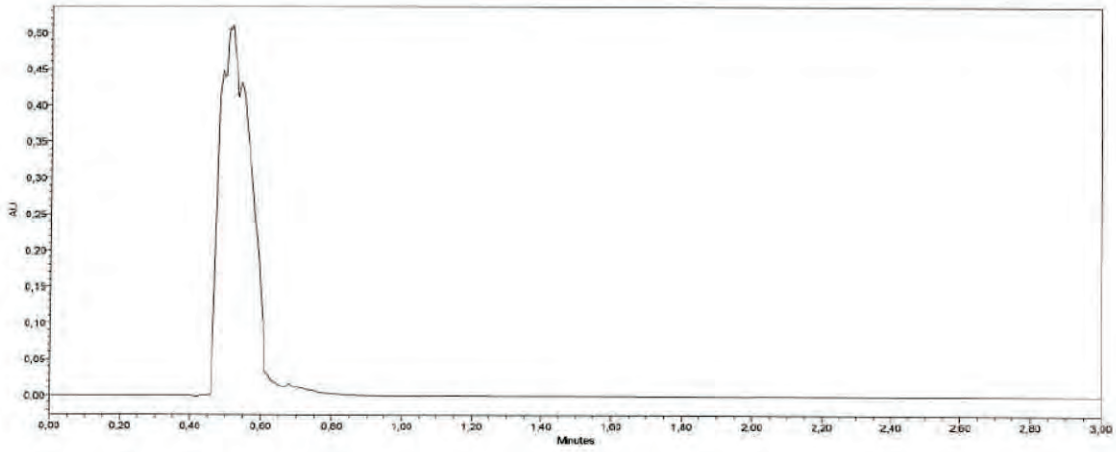


Figure 3. Chromatogram of diluent 3

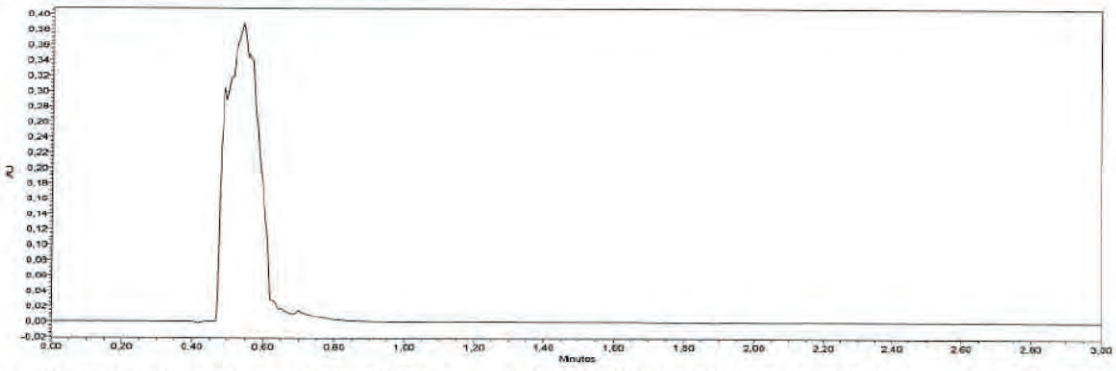


Figure 4. Chromatogram of standard solution

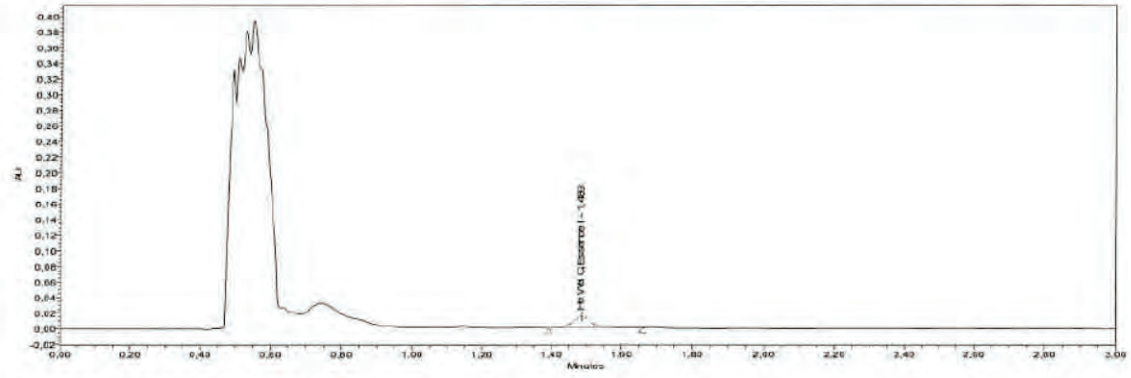


Figure 5. Chromatogram of vehicle

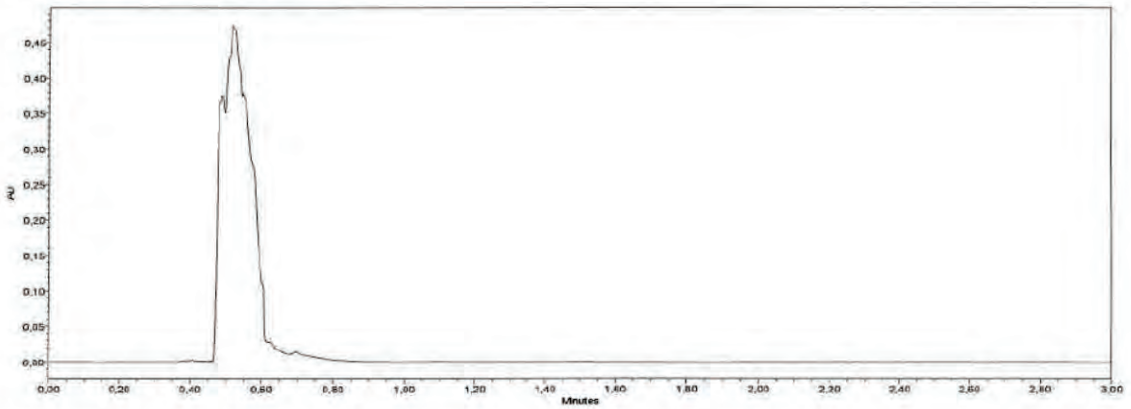
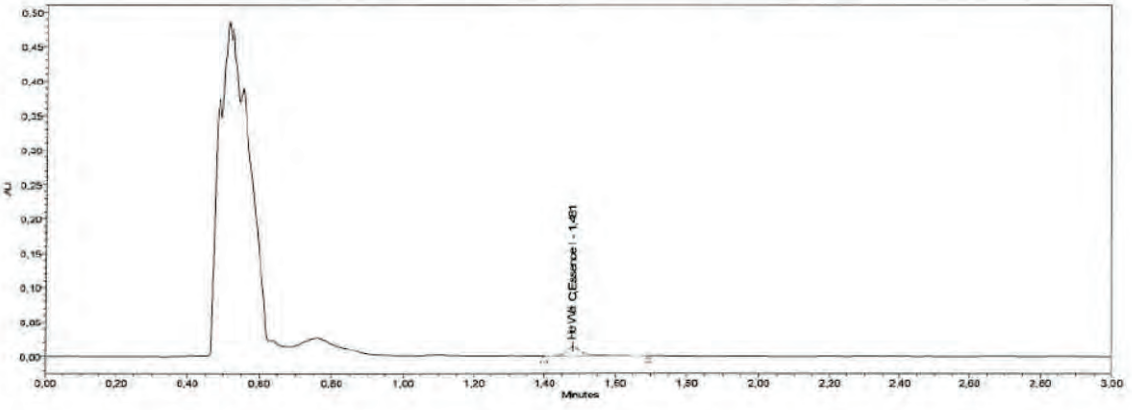


Figure 6. Chromatogram of the low concentrated formulation solution (at 1 mg/mL)



15 SYNOPSIS

Test	Acceptance criteria	Results	Conclusion
Linearity Hewei C.Essence I in diluent 3 from 0.01 To 0.15 mg/mL	$r^2 > 0.99$. residuals % between ± 7% . intercept ≤ 3% of the response at the nominal concentration	$r^2 = 0.9996$ residuals % between -1.0 % and 1.3 % intercept = 0 %	PASS
Carry over Diluent injected after a standard solution	Carry over at the compound retention time ≤ 1% of the standard solution response	No carry over	PASS
Injection repeatability 6 successive injections of a standard solution prepared at the nominal concentration of injection (c = 0.1 mg/mL)	CV ≤ 3%	CV = 1.3 %	PASS
Sensitivity Injection of a solution at 1 µg/mL (1% of a standard solution.) and Injection of a solution at 10 µg/mL (10% of a standard solution.) compared to solvent.	S/N ≥ 10	1 µg/mL S/N = 1.78 10 µg/mL S/N = 16.5	FAIL PASS
Standard solution stability Duplicate standard solutions injected on the day of preparation and after 6 days at +5°C, protected from light	Mean concentration measured for each storage period within 97-103% of the initial concentration	Standard solution (after 6 days) = 101.9%	PASS

CiToxLAB	Pharmaceutical analysis Laboratory	Reference : 43956VAA.MET.V02

Vehicle 1: Water for injection

Test	Acceptance criteria	Results	Conclusion
Specificity . diluted vehicle (10-fold) . diluent 3 . sample solutions diluted from 0.8 and 200 mg/mL reconstituted formulations.	No significant interfering peak in diluent and in vehicle at the compound retention time. No significant peak around the compound retention time	No Interfering peak in diluent and in vehicle No interfering peak around the compound retention time	PASS
Accuracy and Precision Six reconstituted dose formulations prepared at 0.8 and 200 mg/mL, then diluted respectively to 80% and 120% of the nominal concentration of injection (c = 0.1 mg/mL).	Mean recovery: between 95-105% CV ≤ 5%	**0.8 mg/mL:** Mean recovery (%) = 100.6% CV (%) = 2.2 % **200 mg/mL:** Mean recovery (%) = 99.4 % CV (%) = 1.7 %	PASS
Diluted dose formulation stability Sample solutions diluted from reconstituted formulations at 0.8 and 200 mg/mL injected on the day of preparation and after 6 days at +5°C, protected from light	Mean concentration measured for each storage period within 97-103% of the initial concentration	Sample at 0.8 mg/mL (after 6 days) = 105% Sample at 200 mg/mL (after 6days) = 100.5%	FAIL

3. Reference data

IN VITRO MICRONUCLEUS TEST IN CULTURED HUMAN LYMPHOCYTES

Reference Data
(audited Reference data)

Experiments without S9 mix

Parameter	Frequency of micronucleated binucleated cells in 1000 binucleated cells			
Treatment conditions	3 hours treatment + 24 hours recovery		24 hours continuous treatment (no recovery)	
Control items	Vehicle control	COL (0.1 µg/mL)	Vehicle control	MMC (0.1 µg/mL)
n	5	5	5	5
Mean	4.1	31.1	4.3	31.1
SD	2.2	15.4	1.6	10.4
Lower CL 95%	1.4	12.0	2.4	18.2
Upper CL 95%	6.8	50.3	6.2	44.0
5th Percentile	1.5	15.0	3.0	14.5
Median	3.5	28.0	3.5	33.0
95th Percentile	7.0	56.8	6.0	41.0
Min	1.5	15.0	3.0	14.5
Max	7.0	56.8	6.0	41.0

Experiments with S9 mix

Parameter	Frequency of micronucleated binucleated cells in 1000 binucleated cells	
Treatment conditions	3 hours treatment + 24 hours recovery	
Control items	Vehicle control	CPA (6 µg/mL)
n	5	4
Mean	3.1	22.1
SD	2.1	4.1
Lower CL 95%	0.5	15.6
Upper CL 95%	5.7	28.7
5th Percentile	0.0	18.5
Median	3.0	21.0
95th Percentile	5.0	28.0
Min	0.0	18.5
Max	5.0	28.0

COL: Colchicine
MMC: Mitomycin C
CL: Confidence limit
Max: Maximal value

CPA: Cyclophosphamide
SD: Standard deviation
Min: Minimal value
n: number of values

4. Statistical results

43957 MNH - Linear Regression Results

The REG Procedure
Model: Linear_Regression_Model
Dependent Variable: Frequency

Experiment_Name=1-Short treatment without S9 mix (3-h treatment + 24-h recovery)

Number of Observations Read	4
Number of Observations Used	4

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	1.01250	1.01250	0.76	0.4760
Error	2	2.67500	1.33750		
Corrected Total	3	3.68750			

