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

Prepared for Zhejiang Medicine Co., Ltd. (ZMC)

Prepared by:

Expert Panel Members

Susan Cho, Ph.D., AceOne RS, Inc., Clarksville, MD

Michael Falk, Ph.D., LSRO Solutions, LLC, Rockville, MD

George C. Fahey, Jr., Ph.D., University of Illinois-Urbana, Champaign, IL

Yong-Su Jin, Ph.D., University of Illinois-Urbana, Champaign, IL

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

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Abbreviations

ANI	Average Nucleus Identity
ANOVA	analysis of variance
ATCC	American Type Culture Collection
bw	body weight
C	Celsius
CARD	Comprehensive Antibiotic Resistance Database
CAS	Chemical Abstract Service
CFR	Code of Federal Regulations
cfu	colony forming units
cGMP	current Good Manufacturing Practice
CP	cyclophosphamide monohydrate
d	day
dL	deciliter
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
EDIs	Estimated Dietary Intakes
FD&C Act	Federal Food, Drug, and Cosmetic Act
FDA	Food and Drug Administration
FOIA	Freedom of Information Act
FSIS	Food Safety and Inspection Service
g	grams
GD	gestation day
GLP	Good Laboratory Practice
GRAS	Generally Recognized As Safe
H. denitrificans	*Hyphomicrobium denitrificans*
HACCP	Hazard Analysis Critical Control Point
HPLC	high-performance liquid chromatography
i.p.	intraperitoneal
i.v.	intravenous
kg	kilogram
L	liter
LC	liquid chromatography
LD_{50}	lethal dose 50
MCHC	mean corpuscular hemoglobin concentration
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mmol	millimole
MNPCE	micronucleated polychromatic erythrocyte
MTD	maximum tolerated dose
NDI	New Dietary Ingredient
NDIN	New Dietary Ingredient number
ng	nanogram
NHANES	National Health and Nutrition Examination Survey

nm	nanometer
NOAEL	no observed adverse effect level
OD	optical density
PCE	polychromatic erythrocyte
PQQ	pyrroloquinoline quinone
RDW	red cell distribution width
S. typhimurium	*Salmonella typhimurium*
SD	Sprague-Dawley
SPF	Specific-pathogen free
U.S.C.	United States Code
USDA	United Stated Department of Agriculture
USP	United States Pharmacopeia
UV	ultraviolet
V/V	volume/volume
VFDB	Virulence Factors Database
WCB	working cell bank
WGS	whole genome sequence
wk	week
y	years
ZMC	Zhejiang Medicine Co., Ltd.
μg	micrograms
μL	microliter
μm	micrometer

PART 1. SIGNED STATEMENTS AND A CERTIFICATION

1.A. Notice for GRAS Submission

Pursuant to 21 C.F.R. Part 170, subpart E, Zhejiang Medicine Co., Ltd. (hereinafter referred to as 'ZMC') 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 Federal Food, Drug, and Cosmetic Act (FD&C Act) based on its conclusion that the substance is GRAS under the conditions of its intended use.

1.B. Name and Address of the Notifier

Contact person: Ms. Wan Zhang
Company name: Zhejiang Medicine Co., Ltd. (ZMC)
Address:98 East XinchangDadao Road, Xinchang, Zhejiang Province, 312500 P. R. China
Telephone number: +86-575-8602-4939
E-mail address: zhangwan@zmc-china.com

1.C. Common or Trade Name

Pyrroloquinoline quinone (PQQ) disodium salt
Common abbreviation: PQQ

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

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

Intended use and use levels, in terms of per serving size, of ZMC's PQQ disodium salt is the same as those employed in GRN 709. ZMC proposes to use PQQ disodium salt as a food ingredient in selected beverages such as energy, sports, and electrolyte drinks, enhanced and fortified water beverages, bottled water, and non-milk-based meal replacement beverages. ZMC does not intend to use PQQ disodium salt as a component of infant formula or in foods under the United States Department of Agriculture (USDA)'s jurisdiction such as meat, poultry, and egg products.

1.D.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
Beverages and Beverage Bases	Energy drinks	360 mL	12
	Sports and electrolyte drinks	360 mL	8
	Enhanced and fortified water beverages	360 mL	20
	Bottled water	360 mL	8
	Non-milk based meal replacement beverages	240 mL	8

[1] RACC refers to Reference Amounts Customarily Consumed per eating occasion – 21 CFR §101.12 (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.12). Please note that serving size of energy, sport, and electrolyte drinks as well as bottled, enhanced, and fortified water beverages has been changed from 240 mL to 360 mL in 2020.

