GRAS Notice for a Pyrroloquinoline Quinone (PQQ) Disodium Salt

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
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Submitted by:

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255 Oser Avenue
Hauppauge, New York
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February 23, 2017

GRAS Notice for Pyrroloquinoline Quinone (PQQ) Disodium Salt

Table of Contents

List of Figures and Tables

Appendices

GRAS Notice for Pyrroloquinoline Quinone (PQQ) Disodium Salt

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Summit Life Science, Inc hereby informs the United States (U.S.) Food and Drug Administration (FDA) of the view that pyrroloquinoline quinone (PQQ) disoium salt, manufactured by Fuzhou Contay Biotechnology Co., Ltd (Fuzhou Contay hereafter), is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Fuzhou Contay's 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 Summit Life Science, Inc, 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 Fuzhou Contay and pertinent to the evaluation of the safety and GRAS status of PQQ disodium salt as an ingredient for addition to food, as described herein.

Signed,

(b) (6)

2/22/2017

Jimmy Wang
Chief Scientific Officer
Summit Life Science, Inc
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Date

1.1 Name and Address of Notifier

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1.2 Common Name of Notified Substance

Pyrroloquinoline quinone (PQQ) disodium salt

1.3 Conditions of Use

PQQ disodium salt is intended for use in the U.S. in beverages and beverage bases, such as energy, sport, and isotonic drinks, non-milk based meal replacement beverages, and water beverages (bottled, enhanced, fortified) at use levels of up to 0.083%. The intended food categories and use levels at which Fuzhou Contay's PQQ disodium salt will be added are summarized in Table 1.3-1.

Table 1.3-1 Summary of the Individual Proposed Food-Uses and Use Levels for PQQ Disodium Salt in Conventional Food and Beverage Products

Food Category	Proposed Food-Uses	Maximum Intended Use Level of PQQ Disodium Salt (%) in Final Product
Beverages and Beverage Bases	Energy, Sport, and Isotonic Drinks	0.033
	Non-Milk Based Meal Replacement Beverages	0.033
	Water Beverages (Bottled, Enhanced, Fortified)	0.083

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR), PQQ disodiums salt manufactured by Fuzhou Contay, has been concluded to have GRAS status for use as an ingredient for addition to specified conventional beverage and beverage bases as described in Part 1.3, on the basis of scientific procedures (U.S. FDA, 2016a).

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:

Summit Life Science, Inc
255 Oser Avenue
Hauppauge, New York
11788

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, Summit Life Science, Inc will supply these data and information.

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

It is Summit Life Science, Inc 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

Pyrroloquinoline quinone (PQQ) disodium salt is produced *via* fermentation by the non-pathogenic bacteria, *Hyphomicrobium* sp.

2.2 Common or Usual Name

Pyrroloquinoline quinone (PQQ) disodium salt

2.3 Chemical Name

Disodium 4,5-dihydro-4,5-dioxo-1h-pyrrolo(2,3-f) tricarboxylate

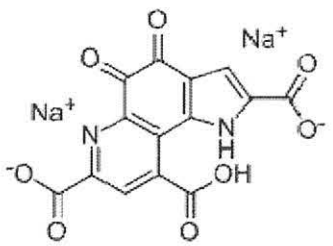
2.4 Chemical Abstract Service (CAS) Number

122628-50-6

2.5 Molecular Weight

374.17 g/mol

2.6 Molecular Structure



2.7 Method of Manufacturing

2.7.1 Production Organism

Fuzhou Contay utilizes a strain of the genus *Hyphomicrobium* in the production of its PQQ disodium salt. The taxonomy of its production strain is shown in Table 2.7.1-1.

Table 2.7.1-1 Taxonomy of Fuzhou Contay's Production Organism

Kingdom	*Bacteria*
Phylum	*Proteobacteria*
Class	*Alphaproteobacteria*
Order	*Rhizobiales*
Family	*Hyphomicrobiaceae*
Genus	*Hyphomicrobium*
Species	*Hyphomicrobium denitrificans*

The genus *Hyphomicrobium* consists of facultatively methylotrophic, non-spore-forming, gram-negative, polarly flagellated, hyphal, budding, rod-shaped organisms that have a Q-9 ubiquinone system (Urakami and Komagata, 1979, 1986, 1987). The 16S ribosomal DNA (rDNA) sequence of the production strain is 99% similar to that of ATCC 5188 (DSM 1869/NCIB 11706/TK 0415). Further morphological and biochemical analyses demonstrate that *Hyphomicrobium denitrificans* is a gram-negative bacterium that forms milky colonies and is positive for nitrate reduction. *H. denitrificans* is not a genetically modified organism.

The *Hyphomicrobium* used for the production of PQQ disodium salt is maintained in-house by Fuzhou Contay and is subject to strict quality control for compliance with established internal specifications and is free of microbial contamination. The specifications for the fermentation organism, *Hyphomicrobium sp.,* are detailed in Table 2.7.1-2

Table 2.7.1-2 Control of Critical Parameters of Strain Used in Production

Item	**Specification**
Appearance	Milky and abundant.
Growth characteristics	Thick and strong, deep staining mycelium, without contamination.
Survival ratio	≥95%
Fermentation potency	≥125 μg/mL
Microbial contamination	Without any other microorganisms.

