GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE OF PYRROLOQUINOLINE QUINONE DISODIUM SALT AS A FOOD INGREDIENT

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GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF Pyrroloquinoline quinone (PQQ) AS A FOOD INGREDIENT

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PART 1. SIGNED STATEMENTS AND A CERTIFICATION

Pursuant to 21 C.F.R. Part 170, subpart E, Shandong Jincheng Bio-Pharmaceutical Co., Ltd. (hereinafter referred to as 'JinCheng') submits a Generally Recognized as Safe (GRAS) notice and claims that the use of pyrroloquinoline quinone (PQQ) disodium salt in foods, as described in Parts 2 through 7 of this GRAS notice, is not subject to premarket approval requirements of the FD&C Act based on its conclusion that the substance is GRAS under the conditions of its intended use.

1.A. Name and Address of the Notifier

Contact person: Lan Jiang
Company name: Shandong Jincheng Bio-Pharmaceutical Co., Ltd.
Address: Zichuan Economic Development Zone, Zibo City,Shandong Province,China
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1.B. Common or Trade Name

Pyrroloquinoline quinone (PQQ) disodium salt;
Common abbreviation: PQQ

1.C. Applicable Conditions of Use of the Notified Substance

1.C.1. Foods in Which the Substance is to be Used

Intended use and use levels of JinCheng's PQQ disodium salt have been modified from GRN 625 (page 6) and GRN 640 (page 5). JinCheng proposes to use PQQ disodium salt as a food ingredient in selected beverages such as energy, sport, and electrolyte drinks, bottled, enhanced and fortified water beverages, and non-milk based meal replacement beverages. JinCheng does not intend to use PQQ disodium salt as a component of infant formula or in foods under the USDA's jurisdiction such as meat, poultry, and egg products.

1.C.2. Levels of Use in Such Foods

Table 1. Intended Use and Maximum Use Levels of PQQ disodium salt, % (w/w)

Food Category	Food-Uses	Serving Size (RACC)[1]	Proposed Use Level	
			(mg/serving)	(%)
	Energy Drinks	240 mL	12	0.005
Beverages and Beverage Bases	Sport and Electrolyte Drinks	240 mL	8	0.00333
	Enhanced and Fortified Water Beverages	240 mL	20	0.008
	Bottled water	240 mL	8	0.00333
	Non-Milk Based Meal Replacement Beverages	240 mL	8	0.00333

[1] RACC refers to Reference Amounts Customarily Consumed per eating occasion – 21 CFR §101.12 (U.S. FDA, 2015). When a range of values is reported for a particular food-use, particular foods within that food-use may differ with respect to their RACC.

As shown in Table 1, PQQ disodium salt is intended for use in selected beverages (energy, sport, and electrolyte drinks, bottled, enhanced and fortified water beverages, and non-milk based meal replacement beverages) at maximum use levels of up to 8 to 20 mg/serving, respectively in these product types.

1.C.3. Purpose for Which the Substance is Used
The substance will be used as a food ingredient.

1.C.4. Description of the Population Expected to Consume the Substance
The population expected to consume the substance consists of members of general population who consume at least one of the products described above.

1.D. Basis for the GRAS Determination: Through scientific procedures.

1.E. Availability of Information
The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting NutraSource, Inc. at the address above. The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

1.F. Availability of FOIA Exemption
Privileged or confidential information such as trade secrets and/or commercial or financial information has been redacted from this document and the information contained in this dossier can be made publicly available if warranted.

None of the data and information in Parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552.

1.G. Certification
JinCheng certifies that, to the best of our knowledge, that this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by JinCheng, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of PQQ disodium salt. JinCheng accepts responsibility for the GRAS determination that has been made for PQQ disodium salt, as described in this dossier.

1.H Name, Position/Title of Responsible Person Who Signs Dossier and Signature

(b) (6)

Name: Lan Jiang Date: 2017/5/19
Title: Sales Manager

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Agent for Shandong Jincheng Bio-Pharmaceutical Co., Ltd.

1.I. FSIS/USDA Statement

JinCheng does not intend to add PQQ disodium salt to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

PART 2. IDENTITY, MANUFACTURING, SPECIFICATION, TECHNICAL EFFECTS OF PYRROLOQUINONE QUINONE (PQQ) DISODIUM SALT

2.A.1. Identity of the Notified Substance

2.A.1.1. Common Name: Pyrroloquinoline quinone (PQQ) disodium salt

Common abbreviation: PQQ

2.A.1.2. Chemical Names

Disodium 4,5-dihydro-4,5-dioxo-1h—pyrrolo(2,3-f) quinolone-2,7,9-tricarboxylate;
Synonyms: Methoxatin disodium salt, Disodium pyrroloquinolinedione tricarboxylate

2.A.1.3. Chemical Abstract Service (CAS) Registry Number

122628-50-6

2.A.1.4. Empirical Formula

$C_{14}H_4N_2Na_2O_8$

2.A.1.5. Structural Formula

Figure 1 shows the structure of PQQ disodium salt.

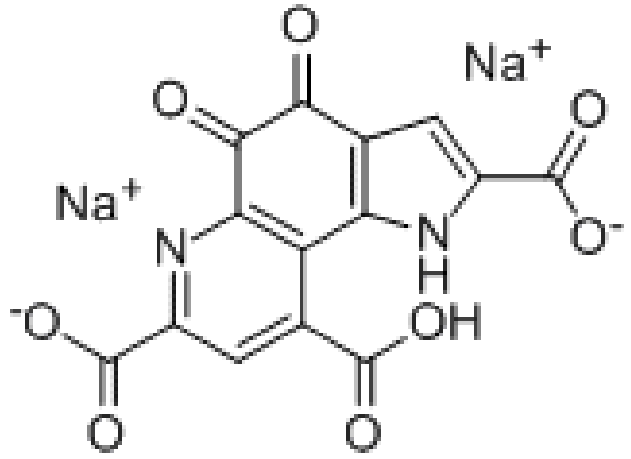


Figure 1. Structure of PQQ disodium salt.

2.A.1.6. Physical Properties
Melting Point: > 300 °C
Solubility: 3 g/L (25 °C) in water; insoluble in organic solvents
PQQ is water soluble and heat stable. It can also be found in the form of PQQ disodium salt.

2.A.1.7. Biological Significance

PQQ was first recognized as a bacterial cofactor. Under appropriate conditions, PQQ is capable of catalyzing continuous redox cycling (the ability to catalyze repeated oxidation and reduction reactions), as well as oxidative deaminations (Rucker et al., 2009), thus, it can serve as an antioxidant (Rucker et al. 2009). There is strong evidence PQQ may play an important role in pathways important to cell signaling. PQQ can also serve as an antioxidant. The importance of PQQ to mammalian health is evident when it is omitted from chemically defined diets. PQQ plays multiple physiological roles, such as promoting growth and reproduction, and providing neural and cardiovascular protection. It also enhances antioxidants, learning, memory, and immune function (Rucker et al., 2009).

PQQ disodium salt is thought to have similar physiological and metabolic effects as PQQ (Rucker et al., 2009).

2.A.2. Potential Toxicants in the Source of the Notified Substance

Potential toxicants have not been identified in PQQ disodium salt. High-performance liquid chromatography (HPLC) reveals that JinCheng's PQQ disodium salt is > 99.0% pure. Based on the purity profile, JinCheng considers the ingredient to meet or exceed food grade quality standards.

2.A.3. Particle Size

Mean particle size of Jincheng PQQ disodium salt is 37-46 μm. Details are presented in Appendix A.

2.B. Method of Manufacture

Hyphomicrobium denitrificans was originally isolated from soil near winery, then strain was successfully screened to produce PQQ disodium salt. JinCheng's strain was identified as a non-pathogenic and non-toxigenic bacterial species. It is not genetically modified.

Preparation and Preservation of the Working Cell Bank

The working cell bank (WCB) is prepared by slant cultivation. The storage condition for the WCB is 2~8 °C, and the validity period for storage is 30 days. The culture medium is prepared by dissolving food grade minerals and agar with purified water in a beaker. Sodium hydroxide solution is then used to adjust to pH. The medium is heat-sterilized at approximately 121 to 125°C for 20 to 22 minutes. Once the medium is cooled, methanol is added and a slant is formed. The WCB is produced by culturing the Master Cell Bank suspension containing the source organism on the blank slant and incubation. The colonies are then isolated from the slant and recovered using glycerol solution prior to quality control testing to ensure that they conform to the internal specifications established for *Hyphomicrobium denitrificans*.

Slant Culture and Shaking Culture

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. The shaking culture medium is prepared by dissolving food grade salts in a beaker. The medium is adjusted using sodium hydroxide solution and is subject to heat sterilization at a temperature of approximately

121 to 125°C. Methanol is added to the medium (control culture temperature is 35°C and shaking speed 200 rpm; culture time is 30 hours) and then cooled.

Fermentation

Pure water is added into the seeding tank as ration. Seeding tank fermentation broth is prepared by adding food grade minerals and an antifoaming agent into the seeding tank. The pH is adjusted using sodium hydroxide. The fermentation broth is heat-sterilized at 118 to 121°C for 25 to 30 minutes. The medium is cooled prior to the addition of methanol. The seed tank fermentation is initiated upon addition of the shaking culture under protected procedures. Fermentation occurs under tightly controlled conditions (*e.g.*, pH temperatures, pressure, agitation speed). Fermentation is terminated when mycelium concentration reaches a defined OD and pH range.

Pure water is added into the propagation tank as ration. The main fermentation broth is produced using the same raw materials and in a similar manner to the seed tank fermentation broth. The fermentation broth is sterilized at 118 to 121°C for 25 to 30 minutes. The fermentation broth is cooled to 30 °C through pipe line transfer into the above seed tank broth. The mycelium solution from the seeding tank fermentation is inoculated into the propagation tank and fermentation occurs under controlled conditions. The pH, mycelium concentration, and amino-nitrogen are tested at 24 hours post-inoculation and the potency is tested daily during fermentation. The pH is maintained by the addition of ammonia water. Methanol solution is continuously added to maintain its levels consistent with the amount within the culture medium. The fermentation is terminated when the mycelia decline, tinting strength is weak, increase of the fermentation potency is slower, pH increases slightly, and potency is reduced.

Extraction and Purification

After the fermentation process, PQQ disodium salt 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 source organism is removed by ceramic membrane filtration with a pore diameter of <200 nm. Sulfuric acid is used to adjust the pH prior to the addition of sodium chloride with stirring. Crystallization then occurs over several hours. The crude product is recovered by filtration prior to dissolving in water using sodium hydroxide to facilitate dissolution. The solution undergoes membrane filtration prior to addition to a crystallizing tank. Ethanol is added and the pH is adjusted using hydrochloric acid with stirring. A second crystallization step is then initiated. The mixture is then filtered to obtain the wet substance.

Drying Sieving and Test and Storage

This is followed by vacuum drying. The wet product of PQQ disodium salt is dried for 6 ± 0.5 hours at a temperature of 45~55 °C under a vacuum degree of not more than -0.090 Mpa. The dried product of PQQ disodium salt contains no more than 12.0% water after drying, milling, and sieving. The finished PQQ disodium salt is then transferred to quarantine storage for quality control testing prior to packaging in aluminum drum storage at room temperatures not to exceed 30°C.

The PQQ disodium salt product is manufactured consistent with the principles of current good manufacturing practices (cGMP). The adsorption polymeric resins (styrene-divinylbenzene cross-linked copolymer) used in the manufacturing process comply with 21 CFR 173.25 (17). Table 2 lists in-process control points in the production of PQQ disodium salt.

Table 2. In-process control points in the production of PQQ disodium salt

Process	Control
Working Cell Bank	Microbiological contamination, appearance, growth characteristics, colony survival number, survival ratio, fermentation potency
Slant Culture	Microbiological contamination, appearance
Shaking Culture	Microbiological contamination, morphological characteristics
Seed Tank Fermentation	Microbiological contamination, appearance of seeding liquid, mycelium content, pH
Main Fermentation	Mycelium content, amino-nitrogen, pH, fermentation potency
Centrifuging and Washing	Mycelium concentration
Filtration	Temperature, time
Crystallization	Temperature, concentration, dropwise time
Drying and sieving	Temperature, time

JinCheng's PPQ disodium salt ingredient is manufactured consistent with the principles of Hazard Analysis and Critical Control Points (HACCP). The manufacturing process, which complies with cGMP, is shown in Figure 2.

Table 3 shows the composition of fermentation medium. Chemicals listed in fermentation medium function as nutrients for fermentation. Chemicals listed in ammonia water have a dual function. They provide balancing pH effects and serve as nutrients for fermentation.

Table 3. Composition of fermentation medium

Composite	g/L	Remarks
Methanol	20	Sterilized by filtration membrane with diameter of 0.22 μm
Ammonium sulfate; $(NH_4)_2SO_4$	5	Nitrogen source for fermentation
Potassium dihydrogen phosphate; KH_2PO_4	1.5	Fermentation nutrient
Sodium phosphate Na_2HPO_4	3	Fermentation nutrient
Magnesium sulfate heptahydrate; $MgSO_4.7H_2O$	1.5	Fermentation nutrient
Ferrous sulfate heptahydrate; $FeSO_4.7H_2O$	0.05	Fermentation nutrient
Zinc sulfate heptahydrate; $ZnSO_4.7H_2O$	0.05	Fermentation nutrient

Manganous sulfate; $MnSO_4.4H_2O$	0.015	Fermentation nutrient
Cupric sulfate pentahydrate; $CuSO_4.5H_2O$	0.02	Fermentation nutrient
Calcium chloride; $CaCl_2.2H_2O$	0.3	Fermentation nutrient
Ammonia water	0.7	pH balancing, fermentation nutrients

Figure 2. Manufacturing process of JinCheng's PQQ disodium salt

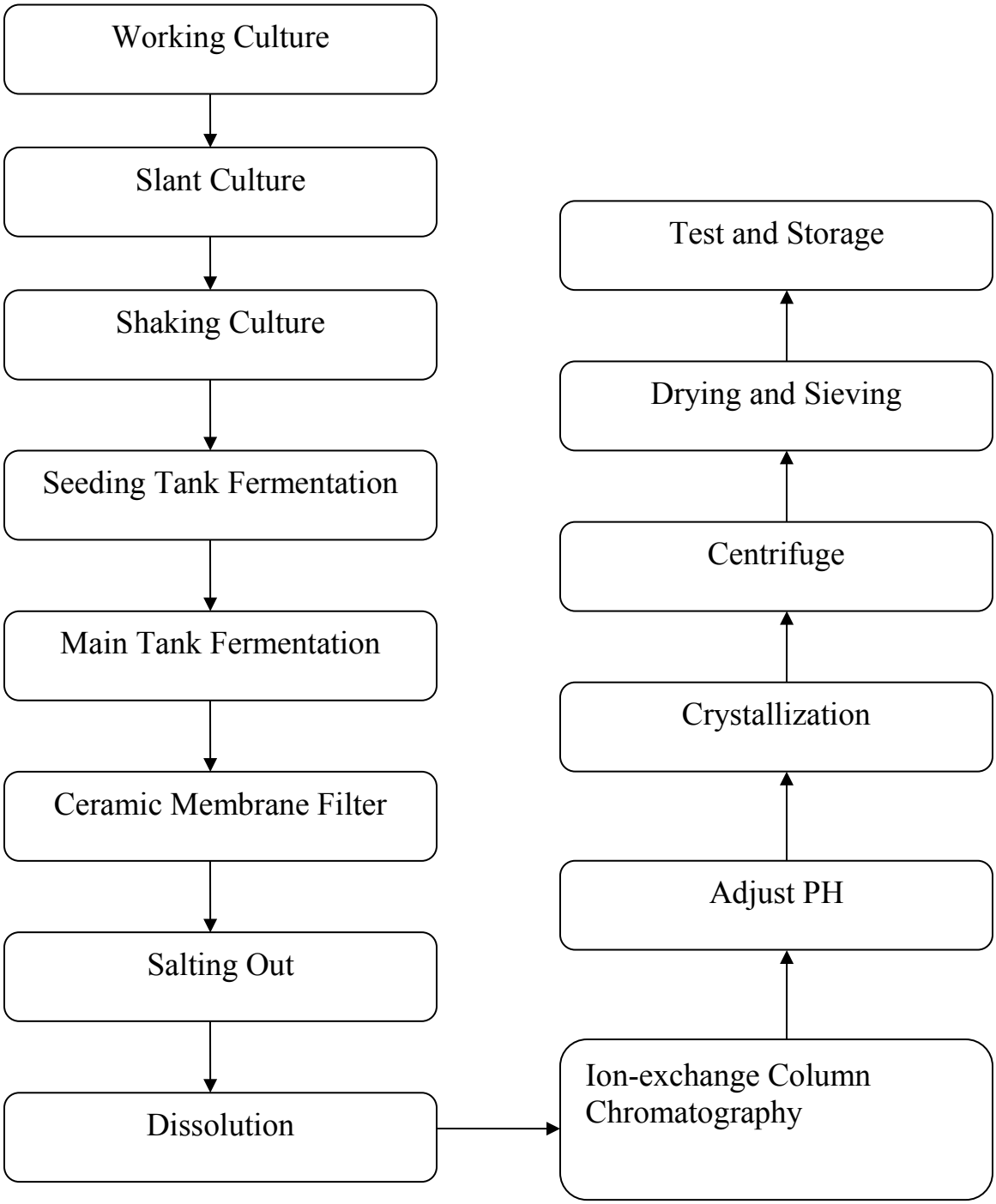


Table 4 presents CAS registry numbers and regulatory status of raw materials used in the manufacture of JinCheng's PQQ disodium salt. The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes. Purchasing specifications of raw materials are included in Appendix B.