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

1.D.3. Purpose for Which the Substance is Used

The substance will be used as a food ingredient.

1.D.4. Description of the Population Expected to Consume the Substance

The population expected to consume the substance consists of members of the general population who consume at least one of the products described above.

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

1.F. Availability of Information

The data and information that are the basis for this GRAS conclusion will be made available to Food and Drug Administration (FDA) upon request by contacting AceOne RS, Inc. at the address below. The data and information will be made available to FDA in a form in accordance with that requested under 21 Code of Federal Regulations (CFR) 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

1.G. Availability of Freedom of Information Act (FOIA) Exemption

None of the data and information in Parts 2 through 7 of this GRAS notice are exempt from disclosure under the FOIA, 5 United States Code (U.S.C.) §552.

1.H. Certification

ZMC certifies that, to the best of our knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by ZMC, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of PQQ disodium salt.

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

Name: Zhang Wan Date: September 12, 2022
Title: Regulatory Affairs, Zhejiang Medicine Co., Ltd.

Address correspondence to
Susan Cho, Ph.D., Lead Expert Panel Member
AceOne RS, Inc.
5903 Hampton Forest Way, Fairfax, VA 22030
scho@aceoners.com or sscho397@yahoo.com
MP: 301-875-6454

1.J. Food Safety and Inspection Service (FSIS)/USDA Statement

ZMC 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.

PART2. IDENTITY, MANUFACTURING, SPECIFICATION, AND TECHNICAL EFFECTSOF 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, Sodium 9-carboxy-4,5-dioxo-4,5-dihydro-1H-pyrrolo[2,3-f]quinoline-2,7-dicarboxylate

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.

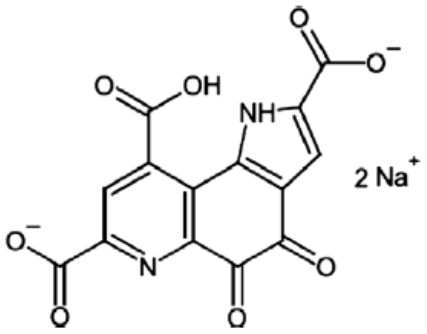


Figure 1. Structure of PQQ Disodium Salt

2.A.1.6. Physical Properties

Melting Point: > 300 °C
Solubility: Slightly soluble in water and practically insoluble in methanol.
PQQ is water-soluble and heat-stable.

2.A.1.7. Biological Significance

PQQ was first recognized as a cofactor for bacterial dehydrogenases in the late 1960s and is now appreciated as part of a family of quinone cofactor-dependent dehydrogenases and oxidases, classified as quinoproteins. PQQ is an aromatic water-soluble quinone. PQQ does not easily self-oxidize or condense into inactive forms. Accordingly, when compared on a molar basis, PQQ can be much more efficient in redox cycling assays than other enediols, such as ascorbic acid and menadione, as well as many polyphenolic compounds. PQQ also serves as a free radical scavenger (Jonscher et al., 2021). 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

No potential toxicants have been identified in PQQ disodium salt.

2.A.3. Particle Size

Approximately 90% of ZMC's PQQ disodium salt is smaller than 60 μm.

2.B. Method of Manufacture

The principle of ZMC's PQQ disodium salt production method (via bacterial fermentation) is similar to those described by other investigators and other companies, such as Mitsubishi Gas Chemical Company, Hisun, Nutraland USA, and Shangdong Jincheng Bio-Pharmaceutical Co., whose production methods for PQQ disodium salt received no objection letters from the FDA (GRN 641, FDA, 2016a; GRN 694, FDA, 2017a; GRN 701, FDA, 2017b; GRN 709, FDA, 2018; FDA, 2007, NDIN RPT 417). The difference is that the above mentioned companies described methods using the *Hyphomicrobium denitrificans* strains in a fermentation process to produce PQQ disodium salt. In this GRAS determination, a non-pathogenic, non-toxigenic strain of Methylotrophic bacteria, *Methylovorus glucosotrophus*, is used to produce PQQ disodium salt.