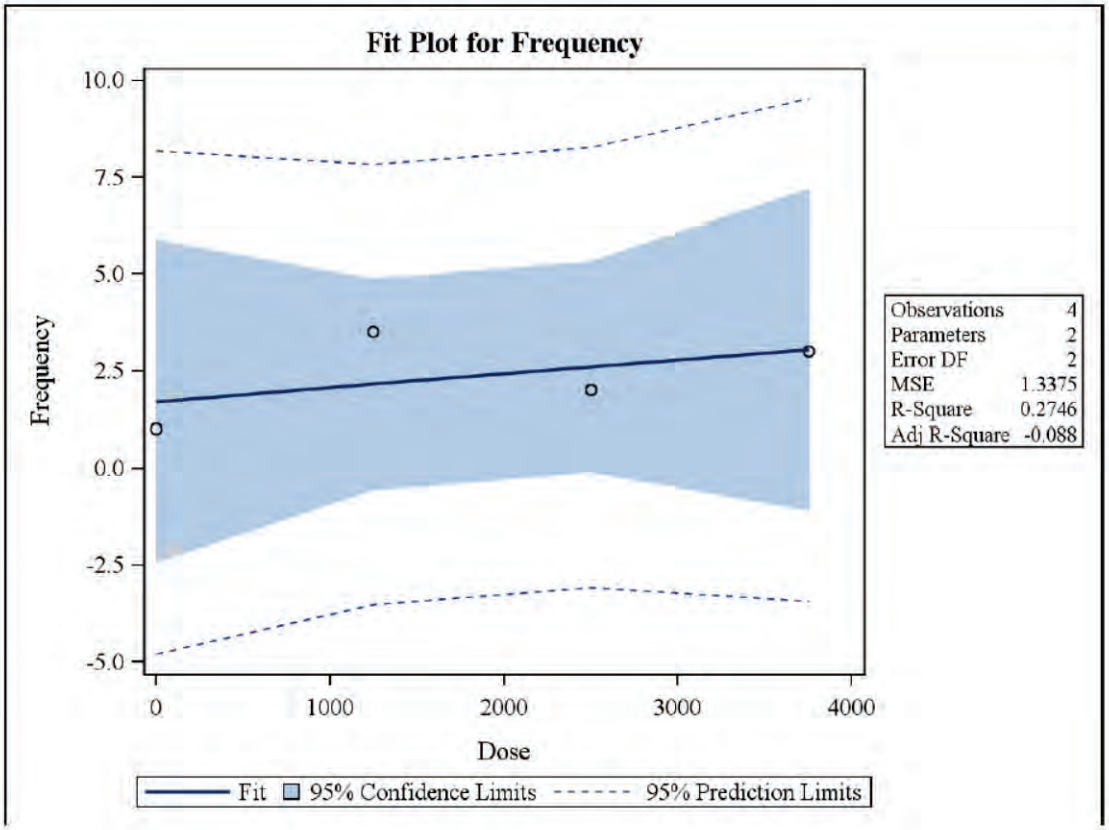
Root MSE	1.15650	**R-Square**	0.2746
Dependent Mean	2.37500	**Adj R-Sq**	-0.0881
Coeff Var	48.69488		

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	1	1.70000	0.96760	1.76	0.2210
Dose	1	0.00036000	0.00041376	0.87	0.4760

43957 MNH - Linear Regression Results

The REG Procedure
Model: Linear_Regression_Model
Dependent Variable: Frequency

Experiment_Name=1-Short treatment without S9 mix (3-h treatment + 24-h recovery)



43957 MNH - Linear Regression Results

The REG Procedure
Model: Linear_Regression_Model
Dependent Variable: Frequency

Experiment_Name=2-Short treatment with S9 mix (3-h treatment + 24-h recovery)

Number of Observations Read	4
Number of Observations Used	4

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	0.11250	0.11250	0.09	0.7954
Error	2	2.57500	1.28750		
Corrected Total	3	2.68750			

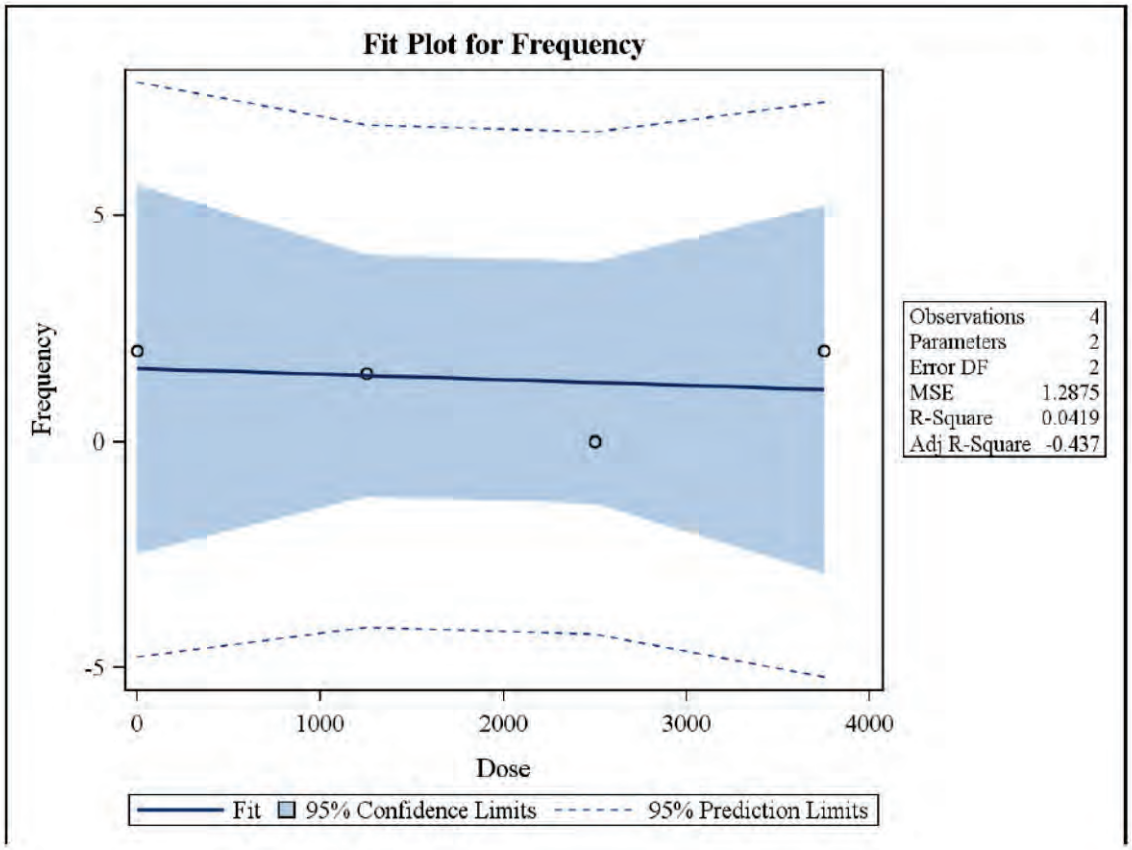
Root MSE	1.13468	**R-Square**	0.0419
Dependent Mean	1.37500	**Adj R-Sq**	-0.4372
Coeff Var	82.52222		

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	1	1.60000	0.94934	1.69	0.2340
Dose	1	-0.00012000	0.00040596	-0.30	0.7954

43957 MNH - Linear Regression Results

The REG Procedure
Model: Linear_Regression_Model
Dependent Variable: Frequency

Experiment_Name=2-Short treatment with S9 mix (3-h treatment + 24-h recovery)



43957 MNH - Linear Regression Results

The REG Procedure
Model: Linear_Regression_Model
Dependent Variable: Frequency

Experiment_Name=3-Long treatment without S9 mix (24-h continuous treatment; no recovery)

Number of Observations Read	4
Number of Observations Used	4

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0	0	.	.
Error	2	0	0		
Corrected Total	3	0			

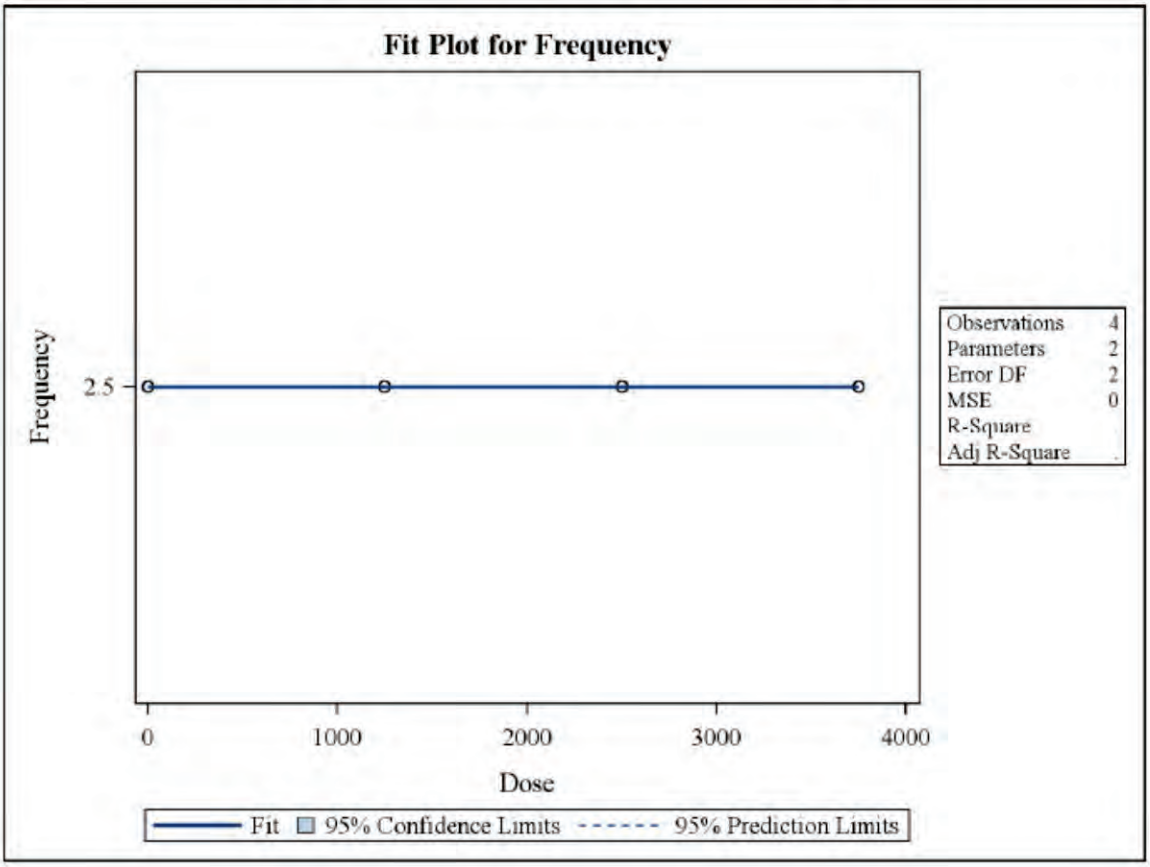
Root MSE	0	R-Square	.
Dependent Mean	2.50000	Adj R-Sq	.
Coeff Var	0		

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.50000	0	Infty	<.0001
Dose	1	0	0	.	.

43957 MNH - Linear Regression Results

The REG Procedure
Model: Linear_Regression_Model
Dependent Variable: Frequency

Experiment_Name=3-Long treatment without S9 mix (24-h continuous treatment; no recovery)



5. CiToxLAB France GLP certificate

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

GROUPE INTERMINISTERIEL DES PRODUITS CHIMIQUES

CERTIFICAT DE CONFORMITE AUX BONNES PRATIQUES DE LABORATOIRE SELON LES DIRECTIVES 2004/9/CE ET 2004/10/CE

CERTIFICATE OF COMPLIANCE WITH GOOD LABORATORY PRACTICES ACCORDING TO DIRECTIVES 2004/9/CE AND 2004/10/CE

Certificat n°: 2015/5

Société ou organisme : / ***Company :*** **CitoxLAB France (ex CIT) - Miseray - BP 563 27005 EVREUX Cedex**

Installation d'essais : / ***Test facilities :*** **CitoxLAB France (ex CIT) - Miseray - BP 563 27005 EVREUX Cedex**

Vu les articles D.523-8 et suivants du code de l'environnement relatifs au groupe interministériel des produits chimiques,
Having regard to the articles D.523-8 and onwards relating to the interministerial group of chemical products (GIPC),

Vu les résultats de l'inspection périodique réalisée par le Comité français d'accréditation (COFRAC) - Section Laboratoires – les :
Having regard to the results of the periodic inspection realised by the French Committee of accreditation (COFRAC) – Laboratory Section – on the :
15, 16 et 17 juillet 2014 / *15,16 and 17 July 2014*

Vu l'avis du GIPC en date du :
Having regard to the GIPC's opinion dated :
24 février 2015 / *24 February 2015*

La conformité aux principes des BPL de l'installation précitée est reconnue dans les domaines suivants :
Compliance with the principles of GLP is recognized for the facility above in the following areas:

2 - études de toxicité (*toxicity testing*)
3 - études de mutagénicité (*mutagenicity testing*)
8 - méthodes de chimie analytique et cliniques (y compris métabolisme) (*analytical and clinical chemistry testing*)

Fait à Ivry, le - 5 MARS 2015

Le Président

[redacted]

Jean- Marc GROGNET

Secrétariat général du GIPC - DGE- SI – 67, rue Barbès – 94201 Ivry-sur-Seine CEDEX
Téléphone : 01 79 84 96 10 –
Adresse mail : gipc.dge@finances.gouv.fr

MINISTÈRE DE L'ÉCONOMIE DE L'INDUSTRIE ET DU NUMÉRIQUE

Appendix B
Expert Panel Consensus Statement

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Savory Base 100 "Corn Sauce" for Use in Food Products

July 21, 2017

INTRODUCTION

Nestec S.A. (Nestec) intends to market Savory Base 100 "Corn Sauce" (Savory Base 100), a savory flavoring derived from fermentation of enzymatically-hydrolyzed corn starch and ammonia salts using *Corynebacterium glutamicum* (*C. glutamicum*) strain American Type Culture Collection (ATCC) 13032 in the United States (U.S.) marketplace. Some of the constituents that contribute to the characteristic savory flavor of Savory Base 100 include glutamic acid, L-alanine, succinic acid, formic acid, and an intrinsic mix of other free and bound amino acids, organic acids, Amadori and Maillard products, and minerals and their salts. Savory Base 100 is intended for use as a savory flavoring ingredient for addition to specified food products, including relishes, mayonnaise, gravies and sauces, herb and spice mixes and seasonings (including mixed dishes containing these ingredients), meat and fish analogues, and soups and broths, at use levels of up to 0.76% of the final food, as consumed (see Table A-1, Attachment A). Savory Base 100 is intended to be used as an alternative to current uses of yeast extract flavoring ingredients, affirmed as Generally Recognized as Safe (GRAS) under 21 CFR §184.1983 (U.S. FDA, 2016).

Nestec convened a panel of independent scientists (the "Expert Panel"), qualified by their scientific training and relevant national and international experience in the safety evaluation of food ingredients, to conduct a critical and comprehensive evaluation of the available data and information related to Savory Base 100 in order to determine whether its intended conditions of use would be GRAS based on scientific procedures. For the purposes of the Expert Panel's evaluation, "safe" or "safety" means that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2016). The Expert Panel consisted of the below-signed qualified scientific experts: Professor Emeritus Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Professor Eric A. Johnson (University of Wisconsin-Madison), and Professor Emeritus John A. Thomas (Indiana University School of Medicine).