Table 4. CAS registry numbers and regulatory status of raw materials and processing aids

Raw material	CAS Registry No.	Regulatory Status
Methanol	67-56-1	21 CFR §182.1; Methanol is permitted for use in foods as a GRAS substance
Ammonium sulfate; $(NH_4)_2SO_4$	7783-20-2	21 CFR §184.1143; Direct food substances affirmed as GRAS; Permitted for use in foods as a dough strengthener, firming agent, and processing aid
Potassium dihydrogen phosphate; KH_2PO_4	7778-77-0	Yeast food-FCC 10th ed.
Sodium phosphate; Na_2HPO_4	7601-54-9	21CFR§182.1778; GRAS when used in accordance with GMP.
Magnesium sulfate heptahydrate; $MgSO_4.7H_2O$	10034-99-8	21 CFR §184.1443; Direct food substances affirmed as GRAS; Permitted for use in foods as a flavor enhancer, nutrient supplement, or processing aid
Ferrous sulfate heptahydrate; $FeSO_4.7H_2O$	7782-63-0	21CFR184.1315; As nutrient supplements as defined in § 170.3(o)(20)
Zinc sulfate heptahydrate; $ZnSO_4.7H_2O$	7446-20-0	21 CFR §182.8997; GRAS when used in accordance with GMP.
Manganous sulfate; $MnSO_4.4H_2O$ or $MnSO_4.5H_2O$	10034-96-5	21CFR184.1461; Direct food substances affirmed as GRAS; Permitted for use in baked goods, nonalcoholic beverages, fish products, meat products, milk products, poultry products and infant formula
Cupric sulfate pentahydrate; $CuSO_4$-$5H_2O$	7758-99-8	21CFR184.1261; Direct food substances affirmed as GRAS; Permitted for use in nutrient supplements and infant formula
Calcium chloride; $CaCl_2.2H_2O$	10035-04-8	CFR §184.1193; Direct food substances affirmed as GRAS; 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
Agar	10035-04-8	FCC 10^{th} ed
Ammonia water		FCC 10^{th} ed

Sulfuric acid	7664-93-9	21 CFR §184.1095; As a pH control agent
Hydrochloric acid	7647-01-0	21 CFR §182.1057; As a buffer and neutralizing agent
Ethanol	64-17-5	21 CFR §184.1293; Direct food substances affirmed as GRAS; Permitted for use as an antimicrobial agent
Sodium hydroxide	1310-73-2	21 CFR §184.1763; Direct food substances affirmed as GRAS; Permitted for use in accordance with cGMP
Sodium chloride	7647-14-5	21 CFR §182.1; GRAS Substance; Permitted for use in accordance with cGMP

CFR = United States Code of Federal Regulations; cGMP = current Good Manufacturing Processes; GRAS = Generally Recognized as Safe.

2. D. Identifications of PQQ Disodium Salt

Figure 3 compares the HPLC chromatograms of JinCheng's PQQ disodium salt product to that of standard PQQ disodium salt. The HPLC chromatograms demonstrate that elution times and peak heights are identical for both reference standard and JinCheng's PQQ disodium salt. The data suggest that the substance is PQQ disodium salt. The absence of additional peaks demonstrates that byproducts are absent in JinCheng's PQQ disodium salt product.

Chromatographic Conditions

a) Instruments：HPLC and UV Detector

b) Chromatographic column: YMC-Pack A-302 ODS or equivalent stainless column, C18, 4.6×150mm, 5μm;

c) Mobile phase：

1. Ratio: 0.1M Acetate acid-0.1M, Ammonium acetate=30:70, pH5.1；

2. Preparation method: Dissolve 1.8g ice acetate acid in 300ml water, dissolve 5.4g ammonium acetate in 700ml water, adjust pH value to 5.1 with acetate acid solution, filtrate through 0.45 μm hydrophilic membrane, ultrasonic wave degassing for 10 minutes;

3. If necessary, prepare deferent volume mobile phase by same ratio, enlarging or reducing according to sample size.

d) Detect wavelength: 259nm；

e) Column temperature: 40 ℃

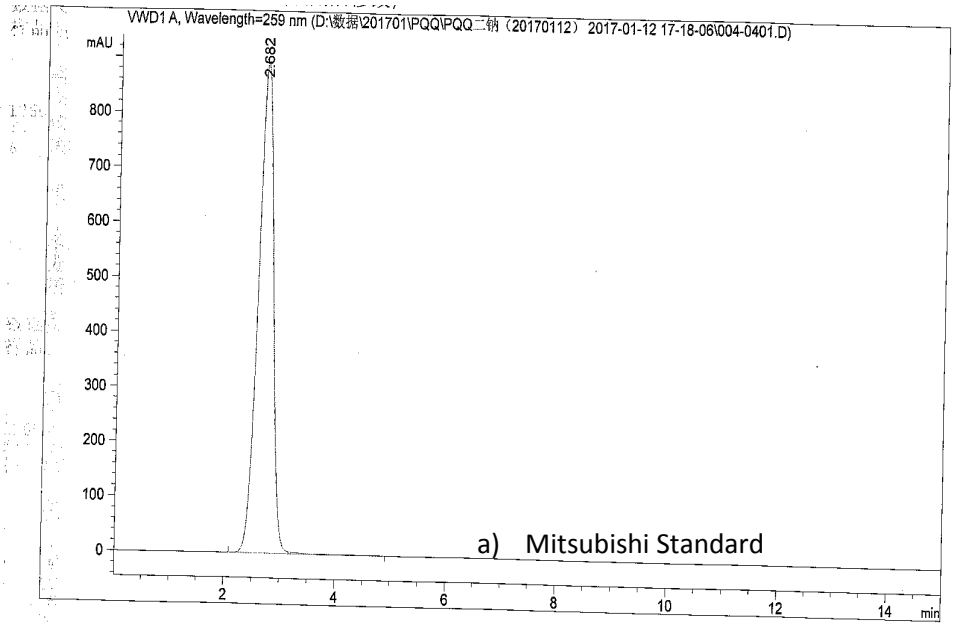
f) Flow rate: 1.5mL/min；

g) Inject volume: 20uL；

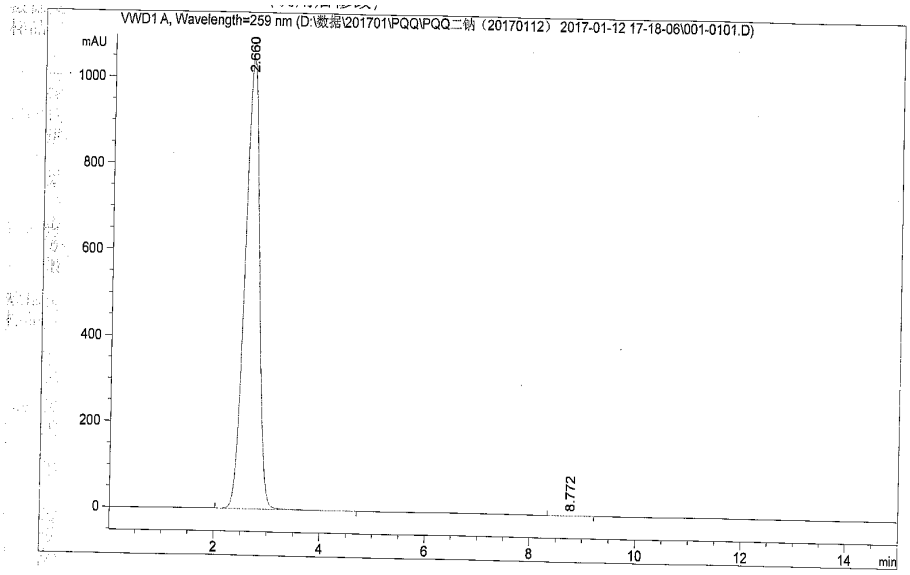
h) Analysis time: 30min.

Figure 3. HPLC chromatograms comparing commercial standard and JinCheng's PQQ disodium salt

a) Mitsubishi Standard



b) Jincheng (160903)



2. C. Composition and Specifications

Table 5 present a typical composition of JinCheng's PQQ disodium salt.

Table 5. Typical Composition of Jincheng's PQQ disodium salt.

Composition:	Limit
PQQ (as-is basis)	≥85%
PQQ disodium salt	≥99%
Sodium	10.0~13.0%
Water content	≤12%
Fat	0
Protein	0

Food grade specifications of PQQ disodium salt have been established by JinCheng and are presented in Table 6. Certificates of analysis from three non-consecutive batches (Appendix C) demonstrate that PQQ disodium salt is consistently manufactured to meet these specifications. Specifications for lead (Pb) and arsenic (As) exceed those established in GRNs 625 and 641.

Table 6. Specifications of JinCheng's PQQ disodium salt

Parameter	Specification	Method of Analysis
Identity		
Appearance	Red crystalline powder	Visual inspection
Affirmation test- UV	A233/A259 = 0.90±0.09 A322/A259 = 0.56±0.03	USP<197U>
PQQ (as-is basis)	≥85%	USP<621>
PQQ disodium salt (chromatography)	≥99%	USP<621>
Water content	≤12%	USP<921>
Ethanol	≤5,000 ppm	Gas chromatography
Heavy metals		
Lead	≤0.5 ppm	ICP-MS BS EN ISO 17294-2004
Arsenic	≤0.5 ppm	ICP-MS BS EN ISO 17294-2004
Cadmium	≤0.3 ppm	ICP-MS BS EN ISO 17294-2004
Mercury	≤0.2 ppm	ICP-MS BS EN ISO 17294-2004
Microbiological analysis		
Total aerobic count	≤10,000 CFU/g	USP<61>
Total mold and yeast	≤1,000 CFU/g	USP<61>
Coliforms	≤100 CFU/g	USP<62>
Escherichia coli	≤10 CFU/g	USP<62>
Salmonella	≤10 CFU/g	USP<62>

2.E Safety of Bacteria Used in Fermentation

The principle of JinCheng's PQQ disodium salt production method (via bacterial fermentation) is similar to those described by other investigators (Urakami et al., 1992) and other companies, such as Mitsubishi Gas Chemical Company and Hisun whose production method for PQQ disodium salt received no objection letter from the FDA (FDA, 2007, RPT 417; FDA, 2016a, GRN 641). Specifically, Mitsubishi Gas Chemical Company and Urakami et al. (1992) describe a method using *Hyphomicrobium denitrificans* American Type Culture Collection (ATCC) 51888 strain (or *Hyphomicrobium* TK0441 strain) in a fermentation process to produce PQQ disodium salt.

This ATCC 51888 strain is classified as Biosafety Level I (BSL-1) (ATCC, 2016). As mentioned in GRN 641 (pages 40-42), the U.S. Centers for Disease Control and Prevention (CDC) define BSL-1 organisms as those "not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment" (CDC, 2009).

Molecular identification *via* 16S ribosomal DNA (rDNA) genomic sequence analysis demonstrates that the JinCheng's source organism of PQQ disodium salt has a 100% sequence similarity with *Hyphomicrobium denitrificans* ATCC 51888 strain.

Hyphomicrobium are facultatively methylotrophic, non-spore forming, gram-negative, rod-shaped bacteria with a unique Q-9 ubiquinone system (FDA, 2016a [GRN 641]). Further morphological and biochemical analyses demonstrate that *H. denitrificans* is a gram-negative bacterium that forms milky colonies and is positive for nitrate reduction. *Hyphomicrobium denitrificans* is not a genetically modified organism. The *Hyphomicrobium* used for the production of PQQ disodium salt is maintained in-house by JinCheng and is subject to strict quality control for compliance with established internal specifications. Table 7 presents taxonomic classification of *Hyphomicrobium denitrificans.*

Table 7. Taxonomic Classification of *Hyphomicrobium denitrificans*

Class	Scientific Classification
Kingdom	*Prokaryota*
Division	*Bacteria*
Subdivision	*Proteobacteria*
Class	*Alphaproteobacteria*
Order	*Rhizobiales*
Family	*Hyphomicrobiaceae*
Genus	*Hyphomicrobium*
Species	*Hyphomicrobium denitrificans*

2.F Shelf-life and Storage Conditions

As the PQQ disodium salt in this NDI notice is similar in specifications and manufacturing process compared to those described in the previous FDA GRAS notice (PQQ disodium salt manufactured by bacterial fermentation with *Hyphomicrobium denitrificans*, filed by Hisun;

GRN 641), it is recognized that the stability data in GRN 641 are pertinent to those of the PQQ disodium salt in this NDI notice. Therefore, this notice incorporates, by reference, the stability studies discussed in the previous GRAS notice.

GRN 641 discussed the stability of PQQ disodium salt as follows:
"The bulk stability of 3 consecutive lots of PQQ (lot no. M130101, M130102, M130103) was assessed under ambient and accelerated storage conditions. These studies were conducted in accordance to testing conditions established by the International Conference on Harmonization (ICH) Q1A guidance (ICH, 2003). At 25±2°C room temperature and 60±5% relative humidity, the PQQ disodium salt was stable for at least 24 months. Under accelerated conditions of 40±2°C and 75±5% relative humidity, PQQ was reported to be stable for a period of 6 months" (from GRN 641 pages 20 and 21). A table presented in GRN 641 (page 21) showed no loss at a room temperature during the 24 month storage period.

In addition, JinCheng has the data to support the stability of PQQ disodium salt at 25℃and at 60% relative humidity for 12 months. Details are found in Appendix D.

PART 3. DIETARY EXPOSURE

3.1. EDI of PQQ Disodium Salt Under the Intended Use

The intended use of PQQ disodium salt of the current notice is in the same food products previously mentioned in GRNs 625 and GRN 641. Table 8 compares use levels of the current notice with those described in previous GRAS notices. With the exception of energy drinks, the higher level was selected for each category, if the different use levels were reported between GRNs 625 and 641 for the same intended use. In the current notice, energy drinks are intended to be used at a maximum use level of 12 mg/serving. Using food intake data reported in the 2011-2014 and 2011-2012 National Health and Nutrition Examination Survey (NHANES), exposure levels to PQQ disodium salt that will result from the intended uses were estimated. The NHANES food codes used in the calculation of EDIs are shown in Appendix E.

Table 8. Comparison of the intended use levels

Food-Uses	Serving Size (RACC)[1]	Proposed Maximum Use Level, mg/RACC		
		GRN 625	GRN 641	Current Notice
Energy Drinks	240 mL	8	5	12
Sport and Electrolyte Drinks	240 mL	8	5	8
Enhanced and Fortified Water Beverages	240 mL	8	20	20
Bottled water	240 mL	8	0	8
Non-Milk Based Meal Replacement Beverages	240 mL	8	0	8

[1] RACC refers to Reference Amounts Customarily Consumed per eating occasion – 21 CFR §101.12 (U.S. FDA, 2015). When a range of values is reported for a particular food-use, particular foods within that food-use may differ with respect to their RACC.