2.7.2 Raw Materials and Processing Aids

The raw materials and processing aids used in the manufacture of the PQQ disodium salt are listed in Table 2.7.2-1. All raw materials and processing aids are food-grade quality and are safe and suitable for use in the manufacture of food ingredients consistent with appropriate U.S. federal regulations, or have previously been determined to be GRAS.

Table 2.7.2-1 Processing Aids/Additives Used During Manufacturing

Material	Function	Regulatory Status
Fermentation Aids		
Ammonium sulfate $(NH_4)_2SO_4$	Processing-aid (nitrogen source for fermentation	§184 – Direct food substances affirmed as generally recognized as safe Permitted for use in foods as a dough strengthener, firming agent, and processing aid in accordance to cGMP (21 CFR §184.1143) (U.S. FDA, 2016a)
Magensium sulfate $MgSO_4 \cdot 7H_2O$	Processing-aid (Fermentation nutrient)	21 CFR §184 – Direct food substances affirmed as generally recognized as safe Permitted for use in foods as a flavor enhancer, nutrient supplement, or processing aid in accordance to cGMP (21 CFR §184.1443) (U.S. FDA, 2016a)
Calcium chloride $CaCl_2 \cdot 2H_2O$	Processing-aid (Fermentation nutrient	21 CFR §184 -Direct food substances affirmed as generally recognized as safe Permitted for use in foods as an anti-caking agent, antimicrobial agent, curing or pickling agent, firming agent, flavor enhancer, humectant, nutrient supplement, pH control agent, processing aid, stabilizer and thickener, surface-active agent, synergist, and texturizer not to exceed cGMP (21 CFR §184.1193) (U.S. FDA, 2016a)
Agar	Processing-aid (preparation of working inoculum)	FCC 9th ed. (FCC, 2014)
Methanol	Processing-aid (carbon source for fermentation)	Methanol is permitted for use in foods as a GRAS substance when used in accordance with cGMP (21 CFR §182.1) (U.S. FDA, 2016a)
Polyether glycol	Anti-foaming	Anti-foaming agent is permitted for use in the processing of foods (21 CFR §173.340) (U.S. FDA, 2016a)
Potassium Phophate K_2HPO_4	Processing-aid (Fermentation nutrient)	This substance is generally recognized as safe when used in accordance with good manufacturing practice (21CFR§182.6285) (U.S. FDA, 2016a)
Ammonia water	Processing-aid (nitrogen source for fermentation)	pH control – FCC 9th ed. (FCC, 2014)
Purification Aids		
Sodium chloride	Crystallization	Sodium chloride is a GRAS substance when used in accordance with cGMP (21 CFR §182.1) (U.S. FDA, 2016a)
Anion exchange resin	Purification	Ion-exchange resin permitted for use in the treatment of food under 21 CFR §173.25 (U.S. FDA, 2016a)
Ethanol	Crystallization	Ethanol is permitted for use in foods as a GRAS substance when used in accordance with cGMP (21 CFR §182.1) (U.S. FDA, 2016a)

Table 2.7.2-1 Processing Aids/Additives Used During Manufacturing		
Material	**Function**	**Regulatory Status**
Sodium hydroxide	pH	GRAS substance and permitted for use in accordance with cGMP (21 CFR §184.1763) (U.S. FDA, 2016a)
Hydrochloric acid	pH	GRAS for use in foods as a buffer and neutralizing agent in accordance with cGMP (21 CFR §182.1057) (U.S. FDA, 2016a).
Activated carbon	Adsorption	FCC 9th ed. (FCC, 2014)

cGMP = current Good Manufacturing Practice; GRAS = Generally Recognized as Safe

2.7.3 Manufacturing Process

The production of Fuzhou Contay's PQQ disodium salt is a modification of the production of PQQ by bacterial fermentation reported by several other investigators (Ameyama *et al.*, 1984; Urakami *et al.*, 1992; Noji *et al.*, 2007) and is similar to that described in GRN 641 (U.S. FDA, 2016c). Fuzhou Contay's PQQ disodium salt is manufactured consistent with the principles of Hazard Analysis and Critical Control Points (HACCP) and current Good Manufacturing Practices (cGMP).

2.7.3.1 Preparation of Working Cell Bank and Slant Culture

The working cell bank (WCB) is prepared by dissolving food-grade raw materials in purified water. Sodium hydroxide solution is added to adjust pH. Following sub-loading in a clean flask, the tube well is packaged with kraft paper and sterilized at 121 ± 2°C for 30 minutes. The medium is cooled and sterile methanol is added. Under aseptic condition, the WCB is drawn from master cell bank with a pipette and transferred to the prepared medium. Following inoculation in a shake flask, the slant culture is produced by inoculating the WCB onto another blank slant using the same culture medium and incubation conditions as the production of the WCB.

2.7.3.2 Culturing in Seed Tank and Fermentation

Food-grade raw materials are poured into seed tank. Purified water is added to dissolve and dilute to scale. Sodium hydroxide solution is added to adjust pH and the mixture is sterilized at 121 to 123°C for 30 minutes. After cooling, sterile and filtered methanol is added. The shaken flask culture is transferred to seed tank under aseptic conditions.