The Expert Panel, independently and collectively, critically evaluated a comprehensive package of scientific information and data compiled from the literature and provided by Nestec. This information was presented in a dossier titled "Documentation to Support the Generally Recognized as Safe (GRAS) Status of Savory Base 100 "Corn Sauce" for Use in Food Products" dated June 21, 2017, and included a review of all publicly available scientific data and information, both favorable and unfavorable, relevant to the safety of the intended food uses of Nestec's Savory Base 100. This information was prepared, in part, based on the available information characterizing the identity and composition of Savory Base 100, manufacturing process, product specifications, supporting analytical data, intended conditions of use in specified food and beverage products, and dietary consumption estimates under the intended uses. Safety studies characterizing the toxicity of Savory Base 100 and its major components were critically evaluated by the Expert Panel.

Following an independent and collaborative critical evaluation of the data and information, the Expert Panel convened *via* teleconference on July 21, 2017 and unanimously concluded that the intended uses described herein of Savory Base 100, meeting appropriate food-grade specifications and manufactured consistent

with current Good Manufacturing Practices (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is presented below.

SUMMARY AND BASIS FOR GRAS

The manufacture of Savory Base 100 involves submerged fermentation of *C. glutamicum* ATCC 13032 in glucose-based media in compliance with the cGMP, incorporates the principles of Hazard Analysis and Critical Control Points (HACCP) and includes appropriate process controls throughout the production process. All raw materials, additives, and processing aids used during the fermentation and manufacturing processes are food-grade quality, and are used in accordance with an appropriate federal regulation, effective food contact notification, or have been determined to be GRAS for their respective uses. The stability of the fermentation organism is ensured by the use of stock and working cultures. The fermentation process is initiated by preparation of a sterilized fermentation broth which contains enzymatically-hydrolyzed corn starch as a carbon source, ammonium salts as a nitrogen source, various mineral nutrients, and pH regulation and anti-foaming aids. Substrates are replenished as needed during fermentation. After fermentation is complete, the broth is heated to kill the bacteria, and then filtered to remove the dead bacterial cells. The broth then undergoes vacuum evaporation, during which sterilized sodium chloride is added to improve shelf-life stability and prevent growth of microbial contaminants, producing the final Savory Base 100.

The Expert Panel reviewed analytical data from 3 non-consecutive batches of Savory Base 100 and concluded that Savory Base 100 is manufactured in a reproducible manner and a consistent product is produced that conforms to the established physical and chemical specifications established by Nestec. Savory Base 100 was analyzed for biogenic amines. Detectable levels of phenethylamine, cadaverine, histamine, spermidine or spermine in Savory Base 100 could not be measured. Low levels of putrescine (1.4 mg/kg), tyramine (5.4 mg/kg) and tryptamine (3.5 mg/kg) were detected at levels below or within concentrations that have been reported in common foods produced by fermentation. For example, sauerkraut is reported to contain the following concentrations of putrescine (87.3 to 222 mg/kg), tyramine (24.7 to 235 mg/kg) and tryptamine (2.4 to 7.2 mg/kg) (Sahu *et al*., 2015). The results of the chemical stability testing of Savory Base 100 demonstrated that Savory Base 100 remained stable for at least 1 year, when stored refrigerated (4°C) or at temperatures of 20, 30, or 37°C and a relative humidity of 50, 70, and 75%, respectively; no significant changes in stability parameters related to water activity, pH, total acidity, and microbial growth were reported.

Savory Base 100 is intended for use in various food products in the United States (U.S.), including relishes, mayonnaise, gravies and sauces, herb and spice mixes and seasonings (including mixed dishes containing these ingredients), meat and fish analogues, and soups and broths at use levels of up to 0.76% of the final food, as consumed. Consumption data pertaining to the individual proposed food-uses of Savory Base 100 were used to estimate the all-person and all-user intakes for specific demographic groups and for the total U.S. population using data from the 2011-2012 National Health and Nutrition Examination Survey (NHANES). For the total population, the mean and 90th percentile consumer-only intakes of Savory Base 100 were determined to be 197 and 477 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of Savory Base 100 on an absolute basis, at 230 and 556 mg/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 105 and 290 mg/person/day, respectively. On a body weight basis, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 8.8 and 23.1 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 2.7 and 6.3 mg/kg body weight/day, respectively.

The absorption, distribution, metabolism and excretion (ADME) of Savory Base 100 have not been investigated because Savory Base 100 is composed mainly of amino acids, minerals, water, sugars and organic acids that are normal components of human diet, which are digested and metabolized by well-established pathways. The Expert Panel critically evaluated published studies characterizing the toxicity of Savory Base 100, which included an acute toxicity study, a 90-day repeated-dose toxicity study, an *in vitro* bacterial reverse mutation assay (Ames test), and an *in vitro* mammalian cell gene mutation assay (Tafazoli *et al*., 2017). These studies were conducted in compliance with the Organization for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) (OECD, 1998), and using international accepted toxicity testing guidelines (OECD).

The results of the acute oral toxicity study demonstrated that Savory Base 100 is of low oral toxicity. Groups of 5 male and 5 female Wistar rats were administered a single dose of 0 (drinking water), 100, 500, or 2,000 mg/kg body weight Savory Base 100 by gavage. At the end of the 14-day observation period, animals were subjected to a macroscopic necropsy, and any organs showing gross pathological changes were subsequently examined microscopically. There were no mortalities or test item-related clinical signs of toxicity. Although a statistically significant (5%) reduction in body weight for males given 500 mg/kg body weight on Day 14 was reported, due to absence of a dose-response, this effect was considered to be toxicologically irrelevant. No macroscopic or microscopic changes related to the test article were reported. White deposits observed in the spleen of 2 females from each of the low and high-dose groups were confirmed microscopically to be slight capsular fibroses. These were isolated instances (only seen for 2 out of 5 females in each of the affected groups) and there was no evidence of a dose-related response. Isolated instances of unilateral pelvic dilatation (1 high-dose male and 1 control female) and red spots on the thymus (1 low dose male) were considered to be unrelated to the test item. Based on the results of the acute toxicity testing in male and female Wistar rats a median lethal dose (LD_{50}) value of >2,000 mg/kg body weight was reported by the authors (Tafazoli *et al*., 2017).

A 90-day repeat dose oral toxicity study was conducted to investigate the subchronic toxicity of NRC Mix (a combination of Savory Base 100 and a related substance, called Savory Base 200 “Corn Sauce” [Savory Base 200], in a 2:1 ratio) in rats (Tafazoli *et al*., 2017). Savory Base 100 and Savory Base 200 are typically expected to be used in combination in foods to provide unique savory flavoring properties; and therefore, the toxicity of these ingredients was evaluated together. The major constituents that contribute to the characteristic savory flavor of Savory Base 200 are disodium 5’ inosine-monophosphate (IMP), glycine, formic acid and an intrinsic mix of other free and bound amino acids, organic acids, Amadori and Maillard products, as well as minerals and their salts. NRC Mix contained 37.8±0.2% glutamic acid (primarily from Savory Base 100) and 14.5±0.4% IMP (primarily from Savory Base 200). Groups of 10 male and 10 female Wistar rats were given 0 (control),1, 2.5, or 7% NRC Mix (equivalent to approximately 0, 500, 1,250, or 3,500 mg/kg body weight/day NRC Mix, which equates to approximately 0, 333, 833, or 2,333 mg/kg body weight/day Savory Base 100, respectively) in the diet for 90 days. An additional 5 males and 5 females were included in the control and high-dose groups receiving the same treatment for 90 days, after which time they were kept untreated for a further 4 weeks to evaluate the reversibility of any effects observed during the treatment period. The concentration levels were selected based on a previous palatability study conducted at the same testing facility.

All except for one, treated and control animals survived the experimental period in good general health. The death of one female in the mid-dose group at the end of the study period (Day 90) was found not to be treatment-related, but no reason for the death was identified upon necropsy. Over the course of the study, all animals consuming the test diets displayed increased body weight development, which was consistent with increased feed consumption by the treated male animals and sporadic increases in female treated animals. The improved growth performance of the animals receiving the test diet was likely related to the

test substance providing significant nutritional value (amino acids and minerals), which was not adjusted for in the control diets. The dose-dependent increase in water consumption in both male and females, which was found to be quite marked in the high-dose groups, is an expected outcome of the high salt content of the savory ingredients. The statistically significant changes in the hematology parameters that were noted in both male and females were considered physiological variations, as the changes were small in magnitude, within historical controls ranges, not consistent between the sexes or findings in the recovery groups, or were considered to be incidental due to the lack of dose-dependency. The sporadic statistically significant findings observed in the clinical chemistry indices between groups were consistently of low magnitude and/or only observed in one sex, as such, considered to be physiological variations.

In the liver, periportal fatty changes observed in males and females of the high-dose group and the corresponding control groups at the end of recovery period were not associated with any findings of necrosis or increases in liver enzyme levels or in absolute or relative liver weights. This indicates that the findings in the liver are likely an adaptive response to the diet *versus* overt toxic effects. Macrovesicular steatosis is the most common form of liver fatty changes, which may be seen sporadically in control animals, and is considered a benign change presumably as a result of nutritional, metabolic or hormonal derangement (Greaves and Faccini, 1992; Thoolen *et al*., 2010; Greaves, 2012).

Tubular mineralization observed only in high-dose female rats during the study and recovery periods was not accompanied by necrosis or inflammation of the kidneys or corroborative changes in kidney function markers in the clinical chemistry or urinalyses parameters. Renal tubular mineralization or nephrocalcinosis is a spontaneous lesion that develops in young and adult rats with high incidences reported in female Sprague-Dawley, Wistar, RlVM-TOX, Zucker and Fischer 344 rats (Peter *et al.*, 1986; NRC, 1995; Rao, 2002). Male rats of any strain were reported to be less susceptible, suggesting an association between female sex hormones and development of such lesions (Rao, 2002). Increased susceptibility to nephrocalcinosis is known to occur from dietary manipulation and it has been reported that imbalances in the calcium and phosphorus content of the diets, calcium:phosphorus ratio in the diet, deficiency of magnesium, deficiency of chloride and high urinary pH can contribute to the development of this lesion (Reeves *et al.*, 1993; Rao, 2002). However, no single mechanism has been identified explaining the association between all of the dietary factors contributing to the incidence of nephrocalcinosis. Considering the high mineral content of the savory ingredients, the likely unbalanced provision of minerals in the test diet relative to the control diet could be responsible for the observed effects in the kidneys. The incidences of renal mineralization were limited to only the female rats, a finding that is consistent with the fact that susceptibility to nephrocalcinosis is a predilection that is specific to females. In general, these mineral deposits are of no pathological significance (Seely and Brix, 2014), and in the absence of correlating markers of kidney impairment were not considered of toxicological significance.

The incidence of chronic focal myocarditis observed in males of the treatment group and a female in the control group was not considered to be toxicologically significant, as these histological observations are similar to the spontaneous lesions commonly observed in test and control rats, with a higher occurrence in males (Gaunt *et al.*, 1967; Jokinen *et al.*, 2011). Likewise, other histopathological findings in the heart, small and large intestines were not considered to be of toxicological significance, as they were sporadic and/or spontaneous, small in magnitude, occurred at similar frequency in treatment and control groups, or only occurred in the control group.

Based on the findings of the 90-day study, a no-observed-adverse-effect level (NOAEL) of 7% Savory Base 100/Savory Base 200 mixture in the diet was established by the authors. This dietary level corresponds to approximately 3,500 mg/kg body weight/day. Based on the 2:1 ratio of Savory Base 100 and Savory Base 200 in the test article mixture, a NOAEL of 7% Savory Base 100/Savory Base 200 mixture in the diet would

correspond to NOAELs of 2,333 and 1,167 mg/kg body weight for Savory Base 100 and Savory Base 200, respectively.

The results of a battery of *in vitro* mutagenicity and genotoxicity tests demonstrated that Savory Base 100 is neither mutagenic nor genotoxic (Chevalier, 2016; Tafazoli *et al*., 2017). In an *in vitro* bacterial reverse mutation test (Ames assay), an initial preliminary range-finding test was conducted using the plate incorporation method at Savory Base 100 concentrations of 5 to 5,000 µg/plate, using *Salmonella* Typhimurium strains TA98 and TA100, in the absence and presence of S9 metabolic activation. Considering that the results of this test were negative, 2 follow-up separate tests (plate incorporation assay and pre-incubation assay) were conducted using strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA, which were incubated with Savory Base 100 at concentrations of 51.2, 128, 320, 800, 2,000, and 5,000 µg/plate in the absence and presence of S9 mix. Three negative control groups (untreated, vehicle [distilled water] and dimethyl sulfoxide) were used, and positive controls were also included in the absence (4-nitro-1,2-phenylene-diamine, sodium azide, 9-aminoacridine and methyl-methanesulfonate) and presence (2-aminoanthracene) of metabolic activation. Savory Base 100 showed no evidence of mutagenicity in any of the tests, in the absence or presence of metabolic activation. In contrast, the positive controls induced biologically relevant increases in revertant colony counts (with metabolic activation where required), which demonstrated the sensitivity of the assay and metabolic activity of the S9 preparations. The results of this study demonstrated that Savory Base 100 is non-mutagenic at concentrations up to 5,000 µg/plate, in the absence or presence of metabolic activation.