The results of the EDI assessment are summarized in the two tables below (Tables 9-1 and 9-2). Under the intended use, approximately 52% of the population are estimated to be the users of PQQ disodium salt. Based on NHANES 2011-2014 dataset, the mean and 90th percentile all-user intakes of PQQ disodium salt were estimated to be 28.2 and 63.1 mg/person/day, respectively. When the NHANES 2011-2012 dataset was used, the intended use resulted in slightly lower values of EDIs: a mean EDI of 26.8 mg/person/day and a 90th percentile EDI of 61.3 mg/person/day. Based on the 2011-2012 NHANES dataset, GRN 625 reported the mean and 90th percentile EDIs of 26.5 and 61.4 mg/person/day, respectively. In GRN 641, corresponding EDIs were 12.8 and 27.8 mg/kg bw/day, respectively. The data show that intended use and use levels in this GRAS notice results in comparable EDIs to those described in GRN 625.

The analysis of 2011-2014 NHANES dataset reveals that males older than 19 years of age would have highest intake among the various age/gender groups, with a 90th percentile value of 72 mg/person/day in all-users. On a body weight basis, children aged 2-5 years had the highest 90th percentile EDI at 1.47 mg/kg bw/day in all-users.

The NOAEL was determined to be 400 mg/kg bw/day in a subchronic toxicity study in rats (details are found in the Part 6.B.3). After applying a safety margin of 100, it can be concluded that doses of up to 4 mg/kg bw/day or 240 mg/person/day would be safe in adults weighing 60 kg. The EDIs under the intended use are less than one-third the estimated safe intake levels in humans.

These estimates are highly amplified since it is not likely that PQQ disodium salt will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered. In addition, short-term surveys, such as the typical 2-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently. Overall, intended use will result in EDIs at levels significantly below those associated with any potential side effects.

Table 9-1. Maximum EDIs of PQQ disodium salt under the intended use*, mg/day

Population	% all-user	N, total population	Per User (g/day)		Per Capita (g/day)	
			Mean	90th Percentile	Mean	90th Percentile
Based on the 2011-2014 NHANES dataset						
2-5 y	45.7	1,262	11.2	23.2	5.1	16.6
6-12 y	53.9	2,206	14.8	32.2	8.0	23.7
13-18 y males	59.0	822	28.2	60.5	16.6	44.7
13-18 y females	64.9	838	23.8	46.5	15.4	41.1
19+ males	49.7	4,294	33.0	72.6	16.4	53.0
19+ females	52.9	4,739	29.7	64.6	15.7	49.0
2-99 y	52.2	14,161	28.2	63.1	14.7	45.9
Based on the NHANES 2011-2012 NHANES dataset						
0-2 y	37.2	605	8.7	21.7	3.2	11.5
3-11 y	49.7	1512	12.7	28.0	6.3	19.7
12-19 y males	60.2	518	25.2	59.4	15.1	44.4
12-19 y females	65.8	533	21.8	45.8	14.4	40.1
20+ males	47.2	2,094	32.9	69.7	15.5	50.9
20+ females	50.6	2,251	28.3	62.9	14.3	44.4
2-99 y	50.2	7,486	26.8	61.3	13.4	41.6

*Assuming All the Foods will be Used at the Maximum Use Levels; NHANES 2011-2014

Table 9-2. Maximum EDIs of PQQ disodium salt, mg/kg bw/day

Population	Per User (g/kg bw/day)		Per Capita (g/kg bw/day)	
	Mean	90th Percentile	Mean	90th Percentile
Based on the 2011-2014 NHANES dataset				
2-5 y	0.66	1.47	0.30	1.01
6-12 y	0.42	0.93	0.23	0.67
13-18 y males	0.39	0.85	0.23	0.66

13-18 y females	0.39	0.80	0.25	0.64
19+ males	0.38	0.87	0.19	0.61
19+ females	0.40	0.85	0.21	0.66
2-99 y	0.41	0.89	0.21	0.65

*Assuming All the Foods will be Used at the Maximum Use Levels; NHANES 2011-2014

3.2. Food Sources of PQQ

PQQ is a naturally occurring in small quantities in food products, particularly vegetables, fruits, and fermented soy and dairy products (Kumazawa et al. 1995, Kumazawa et al., 1995, and Noji et al., 2007). As shown in Table 10, concentrations of PQQ in foods are low, typically in the ppb range.

Table 10. Concentrations of PQQ in Common Foods

Food Item	PQQ Content (ng/g wet weight or ng/mL)	Food Item	PQQ Content (ng/g wet weight or ng/mL)
Broad bean[a]	17.8 ± 6.78	Green soybeans[a]	9.26 ± 3.82
Potato[a]	16.6 ± 7.34	Sweet potato[a]	13.3 ± 3.72
Parsley[a]	34.2 ± 11.6	Cabbage[a]	16.3 ± 3.96
Carrot[a]	16.8 ± 2.81	Celery[a]	6.33 ± 2.41
Green pepper[a,b]	2.12 ± 0.4 to 28.2 ± 13.7	Spinach[a, b]	7.0 ± 2.17 to 21.9 ± 6.19
Tomato[a,b]	ND to 9.24 ± 1.82	Apple[a]	6.09 ± 1.36
Banana[a]	12.6 ± 3.81	Kiwi fruit[a]	27.4 ± 2.64
Orange[a]	6.83 ± 2.20	Papaya[a]	26.7 ± 8.57
Field mustard[b]	5.54 ± 1.50	Broccoli sprout[b]	1.55 ± 0.37
Japanese radish	0.70 ± 0.42	Rape blossom[a]	5.44 ± 0.8
Green tea[a,b]	0.16 ± 0.05 to 29.6 ± 12.9	Miso (bean paste)[a]	16.7 ± 3.30
Coke[a]	20.1 ± 3.17	Fermented soybeans[a] (natto)	61.0 ± 31.3
Wine[a]	5.79 ± 2.73	Fermented soybeans[a]	1.42 ± 0.32
Oolong (tea)[a]	27.7 ± 1.92	Tofu (bean curd)[a]	24.4 ± 12.5
Whiskey[a]	7.93 ± 1.84	Skim milk (dry wt basis)[c]	2.5[1] ± 1.4
Sake[a]	3.65 ± 1.39	Milk[c]	3.4 ± 0.4
Beer[a]	1.66 ± 0.82	Egg yolk[2, c]	7.0 to 19.3
Bread[a]	9.14 ± 3.64	Egg white[2, c]	4.1 to 28.8

PQQ = pyrroloquinoline quinone

Adapted from [a]Kumazawa et al. 1995, [b]Kumazawa et al., 1995, and [c]Noji et al., 2007.

[1] Units for skim milk lyphosilisate are ng/g dry weight.

[2] Eggs were obtained from domestic fowl (*Gallus gallus*) and duck (*Cairina moschata*)

3.3. Estimated Daily Intakes (EDIs) of Naturally Occurring PQQ from the Diet

The PQQ concentration in each food is not listed in the USDA food composition tables or the National Health and Nutrition Examination Survey (NHANES) databases. Using the dietary content of PQQ available from the literature (Table 10), the EDIs from the diet were estimated. NHANES 2011-2012 dietary data for age 2 years and older were used to estimate PQQ exposure from select dietary sources (Table 11-1). The dietary sources being analyzed are 36 common foods that contain PQQ as listed in Part 3.A. Intake used was the average of two-day intakes from subjects with intake over 0 reported on either day's data (N = 7,100).

The mean and 90th percentile EDIs of users are 8.7 and 17.2 µg PQQ/person/day, which correspond to 0.13 and 0.26 µg/kg bw/day. These levels are insignificant compared to EDIs under the intended use.

Table 11-1. EDIs of PQQ from the Diet, µg/day *

		µg/day		µg/kg bw/day	
Population	N	Mean	90th Percentile	Mean	90th Percentile
2-5 y	714	4.14	6.72	0.25	0.43
6-12 y	1,156	4.65	10.31	0.16	0.28
13-18 y males	396	8.31	16.47	0.12	0.22
13-18 y females	396	7.48	15.39	0.12	0.21
19+ males	2,156	10.94	21.20	0.13	0.26
19+ females	2,282	8.06	15.83	0.11	0.20
2-99 y	7,100	8.66	17.02	0.13	0.26

* Based on NHANES 2011-2014; PQQ = pyrroloquinoline quinone.

3.4. EDI of Other Components Under the Intended Use

PQQ disodium salt contains other nutrients such as sodium.

The sodium content of PQQ disodium salt is approximately 12%. Thus, the EDIs of sodium under the intended use were calculated based on the EDI of PQQ disodium salt (Tables 9-1 and 9-2) and the sodium content of PQQ disodium salt. For example, the 90th percentile EDI of PQQ disodium salt is 63.1 mg/person/day in all users aged 2-99 years (Table 9-1). Given that the concentration of sodium in PQQ disodium salt is 12%, the 90th percentile EDI of sodium under the intended use (7.57 mg/person/day) then can be calculated by multiplying 63.1 by 0.12. As shown in Table 11-2, the estimated intakes of sodium under the intended use are negligible compared to usual intakes of sodium from the diet, since an average daily sodium intake for Americans is approximately 4,024 mg per person per day (USDA, 2017). In other words, intended use of PQQ disodium salt would likely have no significant impact on sodium intakes in Americans.

Table 11-2. Maximum EDIs of sodium under the intended use, mg/day

	Per User (mg/day)		Per Capita (mg/day)	
Population	Mean	90th Percentile	Mean	90th Percentile
2-5 y	1.34	2.78	0.61	1.99
6-12 y	1.78	3.86	0.96	2.84
13-18 y males	3.38	7.26	1.99	5.36
13-18 y females	2.86	5.58	1.85	4.92
19+ males	3.96	8.71	1.97	6.36
19+ females	3.56	7.75	1.88	5.88
2-99 y	3.38	7.57	1.76	5.51

*Assuming all the foods assessed will be used at the maximum use levels; NHANES 2011-2014
EDI = Estimated daily intake.

Summary of Consumption Data

Consumption data and information pertaining to the individual proposed beverage-uses of PQQ disodium salt were used to estimate the total population and all-user intakes of PQQ disodium salt for specific age/gender groups. These estimates are highly amplified or unrealistic since it is not likely that PQQ disodium salt will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered. In addition, short-term surveys, such as the typical 2-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently.

Among consumers in the total population, the mean and 90th percentile all-user intakes of PQQ disodium salt were determined to be 28.2 and 63.1 mg/person/day, respectively, when the 2011-2014 NHANES dataset was used for calculation of EDIs. These EDIs are comparable to those reported in GRN 625. Corresponding EDIs reported in GRN 625 were 26.5 and 61.4 mg/person/day, respectively. Males older than 19 years of age would have the highest 90th percentile intake among the various age/gender groups, with a 90th percentile value of 72 mg/person/day in all-users. On a body weight basis, children aged 2-5 years had the highest 90th percentile EDI at 1.47 mg/kg bw/day in all-users.

The NOAEL was determined to be 400 mg/kg bw/day in a subchronic toxicity study in rats (details are found in the Part 6.B.3). After applying a safety margin of 100, it can be concluded that doses of up to 4 mg/kg bw/day or 240 mg/person/day would be safe in adults weighing 60 kg. The EDIs under the intended use are less than one-third the estimated safe intake levels in humans. Overall, intended use will result in EDIs at levels significantly below those associated with any potential side effects.

PART 4. SELF LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with the PQQ disodium salt ingredient although PQQ has a characteristic taste and odor. Excessive amounts of this product are unlikely to be added to food products because the taste is unpleasant when too intense. Additionally, the cost of the product ($5,000-$12,000/kg) will also prohibit the excessive use.

PART 5. THE HISTORY OF CONSUMPTION

EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

The statutory basis for the conclusion of GRAS status of PQQ disodium salt in this document is not based on common use in food before 1958. The GRAS determination is based on scientific procedures. As described above, PQQ is present naturally in food. It is reasonable to conclude that it was present in food prior to 1958, albeit in small quantities.

PART 6. BASIS FOR GRAS DETERMINATION

6.A. Current Regulatory Status

The FDA has previously issued 'no objection' letters on GRAS and New Dietary Ingredient (NDI) notices related to PQQ disodium salt produced by a bacterial fermentation technique (GRN 641 filed by Hisun, FDA, 2016a; NDI notice 417 filed by Mitsubishi Gas Chemical Co., Inc.). In addition, the FDA has issued a 'no question' letter on synthetic PQQ disodium salt (GRN 625, FDA 2016b).

6.B. Review of Safety Data

As the PQQ disodium salt in this GRAS notice has similar specifications compared to the PQQ disodium salt in the previous FDA GRAS and NDI notices (Table 12), it is recognized that the information and data in GRN 641 and NDIN RPT 417 are pertinent to the safety of the PQQ in this GRAS notice. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in the previous GRAS notice, and will not discuss previously reviewed references in detail. Additionally, this notice discusses additional animal studies that have been published since the FDA's last review in 2016. The subject of the present GRAS notice is PQQ disodium salt produced via microbial fermentation (powder form).

Table 12. Comparison of the PQQ disodium salt preparations

Parameter	Source				
	JinCheng	Hisun	Mitsubishi	Shanghai Med Co	Nascent Health
Regulatory status	Current notice	GRN 641	NDIN 417	NA	GRN 625
Manufacturing Method	Fermentation by *Hyphomicrobium sp.*			Not specified; probably synthetic	Synthetic
Appearance	Reddish brown crystalline powder	Henna powder	Reddish brown crystalline powder	Reddish brown crystalline powder	Reddish brown crystalline powder
Purity	>99%	>99% (page13)	>99%	>98%	>98% (page 19)

PQQ = pyrroloquinoline quinine
[1] Urakami et al. (1992); Urakami (1994 -Patent US5344768)

Based on a comparison of the specifications for these products, it is concluded that they are essentially the same.

6.B.1. Metabolism of PQQ

Since the FDA's last review of GRN 625 (pages 15-16 or stamped pages 27-28) and GRN 641 (pages 27-29) in 2016, no new metabolism study has been published.

A human study conducted by Harris et al. (2013) found that levels of PQQ peaked in serum at ~2 h. The rise and clearance of PQQ in serum paralleled changes in urine ($r=0.9$, $p<0.05$) following a single dose of PQQ (0.2 mg PQQ/kg body weight [bw]). In study 1, where PQQ was given in a single dose (0.2 mg PQQ/kg bw), multiple measurements of plasma and urine PQQ levels and changes in antioxidant potential [based on total peroxyl radical-trapping potential and thiobarbituric acid reactive product (TBAR) assays] were made throughout the period of 48 h. Dietary PQQ exposure (Study 1) resulted in apparent changes in antioxidant potential based on malonaldehyde-related TBAR assessments. In study 2, PQQ was administered as a daily dose (0.3 mg PQQ/kg bw). After 76 h, measurements included indices of inflammation (plasma C-reactive protein, interleukin [IL]-6 levels), standard clinical indices (e.g., total cholesterol [TC], glucose, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], triglycerides [TG]) and ^{1}H-nuclear magnetic resonance (NMR) estimates of urinary metabolites related in part to oxidative metabolism. The standard clinical indices were not altered by PQQ supplementation. PQQ supplementation resulted in significant decreases in the levels of plasma C-reactive protein (CRP), IL-6 and urinary methylated amines (e.g., trimethylamine N-oxide), and changes in urinary metabolites consistent with enhanced mitochondria-related functions.

Human tissue contains from 1 to 3 ng of non-derivatized PQQ per gram of tissue or milliliter of fluid. Dietary PQQ (0.1 to 1.0 mg/day) is sufficient to maintain the nanomolar concentrations of PQQ in tissues, and that concentration is responsive to changes in the diet (Kumazawa et al. 1992). Limited data from humans suggest that metabolism would be more in line with what was seen in rats.