2.7.3.3 Fermentation

To prepare fermentation broth, raw materials are added to the fermentation tank. Purified water is dissolved and diluted and sodium hydroxide is added to adjust pH. The broth is sterilized at

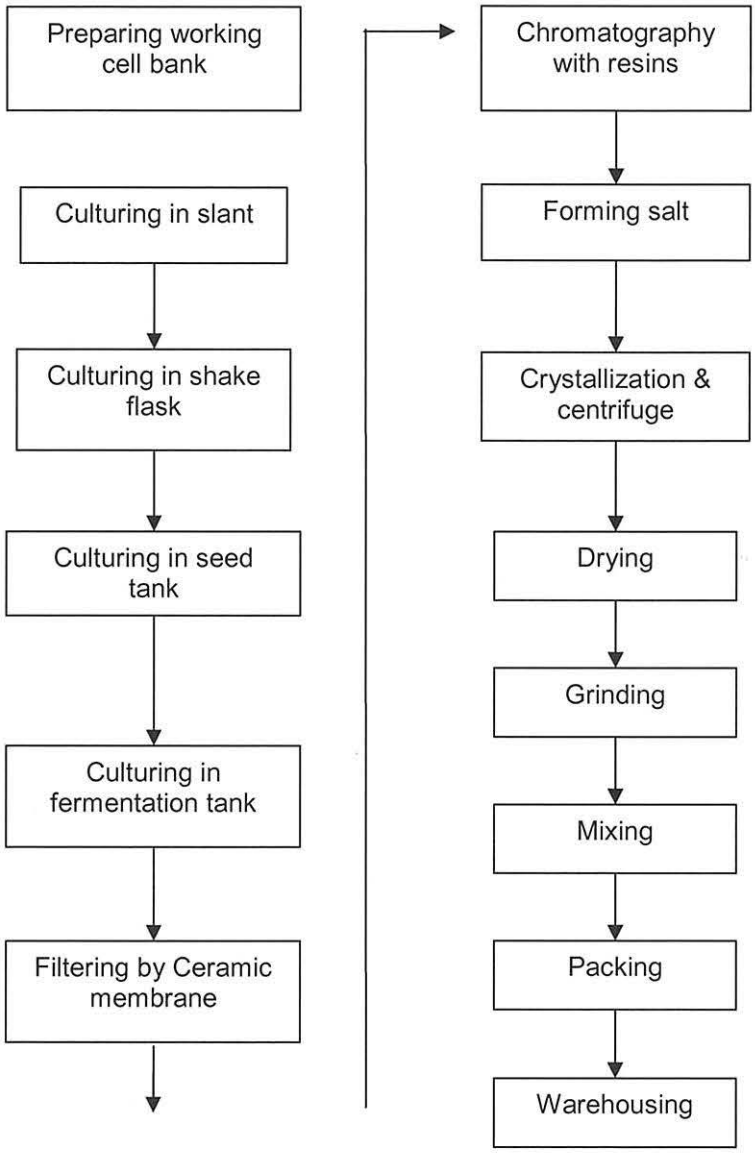
121 to 123°C for 30 minutes. After cooling, methanol is added. The seed tank broth is transferred to fermentation tank by transferring pipeline.

2.7.3.4 Extraction and Purification

PQQ is isolated and purified through a series of filtration steps through a ceramic membrane followed by resin adsorption and elution (with a sodium phosphate buffer solution). The fermentation broth is filtered by ceramic membrane to remove the source organism. The filtrate is collected. The residue is dissolved with phosphate buffer and adsorbed on resin to make a gradient elution. The eluant is collected and PQQ disodium salt is formed by adding sodium chloride to the eluant, adjusting pH with hydrochloric acid, and stirring. Crystals are removed by centrifugation and filtration. The salt is dissolved in purified water and filtered by membrane filtration. Hydrochloric acid is then added to adjust pH. The wet product is obtained after centrifugation and filtration and is then vacuum dried, granulated. Following an additional drying step, the PQQ disodium salt product is sieved, mixed, and packaged.

A schematic overview of the manufacturing process of PQQ disodium salt is provided in Figure 2.7.3-1.

Figure 2.7.3-1 Process Flow Diagram for PQQ Disodium Salt



2.7.4 Quality Control

PQQ disodium salt is manufactured in compliance with cGMP as part of a preventative controls approach. In addition, Fuzhou Contay utilizes quality management systems based on the principles of HACCP standards.

2.8 Product Specifications and Batch Analyses

2.8.1 Proposed Product Specifications

The product specifications for Fuzhou Contay's PQQ disodium salt are detailed in Table 2.8.1-1. All methods of analyses are nationally or internationally recognized or have been validated by Fuzhou Contay. The high performance liquid chromatography purity of PQQ disodium salt is ≥99%. Appropriate limits for heavy metals and microbial impurities have been established. Residual ethanol concentrations are limited by specification to 5,000 ppm.