The mutagenic potential of Savory Base 100 was further investigated in an *in vitro* mammalian cell gene mutation test (Tafazoli *et al*., 2017). A preliminary dose range-finding study (where Savory Base 100 was not cytotoxic at concentrations up to 5,000 µg/mL) was followed by 2 independent experiments (each conducted in duplicate) using V79 Chinese hamster lung (CHL) cells. For both of these experiments, the vehicle (Dulbecco's Modified Eagle's [DME] medium) and dimethyl sulfoxide (DMSO) served as the negative controls and positive controls were included in the absence (ethylmethane sulfonate) and presence (7,12-dimethyl benzanthracene) of S9 metabolic activation. In the first experiment, CHL cells were exposed to Savory Base 100 for 3 hours at concentrations of 312.50, 625, 1,250, 2,500, or 5,000 µg/mL in the absence or presence of S9 metabolic activation. In the second, CHL cells were exposed to Savory Base 100 for 20 hours (in the absence of S9) or 3 hours (in the presence of S9) at concentrations of 156.25 (presence of S9 only), 312.50, 625, 1,250, 2,500, or 5,000 µg/mL. For both experiments, no statistically significant increases in mutation frequency were observed for Savory Base 100 treated cells, compared with that of the negative controls. Based on the results of this study, it was demonstrated that Savory Base 100 is not mutagenic at concentrations up to 5,000 µg/mL, in the absence and presence of metabolic activation.

In a corroborative *in vitro* mammalian cell micronucleus test using human lymphocytes and conducted in accordance with the OECD principles of GLP, the clastogenic and aneugenic potential of Savory Base 100 was further evaluated (Chevalier, 2016 [unpublished]). An initial preliminary cytotoxicity test was conducted using Savory Base 100 at concentrations of 0 to 5,000 µg/mL, in the presence (3-hour treatment) and absence (3 and 24-hour treatments) of S9 metabolic activation; there was no evidence of cytotoxicity reported at any concentration. Cytotoxicity was re-evaluated in the main experiment, where in the absence of S9 (at the same dose levels and under similar conditions to those used in the preliminary test), there was no evidence of cytotoxicity after a 3-hour treatment, but slight to moderate cytotoxicity was reported at concentrations ≥ 2500 µg/mL after 24 hours continuous treatment. However, there was no evidence of cytotoxicity in the presence of S9 after a 3-hour treatment under similar conditions to those described above. In the main experiment, 5,000 µg/mL was considered to produce extreme culture conditions, therefore, human lymphocytes were treated with Savory Base 100 at 312.5, 625, 1,250, 2,500, or 3,750 µg/mL with S9 (3 hours) and without S9 (3 and 24-hour treatments). The vehicle (water for injection)

was used as a negative control and positive controls were included in the absence (colchicine and mitomycin C) and presence (cyclophosphamide) of metabolic activation. A positive result for clastogenicity/aneugenicity was defined by authors as a dose-dependent, statistically significant increase in the frequency of micronucleated binucleated cells (MNBC), with the frequency of MNBC also being above the vehicle background range for at least 1 dose level. Savory Base 100 showed no evidence of clastogenicity or aneugenicity in any of the tests, in the absence or presence of metabolic activation. In contrast, the positive controls induced biologically relevant increases in MNBC (with metabolic activation where required), which demonstrated the sensitivity of the assay and metabolic activity of the S9 preparations. Based on the results of this study, it was demonstrated that Savory Base 100 is neither clastogenic nor aneugenic at concentrations up to 3,750 µg/mL, in the absence or presence of metabolic activation.

In addition to the pivotal studies related to the safety of Savory Base 100, the Panel also reviewed the available data relevant to the safety and regulatory status of major constituents of Savory Base 100. The major constituents of Savory Base 100, including glutamic acid, L-alanine, succinic acid, formic acid have a long history of consumption as part of existing foodstuffs and their dietary intakes occurring from their presence in Savory Base 100 are consistent with levels commonly used in foods, and/or are well below acceptable daily intake (ADI) values that have been derived. Savory Base 100 is intended for use as an alternative to yeast extracts for general food use, and therefore, will not increase dietary intakes of glutamic acid above levels currently occurring by way of existing regulations for glutamic acid and its salts under 21 CFR §184.1983 (U.S. FDA, 2016). Additionally, glutamic acid has been allocated an ADI of 'not specified' by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1988). Based on the results of analysis of 3 batches of Savory Base 100, the glutamic acid content of the product averages about 38%. Considering that the 90th percentile intakes of Savory Base 100 was estimated to be 477 mg/person/day, the daily intakes of glutamic acid is calculated to be 181.26 mg/day. This compares to estimated average daily intakes of 590 mg of monosodium glutamate (equivalent to intakes of ~512.9 mg as glutamic acid) that have been estimated for consumers in the United Kingdom; and therefore, dietary intakes of glutamic acids from the proposed food uses is not a safety concern (FZANZ, 2003).

L-Alanine is permitted for direct addition to foods for nutritive purposes at levels up to 6.1% by weight of total protein (21 CFR §172.320 - U.S. FDA, 2016). L-Alanine has been allocated an ADI of 'acceptable' by JECFA (2004). Based on the results of analysis of 3 batches of Savory Base 100, the L-alanine content of the product averages about 1%. Considering that the 90th percentile intakes of Savory Base 100 (*i.e.,* 477 mg/person/day), the daily intakes of L-alanine, as a component of Savory Base 100, was calculated to be 4.77 mg/day, and this is not expected to raise a safety concern.

Formic acid is permitted for direct addition to food intended for human consumption with no limitations other than GMP (21 CFR §186.1316 - U.S. FDA, 2016). Formic acid has been allocated an ADI of '0 to 3 mg/kg body weight/day' by JECFA (1997). Based on the results of analysis of 3 batches of Savory Base 100, the formic acid content of the product averages about 0.72%. Considering that the 90th percentile consumer-only intakes of Savory Base 100 was estimated to be 477 mg/person/day, the daily intakes of formic acid, as a component of Savory Base 100, was calculated to be 3.43 mg/day (equivalent to 0.049 mg/kg body weight/day for a 70-kg individual.) This intake is well below the ADI of 3 mg formic acid/kg body weight/day as established by JECFA.

In the U.S., succinic acid produced by chemical synthesis or fermentation is GRAS for use as a flavor enhancer, and pH control agent in food at levels consistent with 21 CFR §184.1091 and not to exceed cGMP (U.S. FDA, 2016). In a 13-week subchronic oral toxicity study by Maekawa *et al*. (1990), the toxicity of monosodium succinate was evaluated in groups of 10 male and 10 female F344 rats *via* the drinking water

at concentrations of 0 (control), 0.3, 0.6, 1.25, 2.5, 5, or 10%. No dose-related adverse effects were reported in hematological, biochemical, or histopathological parameters at any dose. The authors concluded that the NOAEL was 1.25% (equivalent to 1,250 mg/kg body weight/day or 1,050 mg/kg body weight/day as succinic acid), based on decreased body weight gain noted at higher doses (Maekawa *et al*., 1990). The food intakes were not measured in this study. In a follow-up 2-year carcinogenicity study, no statistically significant differences were reported between the control and treated animals in overall tumor incidence, or mean survival times in either sex, when groups of 50 male and 50 female F344 rats were administered monosodium succinate through the drinking water at doses up to 2% for 104 weeks, corresponding to daily intakes of up to 1,093 mg/kg body weight/day for males and 773 mg/kg body weight/day for females (Maekawa *et al*., 1990). The results of an *in vitro* reverse mutation assay and a chromosomal aberration test demonstrated that succinic acid was neither mutagenic nor clastogenic (Ishidate *et al*., 1984). Based on the results of analysis of 3 batches of Savory Base 100, the succinic acid content of the product averages about 0.57%. Considering that the 90th percentile consumer-only intakes of Savory Base 100 was estimated to be 477 mg/person/day, and the daily intakes of succinic acid, as a component of Savory Base 100, was calculated to be 2.72 mg/day (equivalent to intakes of 0.039 mg succinic acid/kg body weight/day for a 70-kg individual), which provides a large margin of safety when compared to the NOAEL of 1,050 mg succinic acid/kg body weight/day, as determined in the 13-week oral toxicity study by Maekawa *et al*. (1990).

The Expert Panel also reviewed information on the safety of the source organism used for fermentation of Savory Base 100. The *C. glutamicum* strain used by Nestec in the production of Savory Base 100 is deposited in several international culture collections. Initially deposited as *Micrococcus glutamicus* strain 13032 by Kyowa Ferm. Ind. Co., Ltd., the production organism currently has the strain designation *C. glutamicum* 534 [ATCC 13032^{T}] and represents the type strain for the species (ATCC, 2016; Ikeda and Nakagawa, 2003). The complete genome of *C. glutamicum* ATCC 13032 was sequenced in 1998, which was further characterized and annotated in 2001 and 2002 (reviewed in Ikeda and Nakagawa, 2003) and is also publicly available (NCBI, 2016). The central carbon pathway, physiology and regulation of main and specific metabolic pathways for this strain have been well characterized, as it has significant industrial applications and much interest has been focused on optimizing production performance from this microorganism (Wieschalka *et al*., 2013).

There are no documented case-reports of *C. glutamicum* being pathogenic or toxic to humans or animals. *C. glutamicum* fulfils the requirements for Qualified Presumption of Safety (QPS) when it is used for amino acid production (EFSA, 2013); Savory Base 100 being enriched in amino acids. *C. glutamicum* ATCC 13032 is classified as a Biosafety Level 1 by the ATCC, meaning the microorganism is not known to consistently cause disease in healthy adult humans and is of minimal potential hazard to laboratory personnel and the environment.

C. glutamicum has a long history of use in the food production industry. First isolated in 1956, *C. glutamicum* was initially characterized by its unique natural ability to produce large amounts of glutamic acid (the predominant amino acid in Savory Base 100) from sugar and ammonia (Vertès *et al.*, 2013). Moreover, *C. glutamicum* has been used for the production of glutamic acid in the U.S. since 1961 (Kinoshita *et al*., 1961a,b; Kalinowski *et al*., 2003); in 2005 alone, 1.5 million tons of glutamate were produced using fermentation by *C. glutamicum*, in addition to several thousand tons of threonine, lysine, isoleucine and tryptophan (Smith *et al*., 2010). *C. glutamicum* has also been identified as a surface microflora in cheese during ripening, indicating that this organism has a history of consumption as a species in cheese (Dolci *et al.*, 2009).

Savory Base 100 is free of any residual protein, the source organism and any microbial contamination. Overall, the source organism is not expected to pose any safety concerns in the final Savory Base 100 based on the phenotypic and genotypic properties of the organism, the history of safe use of the organism in food production, its QPS status, as well as implementation of appropriate controls during manufacturing (heat sterilization and filtration) that would prevent carry-over of the source organism into the final product.

CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above, and conclude that Savory Base 100 "Corn Sauce", as described herein, produced in accordance with current Good Manufacturing Practices (cGMP) and meeting food-grade specifications, is safe and suitable and Generally Recognized a Safe (GRAS) based on scientific procedures, for use as a savory flavoring ingredient for addition to specified food products at levels of up to 0.76% of the final food, as consumed.

It is our opinion that other qualified experts would concur with these conclusions.

[redacted]

Professor Emeritus Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

10 August 2017
Date

[redacted]

Professor Eric A. Johnson, Sc.D.
University of Wisconsin-Madison

8/1/17
Date

[redacted]

Professor Emeritus John A. Thomas, Ph.D.
Indiana University School of Medicine

8/4/17
Date

REFERENCES

ATCC (2016). *Corynebacterium glutamicum* (Kinoshita et al.) Abe et al. (ATCC® 13032™). Manassas (VA): American Type Culture Collection (ATCC). Available at: https://www.atcc.org/Products/All/13032.aspx [Last accessed: September 2, 2016].

Chevalier K (2016) [unpublished]. *In Vitro Micronucleus Test in Cultured Human Lymphocytes [He Wei C. Essence I]*. (Laboratory Study Number: 43957 MNH). Prepared by Evreux, France: CiToxLAB, Safety and Health Research Laboratories for Singen, Germany: Nestle PTC Singen Lebensmittelforschung GmbH.

Dolci P, Barmaz A, Zenato S, Pramotton R, Alessandria V, Cocolin L et al. (2009). Maturing dynamics of surface microflora in Fontina PDO cheese studied by culture-dependent and-independent methods. J Appl Microbiol 106(1):278-287.

EFSA (2013). Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update) (EFSA Panel on Biological Hazards/BIOHAZ) (Question no EFSA-Q-2013-00019, adopted: 24 October 2013 by European Food Safety Authority). EFSA J 11(11):3449 [107 pp]. DOI:10.2903/j.efsa.2013.3449. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/3449.

FSANZ (2003). *Monosodium Glutamate: A Safety Assessment* (Technical Report Series No. 20). Canberra, Australia / Wellington, NZ: Food Standards Australia New Zealand (FSANZ). Available at: https://www.foodstandards.gov.au/publications/documents/MSG%20Technical%20Report.pdf.

Gaunt IF, Grasso P, Gangolli SD (1967). Short-term toxicity study of emulsifier YN* in rats. Food Cosmet Toxicol 5(5):623-629.

Greaves P (2012). Liver (chapter 9). In: *Histopathology of Preclinical Toxicity Studies, 4th edition*. New York (NY): Elsevier B.V., pp. 432-544 [see pages 432-481].

Greaves P, Faccini JM (1992). Digestive system. In: *Rat Histopathology: A Glossary for Use in Toxicity and Carcinogenicity Studies, 2nd edition*. Amsterdam, The Netherlands / New York (NY): Elsevier, pp. 105-158 [see pages 107-116].

Ikeda M, Nakagawa S (2003). The *Corynebacterium glutamicum* genome: features and impacts on biotechnological processes. Appl Microbiol Biotechnol 62(2/3):99-109.

Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 22(8):623-636.

JECFA (1988). L-Gluta[m]ic acid and its ammonium, calcium, monosodium and potassium salts. In: *Toxicological Evaluation of Certain Food Additives*. 31st Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Feb. 16-25, 1987, Geneva, Switz. (WHO Food Additives Series no 22). New York (NY) / Geneva, Switz.: Cambridge University Press / World Health Organization (WHO), pp. 97-161. Available at: http://www.inchem.org/documents/jecfa/jecmono/v22je12.htm.