In a study by Smidt et al. (1991), approximately 62% of the PQQ was absorbed through the small intestine and 81% of that was excreted within 24 h via urine when1.5 mg/kg PQQ (radiolabeled with ^{14}C) was administered to 10 male mice by oral gavage. This shows that PQQ is absorbed effectively, with most of it excreted in urine. The radioactive PQQ was detected in the kidneys (10.7%) and skin (1.3%) 24 h after oral administration. The liver retained only a small percentage of the absorbed PQQ (i.e., 5.4% after 6 h and 1.5% after 24 h). Low concentrations were detected in the liver, suggesting that biliary elimination is not a major excretion route in mice. In the blood, nearly all of the PQQ (95 to 97%) was associated with the blood cell fraction at both 6 and 24 h. At 6 h, the blood cell fraction constituted about 10% of the absorbed label. This fell to 1.2% at 24 h.

6.B.2. Mutagenicity and Genotoxicity Studies of PQQ Disodium Salt

Since the FDA's last review in 2016, no new studies have been published. Mutagenicity and genotoxicity studies of PQQ disodium salt are summarized in Table 13. GRN 641 (FDA, 2016a, pages 37-38) and NDI notice 417 (FDA, 2007) reported that PQQ disodium salt preparations obtained from microbial fermentation by *Hyphomicrobium denitrificans* were not mutagenic or genotoxic (Table 13).

Additionally, synthetically manufactured PQQ disodium salt also showed that it lacked mutagenicity and genotoxicity (Table 13; FDA, 2016b, GRN 625, pages 35-38 and 40-41).

Overall, studies consistently show that all preparations of PQQ disodium salt are not mutagenic or genotoxic.

Table 13. Mutagenicity and genotoxicity studies of PQQ disodium salt

Test system	PQQ concentration	Test	Outcome	Reference
S. typhimurium strains TA97, TA98 and TA100	0, 1, 6, 8, 40, 200, or 1,000 µg/plate w/ and w/o S9 activation	Ames test (mutagenicity), *in vitro*	No mutagenic potential	FDA, 2016a (GRN 641)
S. typhimurium strains TA97, TA98, TA100, and TA102	0, 62, 556, 1,667, and 5,000 µg/plate w/ and w/o S9 activation	Ames test (mutagenicity), *in vitro*	No mutagenic potential	FDA, 2016b (GRN 625)
S. typhimurium strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2*uvrA*	0, 10, 20, 39, 78, 156, 313, 625, 1,250, 2,500, or 5,000 µg/plate w/activation or 0, 156, 313, 625, 1,250, 2,500, or 5,000 µg/plate w/o activation	Ames test (mutagenicity), *in vitro*	No mutagenic potential	FDA, 2007 (NDIN RPT 417)
ICR mice	M-0, 530, 1,050, or 2,110 mg/kg bw/day for 4 days; F- 0, 730,1,460, or 2,920 mg/kg bw/day for 4 days	*In vivo* mouse micronucleus assay	Not genotoxic	FDA, 2016a (GRN 641)
Kunming mice	M - 630, 1250, and 2,500; F - 460, 920, and 1,840 mg/kg bw for 1 day	*In vivo* mouse micronucleus assay	Not genotoxic	FDA, 2016b (GRN 625)
30 Crlj:CH1 male mice	0, 250, 500, 1,000, or 2,000 mg/kg bw	*In vivo* mouse micronucleus assay	Not genotoxic	FDA, 2007 (NDIN RPT 417)
Chinese hamster lung fibroblasts	0, 117.2, 234.4, 468.8, 937.5, 1875, or 3750 µg/mL w/activation or 0, 12.5, 25, 50, 100, 200, or 400 µg/mL w/o activation	Chromosomal aberration test, *in vitro*	Very weak positive results	
Human peripheral blood lymphocytes	0, 234.4, 468.8, 937.5, 1,875, or 3,750 µg/mL w/activation, or 0, 117.2, 234.4, 468.8, 937.5, 1,875, or 3,750 µg/mL w/o activation	Chromosomal aberration test, *in vitro*	Not genotoxic	

Abbreviations: bw = body weight; F=female; M=male; PQQ=pyrroloquinline quinone; w/o= without.

6.B.3. Animal Toxicity Studies

3.2.3.1. Study of JinCheng's PQQ Disodium Salt

Acute Oral Toxicity Study in Rats

The aim of this study was to evaluate acute toxicity of PQQ disodium salt after a single oral administration in rats (Gao, 2017). PQQ disodium salt was administered to 50 young rats by oral gavage at a single dose of 2.50, 3.00, 3.60, 4.32 or 5.18 g/kg bw (5 males and 5 females per group). Animals were observed for 14 days to monitor changes in body weight and clinical signs as well as food and water consumption. At the end of the study, all surviving animals were sacrificed and major organs were examined. After PQQ disodium salt administration, a number of animals died on the following days at 3.00, 3.60, 4.32, and 5.18 g/kg BW. On day 1, greenish loose stools were observed in the 3.60, 4.32, and 5.18 g/kg bw groups after PQQ disodium salt administration. Also, a greenish tail was found in the 5.18 g/kg bw group. In addition, necropsy revealed enlarged kidneys in the 3.00 and 3.60 g/kg bw groups, and the coefficient of kidney function was increased in these groups. These differences were not significant. Taken together, orally administered PQQ disodium salt caused dose-dependent mortalities with the median lethal dose (LD_{50}) of 3.47 g/kg bw, with a 95% confidence interval of 3.12-3.84 g/kg bw.

3.2.3.2. Studies on Other Sources of PQQ Disodium Salt

This GRAS notice has summarized existing studies pertaining to the toxicity of PQQ disodium salt in animals (Table 14). The findings of the animal toxicity studies collectively support the safety of PQQ disodium salt supplementation (FDA 2016a, pages 30-33; FDA 2016b, pages 29-34 and 42-43; Liang et al., 2015; Nakano et al., 2014). Specifically, the results from subchronic toxicity studies by Nakano et al. (2014) and Liang et al. (2015) indicate that PQQ disodium salt was safe up to the highest doses tested. These were 100 mg/kg bw/day and 400 mg/kg bw/day, respectively.

Reproductive toxicity study (sperm shape abnormality assay) in mice indicated that there was no treatment-related sperm abnormalities at any dose level (up to 2,000 mg/kg bw/day) of PQQ disodium salt (GRN 625, FDA, 2016b, page 37). Steinberg et al. (2003) reported that PQQ concentration of 6μM per kg diet did not impact reproductive performance.

Conclusion: Based on these studies, for purposes of this evaluation, a NOAEL of 400 mg/kg bw/day was chosen for PQQ disodium salt.

Table 14. Oral toxicity studies of PQQ disodium salt in animals

Animal	Dose, PQQ disodium salt	Duration	Measured Outcome	NOAEL or LD_{50}	Reference
JinCheng 's PQQ disodium salt					
50 male and female Sprague-Dawley rats	2.50, 3.00, 3.60, 4.32, or 5.18 g/kg bw	Single day; 14 d follow-up	Oral median lethal dose (LD_{50})	LD_{50}: 3.47 g/kg bw	Gao, 2017
Studies of Other PQQ disodium salt					
Acute toxicity studies					
50 male and female Sprague-Dawley rats	1, 2.15, 4.64, 10.0, or 21.5 g/kg bw	Single day	LD_{50}	LD_{50}: M, 3.69 g/kg bw; F, 5.01 g/kg bw	GRN 625, FDA 2016b (synthetic)
ICR mice	1-21.5 g/kg bw	Single day	LD_{50}	LD_{50}: M, 4.22 g/kg bw; F, 5.84 g/kg bw	GRN 641, FDA 2016a, pages 30-31, (bacterial fermentation)
80 male and female Sprague-Dawley rats	0, 500, 1,000, or 2,000 mg/kg bw	Acute, 14 day follow-up	LD_{50}	LD_{50}: M, 1- 2 g/kg bw; F, 0.5-1.0 g/kg bw	Nakano et al. (2014)
Subacute toxicity studies					
72 male and female Sprague-Dawley rats	0, 3, 12, 48, 192, or 768 mg/kg bw/d	14 day	Body weight, food consumption, urinalysis, hematology, mortality, serum clinical biochemistry, organ wt, and histopathology	NOAEL: 192 mg/kg bw/d	Nakano et al. (2014); RPT 417 (FDA, 2007) bacterial fermentation)
36 female Sprague-Dawley rats	0, 200, or 700 mg/kg bw/d	28 day		NOAEL: <200 mg/kg bw/d	
Subchronic toxicity studies					
80 male and female Sprague-Dawley rats	0, 100, 200, or 400 mg/kg bw/d	90 days	Body weight, food consumption, hematology, mortality, serum clinical biochemistry, organ wt, and histopathology	NOAEL: 400 mg/kg bw/d	Liang et al., 2015 (synthetic; shown in GRN 625, pages 29-34)
80 male and female Sprague-Dawley rats	0, 3, 20, or 100 mg/kg bw/d	91 day (13 weeks)		NOAEL: 100 mg/kg bw/d, the highest level tested	Nakano et al. (2014)

Teratogenicity study (GRN 625 pages 38-39)					
Wistar rats	0, 78, 310 and 1,250 mg/kg bw/day	7th to the 16th day of gestation	Teratogenicity (embryo survival and development, fetal gross malformations, and fetal bone and organ development)	Not teratogenic	GRN 625, FDA 2016b, pages 38-39 (synthetic)
Reproductive toxicity studies					
Female BALB/c mice	0 or 6μM/kg diet	8 weeks before breeding	Reproductive performance	6 μM/kg diet, the highest level tested	Steinberg et al., 2003
Kunming mice	460, 920, and 1,840 mg/kg bw	5 days	Sperm malformation assay	No abnormality	GRN 625, FDA 2016b, page 37 (synthetic)

Abbreviations: NOAEL= no-observed-adverse-effect-level; LD_{50}= lethal dose 50; bw= body weight; M= male; F= female.

6.D.4. Animal Efficacy Studies

We searched the literature for animal studies that examined the effects of orally administered PQQ disodium salt on various outcomes. Since the FDA review in 2016, three animal efficacy studies of PQQ disodium salt were published (Table 15: Huang et al., 2016; Jonscher et al., 2016; Wang et al., 2016) to confirm the safety PQQ disodium salt. A few animal efficacy studies that were reported in GRN 641 (Table 16: Kilgore et al., 1989; Kumar et al., 2015; Samuel et al., 2015; Wang et al., 2015) also found no adverse effects of PQQ disodium salt. Additional animal efficacy studies that have not been included in previous GRAS notices show that there is no safety concern (Table 15: Bauerly et al. 2011; Hamagishi et al. 1990; Stites et al. 2006a, 2006b; Takeda et al. 2012; Wang et al. 2015).

Although these studies were designed to investigate the efficacy of PQQ disodium salt on various health parameters, several safety related endpoints were obtained during the experiments. Therefore, these studies are reviewed as additional supporting information (Table 15). Measured outcomes reported in these efficacy studies include oxidative stress parameters in the liver and plasma and liver DNA damage (Wang et al., 2016), radioprotective effects (Huang et al., 2016), and nonalcoholic fatty liver disease indices (Jonscher et al., 2016).

Tables 16 and 17 showed that efficacy studies published prior to 2015 include parameters such as serum biochemical indices, mitochondrial content, cardiac ischemia indices, and energy expenditure (Bauerly et al., 2011), effect on mitochondrial complex 1 inhibitor diphenylene iodonium (Stites et al., 2006a), carrageenan-induced edema (Hamagishi et al., 1990), effects on impaired glucose tolerance (Takeda et al., 2012), redox status of plasma and mitochondrial-related metabolism (Wang et al., 2015), mitochondria counts, respiratory indices, and serum biochemical indices (Stites et al., 2006b), brain tissue levels of lipid peroxidation and

hydroperoxidation and the associated hyperglycemia-induced oxidative damage (Kumar and Kar, 2015), growth, carcass yields, antioxidant status (Samuel et al., 2014; Wang et al., 2015), and effect on L-T_4-induced hyperthyroidism (Kumar et al., 2014). No studies showed adverse effects of PQQ or PQQ disodium salt on measured outcomes.

These animal efficacy studies showed that PQQ disodium salt at the level of up to 2 mg/kg diet/day (Stites et al. 2006b) or 30 mg/kg bw/day (Hamagishi et al. 1990) did not cause any adverse effects on measured outcomes and was well tolerated. The results are summarized in Tables 15 through 17.

Table 15. Summary of animal efficacy studies of PQQ disodium salt published since 2016

Animal	Dose	Duration	Measured Outcome	Results	Author
360 53-week-old Hy-Line Gray laying hens	An oxidized sunflower oil diet with 0.08 mg or 0.12 PQQ disodium salt/kg diet	6 wk	Oxidative stress parameters in the liver and plasma and liver DNA damage	These unfavorable changes induced by the oxidized sunflower oil diet were modulated by dietary vitamin E or PQQ disodium salt supplementation to levels comparable to the fresh oil group	Wang et al., 2016
15 female 8-week-old C57BL/6J mice	3 groups: Untreated control (no irradiation); 4 gray (Gy) X-ray irradiation with or without dietary PQQ (4 mg PQQ/kg in normal diet)	4 wk	Radioprotec-tive effects	PQQ could partially rescue irradiation-induced damage to parotid glands via multiple mechanisms, such as promoting proliferation, inhibiting apoptosis and senescence, upregulating antioxidant ability, scavenging reactive oxygen species and reducing DNA damage.	Huang et al., 2016
Mice-Western diet-fed offspring	Study 1- 1.25 mg/l or 7.5 ug/d with high or low fat diet for a prenatal study; Study 2-postnatal study with and w/or PQQ up to 24 ug/d per mouse, with low fat diet or Western diet (PQQ source - NA)	Study 1-prenatal study – from 16 wk of age (or 8 wk before mating) throughout gestation; Study 2-postnatal 21 d (at weaning) or 20 wk of age	Nonalcoholic fatty liver disease indices (NAFLD), such as hepatic ceramide levels, oxidative stress, and expression of pro-inflammatory genes (Nos2, Nlrp3, Il6, and Ptgs2)	Study 1-No effect on maternal body weight gain but increases placental size (by 5-10%, $P<0.05$) in pregnant obese mice. Study 2- Indices of NAFLD including hepatic ceramide levels, oxidative stress and expression of proinflammatory genes were decreased in WD PQQ-fed mice. The authors concluded that PQQ, particularly during pregnancy and lactation, protects offspring from Western diet -induced hepatic lipotoxicity.	Jonscher et al., 2016

Table 16. Key animal efficacy studies of PQQ disodium salt that were included in GRN 641

Animal	Dose	Duration	Measured Outcome	Results	Author
784 male Arbor Acres broiler chicks	0, 0.05, 0.1, 0.2, 0.4, or 0.8 mg/kg diet/d	7 wk	Growth, carcass characteristics, antioxidant status	Improved growth performance, feed efficiency, thymus index, and carcass yield	Samuel et al., 2014
35 female Wistar rats	0, 3, or 5 mg/kg bw/d	16 d	Effect on L-T_4-induced hyperthyroidism	5 mg/kg bw/d of PQQ ameliorated adverse effects of hyperthyroidism	Kumar et al., 2014
Trial 1-490 1 day-old male Arbor Acres (AA) broiler chicks; Trial 2-120 1-day-old male AA chicks	Trial 1-0, 0.05, 0.1, 0.2, or 0.4 mg /kg feed; Trial 2- 0 or 0.2 mg/kg feed	Trial 1-42 d Trial 2-42 d	Trial 1- growth performance, carcass characteristics, and plasma biochemical parameters; Trial 2- the redox status of plasma samples and mitochondrial-related metabolism	Improved feed efficiency, higher anti-oxidative capacity (P = 0.001), lower redox potential (P = 0.008), and higher hepatic citrate synthase activity; no difference in hepatic mitochondrial DNA copy number.	Wang et al., 2015
Mice	PQQ deficient diet or supplemented diet (800 ng PQQ/g diet)	2-3 wk prior to mating, until 8 wk after birth	Mortality, reproductive toxicity and clinical signs of toxicity	By Week 8, the PQQ-deprived group had a significantly higher incidence of mortality than the PQQ-supplemented group (20% vs. 3%). When female mice were given the PQQ-deficient diet 8 to 9 weeks prior to breeding, no litters were produced or offspring were cannibalized upon birth.	Kilgore et al., 1989

D=day; wk=weeks.