Table 2.8.1-1 Product Specifications

Items	Specifications	Method
Description	Henna Powder	Visual
Identification	IR spectrum of test specimen should be consistent with that of reference standard.	USP<197K>
Chromatographic Purity	≥99.0%	In house HPLC
Water content	≤12.0%	USP<731>
Heavy metals	NMT 10 ppm	USP<231>
Arsenic	≤1.5 ppm	In house ICP MS
Lead	≤1.0 ppm	In house ICP MS
Cadmium	≤0.3 ppm	In house ICP MS
Mercury	≤0.2 ppm	In house ICP MS
Ethanol	≤5,000 ppm	In house GC
Total Aerobic Plate count	≤10,000 cfu/gm	USP<61>
Yeast and molds	≤1,000 cfu/gm	USP<61>
Enterrobacteriaceae	≤100 cfu/gm	USP<62>
Escherichia coli	Absent per 10 gm	USP<62>
Salmonella	Absent per 10 gm	USP<62>
Staphylococcus aureus	Absent per 10 gm	USP<62>

cfu = colony forming units; gm = gram; HPLC = high performance liquid chromatography; IR = infrared; NMT = not more than; ppm = parts per million

2.8.2 Batch Analyses

Three (3) non-consecutive batches of the PQQ disodium salt were analyzed to verify that the manufacturing process produces a consistent product that meets the proposed product specifications. The results of the batch analyses are provided in Table 2.8.2-1.

Table 2.8.2-1 Summary of the Chemical Product Analysis for 3 Lots of PQQ Disodium Salt

Item	Specification	Batch		
		VH1606001	VH1607001	VH1608001
Description	Henna Powder	Conforms	Conforms	Conforms
Identification	IR spectrum of test specimen should consistent with that of reference standard.	Conforms	Conforms	Conforms
Chromatographic Purity	≥99.0%	99.86%	99.90%	99.96%
Water content	≤12.0%	9.8%	9.7%	9.8%
Heavy metals	NMT 10 ppm	<10 ppm	<10 ppm	<10 ppm
Arsenic	≤1.5 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm
Lead	≤1.0 ppm	0.37 ppm	<0.1 ppm	0.44 ppm
Cadmium	≤0.3 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm
Mercury	≤0.2 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Ethanol	≤5,000 ppm	1,200 ppm	1,100 ppm	1,000 ppm
Total Aerobic Plate count	≤1,000 cfu/gm	100 cfu/gm	50 cfu/gm	200 cfu/gm
Yeast and molds	≤100 cfu/gm	<50 cfu/gm	<50 cfu/gm	<50 cfu/gm
Enterrobacteriaceae	≤100 cfu/gm	<50 cfu/gm	<50 cfu/gm	<50 cfu/gm
Escherichia coli	Absent per 10 gm	Not detected	Not detected	Not detected
Salmonella	Absent per 10 gm	Not detected	Not detected	Not detected
Staphylococcus aureus	Absent per 10 gm	Not detected	Not detected	Not detected

cfu = colony forming units; gm = gram; IR = infrared; NMT = not more than; ppm = parts per million; PPQ = pyrroloquinoline quinone

2.8.3 Additional Analytical Information

2.8.3.1 Protein Residue

Protein from the raw materials and cell substrates could be removed by many steps (*i.e.,* filtration, absorption & desorption, and ultra-filtration). As shown in Table 2.8.3-1, analysis of the finished product using the Bradford assay demonstrate the levels of residual protein present in 3 batches of the finished product are below the 5,000 ppm.

Table 2.8.3-1 Residual Protein Levels in the Finished Product				
Test sample	**Limit**	**Results of Analysis**		
		Batch No.: **VH1606001**	Batch No.: **VH1607001**	Batch No.: **VH1608001**
PQQ	≤5,000 ppm	3546	3824	3627

2.9 Stability of PQQ Disodium Salt

The stability of Fuzhou Contay's PQQ disodium salt was evaluated under accelerated storage conditions (temperature 40 ± 2°C, relative humidity 75 ± 5% for 6 months). As shown in Table 2.9-1, all parameters remain within acceptable limits under both accelerated and long-term storage. In addition, long-term stability tests (temperature 25 ± 2°C, relative humidity 60 ± 5%) are currently underway. All values remain within acceptable specifications at 6 months.

Table 2.9-1 Stability of PQQ Disodium Salt				
Items	**Specifications**	**Batch**		
		S1601001	**S1601002**	**S1601003**
Accelerated stability testing at 6 months (Temperature 40 ± 2°C, humidity 75 ± 5% RH)				
Description	Henna Powder	Conforms	Conforms	Conforms
Identification	IR spectrum of test specimen should consistent with that of reference standard.	Conforms	Conforms	Conforms
Chromatographic Purity	≥99.0%	99.94%	99.97%	99.85%
Water content	≤12.0%	10.1%	9.9%	10.1%
2 Long-term Stability Testing at 6 months (Temperature 25±2°C, humidity 60±5%RH)				
Description	Henna Powder	Conforms	Conforms	Conforms
Identification	IR spectrum of test specimen should consistent with that of reference standard.	Conforms	Conforms	Conforms
Chromatographic Purity	≥99.0%	99.96%	99.97%	99.86%
Water content	≤12.0%	9.8%	9.8%	9.8%

IR = infrared ; PPQ = pyrroloquinoline quinone

Part 3. §170.235 Dietary Exposure

3.1 Probable Consumption

Background Dietary Intakes of PQQ Disodium Salt

The ingredient, PQQ disodium salt, disassociates to PQQ. PQQ is reportedly ubiquitous in the soil, bacteria, and plants. It has reportedly been found in all plant foods analyzed to date, suggesting humans have a long history of exposure to PQQ. Kumazawa *et al.* (1995) detected nanogram quantities of PQQ in various fruits, vegetables, and beverages. PQQ was also found in human milk at concentrations between 140 and 180 ng/mL (Mitchell *et al.*, 1999). Based on the values reported by Kumazawa *et al.* (1995), Based on these data, Harris *et al.* (2013) estimated the daily consumption of PQQ and its derivatives in humans to be 0.1 to 1.0 mg/day.