JECFA (1997). Formic acid. In: *Summary of Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 1956-2014 (1st through 79th Meetings) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA) / Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO) / International Programme on Chemical Safety (IPCS). Available at: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1561.

JECFA (2004). DL- Alanine. In: *Summary of Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 1956-2014 (1st through 79th Meetings) [Database]*. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA) / Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO) / International Programme on Chemical Safety (IPCS). Available at: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=5314.

Jokinen MP, Lieuallen WG, Boyle MC, Johnson CL, Malarkey DE, Nyska A (2011). Morphologic aspects of rodent cardiotoxicity in a retrospective evaluation of National Toxicology Program studies. Toxicol Pathol 39(5):850-860.

Kalinowski J, Bathe B, Bartels D, Bischoff N, Bott M, Burkovski A et al. (2003). The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. J Biotechnol 104(1/3):5-25.

Kinoshita S, Tanaka T and Akita S, inventors; Chiyoda-ku, Tokyo, Japan: Kyowa Hakko Kogyo Co., Ltd., assignee (1961a). *Method of Producing L-Glutamic Acid.* US Patent 3,002,889 [Oct. 3, 1961]. Available at: http://www.freepatentsonline.com/3002889.html.

Kinoshita S, Udaka S, Akita S, inventors; Tokyo, Japan: Kyowa Hakko Kogyo Co., Ltd., assignee (1961b). *Method of Producing L-Glutamic Acid by Fermentation.* US Patent 3,003,925 [Oct. 10, 1961]. Available at: http://www.freepatentsonline.com/3003925.html.

Maekawa A, Todate A, Onodera H, Matsushima Y, Nagaoka T, Shibutani M et al. (1990). Lack of toxicity/carcinogenicity of monosodium succinate in F344 rats. Food Chem Toxicol 28(4):235-241.

NCBI (2016). *Corynebacterium glutamicum ATCC 13032 DNA, complete genome.* (GenBank BA000036.3). In: *NCBI Protein Database*. Bethesda (MD): National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine (NLM). Available at: http://www.ncbi.nlm.nih.gov/nuccore/BA000036 (Last accessed: September 2, 2016).

NRC (1995). Nutrient requirements of the laboratory rat. In: *Nutrient Requirements of Laboratory Animals, 4th revised edition*. Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council (NRC). Washington (DC): National Academy Press (NAP), pp. 27-38. Available at: http://www.nap.edu/catalog/4758.html.

OECD (1998). *OECD Principles of Good Laboratory Practice*. (Series on Principles of Good Laboratory Practice and Compliance Monitoring, no. 1 [ENV/MC/CHEM(98)17]). Paris, France: Organisation for Economic Co-Operation & Development (OECD), Environment Directorate, Chemicals Group and Management Committee, OECD Environmental Health and Safety Publications. Available at: http://www.oecd-ilibrary.org/environment/oecd-principles-on-good-laboratory-practice_9789264078536-en [As revised in 1997].

Peter CP, Burek JD, van Zwieten MJ (1986). Spontaneous nephropathies in rats. Toxicol Pathol 14:91-100.

Rao GN (2002). Diet and kidney diseases in rats. Toxicol Pathol 30(6):651-656.

Reeves PG, Rossow KL, Lindlauf J (1993). Development and testing of the AIN-93 purified diets for rodents: Results on growth, kidney calcification and bone mineralization in rats and mice. J Nutr 123(11):1923-1931.

Sahu L, Panda SK, Paramithiotis S, Zdolec N, Ray RC (2015). Biogenic amines in fermented foods: overview (chapter 16). In: Montet D, Ray RC, editors. *Fermented Foods. Part I: Biochemistry and Biotechnology*. (Food Biology Series). Boca Raton (FL): CRC Press (Taylor & Francis Group), pp. 303-317.

Seely JC, Brix A (2014). Kidney – mineralization. In: Cesta MF, Herbert RA, Brix AL, Malarkey DE and Sills RC, editors. *Nonneoplastic Lesion Atlas: A Guide for Standardizing Terminology in Toxicologic Pathology for Rodents*. Research Triangle Park (NC): National Toxicology Program (NTP). Available at: https://ntp.niehs.nih.gov/nnl/urinary/kidney/mineral/index.htm [Last updated: Jan. 14, 2014].

Smith KM, Cho KM, Liao JC (2010). Engineering *Corynebacterium glutamicum* for isobutanol production. Appl Microbiol Biotechnol 87(3):1045-1055.

Tafazoli S, Vo TD, Petersen A, Constable A, Coulet M, Phothirath P et al. (2017). Genotoxicity, acute and subchronic toxicity evaluation of savory food ingredients. Regul Toxicol Pharmacol 87:71-87.

Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T et al. (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicol Pathol 38(7, Suppl.):5S-81S.

U.S. FDA (2016). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs*. (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	§	Section Title
170—Food additives	170.3	Definitions
172—Food additives permitted for direct addition to food for human consumption	172.320	Amino acids
184—Direct food substances affirmed as generally recognized as safe	184.1091	Succinic acid
	184.1983	Bakers yeast extract
186—Indirect food substances affirmed as generally recognized as safe	186.1316	Formic acid

Vertès AA, Inui M, Yukawa H (2013). The biotechnological potential of *Corynebacterium glutamicum*, from *Umami* to *Chemurgy*. In: Tatsuni N, Inui M, editors. *Corynebacterium* glutamicum*: Biology and Biotechnology*. (Microbiology Monographs, vol 23). Heidelberg, Germany / New York (NY): Springer, pp. 1-49.

Wieschalka S, Blombach B, Bott M, Eikmanns BJ (2013). Bio-based production of organic acids with *Corynebacterium glutamicum*. Microb Biotechnol 6(2):87-102.

ATTACHMENT A

Proposed Uses and Use-Levels of Savory Base 100

Table A-1 Summary of the Individual Proposed Food Uses and Use Levels of Savory Base 100 "Corn Sauce" (Savory Base 100) in the United States

Food Category[a]	Proposed Food Use[b]	Maximum Proposed Use Level of Savory Base 100 (g/100 g)	Maximum Proposed Use Level of Savory Base 100 (g/100 g, expressed on a dwb)[c, d]
Condiments and Relishes	Relishes	0.76	0.51
Fats and Oils	Mayonnaise[e]	0.76	0.51
Gravies and Sauces	Gravies and sauces[f]	0.38	0.25
Herbs, Seeds, Spices, Seasonings, Blends, Extracts, and Flavorings	Herb and spice mixes, and seasonings[f]	0.60	0.40
Plant Protein Products	Meat and fish analogues	0.40	0.27
Soups and Soup Mixes	Soups and broths (all types)	0.38	0.25

dwb = dry weight basis.

[a] Food categories established under 21 CFR §170.3(n) (U.S. FDA, 2017).

[b] This table lists the direct proposed food uses of Savory Base 100. The exposure assessment conducted has accounted for final products as consumed, whereby if the proposed uses are a component of a final food, *e.g.*, mixed dish containing spices, an ingredient fraction was applied to the final product as consumed.

[c] The dry weight content of Savory Base 100 is 67%, assuming typical moisture content of 33%.

[d] Values used in the exposure assessments.

[e] This food-use represents non-standardized mayonnaise. As there were a limited number of food codes identified for non-standardized mayonnaise, food codes of standardized mayonnaise were also selected as surrogate food codes in order to provide a more robust intake estimate.

[f] These food uses may fall under the USDA's jurisdiction, as some of the finished food products to which Savory Base 100 is intended to be added can contain meat/poultry products (*e.g.*, ham, sausage).

Viebrock, Lauren

From: Petersen,Anne,SINGEN,NPTC Food - Regulatory <Anne.Petersen@rdsi.nestle.com>
Sent: Tuesday, November 27, 2018 11:53 AM
To: Viebrock, Lauren
Cc: Winters,Robert,ARLINGTON,Nestlé Legal
Subject: RE: GRN 793 Questions
Attachments: GRN 792 793 Answers final.docx

Dear Ms. VieBrock,

Thank you very much for your E-mail!

Please find attached a document with our response to the questions you raised. I hope this helps to clarify. Given the similarity of the two corn sauces filed under GRN 792 and 793, we have taken the liberty to combine the response in one document, which is valid for both GRAS notices.

Please feel free to contact me again, if anything remains unclear or if you need any further information from our side.

Kind regards,
Anne

Anne Petersen
Regulatory & Scientific Affairs Manager
SBU FOOD & NPTC FOOD
Phone: +49 7731 14 1235
e-mail: anne.petersen@rdsi.nestle.com

Nestlé Product Technology Centre Lebensmittelforschung GmbH | Lange Str. 21 | 78224 Singen | Germany
GESCHÄFTSFÜHRUNG: Sean Westcott; REGISTERGERICHT: Amtsgericht Freiburg im Breisgau, HRB 542008

From: Viebrock, Lauren [mailto:Lauren.Viebrock@fda.hhs.gov]
Sent: Montag, 12. November 2018 20:59
To: Petersen,Anne,SINGEN,NPTC Food - Regulatory <Anne.Petersen@rdsi.nestle.com>
Subject: GRN 793 Questions

Dear Ms. Petersen,

During our review of GRAS Notice No. 000793, we noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock, Ph.D.
Consumer Safety Officer/Microbiology Reviewer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
(301) 796-7454

Response to FDA Questions on GRN 792 and 793

1. **Please confirm that the enzyme used to hydrolyze the corn starch is a safe and suitable enzyme for such use.**

   The following enzymes are used for hydrolysis of the corn starch and are safe and suitable for this use. The enzymes comply with the recommended purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemical Codex (FCC).

Enzymes	Microbial Source of Enzymes
Glucoamylase	*Aspergillus niger, Bacillus subtilis*
alpha-Amylase	*Bacillus licheniformis*
Glucose isomerase	*Streptomyces murinus*

2. **Please confirm that the fermentation is monitored for contamination.**

   The fermentation is monitored for contamination in accordance with the attached HACCP plan. Specifically, the 4th operational pre-requisite program (OPRP4) is focused on preventing external microbial contamination during the fermentation process. Consistent with OPRP4, samples of the fermentation broth are taken for microscopic examination to confirm the absence of foreign microorganisms.
   More specifically, each batch is analyzed according to the parameters outlined in table 3.1.1 in the attached HACCP plan for microbial contamination. Analysis for mycotoxins as listed in the above mentioned table is performed for each raw material batch as well as for the final product on a monthly basis.
   Please note that the corn sauces are referred to as Corn Savory Bases 100 and 200 in these attachments.

   HACCP plan for Corn Savory Base pr

3. **Please provide a complete breakdown of the constituents of the sauce once it is finished processing if Table 2.3.1 "Compositional Parameters" is not comprehensive. Please provide more information on the other free and bound amino acids, organic acids, Amadori and Mailliard products, and minerals and their salts.**

   Please see attached compositional breakdowns for three representative lots each of Savory Base 100 and Savory Base 200. These breakdowns include information on total and free amino acids, minerals, organic acids, and sugars for both bases as well as the ribonucleotide profile for Savory Base 200.

   Savory Base 100 & 200 - Compositional

4. **Please clarify whether the sugars are completely consumed by the fermentation process. If they are not completely consumed, please provide information on how much of the original sugars remain in the final product.**

   As demonstrated in the attached compositional breakdowns, the sugars are completely consumed by the fermentation.

5. **Please state whether any preservatives are added to the product.**

   No chemical preservatives are added to the corn sauces. Shelf stability is achieved primarily through controlling water activity, which is lowered through the addition of sterilized salt (sodium chloride).

6. **Please explain the function of each of the processing aids used (ammonia gas, sodium hydroxide, and PPE).**

   Ammonia gas and sodium hydroxide are added as pH regulators to maintain a pH range between 5 and 9 for optimal growth of the microorganisms.
   PPE (Polyoxyethylene Polyoxypropylene Pentaerythritol Ether) is used as an antifoaming agent during fermentation and is not analytically detectable in the final product.

7. **Please state how you intend to sublist this product.**

   We met with Felicia B. Billingslea, Director of CFSAN's Division of Food Labelling and Standards, and her staff in February 2018 and shared our intention to label the multi-ingredient corn sauces as follows on the labels of foods in which they are incorporated: "corn sauce (cultured corn starch, water, salt)". We received no objections to the use of "corn sauce" or the sublisting of ingredients in a parenthetical listing. We also believe it is appropriate, consistent with the "designation of ingredients" regulation at 21 CFR 101.4(b)(2), to list the corn sauce ingredients in composite format – i.e., by incorporating "cultured corn starch," "water," and "salt" into the statement of ingredients in descending order or predominance in the finished food without listing "corn sauce" itself.

8. **Please describe how the flavor of the ingredient compares to yeast extract flavoring.**

   Please see attached a sensory monadic profile of the corn sauces.

   Sensory data SB100 and SB200 march 20

   We have not directly compared the corn sauces to yeast extracts. However, please see attached a compilation of taste / flavour profiles of several different commercially available yeast extracts. This exemplifies that a vast variety of yeast extracts exist, each of which has an individual flavour and taste profile (and composition). The general flavour direction can be described as savoury, umami, xian for all of these products with different more typified directions for each individual product, similar to the two corn sauces.