Table 17. Animal efficacy studies that were not included in GRN 641 and 625

Animal	Dose	Duration	Measured Outcome	Results	Author
32 male Sprague-Dawley (SD) rats	0 or 2 mg/kg diet	3 d	Serum biochemical indices, standard clinical indices, mitochondrial content, cardiac ischemia indices, and energy expenditure	PQQ deficient rats had lower energy expenditure, mitochondrial content, higher lipids, and cardiac injury from ischemia	Bauerly et al., 2011
15 male BALB/c mice from PQQ deficient dams	0, 0.4, or 4 mg/g bw/d	14 d	Effect on mitochondrial complex 1 inhibitor diphenylene iodonium (DPI)	PQQ prevented weight loss and lower plasma glucose levels found in deficient mice	Stites et al., 2006a
46 male SD rats	0, 10, or 30 mg/kg bw	2 h	Carrageenan-induced edema	PQQ reduced edema by 33-39.4%	Hamagishi et al., 1990
18 male type-2 diabetic KK-A^y/TaJcl mice	0, 5, or 20 mg/kg bw/d	14 d	Effect on impaired glucose tolerance	PQQ decreased peak blood glucose levels and increased oral glucose tolerance	Takeda et al., 2012
120 male Arbor Acres broiler chicks	0 or 0.2 mg/kg diet	7 wk	Redox status of plasma and mitochondrial-related metabolism	PQQ increased feed efficiency, anti-oxidative capacity, hepatic citrate synthase activity, and lowered redox potential.	Wang et al., 2015
Pups from 24 female BALB/c mice	0 or 2 mg/kg diet/d	8 wk	Mitochondria counts, respiratory indices, and serum biochemical indices	Pups from PQQ deficient mice had 20-30% lower mitochondrial counts, reduced respiratory control ratios and quotients, and elevated serum glucose, Ala, Gly, and Ser.	Stites et al., 2006b
Streptozotocin-induced diabetes	20 mg/kg body mass/d	15 d	Brain tissue levels of lipid peroxidation and hydroperoxidation and the associated hyperglycemia-induced oxidative damage	Decreased serum levels of glucose and lipid peroxidation products, and increased activities of antioxidants in the diabetic mouse brain	Kumar and Kar, 2015

Abbreviations: bw= body weight, d=day, PQQ=pyrroloquinioline quinone, wk = week.

6.B.5. Human Clinical Studies

Since the FDA's last review of GRN 625 (pages 43-44) and GRN 641 (pages 39-40) in 2016, one human clinical study has been published (Nakano et al., 2016). This study did not find any adverse effects of PQQ disodium salt when elderly, healthy subjects consumed 20 mg per day for 12 weeks. GRNs 641 and 625 reported that no human clinical studies reported adverse effects of PQQ disodium salt (Harris et al., 2013; Itoh et al., 2016; Nakano et al., 2009, 2012, 2015a, 2015b; Rucker et al., 2009). Daily doses up to 60 mg per person were well tolerated and did not results in any adverse effects (Rucker et al., 2009). For these 'pivotal' studies, the dose levels represent the maximum doses administered, rather than absolute safety endpoints. The results are summarized in Table 18.

Table 18. Human clinical studies of PQQ disodium salt

Subjects	Dose	Duration	Measured Outcome	Results	Author
A study published since FDA's 2016 review					
20 elderly healthy subjects aged 50-70 years	20 mg	12 wk	Regional cerebral blood flow and oxygen metabolism in prefrontal cortex; Hb concentration and absolute tissue oxygen saturation in the bilateral prefrontal cortex	PQQ resulted in increased activity in the right prefrontal cortex associated with increases in regional cerebral blood flow and oxygen metabolism, resulting in enhanced cognitive function.	Nakano et al., 2016
Studies reviewed in the GRNs 625 (pages 43-44) and 641 (pages 39-40)					
41 elderly healthy subjects	20 mg	12 wk	Cognitive function	PQQ increased cerebral blood flow in the prefrontal cortex	Itoh et al., 2016
29 healthy subjects, 40-57 y	20 mg	6 and 12 wk	Serum lipid profile	PQQ marginally significantly decreased serum LDL-cholesterol concentration.	Nakano et al., 2015a
22 healthy women with mildly dry skin, 20-49 y	20 mg	8 wk	Skin health	PQQ improved skin conditions.	Nakano et al., 2015b
17 healthy adults	20 mg	8 wk	Subjective symptoms, body weight, heart rate, blood pressure	No adverse effects related to PQQ disodium salt, as measured by subjective symptoms, objective findings and abnormal changes in the measured values.	Nakano et al., 2012
20 healthy adults	0, 20, or 60 mg/d	4 wk	Liver toxicity, urinary biomarkers, serum biochemistry,	No adverse effects were observed in standard clinical tests	Rucker et al., 2009 (Cited

			and adverse events	(glucose, triglycerides, lipoprotein fractions). Functional tests for liver toxicity were also normal. Urinary N-acetyl-β-(D)-glucosaminidase activity was also normal. No adverse effects of PQQ were noted.	Tsuji et al., 1998; Urakami et al., 1994)
71 healthy adults, 45-65 y	20 mg PQQ+ 300 mg CoQ10	4, 8, and 12 wk	Memory and attention	PQQ significantly increased word memorization, recall task, and attention (no data shown).	Nakano et al., 2009
10 male and female subjects	0.2 mg/kg bw	Single dose	Plasma/urine PQQ levels, antioxidant potential, standard clinical indices	PQQ slightly increased antioxidant potential. Serum TC, LDL-C, HDL-C, TG, creatinine, glucose, uric acid, total protein, and AAT isozyme activity were within normal range. Levels of PQQ peaked in serum at about 2 hours.	Harris et al., 2013
10 male and female subjects	0.3 mg/kg bw/d	3 days	Inflammation indices, standard clinical indices, estimates of urinary metabolites	PQQ decreased levels of CRP, IL-6, and urinary methylated amines. PQQ increased mictochondria-related functions. Serum TC, LDL-C, HDL-C, TG, uric acid, creatinine, glucose, total protein, and AAT isozyme activity were within normal ranges.	Harris et al., 2013

6.C. Safety determination

Numerous human and animal studies have reported health benefits of PQQ disodium salt with no major adverse effects. There is broad-based and widely disseminated knowledge concerning the chemistry of PQQ disodium salt. This GRAS determination is based on the data and information generally available and consented opinion about the safety of PQQ disodium salt. The literature indicates that PQQ disodium salt offers consumers health benefits without serious adverse effects.

The following safety evaluations fully consider the composition, intake, nutritional, microbiological, and toxicological properties of PQQ disodium salt as well as appropriate corroborative data.

1. JinCheng's PQQ disodium salt (powder form) is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. JinCheng uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

2. Analytical data from multiple lots indicate that PQQ disodium salt reliably complies with established specifications and meets all applicable purity standards.

3. In response to GRAS notifications on PQQ disodium salt (GRN 625 and 641), the FDA did not question the safety of PQQ disodium salt for the specified food uses.

4. JinCheng's PQQ disodium salt will be used as a food ingredient (nutrient) at concentrations of 8-20 mg per serving (reference amounts customarily consumed, 21CFR 101.12) in selected beverages (energy, sport, and electrolyte drinks; bottled, enhanced and fortified water beverages; and non-milk-based meal replacement beverages) at maximum use levels of up to 8 to 20 mg/serving, respectively, in these product types.

5. Among consumers in the total population, the mean and 90th percentile all-user intakes of PQQ were determined to be 26.8-28.2 and 61.3-63.1 mg/person/day, respectively. Corresponding EDIs reported in GRN 625 were 26.5 and 61.4 mg/person/day, respectively. It is assumed that JinCheng's PQQ disodium salt will replace currently marketed PQQ disodium salt or other POQQ sources. Thus, cumulative exposures are not expected to change.

6. In the previous GRAS notices to the FDA, the safety of PQQ disodium salt has been established in toxicological studies in animals, mutagenicity studies, and is further supported by clinical studies in human. The NOAEL was determined to be 400 mg/kg bw/day in a subchronic toxicity study in rats. After applying a safety margin of 100, it can be concluded that doses up to 4 mg/kg bw/day or 240 mg/person/day would be safe in adults weighing 60 kg. The EDIs under the intended use are less than one thirds of the estimated safe intake levels in humans.

7. Furthermore, historical consumption of disodium salt supports the safety of PQQ disodium salt. Additional studies published subsequent to the FDA GRAS notices continue to support safety of PQQ disodium salt as a food ingredient.

6.D. Conclusions and General Recognition of the Safety of PQQ

Several sources of PQQ disodium salt have been evaluated by the FDA and other global regulatory agencies over the past 10 years for proposed incorporation of PQQ disodium salt in foods for human

consumption. Relevant US GRAS notifications include GRNs 625 and 641 (FDA, 2016a, 2016b) and NDI notice 417 (FDA, 2007). All of the GRAS and NDI notices provided information/clinical study data that supported the safety of the proposed PQQ disodium salt ingredients for use in human foods and dietary supplements. In all of the studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues attributable to PQQ disodium salt. Because this safety evaluation was based on generally available and widely accepted data and information, it satisfies the so-called "common knowledge" element of a GRAS determination.

In addition, the intended uses of PQQ disodium salt have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. The specifications of the proposed GRAS substance, JinCheng's PQQ disodium salt, is almost identical to those that have received FDA no question letters.

The PQQ disodium salt product that is the subject of this GRAS determination is produced by non-toxigenic bacteria, *Hyphomicrobium denitrificans,* and its purity is over 99%. The PQQ disodium salt product is manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes. Literature searches did not identify safety/toxicity concerns related to PQQ disodium salt. Toxicity studies of PQQ disodium salt include acute, subacute, and subchronic toxicity, a battery of genotoxicity studies, and developmental and reproductive toxicity studies. In all of these reports, no evidence of toxicity was noted at up to 400 mg/kg bw/day, the highest dose levels tested. The publicly available scientific literature on the consumption and safety of PQQ disodium salt in human clinical studies is extensive and sufficient to support the safety and GRAS status of the proposed PQQ disodium salt.

Determination of the safety and GRAS status of the PQQ disodium salt that is the subject of this self-determination has been made through the deliberations of an Expert Panel convened by NutraSource and comprised of Robert L. Martin, Ph.D., Joanne Slavin, Ph.D., and Susan Cho, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that the proposed PQQ disodium salt, produced consistent with cGMP and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concluded that these uses of the PQQ disodium salt are GRAS based on scientific procedures, and that other experts qualified to assess the safety of foods and food additives would concur with these conclusions. The Executive Summary of the Panel's GRAS opinion is included as Appendix G to this document.

JinCheng also has concluded that PQQ disodium salt is GRAS under the intended conditions of use on the basis of scientific procedures. Therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

JinCheng is not aware of any information that would be inconsistent with a finding that the proposed use of PQQ disodium salt meets appropriate specifications, and its use according to cGMP, is GRAS.

PART 7. REFERENCES

7.A. A List of the Data and Information that are Generally Available, References

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7.B. A List of the Data and Information that are Not Generally Available

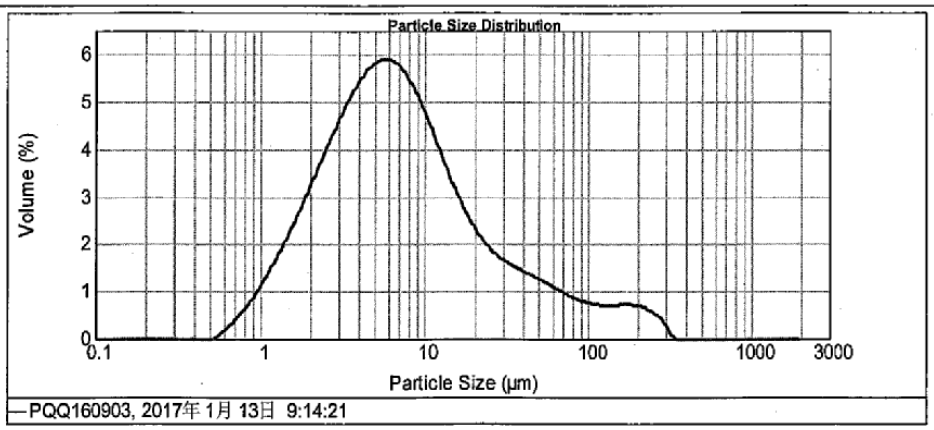
Gao Y. 2017. Oral acute toxicity study of pyrroloquinoline quinone (PQQ) disodium salt in rats

Appendix A. Particle Size Analysis

The particle size analysis of 3 non-consecutive batches show that 90% of Jincheng PQQ disodium is less than 50μm. The particle size distribution is shown below.

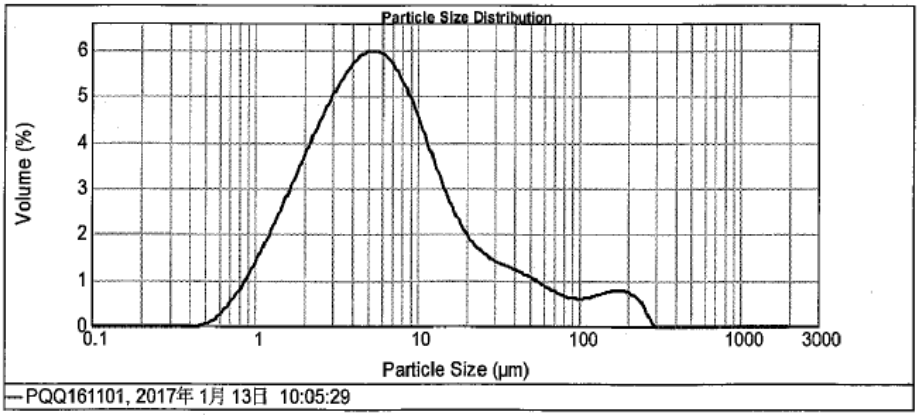
Concentration:	Span :	Uniformity:	Result units:
0.0006 %Vol	6.714	2.51	Volume
Specific Surface Area:	**Surface Weighted Mean D[3,2]:**	**Vol. Weighted Mean D[4,3]:**	
1.38 m?g	4.355 um	19.999 um	

d(0.1): 1.864 um **d(0.5): 6.583 um** **d(0.9): 46.064 um**



Concentration:	Span :	Uniformity:	Result units:
0.0006 %Vol	6.174	2.46	Volume
Specific Surface Area:	**Surface Weighted Mean D[3,2]:**	**Vol. Weighted Mean D[4,3]:**	
1.54 m?g	3.908 um	17.440 um	

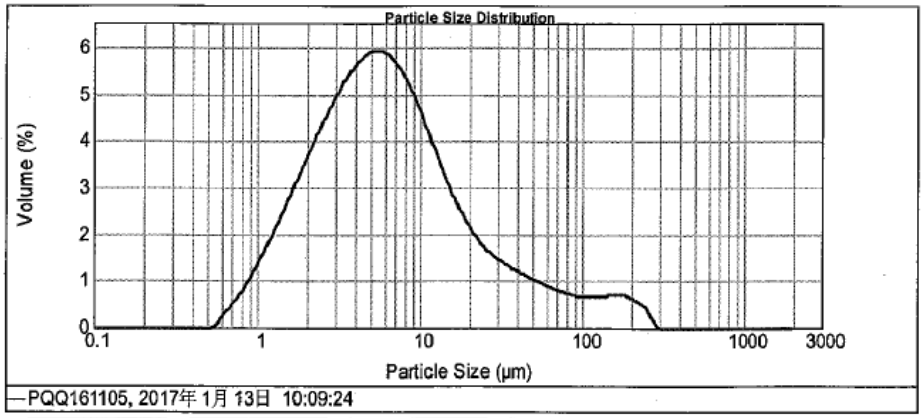
d(0.1): 1.681 um **d(0.5): 5.826 um** **d(0.9): 37.653 um**