The original strain (*Methylovorus glucosotrophus* strain) producing PQQ was isolated from soil samples collected at Beijing, Hebei, HeiLongjiang, LiaoNing, and ShangDong Provinces, Peoples' Republic (P.R.) China, and then a strain producing PQQ disodium salt was successfully screened. ZMC's strain was identified as a non-pathogenic and non-toxigenic bacterial species. It is not genetically modified. The whole gene sequencing and bioinformatics analysis revealed that ZMC's *Methylovorus glucosotrophus* strain does not contain virulence or pathogenic genes. Details are described in Appendix A.

Preparation of the Working Cell Bank (WCB)

The working cell bank (WCB) is prepared by slant cultivation. The storage condition for the WCB is -30℃, and the validity period for storage is not more than 12 months. The freshly prepared slant culture is rinsed with 50 mL of 15% glycerol solution and is incubated. The prepared suspension is separated into each sterilized tube with 60-80% loading amount and the batch number is marked. The colonies are sent for testing to ensure that they conform to the established specifications.

Shaking Flask Culture

Drinking water is added to the culture medium, and then sub-packed with the amount of 200mL/750mL or 100mL/250mL each. The medium is moist heat-sterilized at 119 to 123℃ for 30 to 35 minutes. On the super clean workbench, inoculated WCB is added into each shaking culture medium according to the proportion of 0.025-1.000% of the inoculation amount; then 8.00-12.00 mL/L sterile methanol is added and shaken well. It is cultured at 26-32℃ and the shaking speed of 180-220 rpm for 20-56 hours.

Preparation of Inoculum and Fermentation

Drinking water is added to the seeding tank. The culture medium is moist heat-sterilized at 118 to 125℃ for 10 to 30 minutes. After sterilization, the medium is cooled; the pH is adjusted with ammonium hydroxide solution (≥25%). After methanol (≥99.0%) is added, the inoculum is incubated, stirred, and cultured for 15 to 40 hours with air flow at 26 to 32°C. Fermentation is terminated when mycelium concentration reaches a defined optical density (OD) and pH range.

Drinking water is added to the fermentation tank. The culture medium is moist heat-sterilized at 118 to 125°C for 10 to 30 minutes. After the sterilization, the medium is cooled. The pH is adjusted by ammonium hydroxide solution (≥25%). Methanol (≥99.0%) is added, and then the inoculum is incubated, stirred, and cultured for 65 to 180 hours at 26 to 32°C. The fermentation is terminated when the mycelia decline, tinting strength is weak, the increase in fermentation potency is slower and pH increases slightly

Extraction and Purification

After the fermentation process, PQQ disodium salt is isolated and purified through a filtration step to remove the source microorganism, followed by ion exchange chromatography. Nanofiltration is performed when the concentration of the mixed eluate is less than 2,000μg/mL (for the purpose of concentration). The eluate is collected and sodium chloride is added with stirring at a concentration of 5-15%. Crystallization then occurs over several hours at ≤ 30°C. The first crude product is dissolved by de-ionized water, subjected to ion exchange chromatography, washed with de-ionized water and then eluted with sodium chloride. The eluate is collected, and sodium chloride is added with stirring at a concentration of 5-15%. Crystallization then occurs

over several hours at ≤30°C. The mixture then is filtered to obtain the second PQQ disodium salt (crude).

Preparation of PQQ

The second PQQ disodium salt (crude) is dissolved in deionized water, the pH is adjusted with hydrochloric acid, then cooled to ≤30°C for crystallization, and filtered to get wet product. This is followed by vacuum drying. The wet product of PQQ disodium salt is first dried for 8-48 hours at 70-90°C and under a vacuum ≤0.02MPa. The product is dried again for 2-16 hours after milling. The dried product of PQQ disodium salt contains no more than 12.0% water after drying, milling, and sieving. The finished PQQ disodium salt then is transferred to quarantine storage for quality control testing prior to packaging in aluminum foil bag storage containers, and stored at a temperature not to exceed 30°C.

The PQQ disodium salt product is manufactured consistent with the principles of current Good Manufacturing Practices (cGMP). Figure 2 presents the manufacturing process of PQQ disodium salt.