   Yeast extract taste profiles.docx

Star Lake Bioscience Co., Inc. Zhaoqing Guangdong

HACCP Plan

Corn Savory Base Products

Document No.：**SLH-QA-4-01.01**

Prepared by：Lu Qinying

Reviewed by：

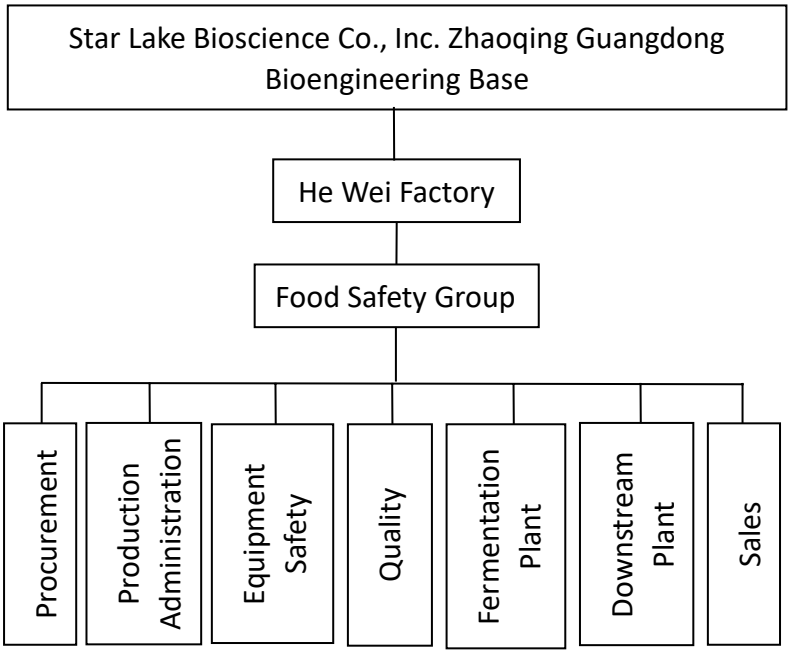
Approved by：

Controlled status：

Issue No.：

Issued on：2018.05.20 **Implemented on：2018.06.01**

1. Organization chart



2. Food Safety (HACCP) Group and Responsibility

The following persons are appointed as members of the Food Safety team:

Team leader：Cai Youhua

Team member：Lu Chenying, Wan Luming, Wang Weigui, Zhang Xiaofan, Chen Wenyan, Shi Jinling, Xiao Zhiquan, Liu Jieting

Responsibility of each member of the Food Safety (HACCP) Team：

Name	Department	Position title	Specialty	Qualifications	Main responsibility
Cai Youhua	He Wei Factory Line	Executive Director/ PhD	Industrial fermentation	Has entered service since 2011 and engaged in the research and development of new products, including strain breeding, industrial	Implementation of Corn Savory Base production. Approve production process and SOP. Approve and confirm HACCP Plan. Assign production task.

				fermentation, and extraction.	
Qinying Lu	Quality Department	Department Head/ Engineer	Biochemical Engineering	Has entered service since 2000 and engaged in products analysis	Lead the Food Safety (HACCP) Team and responsible for the implementation of HACCP, organize and guide the HACCP members to carry out tasks. Make HACCP implementation plan, organize and coordinate related work, compile and review the relevant documents such as HACCP, GMP, SSOP, test list etc. Responsible for the implementation of HACCP Plan, GMP, and SSOP, production monitoring plan etc. Verification of HACCP Plan.
Wan Luming	Production Department	Department Head/ Engineer	Food Engineering	Has entered service since 1995 and engaged in production management	Implementation of Corn Savory Base production. Approve production process and SOP. Approve and confirm HACCP Plan. Assign production task.
Wang Weigui	Equipment Department	Department Head/ Engineer	Applied Bio-technology	Has entered service since 2002 and engaged in instrument and equipment management	Responsible for the management and maintenance of plant, equipment and facilities, and the departmental documents establishment and revision, own department to implement HACCP Plan, GMP, and SSOP. Verification of HACCP Plan.
Zhang Xiaofan	Fermentation plant	Plant Manager	Applied Bio-technology	Has entered service since 1986 and engaged in microorganism fermentation and extraction.	Carry out hazard analysis for each processing step of Corn Savory Base products and develop preventive measures, responsible for the implementation of HACCP Plan, training plan, GMP, SSOP, pest control etc.

					Compile relevant operation documents as well as cleaning and disinfection document. Verification of the HACCP Plan.
Chen Wenyan	Downstream plant	Plant Manager /Engineer	Industrial fermentation	Has entered service since 1987 and engaged in fermentation and extraction production	Carry out hazard analysis for each processing step of Corn Savory Base products and develop preventive measures, responsible for the implementation of HACCP Plan, training plan, GMP, SSOP, pest control etc. Compile relevant operation documents as well as cleaning and disinfection document. Verification of the HACCP Plan.
Shi Jinling	Quality Department	Supervisor/ Engineer	Preventive Medicine and Hygiene Test	Began to work in Star Lake Bioscience Co., Inc. in 2000 and has engaged in quality management	Responsible for the assessment and review of suppliers; participate in implementations of the HACCP Plan that are relevant to the department. Verification of the HACCP Plan.
Xiao Zhiquan	Procurement Department	Head of Department /Engineer	Industrial fermentation	Has entered service since 1986 and engaged in production management	Responsible for communication with the suppliers, verification of the specification/ requirement of the raw materials purchased, and the signing of purchasing contract. Execute the relevant provisions of procurement control. Verification of the HACCP Plan.
Liu Jieting	Sales Department	Deputy General Manager	Marketing Management / Admin Management / E-commerce	Has entered service in 2003, and engaged in sales in 2004	Responsible for communication with the customers, verification of the specification/requirement of products, and the signing of sales contracts. Approve the product sales plan. Feedback of communication outcome to the group leader, participate in product recalls and the

					implementations of HACCP Plan that are relevant to the department. Verification of the HACCP Plan.

3. Corn Savory Base Products Descriptions

3.1 Product Characteristic

Product names：

a) Corn Savory Base 100 Paste
b) Corn Savory Base 200 Paste (D)
c) Corn Savory Base 200 Paste (L)
d) Corn Savory Base 110 Powder
e) Corn Savory Base 210 Powder (D)
f) Corn Savory Base 210 Powder (L)

Composition: Specific intrinsic mix of compounds, like free and bound amino acids, nucleotides, organic acids, Amadori and Maillard products, minerals and their salts, which have individually a more or less intense impact on overall taste.

Product description: A beige to brown uniform paste or powder. They are produced from liquid fermentation using bacteria strain. The enzymatically hydrolyzed corn starch is used as a primary substrate. It provides brothy taste and can be applied to a wide range of savory foods.

Production Method: Bacterial fermentation.

Raw materials: Substrates (corn starch), drinking water, salt (sodium chloride).

Processing aids: Amylase enzymes, pH regulation agent (liquid ammonia, sodium hydroxide), defoamer (PPE).

Culture nutrients: Glucose, corn steep powder, yeast extract, potassium chloride, magnesium sulfate, ammonium sulfate, manganese sulfate, ferrous sulfate, zinc sulfate, copper sulfate, succinic acid, β-alanine, plant polypeptides, vitamins, potassium dihydrogen phosphate, sodium dihydrogen phosphate, potassium hydroxide.

Packaging materials:

- For paste product, the inner packaging is food grade polyethylene film (PE) bag, Outer packaging is polypropylene (PP) drum.
- For powder product, the inner packaging is food grade aluminum coated metalized bag, covered by High density polyethylene (HDPE) bag, outer packaging is carton box or bag.

Packaging specification: Paste product 20kg/ drum; powder product 15 or 20kg/ cs.

Storage Condition: Store in a cool, dry, ventilated and clean warehouse, protect from direct sunlight and heat sources.

Transportation: Transport at ambient temperature, pest control, measures preventing exposure to dust, flies, the sun, and rain shall be taken during the transportation process. Mixed loading and transportation with toxic, harmful, and corrosive substances and their pollutants are strictly prohibited.

Sales method：Supply to manufacturers of food, condiment etc. as flavor or seasoning.

Shelf life：18 months for paste and powder products

Product labelling: Comply with the requirement of National Food Safety Standard GB7718 "General Standard for the Labeling of Prepackaged Foods"

Product quality：Comply with the requirements of Enterprise Standard Q/ZXH 0040 S “Corn Savory Base Paste” and Q/ZXH 0041 S “Corn Savory Base Powder”.

Relevant product safety indicators:

3.1.1 Paste product

Item	Specifications	
	Corn Savory Base 100 Paste	Corn Savory Base 200 Paste (D) and (L)
1. Loss in dying，%	27-34	27-32
2. pH value	5.0-7.0	
3. Water activity	≤0.75	
4. Heavy Metal (Pb)，mg/kg	≤10	
5. Arsenic (As)，mg/kg	≤0.5	
6. Microbial Limit	Total plate count ≤10000 CFU/g	
	Yeast and mold count ≤100 CFU/g	
	Enterobacteriaceae ≤10 CFU/g	
	Salmonella should not be detected in 25g	
7. Mycotoxins	Aflatoxin B+G ≤4 μg/kg	
	Deoxynivalenol ≤50 μg/kg	
	Zearalenone ≤20 μg/kg	
	Fumonisins B1+B2 ≤100 μg/kg	
	Ochratoxin A ≤0.5 μg/kg	

3.1.2 Powder product

Item	Specification	
	Corn Savory Base 110 Powder	Corn Savory Base 210 Powder (D) and (L)
1. Loss in dying，%	≤2.0	≤3.0

2. pH value	5.0-7.0
3. Heavy Metal (Pb)， mg/kg	≤10
4. Arsenic (As)， mg/kg	≤0.5
6. Microbial limit	Total plate count ≤100000 CFU/g
	Bacillus cereus ≤1000 CFU/g
	Enterobacteriaceae ≤100 CFU/g
	Yeast and mold count ≤100 CFU/g
	Salmonella should not be detected in 25g
7. Mycotoxin	Aflatoxin B+G ≤4 μg/kg
	Deoxynivalenol ≤50 μg/kg
	Zearalenone ≤20 μg/kg
	Fumonisins B1+B2 ≤100 μg/kg
	Ochratoxin A ≤0.5 μg/kg

3.2 Intended Use

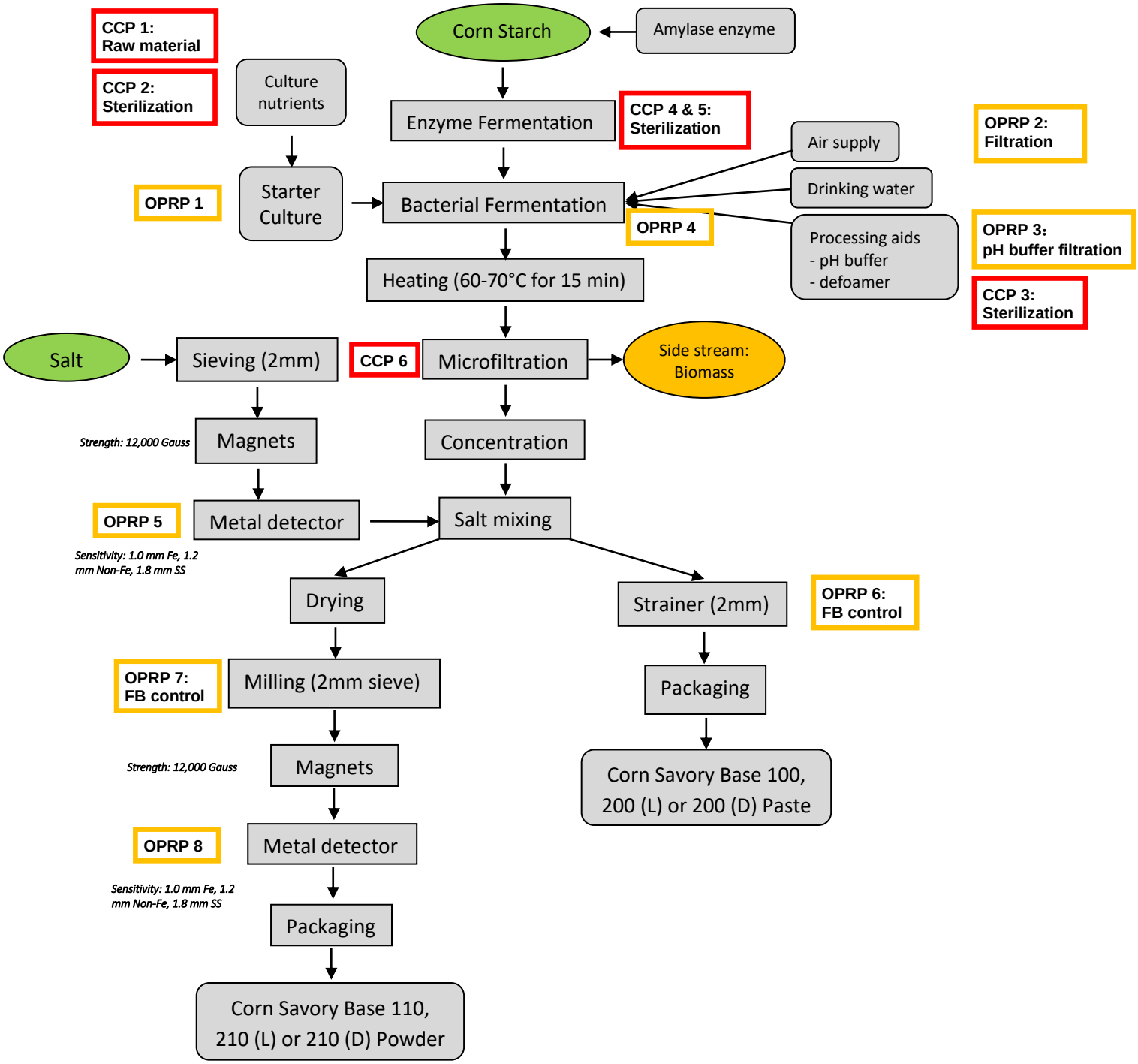
Corn Savory Base products are fermented preparation for use as food ingredient that provides savory taste and mouthfeel to culinary products. It complies with the market requirement for green and natural seasoning and improves the taste of food with the effect of increasing flavor and savory taste, it can be widely used in food, cooking and food processing.