Concentration:	Span :	Uniformity:	Result units:
0.0002 %Vol	5.960	2.35	Volume

Specific Surface Area:	Surface Weighted Mean D[3,2]:	Vol. Weighted Mean D[4,3]:
1.51 m?g	3.965 um	17.079 um

d(0.1): 1.698 um **d(0.5): 5.934 um** **d(0.9): 37.068 um**

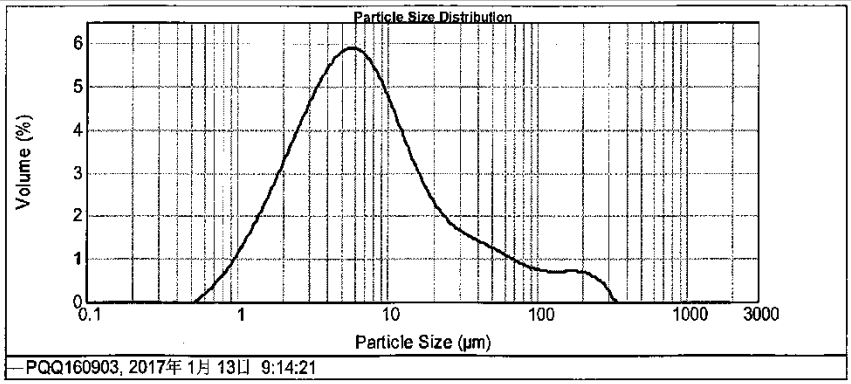


MASTERSIZER 2000

Result Analysis Report

Sample Name: PQQ160903	**SOP Name:** 外检样品	**Measured:** 2017年1月13日 9:14:21	
Sample Source & type: Paris	**Measured by:** jcs20	**Analysed:** 2017年1月13日 9:14:23	
Sample bulk lot ref:	**Result Source:** Measurement		
Particle Name: Polystyrene latex	**Accessory Name:** Scirocco 2000	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 1.590	**Absorption:** 0	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 4.74 %
Dispersant Name:	**Dispersant RI:** 1.000	**Weighted Residual:** 0.159 %	**Result Emulation:** Off
Concentration: 0.0006 %Vol	**Span :** 6.714	**Uniformity:** 2.51	**Result units:** Volume
Specific Surface Area: 1.38 m?g	**Surface Weighted Mean D[3,2]:** 4.355 um	**Vol. Weighted Mean D[4,3]:** 19.999 um	

d(0.1): 1.864 um **d(0.5):** 6.583 um **d(0.9):** 46.064 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	1.36	11.482	3.61	120.226	0.64	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	1.72	13.183	3.12	138.038	0.64	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	2.11	15.136	2.68	158.489	0.65	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	2.52	17.378	2.29	181.970	0.64	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	2.94	19.953	1.96	208.930	0.59	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	3.38	22.909	1.68	239.883	0.47	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	3.81	26.303	1.74	275.423	0.32	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	4.23	30.200	1.57	316.228	0.22	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	4.61	34.674	1.44	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	4.93	39.811	1.34	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	5.18	45.709	1.25	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	5.31	52.481	1.15	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.13	5.754	5.32	60.256	1.05	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.29	6.607	5.19	69.183	0.95	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.51	7.586	4.93	79.433	0.85	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.75	8.710	4.56	91.201	0.76	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	1.04	10.000	4.10	104.713	0.69	1096.478	0.00		
0.105	0.00	1.096		11.482		120.226	0.65	1258.925	0.00		

Operator notes:

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Mastersizer 2000 Ver. 5.61
Serial Number : MAL1110077

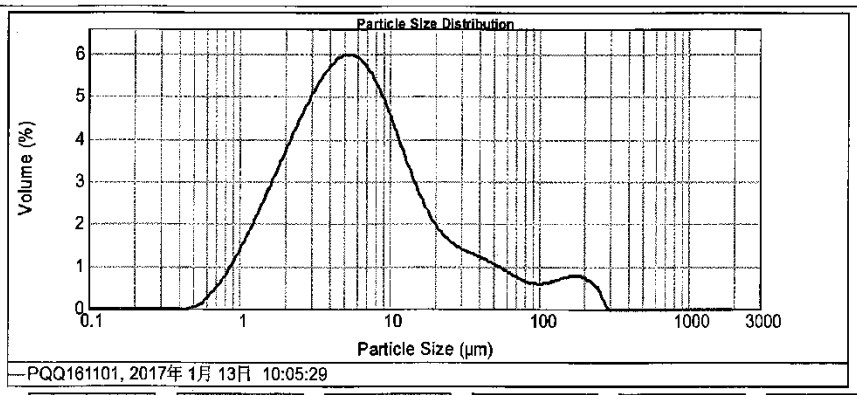
File name: 外检样品
Record Number: 77
2017/1/13 10:16:11

MASTERSIZER 2000

Result Analysis Report

Sample Name: PQQ161101	**SOP Name:** 外检样品	**Measured:** 2017年1月13日 10:05:29	
Sample Source & type: Paris	**Measured by:** jcs20	**Analysed:** 2017年1月13日 10:05:31	
Sample bulk lot ref:	**Result Source:** Measurement		
Particle Name: Polystyrene latex	**Accessory Name:** Scirocco 2000	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 1.590	**Absorption:** 0	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 5.94 %
Dispersant Name:	**Dispersant RI:** 1.000	**Weighted Residual:** 0.235 %	**Result Emulation:** Off
Concentration: 0.0006 %Vol	**Span :** 6.174	**Uniformity:** 2.46	**Result units:** Volume
Specific Surface Area: 1.54 m?g	**Surface Weighted Mean D[3,2]:** 3.908 um	**Vol. Weighted Mean D[4,3]:** 17.440 um	

d(0.1): 1.681 um **d(0.5):** 5.826 um **d(0.9):** 37.653 um



Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %
0.010	0.00	0.105	0.00	1.096	1.87	11.482	3.34	120.226	0.60	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	2.09	13.183	2.82	138.038	0.66	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	2.51	15.136	2.36	158.489	0.71	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	2.95	17.378	1.98	181.970	0.68	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	3.38	19.953	1.69	208.930	0.57	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	3.81	22.909	1.47	239.883	0.37	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	4.21	26.303	1.33	275.423	0.01	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	4.59	30.200	1.23	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	4.91	34.674	1.15	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	5.17	39.811	1.07	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	5.35	45.709	0.97	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	5.41	52.481	0.87	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.05	5.754	5.34	60.256	0.75	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.17	6.607	5.15	69.183	0.64	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.39	7.586	4.81	79.433	0.57	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.63	8.710	4.38	91.201	0.63	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.84	10.000	3.87	104.713	0.55	1096.478	0.00		
0.105	0.00	1.096	1.29	11.482		120.226		1258.925			

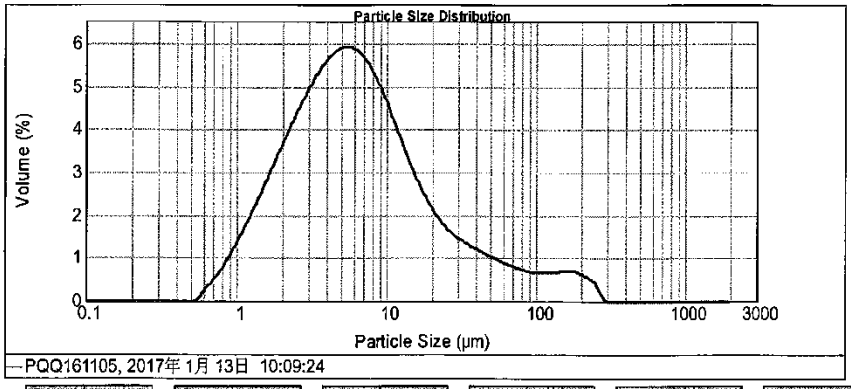
Operator notes:

MASTERSIZER 2000

Result Analysis Report

Sample Name: PQQ161105	**SOP Name:** 外检样品	**Measured:** 2017年1月13日 10:09:24	
Sample Source & type: Paris	**Measured by:** jcs20	**Analysed:** 2017年1月13日 10:09:26	
Sample bulk lot ref:	**Result Source:** Measurement		
Particle Name: Polystyrene latex	**Accessory Name:** Scirocco 2000	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 1.590	**Absorption:** 0	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 2.20 %
Dispersant Name:	**Dispersant RI:** 1.000	**Weighted Residual:** 0.199 %	**Result Emulation:** Off
Concentration: 0.0002 %Vol	**Span :** 5.960	**Uniformity:** 2.35	**Result units:** Volume
Specific Surface Area: 1.51 m?g	**Surface Weighted Mean D[3,2]:** 3.965 um	**Vol. Weighted Mean D[4,3]:** 17.079 um	

d(0.1): 1.698 um **d(0.5):** 5.934 um **d(0.9):** 37.068 um



Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %
0.010	0.00	0.105	0.00	1.096	1.85	11.482	3.44	120.226	0.60	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	2.05	13.183	2.94	138.038	0.82	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	2.18	15.136	2.50	158.489	0.63	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	2.91	17.378	2.11	181.970	0.59	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	3.34	19.953	1.81	208.930	0.49	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	3.75	22.909	1.57	239.883	0.31	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	4.18	26.303	1.40	275.423	0.01	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	4.52	30.200	1.26	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	4.85	34.674	1.15	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	5.11	39.811	1.05	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	5.28	45.709	0.95	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	5.35	52.481	0.86	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.15	5.754	5.30	60.256	0.78	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.62	6.607	5.12	69.183	0.70	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.36	7.586	4.82	79.433	0.64	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.62	8.710	4.42	91.201	0.60	954.993	0.00	10000.000	
0.091	0.00	0.955	0.93	10.000	3.94	104.713	0.59	1096.478	0.00		
0.105	0.00	1.096	1.27	11.482		120.226		1258.925			

Operator notes:

Malvern Instruments Ltd.
Malvern, UK
Tel :- +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 5.61
Serial Number : MAL1110977

File name: 外检样品
Record Number: 85
2017/1/13 10:15:07

APPENDIX B. Specifications of Raw Materials

Ferrous sulfate (Reagent)
Molecular formula: FeSO4·7H2O
Molecular weight: 278.02

Item	Spec
Appearance	Light blue green crystal
Assay	99.0-101.0%
Pb	≤0.001%

zinc sulfate (Reagent)
Molecular formula: ZnSO4·7H20)
Molecular weight: 287.58

Item	Spec
PH	4.4-6.0
Assay	≥99.5%
Pb	≤0.001%

Manganese sulfate (Reagent)
Molecular formula: $MnSO_4 \cdot H_20$
Molecular weight: 169.02

Item	Spec
Appearance	Pink crystal
Assay	≥99.0%
Pb	≤0.0002%

Copper sulfate (Reagent)
Molecular formula: CuSO4·5H2O
Molecular weight: 249.69

Item	Spec
Appearance	Blue crystal
Assay	≥99.0%
Pb	≤0.0002%

NaCl
Molecular formula: NaCl
Molecular weight: 58.44

Item	Spec
Assay	≥99.5%
Pb	≤0.0005%

Ammonium sulfate ((Reagent)
Molecular formula: (NH4)2SO4
Molecular weight: 132.14

Item	Spec
Appearance	Colorless crystal
Assay	≥99.0%
pH(50g/L，25 ℃)	4.8-6.0
Pb	≤0.0005%

$CaCl_2$ ((Reagent)
Molecular formula: $CaCl_2$
Molecular weight: 110.99

Item	Spec
Assay	≥96.0%
Pb	≤0.0005%

Sodium phosphate dibasic ((Reagent)
Molecular formula: (Na2HPO4)
Molecular weight: 141.96

Item	Spec
Appearance	White crystal
Assay	≥99.0%
Loss on drying	≤0.2%
Pb	≤0.0001%

Potassium phosphate monobasic

Item	Spec
Appearance	White crystal
Assay	≥99.0%
Loss on drying	≤0.2%
Pb	≤0.0001%

Agar

Item	Spec
Appearance	White powder
Ash	≤5%
Loss on drying	≤18%
Pb	≤0.0001%

Methanol

Item	Spec
Appearance	Colorless liquid
water	≥99.0%
density	0.791g/cm^3～0.793g/cm^3
Pb	≤0.0001%

C. Certificate of Analysis of PQQ Disodium Salt

COA of Batch160903

Product Name	PQQ Disodium Salt	Batch No.:	160903
Manufacture Date	Sep 03, 2016	Expiry Date	Aug , 2018

Parameter	Specification	Result
Identity		
Appearance	Red crystalline powder	Comply
Identification UV	A233/A259 = 0.90±0.09 A322/A259 = 0.56±0.03	Comply
PQQ (as-is basis)	≥85%	91.3%
PQQ disodium salt	≥99%	100.0%
Sodium	10.0~13.0%	12.16%
Water content	≤12%	10.6
Ethanol	≤5,000 ppm	148 ppm
Heavy Metals		
Lead	≤0.5 ppm	<0.05
Arsenic	≤0.5 ppm	<0.1
Cadmium	≤0.3 ppm	0.03 ppm
Mercury	≤0.2 ppm	<0.005
Microbiological Analysis		
Total Aerobic Count	≤10,000 CFU/g	Comply
Total Mold and Yeast	≤1,000 CFU/g	Comply
Coliforms	≤100 CFU/g	< 10 CFU/g
Escherichia coli	≤10 CFU/g	< 10 CFU/g
Salmonella	≤10 CFU/g	Note detected

COA of Batch161101

Product Name	PQQ Disodium Salt	Batch No.:	161101
Manufacture Date	Nov 01, 2016	Expiry Date	Oct , 2018
Parameter	Specification	Result	
Identity			
Appearance	Red crystalline powder	Comply	
Identification UV	A233/A259 = 0.90±0.09 A322/A259 = 0.56±0.03	Comply	
PQQ (as-is basis)	≥85%	91.0%	
PQQ disodium salt	≥99%	99.9%	
Sodium	10.0~13.0%	12.43%	
Water content	≤12%	10.3	
Ethanol	≤5,000 ppm	153 ppm	
Heavy Metals			
Lead	≤0.5 ppm	<0.05	
Arsenic	≤0.5 ppm	<0.1	
Cadmium	≤0.3 ppm	0.03 ppm	
Mercury	≤0.2 ppm	<0.005	
Microbiological Analysis			
Total Aerobic Count	≤10,000 CFU/g	Comply	
Total Mold and Yeast	≤1,000 CFU/g	Comply	
Coliforms	≤100 CFU/g	< 10 CFU/g	
Escherichia coli	≤10 CFU/g	< 10 CFU/g	
Salmonella	≤10 CFU/g	Note detected	

COA of Batch161103

Product Name	PQQ Disodium Salt	Batch No.:	161103
Manufacture Date	Nov 03, 2016	Expiry Date	Oct , 2018
Parameter	Specification	Result	
Identity			
Appearance	Red crystalline powder	Comply	
Identification UV	A233/A259 = 0.90±0.09 A322/A259 = 0.56±0.03	Comply	
PQQ (as-is basis)	≥85%	89.8.0%	
PQQ disodium salt (chromatography)	≥99%	99.9%	
Sodium	10.0~13.0%	12.61%	
Water content	≤12%	10.1	
Ethanol	≤5,000 ppm	139 ppm	
Heavy Metals			
Lead	≤0.5 ppm	<0.05	
Arsenic	≤0.5 ppm	<0.1	
Cadmium	≤0.3 ppm	0.03 ppm	
Mercury	≤0.2 ppm	<0.005	
Microbiological Analysis			
Total Aerobic Count	≤10,000 CFU/g	Comply	
Total Mold and Yeast	≤1,000 CFU/g	Comply	
Coliforms	≤100 CFU/g	< 10 CFU/g	
Escherichia coli	≤10 CFU/g	< 10 CFU/g	
Salmonella	≤10 CFU/g	Not detected	

Appendix D. Stability Test

Temperature: 25±2℃; Relative humidity: 60%±5%

Lot number (b) (6)