PQQ disodium salt has been the subject of 2 previous GRAS Notices (GRN No. 625 & GRN No. 641) (U.S. FDA, 2016b,c). Nascent Health Sciences informed FDA that it had determined its PQQ disodiums salt is GRAS for use as an ingredient in energy, sport, and isotonic drinks; non-milk based meal replacement beverages; water (bottled, enhanced, fortified); milk-based meal replacement beverages; at levels intended to provide 8 milligrams PQQ per serving (U.S. FDA, 2016b,c). Similarly, Zhejiang Hisun Pharmaceutical Co. Ltd. notified FDA of its conclusion that PQQ disodium salt is GRAS for use as an ingredient in energy, sport, and electrolyte drinks; and enhanced and fortified water at a maximum use level of up to 5 and 20 milligrams per serving, respectively (Zhejiang Hisun Pharmaceutical Co., Ltd., 2012). Fuzhou Contay's PQQ ingredient is intended as an alternative to these PQQ disodium salt products and not anticipated to result in significant increases in consumer intakes. PQQ is also available in dietary supplements, with typical recommended intakes of 10 to 20 mg PQQ/day.

Dietary Intake in General U.S. Population from all Proposed Food Uses

The estimates for the intake of PQQ disodium salt were 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 (USDA, 2014; CDC, 2015).

A summary of the estimated daily intake of PQQ disodium salt from proposed food-uses is provided in Table 3.1-1 on an absolute basis (g/person/day), and in Table 3.1-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was relatively low among all age groups evaluated in the current intake assessment; ranging from 6.9 (infants and young children) to 27.3% (male teenagers) of the population groups consisted of consumers of those food products in which PQQ disodium salt is currently proposed for use (Table 3.1-1). The consumer-only intakes are more applicable

to the assessment of safety as they are more likely to represent exposure in the target populations. Consequently, only the consumer-only intake results will be discussed in detail.

Among the total population, the mean and 90th percentile consumer-only intakes of PQQ disodium salt were determined to be 19.2 and 42.7 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 PQQ disodium salt on an absolute basis, respectively, at 22.5 and 47.9 mg/person/day, respectively. Infants and young children had the lowest mean consumer-only intake of 8.6 mg/person/day, whereas male teenagers had the lowest reliable consumer-only 90th percentile intake of 22.1 mg/person/day (Table 3.1-1).

Table 3.1-1 Summary of the Estimated Daily Intake of PQQ Disodium Salt from Proposed Beverage-Uses in the United States 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	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	0.6	na	6.9	45	8.6	18.4*
Children	3 to 11	1.5	5.0	14.1	200	10.8	24.6
Female Teenagers	12 to 19	2.9	10.0*	17.2	76	17.0	32.2*
Male Teenagers	12 to 19	3.9	15.8	27.3	111	14.4	22.1
Female Adults	20 and up	2.8	8.8	13.9	252	20.1	44.2
Male Adults	20 and up	4.2	14.6	18.7	336	22.5	47.9
Total Population	All Ages	3.1	10.1	16.3	1,020	19.2	42.7

NHANES = National Health and Nutrition Examination Survey; na = not available; PQQ = pyrroloquinoline quinone
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements

On a body weight basis, the total population mean and 90th percentile consumer-only intakes of PQQ disodium salt were determined to be 0.29 and 0.62 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean consumer-only intake of any population group, of 0.66 mg/kg body weight/day, while children were determined to have the highest reliable consumer-only 90th percentile intake of 0.88 mg/kg body weight/day. Male teenagers had the lowest mean and 90th percentile consumer-only intakes of 0.22 and 0.46 mg/kg body weight/day, respectively (Table 3.1-2).

Table 3.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Proposed Beverage-Uses in the United States by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	0.05	na	6.9	44	0.66	1.45*
Children	3 to 11	0.06	0.17	14.1	200	0.40	0.88
Female Teenagers	12 to 19	0.05	0.16*	17.2	72	0.30	0.58*
Male Teenagers	12 to 19	0.06	0.22	27.4	111	0.22	0.46
Female Adults	20 and up	0.04	0.13	13.9	248	0.29	0.70
Male Adults	20 and up	0.05	0.16	18.7	333	0.27	0.53
Total Population	All Ages	0.05	0.15	16.3	1,008	0.29	0.62

bw = body weight; NHANES = National Health and Nutrition Examination Survey; na = not available;
PQQ = pyrroloquinoline quinone
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements

Consumption data from the 2011-2012 NHANES dataset and information pertaining to the individual proposed beverage-uses of PQQ disodium salt were used to estimate the *per capita* and consumer-only intakes for specific demographic groups and for the total U.S. population. Conservative assumptions have been included in the present assessment, which render exposure estimates that may be considered suitably conservative. For example, it was assumed that all food products within a food category contain the ingredients at the maximum specified level of use. In reality, the levels of PQQ disodium salt added to specific foods will vary and are unlikely to have 100% market penetration. Furthermore, Fuzhou Contay's ingredient would be intended as a replacement for other sources of PQQ in the proposed food categories and thus would not significantly increase overall intake.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of PQQ disodium salt by the total U.S. population from all proposed beverage-uses in the U.S. were estimated to be 19.2 mg/person/day (0.29 mg/kg body weight/day) and 42.7 mg/person/day (0.62 mg/kg body weight/day), respectively. Among the individual population groups, the highest absolute mean and 90th percentile intakes of PQQ disodium salt were determined to be 22.5 mg/person/day (0.27 mg/kg body weight/day) and 47.9 mg/person/day (0.53 mg/kg body weight/day), respectively, as identified among male adults. When intakes were expressed on a body weight basis, infants and young children had the highest mean consumer-only intake of 0.66 mg/kg body weight/day, while children were identified as having the highest reliable 90th percentile consumer-only intake of 0.88 mg/kg body weight/day. While the younger populations were identified as the groups having higher exposures to PQQ disodium salts on a body weight basis, it should be noted that products containing PQQ will not be targeted towards infants and young children, and estimates described herein assume all products, including those consumed

by younger individuals would contain the ingredient at the maximum intended use levels. In actuality, these products would, in the worst case, only be consumed incidentally and intakes described in the older populations (*i.e.*, not more than 0.30 and 0.70 mg/kg body weight/day at the mean and 90th percentile, respectively) are expected to be more accurate estimates of dietary exposure among the intended target consumer group.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with Fuzhou Contay's PQQ disodium salt.

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

Not applicable.

Part 6. §170.250 Narrative

6.1 Pivotal Safety Data

The conclusion that PQQ disodium salt, as described herein, is GRAS under the conditions of its intended use in specified conventional beverage products is based on scientific procedures using generally available data from safety studies on the material. Much of the information related to the absorption, distribution, metabolism, and excretion and safety of PQQ Disodium Salt has been previously reviewed (see GRAS notices GRN No. 625 & GRN No. 641) (U.S. FDA, 2016b,c). A summary of the main findings is provided below. Furthermore, details of pivotal safety data are included in Table 6.1-1.

- In a 13-week oral, repeated dose study in Sprague-Dawley rats, no unscheduled deaths occurred and there were no treatment-related changes in food consumption or body weight gain. The observed histopathologic changes did not appear to be dose-related and the incidence of lesions in the various control groups renders the results to be spurious. The no-observed-adverse-effect level (NOAEL) of PQQ disodium salt was considered to be 400 mg/kg body weight/day (Liang *et al.*, 2015).

- PQQ was not genotoxic or mutagenic. No significant incidences of structural aberrations (excluding gaps) and polyploidy were observed (Nakano *et al.*, 2013).

- In an acute oral toxicity study, at 2,000 mg/kg dose level, 17 rats died in a 7-day period and hypothermia was observed in 3 female rats and 3 male rats at the same dose level. Decrease in body weight gain was observed in all animals at the 1,000 and 2,000 mg/kg body weight dose levels. Necropsy revealed enlarged kidneys and livers in all animals

in those dose levels. Soft feces and diarrhea was observed in all animals at all dose levels. Decrease in defecation was observed in all animals at the 1,000 and 2,000 dose levels (Nakano *et al.*, 2014).

- In a 14-day repeated dose study, urinalysis revealed increased sodium levels in the high dose group as well as green colored content in the cecum of 3 animals of each sex. An increase in relative kidney weight was observed in females of the high-dose group. Histopathological examination revealed focal basophilic changes and atrophy of the renal tubules in females of the high-dose group (Nakano *et al.*, 2014)

- The NOAEL in the 90-day study by Nakano *et al.* (2014) was considered to be 100 mg/kg body weight/day.