3.3 Labelling

Labeling must be applied according to local legislation:

a) General recommendation is to label as food ingredient: Corn sauce (corn starch, salt)
b) Flavoring or natural flavoring if local regulation allows. Labelling in US: Corn sauce (cultured corn starch, salt).

4. Corn Savory Base Paste and Powder production flow chart:



Process description for the production of Corn Savory Base:

The continuous bacterial fermentation process is started similar to other food fermentation processes with the provision of the optimum grow media for the bacterial strains. In order to achieve this the food grade raw materials are pumped into a vessel, which has been sterilized before the start of the fermentation. The key substrate component, sterilized enzymatically hydrolyzed corn starch, which is produced prior through corn starch amylolytic hydrolysis, is added to the fermentation broth. The sterilized fermentation vessel, containing the nutrients and the substrate enzymatically hydrolyzed corn starch, is waiting to receive the bacterial culture to start to fermentation.

The starter culture itself is upfront undergoing a propagation period, in which biomass is produced to inoculate the fermentation vessel mentioned above at a sufficient high living cell number.

The fermentation starts at 35-37°C using some processing aids controlling the pH (e.g. ammonia gas, lye and acid) and controlling the foam, which is commonly built up during aerobic fermentation processes.

After end of fermentation time is reached the fermentation broth undergoes a heating step of 60-70°C for 15 minutes in order to inactivate the strain. Subsequently a filtration step is applied at 0.22 micrometer to remove the cell mass.

The broth is submitted to vacuum concentration (evaporation) at ≤20 mbar at 60°C in order to obtain a paste finally. During evaporation table salt is added to improve shelf life stability and microbial resistance against contaminants for global shipment. The paste is packed into 20 kg bucket and palletized for dispatch.

There is subsequent vacuum drying of the paste to produce dried powder. The powder is packed into 15 or 20 kg in box/ bag and palletized for dispatch.

There is the same process and ingredients used on both paste products. However, the products can be distinguished by different bacteria strain and fermentation duration as shown below:

Products	Corn Savory Base 100 Paste / Corn Savory Base 110 Powder	Corn Savory Base 200 Paste (L) and (D) / Corn Savory Base 210 Powder (L) and (D)
Bacteria strain code	540	560
Fermentation duration	30 - 35 hours at 35-37°C	90 - 96 hours at 35-37°C

5. CCP & OPRP Control and Verification Plan

CCP/OPRP	Hazard Description	Control measures	Critical Limiting Value	Monitoring Method	Frequency	Responsible Personnel	Corrective action	Record	Verification Procedure
CCP 1 **Inspect and accept the raw material**	Corn steep powder and plant polypeptides exceed mycotoxins limit	Choose qualified supplier	1. Corn steep powder DON ≤15000ppb ZEN ≤500ppb 2. Plant polypeptides AFT B+G ≤4ppb DON ≤1000ppb ZEN ≤1000ppb FUM ≤6000ppb OTA ≤10ppb	1. Ensure that the raw materials are from qualified supplier; 2. Ensure that each batch of raw material COA meets the specification requirements.	Each batch	Warehouse keeper Analyst QA	1. The QA submits the unqualified test report to the warehouse keeper, and the warehouse keeper will hang a red status card for this batch of material; 2. The general office will notify the procurement department, and the return process is initiated for the unqualified raw materials.	1. Test report of the raw materials 2. Test report of mycotoxins	1. Mycotoxins is tested for every batches of raw materials; 2. The raw materials can only be picked up after the warehouse keeper receives the qualified test report; 3. Mycotoxins is tested at one fermentation batch per month of the finished product for verification purpose.
CCP 2 **Culture nutrient sterilization**	Microbial contaminatio n from raw materials (pathogens and spore-derived pathogens)	Sterilization	Sterilization temperature: 135 – 145°C in steam heat exchanger system	Check the sterilization temperature	Each batch	Person on duty	1. Person on duty to report Plant manager and QA manager; 2. Person on duty immediately maintain the tank inlet steam pressure, and then open the return valve to allow the	1. Continuous sterilization data records 2. Foreign micro test records 3. Microscopic examination records	1. After the end of sterilization, take sample for plate count, to confirm sterility; 2. Take sample of inoculum (OPRP1) of the fermentation broth for microscopic examination, to confirm no foreign microorganism; 3. Take sample of

							remaining material back to the volumetric tank, re-adjust the steam pressure and liquid flow rate, so that the liquid sterilization temperature reaches 135-145 ℃.		fermentation broth when inoculation for plate count, to confirm no foreign microorganism.
CCP 3 **Defoamer sterilization**	Microbial contamination from defoamer (pathogens and spore-derived pathogens)	Sterilization	Sterilization temperature: 125 – 132°C Sterilization time: 45-60 minutes	Check the sterilization temperature and time	Each batch	Person on duty	1. Person on duty to report Plant manager and QA manager; 2. When the sterilization temperature is lower than 125 °C, person on duty immediately open the steam inlet valve much so as to increase the sterilization temperature higher than 125 °C, then start the sterilization time again; when the sterilization temperature is higher than 132 °C, personnel immediately close the steam inlet valve much so as to decrease the sterilization temperature lower than 132 °C.	1. Sterilization reading data records 2. Microscopic examination records	1. Take sample of the fermentation broth (OPRP4) for microscopic examination, to confirm no foreign microorganism.

CCP 4 **Substrate sterilization**	Microbial contamination from raw materials (pathogens and spore-derived pathogens)	Sterilization	Sterilization temperature: 135 – 145°C in steam heat exchanger system	Check the sterilization temperature	Each batch	Person on duty	1. Person on duty to report Plant manager and QA manager; 2. Person on duty immediately maintain the tank inlet steam pressure, and then open the return valve to allow the remaining material back to the volumetric tank, re-adjust the steam pressure and liquid flow rate, so that the liquid sterilization temperature reaches 135-145 ℃.	1. Continuous sterilization data records 2. Microbial test records 3. Microscopic examination records	1. At the end of sterilization, take sample for plate count, to confirm sterility; 2. Take sample of the fermentation broth (OPRP4) for microscopic examination, to confirm no foreign microorganism.
CCP 5 **Feed substrate sterilization**	Microbial contamination from raw materials (pathogens and spore-derived pathogens)	Sterilization	Sterilization temperature: 135 – 145°C in steam heat exchanger system	Check the sterilization temperature	Each batch	Person on duty	1. Person on duty to report Plant manager and QA manager; 2. Person on duty immediately maintain the tank inlet steam pressure, and then open the return valve to allow the remaining material back to the volumetric tank,	1. Continuous sterilization data records 2. Microscopic examination records	1. Take sample of the fermentation broth (OPRP4) for microscopic examination, to confirm no foreign microorganism.

							re-adjust the steam pressure and liquid flow rate, so that the liquid sterilization temperature reaches 135-145 ℃.		
CCP 6 **Microfiltration**	The targeted bacteria from the fermentation broth are non-direct edible microorganism Inorganic membrane shedding, free stone, metal impurity, glass	Micro-filtration	1. membrane pore sizes: 0.22μm 2. Membrane Inlet pressure: ≤0.40MPa	1. Check the precision of the membrane tube; 2. Record the membrane inlet pressure gauge values	1. Upon installation or replacement 2. Every hour	Person on duty	1. Person on duty to report Plant manager and QA manager. 2. Stop running the membrane filter, empty the material after dismantling and examine the filter to inspect the integrity of the filter element, and carry out the corresponding filter element cleaning or replacement. 3. Ensure that the material liquid that deviates from the critical limiting value and has entered the concentration system is re-cycled into the pre-filtration tank and re-filter again.	1. Precision certificate of membrane tube; 2. Pressure gauge calibration certificate; 3. Fermentation broth filtration operation record	1. Verification of membrane filtration efficiency; 2. The pressure gauge is calibrated once a year; 3. Check the clarity of the filtrate and confirm that it must not exceed the reference.

OPRP 1 **Inoculum cultivation**	External microbial contamination (pathogens and spore-derived pathogens)	Maintain the positive pressure of the cultivation tank	Cultivation tank pressure value: 0.03 - 0.10MPa	1. Computer automatic control system to record tank pressure; 2. Manually record the tank pressure value.	1. Every hour 2. The entire process	Person on duty	1. Person on duty to report Plant manager and QA manager; 2. When the tank pressure is lower than 0.03 MPa, the operator immediately fully shut down the exhaust valve manually or through computer-controlled system, and open the large air inlet valve, until tank pressure rises back to the critical limit level after re-open the exhaust valve; when the tank pressure is higher than 0.10 MPa, immediately increase the exhaust valve, increase the exhaust volume from fermentation tank manually or through computer-controlled system.	1. Pressure gauge calibration certificate 2. Fermentation batch reports 3. Microscopic examination records	1. The pressure gauge is calibrated once a year; 2. Take sample of the cultivate broth for microscopic examination, to confirm no foreign microorganism.
OPRP 2 **Ultra-filtration of compressed**	Microbial contamination from air (pathogens and spore-derived	Filtration	Filter pore size 0.01µm	1. Examine the filter precision certificate; 2. Regular	1. Upon Installation 2. Every 2 months	Person on duty	Person on duty to replace a new fine filter with pore size of 0.01µm.	1. Precision certificate of filtration unit; 2. Public	Take sample of the inoculum culture (OPRP1), fermentation broth (OPRP4) and fermentation filtrate, to

air (0.01µm)	pathogens)			dismantling for inspection				system periodical working condition record	confirm no foreign microorganism.
OPRP 3 **Ultra-filtration of pH buffer (0.01µm)**	Foreign body from ammonia liquid (sand and stone)	Filtration	Filter pore size 0.01µm	1. Examine the filter precision certificate; 2. Regular dismantling for inspection	1. Upon Installation 2. Every 2 months	Person on duty	Person on duty to replace a new fine filter with pore size of 0.01µm.	1. Precision certificate of filtration unit; 2. Public system periodical working condition record	Take sample of the inoculum culture (OPRP1), fermentation broth (OPRP4) and fermentation filtrate, to confirm no foreign microorganism.
OPRP 4 **Fermentation**	External Microbial contamination (pathogens and spore-derived pathogens)	Maintain the positive pressure of the fermentation tank	Fermentation tank pressure value: 0.03 - 0.10MPa	1. Computer automatic control system to record tank pressure; 2. Manually record the tank pressure value.	1. Every hour 2. The entire process	Person on duty	1. Person on duty to report Plant manager and QA manager; 2. When the tank pressure is lower than 0.03 MPa, the operator immediately fully shut down the exhaust valve manually or through computer-controlled system, and open the large air inlet valve, until tank pressure rises back to the critical limit level after re-open the exhaust	1. Pressure gauge calibration certificate; 2. Fermentation batch reports 3. Microscopic examination records	1. The pressure gauge is calibrated once a year; 2. Take sample of the fermentation broth for microscopic examination, to confirm no foreign microorganism.

							valve; When the tank pressure is higher than 0.10 MPa, immediately increase the exhaust valve, increase the exhaust volume from fermentation tank manually or through computer-controlled system.		
OPRP 5 **Metal detector for salt**	Metallic foreign body from the equipment and salt	Online elimination	Iron Φ 1.0 mm, non-iron Φ 1.2 mm, stainless steel Φ 1.8 mm.	Each batch of products before and after packaging; the sensitivity is tested by standard test method; functional check and debug when metal detector fails.	Throughout whole production	Person on duty	1. Person on duty to report Plant manager and QA manager; 2. When the metal detector is not working in normal condition, the affected product should be detained, and the affected product will be re-tested after maintenance qualifying and the system back to normal; 3. Detain the products with triggered metal detection, unpack and sieve out the metal foreign body, re-pack and pass through metal	Metal detector sensitivity correction record	Shift supervisor to review the monitoring verification record

							detector again. Meanwhile, fill in the investigation and processing report of deviation from the critical limit, to investigate the root cause of deviation, identify the type of foreign bodies, sources, and make appropriate corrective and preventive measures. 4. Educate and train operators to strictly follow the SOP, all deviations from the critical limit during operation must be immediately rectified and restored to the scope of the critical limits.		
OPRP 6 **Paste sieving (10 mesh)**	Screws from the equipment, view glass	sieving	No external hard and sharp foreign body (≥2 mm)	Visual inspect the screen to ensure correct pore size without defect.	1. Before packaging 2. After packaging	Person on duty	1. Person on duty to report Plant manager and QA manager; 3. To replace the sieve, the sieved materials will be dissolved, evaporated, and/or dried again and then packing.	Paste Product Packaging Record	Visual inspect on sieve integrity.

OPRP 7 **Milling and sieving (10 mesh)**	Screws from the equipment, dryer belt	sieving	No external hard and sharp foreign body (≥2 mm)	Visual inspect the screen to ensure correct pore size without defect.	1. Before packaging 2. After packaging	Person on duty	1. Person on duty to report Plant manager and QA manager; 3. To replace the sieve, the sieved materials will be dissolved, evaporated, and/or dried again and then packing.	Powder Product Packaging Record	Visual inspect on sieve integrity.
OPRP 8 **Metal detector for powder**	Metallic foreign body from the equipment	Online elimination	Iron Φ 1.0 mm, non-iron Φ 1.2 mm, stainless steel Φ 1.8 mm.	Each batch of products before and after packaging; the sensitivity is tested by standard test method; functional check and debug when metal detector fails.	Throughout whole production	Person on duty	1. Person on duty to report Plant manager and QA manager; 2. When the metal detector is not working in normal condition, the affected product should be detained, and the affected product will be re-tested after maintenance qualifying and the system back to normal; 3. Detain the products with triggered metal detection, unpack and sieve out the metal foreign body, re-pack and pass through metal detector again. Meanwhile, fill in the	Metal detector sensitivity correction record	Shift supervisor to review the monitoring verification record

							investigation and processing report of deviation from the critical limit, to investigate the root cause of deviation, identify the type of foreign bodies, sources, and make appropriate corrective and preventive measures. 4. Educate and train operators to strictly follow the SOP, all deviations from the critical limit during operation must be immediately rectified and restored to the scope of the critical limits.		