Item	**Specification**	**Initial**	**1 month**	**2 months**
Color/physical form	Red crystalline powder	Red crystalline powder	Red crystalline powder	Red crystalline powder
Identify	Comply	Comply	/	/
Loss on drying	≤12.0%	10.6	10.9	10.7
Residue ignition	30.0-35.0%	32.8	/	/
Purity	≥99.0%	100.0	99.9	99.9
Assay	≥85.0%	91.3	91.5	91.7
Item	**3 months**	**6 months**	**9 months**	**12 months**
Color/physical form	Red crystalline powder	Red crystalline powder	Red crystalline powder	Red crystalline powder
Identify	/	/	/	/
Loss on drying	10.5	10.6	10.7	10.8
Residue ignition	/	/	/	/
Purity	100.0	99.9	99.9	99.9
Assay	92.5	92.0	91.8	92.0

Lot Number (b) (6)

Item	**Specification**	**Initial**	**1 month**	**2 months**
Color/physical form	Red crystalline powder	Red crystalline powder	Red crystalline powder	Red crystalline powder
Identify	Comply	Comply	/	/
Loss on drying	≤12.0%	10.1	10.2	10.5
Purity	≥99.0%	100.0	99.9	99.9
Item	**3 months**	**6 months**	**9 months**	**12 months**
Color/physical form	Red crystalline powder	Red crystalline powder	Red crystalline powder	Red crystalline powder

Identify	/	/	/	/
Loss on drying	10. 4	10.3	10.4	10.3
Purity	100.0	99.9	99.9	99.9

Lot number (b) (6)

Item	**Specification**	**Initial**	**1 month**	**2 months**
Color/physical form	Red crystalline powder	Red crystalline powder	Red crystalline powder	Red crystalline powder
Identify	Comply	Comply	/	/
Loss on drying	≤12.0%	10. 3	10. 5	10. 7
Purity	≥99.0%	100.0	99.9	99.9
Item	**3 months**	**6 months**	**9 months**	**12 months**
Color/physical form	Red crystalline powder	Red crystalline powder	Red crystalline powder	Red crystalline powder
Identify	/	/	/	/
Loss on drying	10. 2	10.5	10.6	10.7
Purity	100.0	99.9	99.9	99.9

Appendix E. NHANES Food Codes for EDI Calculation under the Intended Use

95310200	Full Throttle Energy Drink	4
95310400	Monster Energy Drink	43
95310500	Mountain Dew AMP Energy Drink	1
95310550	No Fear Energy Drink	0
95310555	No Fear Motherload Energy Drink	0
95310560	NOS Energy Drink	3
95310600	Red Bull Energy Drink	34
95310700	Rockstar Energy Drink	8
95310750	SoBe Energize Energy Juice Drink	2
95310800	Vault Energy Drink	0
95311000	Energy Drink	11
95312400	Monster Energy Drink, Lo Carb	15
95312500	Mountain Dew AMP Energy Drink, sugar-free	2
95312550	No Fear Energy Drink, sugar-free	0
95312555	NOS Energy Drink, sugar-free	0
95312560	Ocean Spray Cran-Energy Cranberry Energy Juice Drink	0
95312600	Red Bull Energy Drink, sugar-free	7
95312700	Rockstar Energy Drink, sugar-free	9
95312800	Vault Zero Energy Drink	0
95312900	XS Energy Drink	3
95312905	XS Gold Plus Energy Drink	0
95313200	Energy drink, sugar free	13
95320200	Gatorade G sports drink	368
95320500	Powerade sports drink	114
95321000	Sports drink, NFS	1
95322200	Gatorade G2 sports drink, low calorie	49
95322500	Powerade Zero sports drink, low calorie	19
95323000	Sports drink, low calorie	5
95330100	Fluid replacement, electrolyte solution	9
95330500	Fluid replacement, 5% glucose in water	0
92900300	Sports drink, dry concentrate, not reconstituted	0
95101000	Boost, nutritional drink, ready-to-drink	8
95101010	Boost Plus, nutritional drink, ready-to-drink	1
95104000	Glucerna, nutritional shake, ready-to-drink	13
95105000	Kellogg's Special K Protein Shake	2
95110000	Slim Fast Shake, meal replacement, regular, ready-to-drink	15
95110010	Slim Fast Shake, meal replacement, sugar free, ready-to-drink	3
95110020	Slim Fast Shake, meal replacement, high protein, ready-to-drink	7
95120000	Nutritional drink or meal replacement, ready-to-drink, NFS	10
95120010	Nutritional drink or meal replacement, high protein, ready-to-drink, NFS	19
95120020	Nutritional drink or meal replacement, high protein, light, ready-to-drink, NFS	13
95120050	Nutritional drink or meal replacement, liquid, soy-based	1
95201300	EAS Soy Protein Powder	0
95201500	Herbalife, nutritional shake mix, high protein, powder	10
95201600	Isopure protein powder	0
95201700	Kellogg's Special K20 Protein Water Mix	1
95210000	Slim Fast Shake Mix, powder	2
95210010	Slim Fast Shake Mix, sugar free, powder	0

95210020	Slim Fast Shake Mix, high protein, powder	1
95220000	Nutritional drink mix or meal replacement, powder, NFS	2
95220010	Nutritional drink mix or meal replacement, high protein, powder, NFS	17
95230010	Protein powder, soy based, NFS	8
95230020	Protein powder, light, NFS	5
95230030	Protein powder, NFS	50
94100200	Water, bottled, sweetened, with low calorie sweetener	61
94100300	Capri Sun Roarin' Waters	13
94210100	Propel Water	5
94210200	Glaceau Vitamin Water	58
94210300	SoBe Life Water	3
94220100	Propel Zero Water	5
94220110	Propel Zero Calcium Water	0
94220215	Glaceau Vitamin Water Zero	18
94220310	SoBe Life Water Zero	7
94100100	Water, bottled, unsweetened	3,680

APPENDIX F. TOXICOLOGY STUDY REPORT

Title of Study	Oral Acute Toxicity Study of pyrroloquinoline quinone (PQQ) disodium salt in Rats
Study Number	A2017-T002
Entrustment Company	Shandong Jincheng Bio-pharmaceutical Co., Ltd.
Address of Entrustment Company	Zichuan Economic Development Zone, Zibo City,Shandong Province,China
Contact person	Mr. Joe Lan
Contact Tel. and E-mail	Tel.: +86-533-5415882; Fax: +86-535-6902501 Mobile: +86-13969374086; E-mail: lanjiang@jinchengpharm.com
Primary Test Facility	School of Life Sciences, Yantai University
Address of Research Institute	32, Qingquan RD, Laishan District, Yantai, China
Contact person	Yonglin Gao (b) (6)
Contact Tel. and E-mail	Tel: +86-15854569558; Fax: +86-533-6725485 E-mail: gylbill@163.com (gaoyonglin@ytu.edu.cn)
Study Director	Yonglin Gao
Study Participants	Yonglin Gao *Operator* Xiaochen Fan *Test products management* Yunzhi Wang *Animal management*
Study Start and End Dates	Jan 2017 - Feb 2017

ABSTRACT

The aim of this study was to evaluate the acute toxicity of pyrroloquinoline quinone (PQQ) disodium salt after a single oral administration in rats. PQQ disodium salt was administered to 50 young rats by oral gavage at a single dose of 2.50, 3.00, 3.60, 4.32, or 5.18 g/kg body weight (BW) (5 males and 5 females per group). Animals were observed for 14 days to monitor changes in BW, clinical signs, and food and water consumption. At the end of the study, all surviving animals were sacrificed and major organs were examined. A number of animals died in the days following PQQ disodium salt administration at 3.00, 3.60, 4.32, and 5.18 g/kg BW. On day 1, greenish loose stools were observed in the 3.60, 4.32, and 5.18 g/kg BW groups after PQQ disodium salt administration. Also, greenish tails were found in the 5.18 g/kg BW group. Necropsy revealed enlarged kidneys in the 3.00 and 3.60 g/kg BW groups, with an increased coefficient of kidney (organ weight/body weight). However, no significant differences were found. Outcomes showed that orally administered PQQ disodium salt caused dose-dependent mortalities with the median lethal dose (LD_{50}) of 3.47 g/kg BW, with a 95% confidence interval (CI) estimate of 3.12~3.84 g/kg BW. The no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) were 2.50 g/kg BW and 3.00 g/kg BW, respectively. According to acute toxicity classification (World Health Organization), PQQ disodium salt demonstrates low toxicity in rats.

1. Study design

The study was performed in accordance with the Food and Drug Administration (FDA) Redbook 2000 Chapter IV.C.3., Short-Term Toxicity Studies with Rodents. PQQ disodium salt was administered by oral gavage to 60 rats (0, 2.50, 3.00, 3.60, 4.32 or 5.18 g/kg body weight [BW]; 5 males and 5 females per group). The animals were observed for 2 weeks. Clinical signs, body weight, food and water consumption, and death rates were documented. On day 15, all surviving animals were sacrificed and organs (i.e., brain, heart, kidney, liver, and spleen) in the control and the highest dose group were weighed. The Bliss method was used to conduct analyses of LD_{50} and estimate its 95% confidence intervals (CI).

2. Animals

Sprague-Dawley rats, 6 weeks of age, were housed in cages under hygienic conditions and placed in a controlled environment with a 12 h light/dark cycle at 23±3 °C and 40-60% humidity. Animals were allowed a commercial standard rat cube diet and water *ad libitum*. All procedures involving the use of laboratory animals were in accordance with The Guidelines of Animal Care.

3. Treatment

Rats were divided into five groups (each included 10 rats, 5 male and 5 female) by stratified randomization based on body weights before treatment to receive either control (0.5% CMCNa) or PQQ disodium salt (Batch No. 161101 from Shandong Jincheng Bio-pharmaceutical Co., Ltd.). Single oral doses of 2.50, 3.00, 3.60, 4.32, or 5.18 g/kg BW were administered by gavage.

Group assignments are outlined in Table 1.

Table 1. Experimental design of a 14-day rat acute toxicity study

Groups	PQQ disodium salt dose levels (g/kg BW)	Number of animals
1	0 (Control)	**10（♀:5+♂:5）**
2	2.50	**10（♀:5+♂:5）**
3	3.00	**10（♀:5+♂:5）**
4	3.60	**10（♀:5+♂:5）**
5	4.32	**10（♀:5+♂:5）**
6	5.18	**10（♀:5+♂:5）**

The control animals received with the same volume of 0.5% CMCNa. Abbreviations: PQQ = pyrroloquinoline quinone; BW = body weight.

4. Observations and clinical tests

All animals were observed once daily for clinical signs of toxicity and twice daily for mortality and morbidity. The body weight of each rat was measured pre-test, weekly thereafter, and at sacrifice. Food and water consumption were noted.

5. Organ weights and gross necropsy

At the end of treatment, all surviving animals were fasted overnight. Body and main organ weights (including the liver, kidneys, spleen, heart, and lungs) were measured. The coefficient was reported as organ weight/body weight.

6. Statistical analysis

We used SPSS 11.5 software for Windows to perform all analyses. One-way ANOVA with Dunnett's post hoc test was used to compare treatment and control group data. A P-value less than 0.05 was considered statistically significant. The Bliss method was used to conduct analyses of LD_{50} and estimate its 95% CIs.

7. Results

A number of animals died in the days following PQQ disodium salt administration at 3.00, 3.60, 4.32, or 5.18g/kg BW (Table 2). Orally administered PQQ disodium salt caused dose-dependent mortalities with an LD_{50} of 3.47g/kg BW; the 95% confidence interval (CI) was 3.12~3.84 g/kg BW. The no observed adverse

effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) were 2.50 g/kg BW and 3.00 g/kg BW, respectively.

Table 2. Oral acute toxicity study of PQQ disodium salt in rats

Groups (g/kg BW)	Dead animals					
	0	2.50	3.00	3.60	4.32	5.18
0 day	0	0	0	0	0	0
1 day	0	0	0	2	3	3
2 day	0	0	1	1	2	2
3 day	0	0	2	2	1	2
4 day	0	0	1	0	2	3
5 day	0	0	0	0	0	0

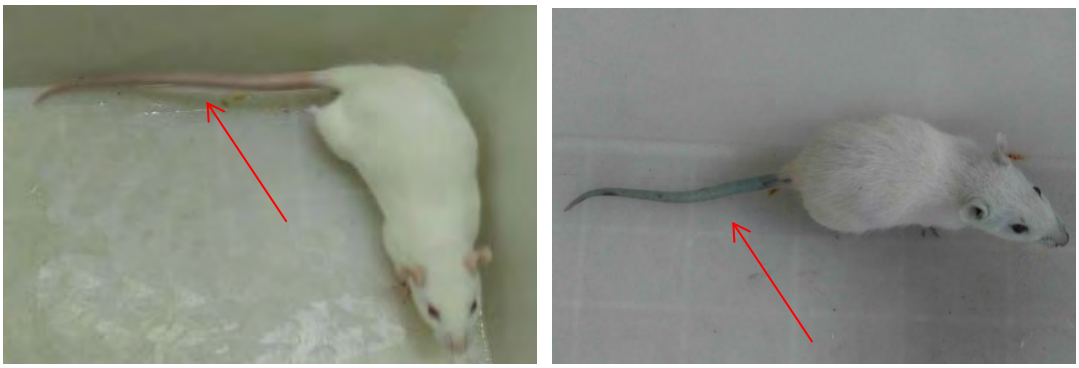
Abbreviations: PQQ = pyrroloquinoline quinone; BW = body weight.

On day 1, greenish loose stools were observed in the 3.60, 4.32, and 5.18 g/kg BW groups after PQQ disodium salt administration (Fig.1). Also, the tails of animals in the 5.18 g/kg BW group were greenish in color (Fig.2). When compared with the control group, there were no significant differences in body weight gain, food consumption, or water intake (Table 3,4,5; Fig. 3,4,5). Necropsy revealed enlarged kidneys in the 3.00 and 3.60 g/kg BW groups, with increased coefficients of kidney. However, no significant differences were found (Table 6; Fig. 6).

Normal feces

Greenish loose stools;

Figure 1. The greenish loose stools were observed after PQQ disodium salt administration



Control group 5.18 g/kg BW group

Figure 2. The animals' tails appeared greenish in the 5.18 g/kg BW group

Table 3. Body weight change of rats during a 14-day study (g)

Groups	PQQ disodium salt (g/kg BW)	Before	1st week	2nd week
1	0	121.90±14.49 （n=10）	154.20±24.55 （n=10）	184.90±27.82 （n=10）
2	2.50	123.60±12.79 （n=10）	147.50±20.33 （n=10）	171.10±19.30 （n=10）
3	3.00	122.40±13.04 （n=10）	145.17±16.45 （n=6）	173.00±25.51 （n=6）
4	3.60	121.80±13.55 （n=10）	147.60±15.60 （n=5）	175.20±15.67 （n=5）
5	4.32	120.60±14.35 （n=10）	160.50±14.85 （n=2）	193.00±8.49 （n=2）
6	5.18	124.20±15.57 （n=10）	-	-

Abbreviations: PQQ = pyrroloquinoline quinone; BW =body weight.

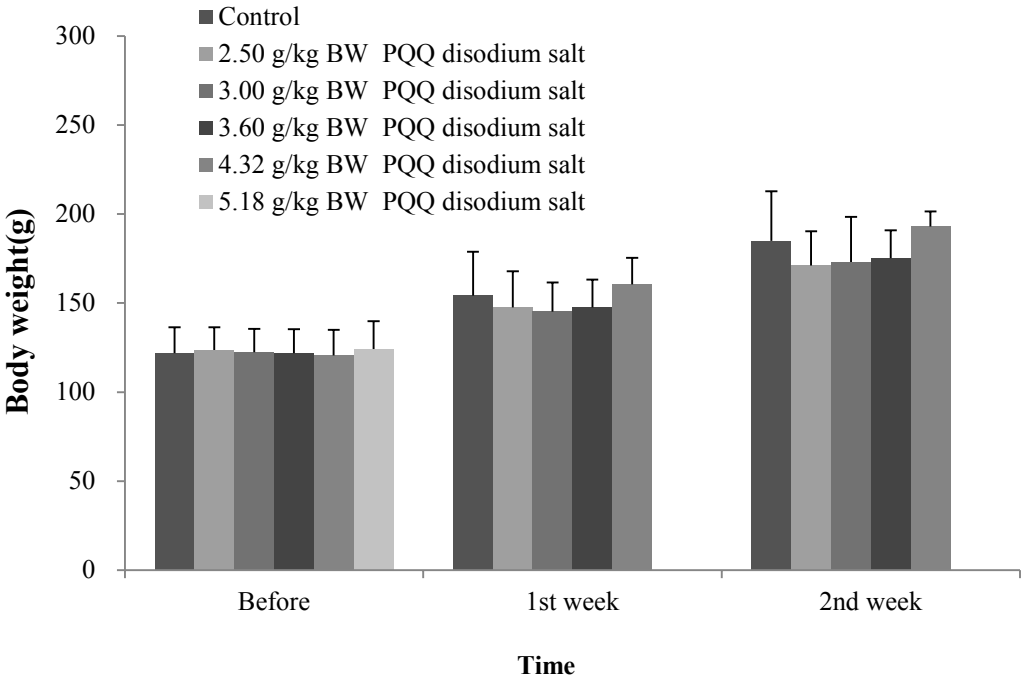


Figure 3. Body weight change of rats during a 14-day study.