Table 6.1-1 Summary of Pivotal PQQ Safety Data				
Reference	**Study Type**	**Test System**	**Exposure**	**Findings/Comments**
Nakano *et al.* (2013)	Ames/Reverse Mutation Assay	*Salmonella typhimurium* test strains (TA98, TA100, TA1535, and TA1537), and *Escherichia coli* WP2*uvr*A	Absence of metabolic activation: PQQ disodium salt (99.6% purity in DMSO) at concentrations of 0,10, 20, 39, 78,156, 313, 625,1,250, 2,500, or 5,000 µg/plate. Incubated 48 hours.	No evidence of test article precipitation. No increase in the number of revertant colonies or dose-response observed. Growth inhibition noted in some plates treated with 313 µg or higher.
			Presence of metabolic activation (S9 mix): PQQ disodium salt (99.6% purity in DMSO) at concentrations of 0, 156, 313, 625, 1,250, 2,500, or 5,000 µg/plate. Incubated 48 hours.	No evidence of test article precipitation. No increase in the number of revertant colonies or dose-response observed. Growth inhibition noted in some plates treated with 2,500 µg or higher.
Nakano *et al.* (2013)	*In vitro* chromosomal aberration test in cultured mammalian cells	Cell suspensions of lung fibroblast derived from newborn female Chinese hamsters	Absence of metabolic activation: PQQ disodium salt (99.3 - 99.7% purity in distilled water) at concentrations of 0, 12.5, 25, 50, 100, 200, 400 µg/mL for 6 and 24 h.	No evidence of test article precipitation. Cell growth inhibition exceeded 50% for cells treated with 100 µg or higher. No significant differences in polyploidy was observed. A statistically significant increase in the incidence of structural chromosome aberrations was observed at 200 µg/mL (6 h treatment), but is below the threshold to be classified as "positive".
			Presence of metabolic activation (S9 mix): PQQ disodium salt (99.3- 99.7% purity in distilled water) at concentrations of 0, 117.2, 234.4, 468.8, 937.5, 1,875, and 3,750 µg/mL for 6 and 24 h.	Precipitation noted at all concentrations tested. pH slightly elevated (8) at concentrations of 3750 µg/mL; these treatments were excluded from analysis , but used in the overall assessment. Data indicated a "weakly positive" result of chromosome damaging effect. No significant differences in incidence of structural chromosome aberrations or polyploidy was observed. Study was repeated and no significantly different results were reported.
Nakano *et al.* (2013)	*In vitro* chromosomal aberration test in human peripheral blood lymphocytes	Human peripheral blood lymphocytes (from healthy, non-smoking, male and female adult volunteers under 40 years of age).	Absence of metabolic activation: PQQ disodium salt (99.3% purity dissolved in water) at concentrations of 0, 234.4, 468.8, 937.5, 1,875, 3,750 µg/mL for 3 and 24 h.	Precipitation of the test article observed at concentrations of 1875 and 3750 µg/mL. No significant incidences of structural aberrations (excluding gaps) and polyploidy were observed in treated cells compared to negative control group.
			Presence of metabolic activation (S9 mix): PQQ disodium salt (99.3% purity dissolved in water) at concentrations of 0, 117.2, 234.4, 468.8, 937.5, 1,875, 3,750 µg/mL (3-hour test).	Precipitation of the test article observed at concentrations of 1,875 and 3,750 µg/mL. No significant incidences of structural aberrations (excluding gaps) and polyploidy were observed in treated cells compared to negative control group. No clear inhibition of the MI of below 50% was noted in any dose group.

Table 6.1-1 Summary of Pivotal PQQ Safety Data				
Reference	**Study Type**	**Test System**	**Exposure**	**Findings/Comments**
Nakano *et al.* (2013)	*In vivo* micronucleus test in mice	Crlj: CD1 (ICR) male mice (6/group, 5 groups). Cells from bone marrow collected.	Administered PQQ disodium salt (99.7% purity dissolved in .5% methylcellulose solution) at a total dose of 0, 250, 500, 1,000, or 2,000 mg/kg over 2 doses. Doses given by gastric intubation and separated by 24 h. Mice sacrificed 24 h after final dose.	All animals survived until terminal sacrifice. Blackish stools noted from all animals administered 1,000 or 2,000 mg/kg doses. No clinical signs of toxicity observed. No significant differences in body weights observed between groups. No significant increases in mean percentage of micronucleated polychromatic erythrocytes and no significant difference in percentage of polychromatic erythrocytes was observed.
Liang *et al.* (2015)	Subchronic oral toxicity study	Sprague-Dawley Rats, 10/sex/group (4 groups)	13 weeks – administered PQQ disodium salt at doses of 0, 100, 200, and 400 mg/kg bw/day by oral gavage.	No mortality or toxicologically significant changes in clinical signs, body weight, food consumption, necropsy findings, or organ weights observed. Hematological and serum biochemical examination differences between treated and control groups were not considered treatment related. The NOAEL of PQQ disodium in rats was considered to be 400 mg/kg bw/day.
Nakano *et al.* (2014)	Acute Oral Toxicity Study	5-week-old Sprague Dawley (SD) rats. 10 animals of each sex/group. 30 animals total.	500, 1,000, and 2,000 mg/kg bw doses	At the 2,000 dose level, 3/10 males and 3/10 females died on day 2, 1/10 female at the 1,000 dose level and 3/10 males and 7/10 females at the 2,000 dose level on day 3 and 1/10 male at the 2,000 dose level on day 7. Soft feces and/or diarrhea were observed in all dose groups after 1 hour. On day 1, an increase of greenish feces and/or decreased defecation was observed in the 1,000 and 2,000 dose levels. Hypothermia was observed in 3 males and 3 females at the 2,000 dose level. Significant decreases in body weight gain were observed in the top 2 dose levels. Necropsy revealed pale and enlarged kidneys in all dead and surviving animals at the 1,000 and 2,000 dose levels.