（Note：AFT B+G is Aflatoxin B+G，DON is Deoxynivalenol，ZEN is Zearalenone，FUM is Fumonisin B1+B2，OTA is Ochratoxin A）

Compositional breakdown for 3 Non-Consecutive Lots of Savory Base 100			
Parameter (values given on a dry weight basis)	**Manufacturing Lot**		
	G151002[a]	**G160302[b]**	**G160304[c]**
Amino acid profile (total)			
L-Glutamic acid (%)	39.50	38.30	40.40
L-Aspartic acid (%)	<0.09	0.11	<0.09
L-Threonine (%)	<0.09	<0.09	<0.09
L-Serine (%)	0.10	0.10	<0.09
Glycine (%)	<0.09	0.16	<0.09
L-Alanine (%)	1.37	1.22	0.84
L-Cysteine (%)	<0.03	<0.03	<0.03
L-Valine (%)	<0.09	0.12	<0.09
L-Methionine (%)	<0.03	<0.03	<0.03
L-Isoleucine (%)	<0.09	<0.09	<0.09
L-Leucine (%)	<0.09	<0.09	<0.09
L-Tyrosine (%)	<0.09	<0.09	<0.09
L-Phenylalanine (%)	<0.09	<0.09	<0.09
L-Lysine (%)	<0.09	0.11	0.10
L-Histidine (%)	<0.09	<0.09	<0.09
L-Tryptophan (%)	0.00	0.01	0.01
L-Proline (%)	1.24	0.61	0.68
L-Arginine (%)	<0.09	<0.09	<0.09
Total amino acids (%)	**42.21**	**40.73**	**42.03**
Amino acid profile (free)			
L-Glutamic acid (%)	37.00	37.20	39.70
L-Aspartic acid (%)	0.02	0.03	0.03
L-Threonine (%)	<0.01	<0.01	<0.01
L-Serine (%)	0.01	<0.01	0.02
Glycine (%)	0.02	0.03	0.02
L-Alanine (%)	1.23	0.98	0.82
L-Cystine (%)	<0.01	<0.01	<0.01
L-Valine (%)	0.02	0.02	0.01
L-Methionine (%)	<0.01	0.05	0.05
L-Isoleucine (%)	<0.01	<0.01	<0.01
L-Leucine (%)	0.01	0.01	0.01
L-Tyrosine (%)	<0.01	<0.01	<0.01
L-Phenylalanine (%)	<0.01	<0.01	<0.01
L-Lysine (%)	0.02	0.08	0.10
L-Histidine (%)	<0.01	<0.01	<0.01
L-Tryptophan (%)	0.00	0.00	0.00
L-Asparagine (%)	<0.001	<0.001	<0.001
L-Glutamine (%)	<0.001	<0.001	<0.001
L-Proline (%)	1.05	0.43	0.65
L-Cysteine (%)	<0.03	<0.03	<0.03
L-Arginine (%)	<0.01	<0.01	<0.01

Compositional breakdown for 3 Non-Consecutive Lots of Savory Base 100			
Parameter (values given on a dry weight basis)	**Manufacturing Lot**		
	G151002[a]	**G160302[b]**	**G160304[c]**
Total free amino acids (%)	**39.39**	**38.83**	**41.41**
Mineral profile			
Sodium (%)	4.03	4.79	4.57
Potassium (%)	0.94	1.00	0.89
Magnesium (%)	0.06	0.07	0.06
Calcium (%)	0.02	0.02	0.02
Chloride (%)	3.32	3.65	4.11
Phosphate (%)	0.49	0.54	0.45
Sulphate (%)	0.15	0.20	0.14
Total minerals (%)	**9.00**	**10.27**	**10.23**
Organic acid profile			
Citric acid (%)	<0.04	<0.04	<0.04
Malic acid (%)	<0.04	<0.04	<0.04
Succinic acid (%)	0.56	0.61	0.55
Lactic acid (%)	<0.04	<0.04	<0.04
Formic acid (%)	1.00	0.73	0.42
Acetic acid (%)	<0.04	<0.04	<0.04
Total organic acids (%)	**1.56**	**1.34**	**0.97**
Sugar profile			
Fructose (%)	<0.10	<0.10	<0.10
Glucose (%)	<0.10	<0.10	<0.10
Sucrose (%)	<0.10	<0.10	<0.10
Maltose (%)	<0.10	<0.10	<0.10
Total sugars (%)	**<0.10**	**<0.10**	**<0.10**

[a] Manufacturing date: October 18, 2015;
[b] Manufacturing date: March 2, 2016;
[c] Manufacturing date: March 3, 2016

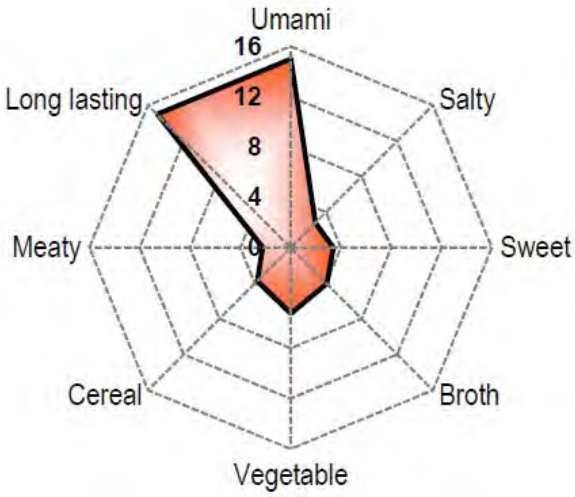
Compositional breakdown for 3 Representative Lots of Savory Base 200			
Parameter (values given on a dry weight basis)	**Manufacturing Lot**		
	I151203[a]	**I160305[b]**	**I160306[c]**
Amino acid profile (total)			
L-Glutamic acid (%)	1.91	2.06	2.35
L-Aspartic acid (%)	0.16	0.23	0.22
L-Threonine (%)	<0.08	<0.09	<0.09
L-Serine (%)	<0.08	0.12	0.11
Glycine (%)	4.71	4.08	4.09
L-Alanine (%)	0.32	0.49	0.49
L-Cystine (%)	0.09	0.07	0.07
L-Valine (%)	<0.08	0.42	0.16
L-Methionine (%)	0.25	0.22	0.27
L-Isoleucine (%)	<0.08	<0.09	<0.09
L-Leucine (%)	<0.08	0.12	0.09
L-Tyrosine (%)	<0.08	<0.09	<0.09
L-Phenylalanine (%)	<0.08	<0.09	<0.09
L-Lysine (%)	<0.08	<0.09	<0.09
L-Histidine (%)	<0.08	<0.09	<0.09
L-Tryptophan (%)	0.02	0.02	0.02
L-Proline (%)	<0.4	0.13	0.20
L-Arginine (%)	<0.08	<0.09	<0.09
Total amino acids (%)	**7.46**	**7.95**	**8.07**
Amino acid profile (free)			
L-Glutamic acid (%)	0.72	0.63	0.62
L-Aspartic acid (%)	0.02	0.02	0.02
L-Threonine (%)	0.01	0.02	0.01
L-Serine (%)	0.01	0.02	0.02
Glycine (%)	0.03	0.02	0.03
L-Alanine (%)	0.08	0.09	0.11
L-Cystine (%)	<0.01	<0.01	<0.01
L-Valine (%)	<0.01	0.28	0.06
L-Methionine (%)	<0.01	0.01	<0.01
L-Isoleucine (%)	<0.01	0.02	<0.01
L-Leucine (%)	0.01	0.04	0.02
L-Tyrosine (%)	<0.01	0.01	<0.01
L-Phenylalanine (%)	0.03	0.02	0.01
L-Lysine (%)	<0.01	0.01	<0.01
L-Histidine (%)	<0.01	<0.01	<0.01
L-Tryptophan (%)	0.00	0.01	0.00
L-Asparagine (%)	<0.01	<0.01	<0.01
L-Glutamine (%)	0.09	0.03	0.05
L-Proline (%)	<0.03	0.07	0.15

Compositional breakdown for 3 Representative Lots of Savory Base 200			
Parameter (values given on a dry weight basis)	**Manufacturing Lot**		
	I151203[a]	**I160305[b]**	**I160306[c]**
L-Cysteine (%)	<0.03	<0.03	<0.03
L-Arginine (%)	<0.01	<0.01	<0.01
Total free amino acids (%)	**1.02**	**1.29**	**1.10**
Organic acid profile			
Citric acid (%)	<0.04	<0.04	<0.04
Malic acid (%)	<0.04	<0.04	<0.04
Succinic acid (%)	<0.02	<0.02	<0.02
Lactic acid (%)	<0.04	<0.04	<0.04
Formic acid (%)	0.79	0.69	0.63
Acetic acid (%)	<0.04	<0.04	<0.04
Total organic acids (%)	**0.79**	**0.69**	**0.63**
Sugar profile			
Fructose (%)	<0.10	<0.10	<0.10
Glucose (%)	<0.10	<0.10	<0.10
Sucrose (%)	<0.10	<0.10	<0.10
Maltose (%)	<0.10	<0.10	<0.10
Total sugars (%)	**0.00**	**0.00**	**0.00**
Ribonucleotide profile			
IMP anhydrous (%)	21.50	21.40	21.80
AMP (%)	0.55	0.64	0.46
GMP (%)	0.11	0.11	0.81
CMP (%)	0.44	0.35	0.51
UMP (%)	0.12	0.81	0.15
Total ribonucleotides (%)	**22.72**	**23.31**	**23.73**
Mineral profile			
Sodium (%)	4.68	6.25	6.09
Potassium (%)	3.29	2.43	2.05
Magnesium (%)	0.14	0.13	0.14
Calcium (%)	0.02	0.02	0.02
Chloride (%)	3.19	4.24	4.34
Phosphate (%)	11.30	10.10	10.10
Sulfate (%)	1.98	1.41	1.05
Total minerals (%)	**24.60**	**24.58**	**23.79**

[a] Manufacturing date: December 18, 2015
[b] Manufacturing date: March 12, 2016
[c] Manufacturing date: March 16, 2016

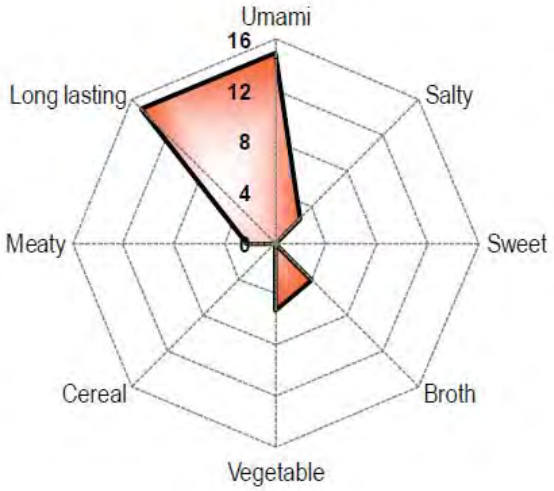
FLAVOUR PROFILE

Taste evaluation: 1 % in hot water.



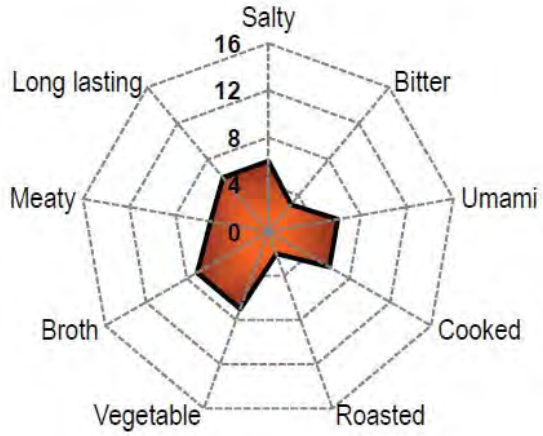
FLAVOUR PROFILE

Taste evaluation: 1 % in hot water.

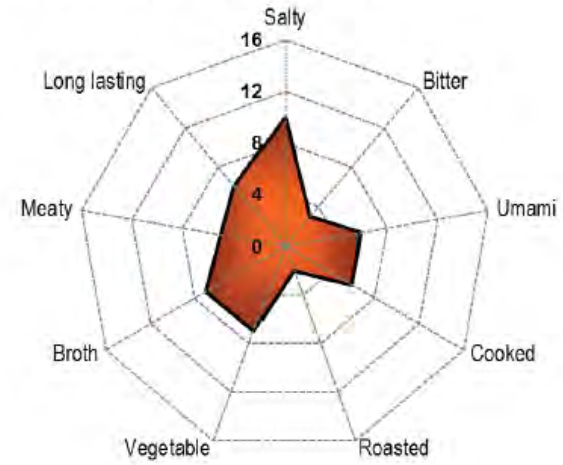


FLAVOUR PROFILE

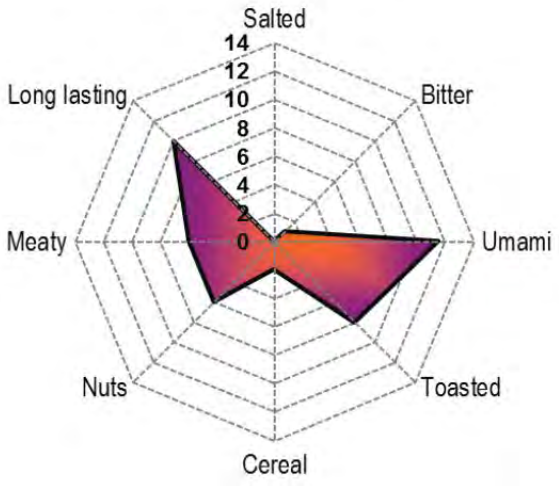
Taste evaluation: 1 % in hot water.



Taste evaluation: 1 % in hot water.



Taste evaluation: product as it is.



FLAVOUR PROFILE

Taste evaluation: 1 % in hot water.