Abbreviations: PQQ = pyrroloquinoline quinone; BW = body weight.

Table 4. Water intake of rats during a 14-day study (ml/100g BW/day)

Groups	PQQ disodium salt (g/kg BW)	1st week	2nd week
1	0（n=10）	13.56±2.15	13.82±2.09
2	2.50（n=10）	13.46±2.15	13.33±2.19
3	3.00（n=6）	13.98±2.84	13.67±2.06
4	3.60 （n=5）	12.74±1.49	12.55±2.12
5	4.32（n=2）	14.23±2.58	14.90±1.53
6	5.18（n=0）	-	-

Abbreviations: PQQ = pyrroloquinoline quinone; BW = body weight.

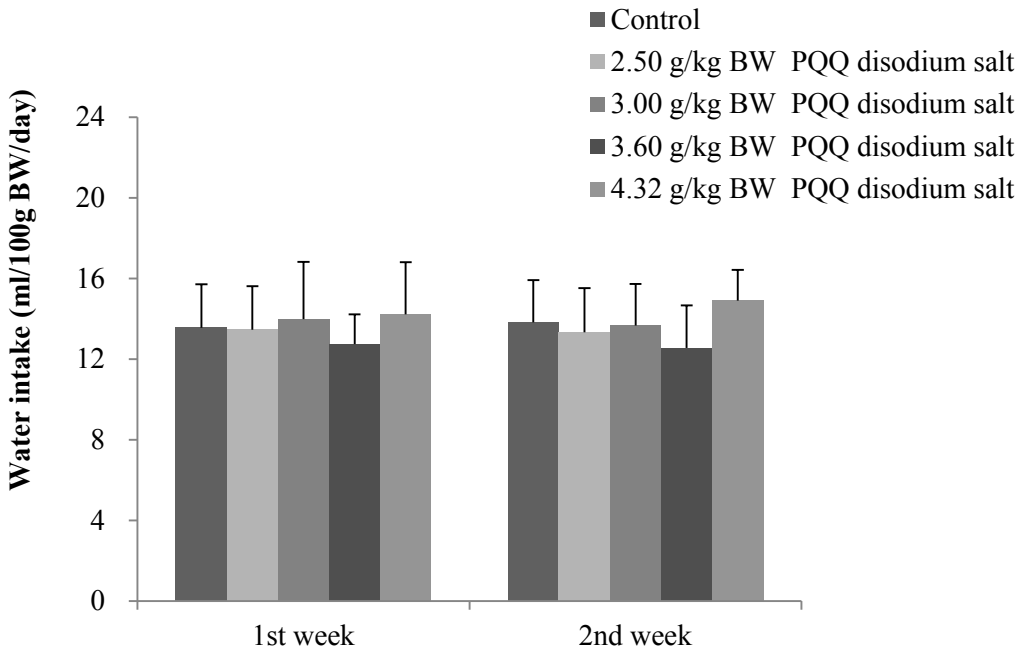


Figure 4. Water intake of rats during a 14-day study.

Abbreviations: PQQ = pyrroloquinoline quinone; BW = body weight.

Table 5. Food consumption of rats during a 14-day study (g/100g BW/day)

Groups	PQQ disodium salt (g/kg BW)	1st week	2nd week
1	0（n=10）	10.46±2.18	10.57±2.06
2	2.50（n=10）	9.87±2.34	10.12±2.12
3	3.00（n=6）	9.79±2.56	10.10±2.45
4	3.60 （n=5）	9.08±1.54	9.64±1.12
5	4.32（n=2）	9.78±1.18	10.14±1.01
6	5.18（n=0）	-	-

Abbreviations: PQQ = pyrroloquinoline quinone; BW = body weight.

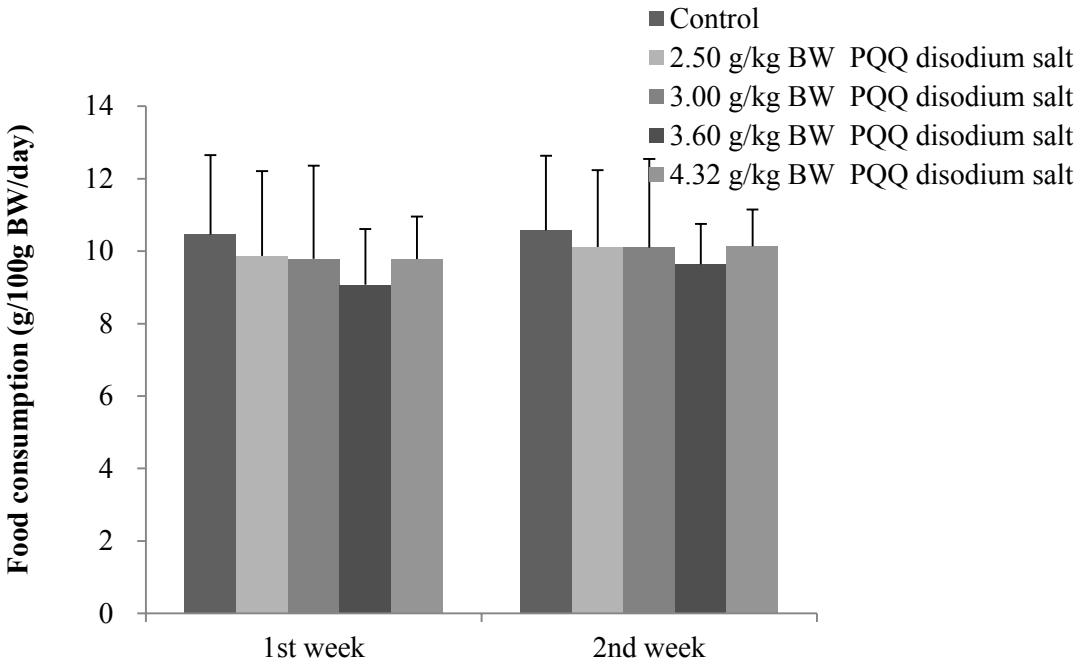


Figure 5. Food consumption of rats during a 14-day study.

Abbreviations: PQQ = pyrroloquinoline quinone; BW = body weight.

Table 6. The organ coefficient of rats after a 14-day study (% BW).

PQQ disodium salt Dose Groups	Heart	Liver	Spleen	Lung	Kidney
0（n=10）	0.49±0.07	4.05±0.39	0.33±0.04	0. 65±0. 11	1. 00±0. 12
2.50（n=10）	0.51±0.09	3.91±0.38	0.32±0.03	0.69±0.11	0.97±0.14
3.00（n=6）	0.49±0.07	4.37±0.28	0.31±0.09	0.61±0.03	1.11±0.13
3.60 （n=5）	0.48±0.07	4.27±0.31	0.34±0.05	0.56±0.05	1.15±0.26
4.32（n=2）	0.50±0.04	4.08±0.53	0.34±0.02	0.57±0.14	1.05±0.02
5.18（n=0）	-	-	-	-	-

Abbreviations: PQQ = pyrroloquinoline quinone; BW =body weight.

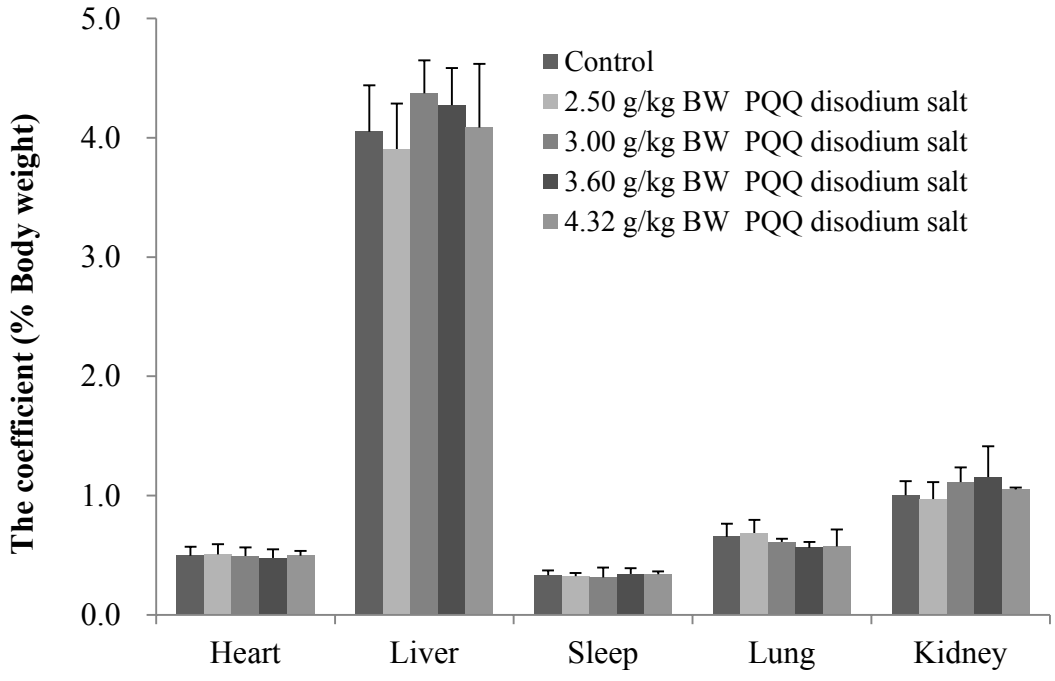


Figure 6. The organ coefficient of rats after a 14-day study.

Abbreviations: PQQ = pyrroloquinoline quinone; BW = body weight.

8. Conclusion

Under our test conditions, orally administered PQQ disodium salt caused dose-dependent mortalities with an LD_{50} of 3.47g/kg BW and 95% CI of 3.12~3.84 g/kg BW. The NOAEL and the LOAEL were 2.50 g/kg BW and 3.00 g/kg BW, respectively. According to acute toxicity classification (World Health Organization), PQQ disodium salt demonstrates low toxicity in rats.

APPENDIX G. PART 1. EXECUTIVE SUMMARY

GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF PYRROLOQUINOLINE QUINONE (PQQ) AS A FOOD INGREDIENT

1. A. Safety determination

Numerous human and animal studies have reported health benefits of PQQ disodium salt with no major adverse effects. There is broad-based and widely disseminated knowledge concerning the chemistry of PQQ disodium salt. This GRAS determination is based on the data and information generally available and consented opinion about the safety of PQQ disodium salt. The literature indicates that PQQ disodium salt offers consumers health benefits without serious adverse effects.

The following safety evaluations fully consider the composition, intake, nutritional, microbiological, and toxicological properties of PQQ disodium salt as well as appropriate corroborative data.

1. JinCheng's PQQ disodium salt (powder form) is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. JinCheng uses a Hazard Analysis and Critical Control Points (HACCP)-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

2. Analytical data from multiple lots indicate that PQQ disodium salt reliably complies with established specifications and meets all applicable purity standards.

3. In response to GRAS notifications on PQQ disodium salt (GRN 625 and 641), the FDA did not question the safety of PQQ disodium salt for the specified food uses.

4. As shown in Table 1, As shown in Table 1, PQQ disodium salt is intended for use in selected beverages (energy, sport, and electrolyte drinks, bottled, enhanced and fortified water beverages, and non-milk based meal replacement beverages) at maximum use levels of up to 8 to 20 mg/serving, respectively in these product types.

Table 1. Intended Use and Maximum Use Levels of PQQ disodium salt, % (w/w)

Food Use	Serving Size (RACC)[1]	Proposed Use Level	
		(mg/serving)	(%)
Energy Drinks	240 mL	12	0.005
Sport and Electrolyte Drinks	240 mL	8	0.0033
Enhanced and Fortified Water Beverages	240 mL	20	0.008
Bottled water	240 mL	8	0.00333
Non-Milk Based Meal Replacement Beverages	240 mL	8	0.00333

[1] RACC refers to Reference Amounts Customarily Consumed per eating occasion – 21 CFR §101.12 (U.S. FDA, 2015). When a range of values is reported for a particular food-use, particular foods within that food-use may differ with respect to their RACC.

5. Among consumers in the total population, the mean and 90th percentile all-user intakes of PQQ disodium salt were determined to be 28.2 and 63.1 mg/person/day, respectively. Corresponding EDIs reported in GRN 625 were 26.5 and 61.4 mg/person/day. The data show that intended use and use levels in this GRAS notice results in comparable EDIs to those described in GRN 625. It is assumed that JinCheng's PQQ disodium salt will replace currently marketed PQQ disodium salt or other POQ sources. Thus, cumulative exposures are not expected to change.

6. In the previous GRAS notices to the FDA, the safety of PQQ disodium salt has been established in toxicological studies in animals, mutagenicity studies, and is further supported by clinical studies in human. The NOAEL was determined to be 400 mg/kg bw/day in a subchronic toxicity study in rats. After applying a safety margin of 100, it can be concluded that doses up to 4 mg/kg bw/day or 240 mg/person/day would be safe in adults weighing 60 kg. The EDIs under the intended use are less than one thirds of the estimated safe intake levels in humans.

7. Furthermore, historical consumption of disodium salt supports the safety of PQQ disodium salt. Additional studies published subsequent to the FDA GRAS notices continue to support safety of PQQ disodium salt as a food ingredient.

1.B. Conclusions and General Recognition of the Safety of PQQ Disodium Salt

Several sources of PQQ disodium salt have been evaluated by the FDA and other global regulatory agencies over the past 10 years for proposed incorporation of PQQ disodium salt in foods for human consumption. Relevant US GRAS notifications include GRNs 625 and 641 (FDA, 2016a, 2016b) and NDI notice 417 (FDA, 2007). All of the GRAS and NDI notices provided information/clinical study data that supported the safety of the proposed PQQ disodium salt ingredients for use in human foods and dietary supplements. In all of the studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues attributable to PQQ disodium salt. Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

In addition, the intended uses of PQQ disodium salt have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. The specifications of the proposed GRAS substance, JinCheng's PQQ disodium salt, is almost identical to those that have received FDA no question letters.

The PQQ disodium salt product that is the subject of this GRAS determination is produced by non-toxigenic bacteria, *Hyphomicrobium denitrificans,* and its purity is over 99%. The PQQ disodium salt product is manufactured consistent with cGMP for food (21

CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes.

Literature searches did not identify safety/toxicity concerns related to PQQ disodium salt. Toxicity studies of PQQ disodium salt include acute, subacute, and subchronic toxicity, a battery of genotoxicity studies, and developmental and reproductive toxicity studies. In all of these reports, no evidence of toxicity was noted at up to 400 mg/kg bw/day, the highest dose levels tested. The publicly available scientific literature on the consumption and safety of PQQ disodium salt in human clinical studies is extensive and sufficient to support the safety and GRAS status of the proposed PQQ disodium salt.

The Panel further unanimously concluded that these uses of the PQQ disodium salt are GRAS based on scientific procedures, and that other experts qualified to assess the safety of foods and food additives would concur with these conclusions.

The Panel also has concluded that PQQ disodium salt is GRAS under the intended conditions of use on the basis of scientific procedures. Therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR. The Panel is not aware of any information that would be inconsistent with a finding that the proposed use of PQQ disodium salt meets appropriate specifications, and its use according to cGMP, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concern.

(b) (6)

May 5, 2017

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May 2, 2017

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Date