Table 6.1-1 Summary of Pivotal PQQ Safety Data				
Reference	**Study Type**	**Test System**	**Exposure**	**Findings/Comments**
Nakano *et al.* (2014)	14-Day dose-range	6-week-old SD rats. 6/sex/group 36 animals total	3, 12, 48, 192, 768 mg/kg bw/day by oral gavage for 14 days	No deaths or significant clinical findings were noted. No statistically significant differences in body weight were observed. Urinalysis showed a significant increase in sodium levels in males and females of the high-dose group. Gross necropsy revealed green colored contents in the cecum of 4 males and 5 females in the high-dose group. A significant increase in relative kidney weight was observed in females in the high-dose group. Histopathological examination revealed focal basophilic changes and atrophy of the renal tubules in females of the high-dose group.
Nakano *et al.* (2014)	28-Day Renal Toxicity Study	6-week-old SD rats. 36 animals of both sexes, 12/group	200 and 700 mg/kg bw/day for 28 days	No deaths or significant clinical findings were noted in any group throughout the study. No statistically significant differences in clinical biochemistry were observed. Urinalysis showed an increased incidence of crystals in urinary sediment in both dose groups. This finding was only observed in 1 male at the end of the recovery period. Protein positive detections were noted in 6/12 low dose animals (5/12 slight reaction 1/12 moderate reaction) and 3/12 high dose (slight reaction) at week 2 and then 5/12 low dose and 6/12 high-dose rats at week 4 all with slight reactions. At the end of the 4-week recovery period, minimum protein was detected in 1/6 rats in the low-dose group and 2/6 rats in the high-dose group.
Nakano *et al.* (2014)	13-Week Subchronic Oral Toxicity	6-week-old SD rats. 40 animals of each sex	3, 20, 100 mg/kg bw/day by oral gavage for 13 weeks	No deaths occurred throughout the study and there were no significant clinical findings. There were no significant differences in body weight gain or food consumption and no ophthalmological abnormalities. There were no significant differences in hematology or urinalysis parameters. Increased AST activity in the low dose female group, decreased total cholesterol concentration in the mid-dose female group and decreased triglyceride concentration in the high-dose female group was observed. Gross necropsy revealed green-colored contents in the intestines and the ceca of 3 males and 1 female in the high-dose group.

bw = body weight; DMSO = dimethylsulfoxide; PPQ = pyrroloquinoline quinone

6.2 Updated Discussion of PQQ Disodium Salt Safety

An updated search of the published scientific literature was conducted through August 2016 using the search program Proquest to identify published studies relevant to the safety of PQQ disodium salt and the source organism, *Hyphomicrobium*. The search was conducted on databases including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and Toxfile®. No additional studies addressing the preclinical safety of PQQ were identified through these searches.

6.3 Clinical Studies with PQQ

As discussed in greater detail in GRN 625 and 641 (U.S. FDA, 2016b,c), PQQ is reportedly well tolerated in clinical studies, with no adverse effects reported following consumption of PQQ at does up to 60 mg/kg/day for 4 weeks or 20 mg/kg body weight/day for 24 weeks (Nakano *et al.*, 2009, 2013; Rucker *et al.*, 2009; Koikeda *et al.*, 2011). No additional studies addressing the clinical safety of PQQ were identified through updated literature searches.

6.4 Expert Panel Evaluation

Fuzhou Contay has concluded that PQQ disodium salt, produced through fermentation using a strain of the genus *Hyphomicrobium*, manufactured consistent with cGMP and meeting appropriate food grade specifications, is GRAS for use as an ingredient in specified beverage products, as described in Part 1.3, on the basis of scientific procedures.

Fuzhou Contay's conclusion on the GRAS status of PQQ disodium salt under the conditions of its intended use is based on data from traditional toxicology studies generally available in the public domain.

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients unanimously concluded on the GRAS status of the PQQ disodium salt under conditions of its intended use. The Expert Panel consisted of the following qualified scientific experts: Dr. John Thomas (Adjunct Professor, Indiana University School of Medicine), Dr. Robert Nicolosi (Professor Emeritus, University of Massachusetts Lowell) and Dr. David Bechtel (Bechtel Consulting, Inc.).[1]

The Expert Panel, convened by Fuzhou Contay, independently and critically evaluated all data and information presented herein and concluded that PQQ disodium salt, meeting appropriate food-grade specifications and manufactured consistent with cGMP, is safe and suitable for use

[1] The panelists participated in their individual capacities. Institutional affiliations are provided for identification purposes only.

as an ingredient in specified conventional beverage products, as described in Part 1.3, and is GRAS based on scientific procedures. A summary of data and information reviewed by the Expert Panel is presented in Appendix A.

6.5 Conclusion

Based on the above data and information presented herein, Fuzhou Contay has concluded that the intended uses of PQQ disodium salt specified conventional food and beverage products, as described in Part 1.3, are GRAS based on scientific procedures. The GRAS status of PQQ disodium salt is further supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended use of PQQ disodium salt in conventional beverage products, as described herein, is GRAS.

PQQ disodium salt, therefore. may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations (U.S. FDA, 2016a).

Part 7. §170.255 List of Supporting Data and Information

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Part	§	Section Title
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
173—Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins
	173.40	Molecular sieve resins
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe
	182.1057	Hydrochloric acid
	182.6285	Dipotassium phosphate
184—Direct food substances affirmed as generally recognized as safe	184.1143	Ammonium sulfate
	184.1193	Calcium chloride
	184.1443	Magnesium sulfate
	184.1763	Sodium hydroxide

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