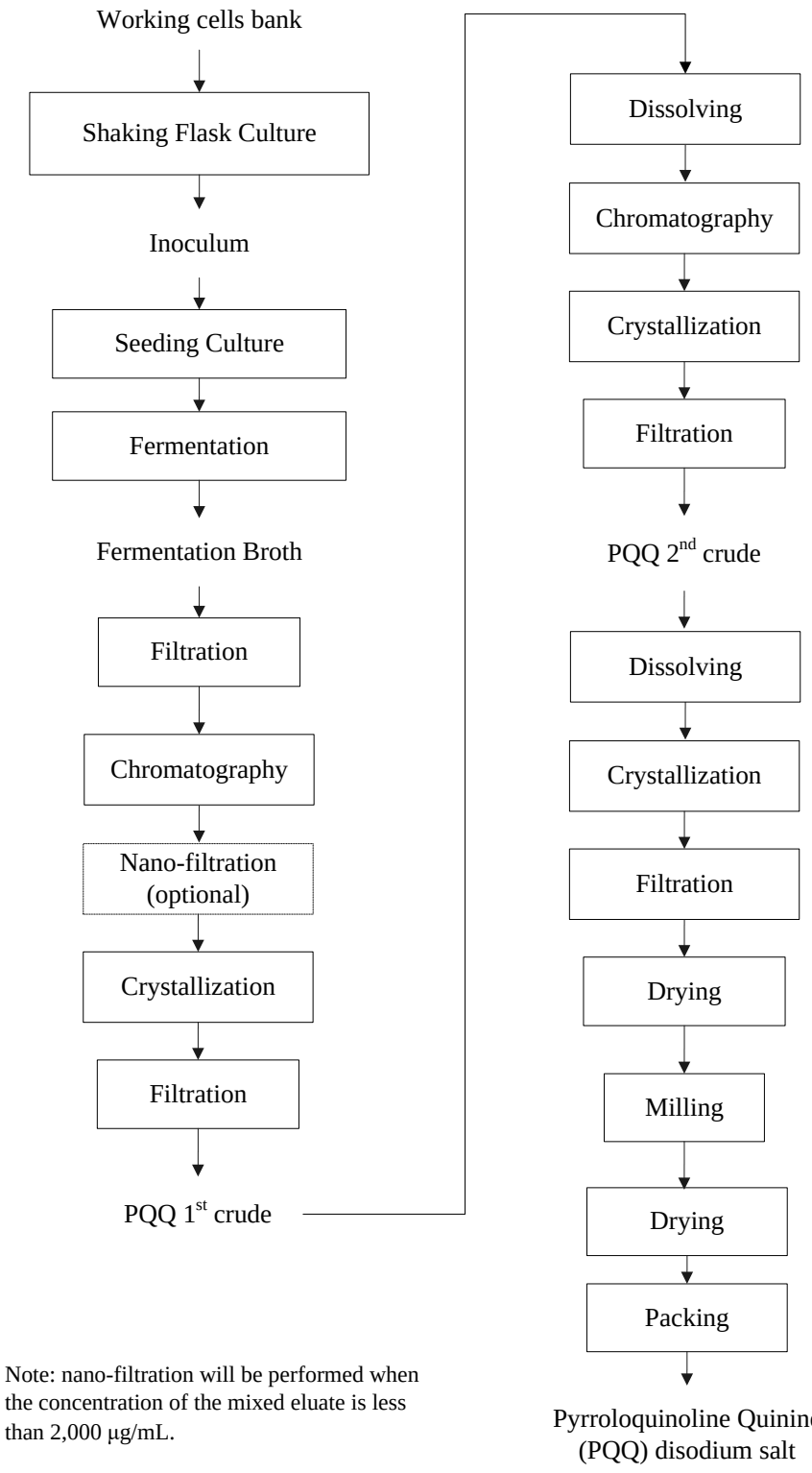


Figure 2: Manufacturing Process of ZMC's PQQ Disodium Salt

Table 2 shows the composition of fermentation medium. Raw materials listed in fermentation medium function as nutrients for fermentation.

Table 2. Composition of Fermentation Medium

Composite	Remarks
Magnesium sulfate	Fermentation nutrient
Ammonium sulfate	Nitrogen source for fermentation
Potassium dihydrogen phosphate	Fermentation nutrient
Disodium hydrogen phosphate	Fermentation nutrient
Ferric citrate	Fermentation nutrient
Zinc sulfate heptahydrate	Fermentation nutrient
Cupric sulfate pentahydrate	Fermentation nutrient
Manganese chloride tetrahydrate	Fermentation nutrient
Methanol	Carbon source for fermentation
Ammonium hydroxide	pH balancing, nitrogen source for fermentation

Table 3 presents Chemical Abstract Service (CAS) registry numbers and regulatory status of raw materials used in the manufacture of ZMC'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.

Table 3. CAS Registry Numbers and Regulatory Status of Raw Materials

Raw material	CAS Registry No.	Regulatory Status
Magnesium sulfate ($MgSO_4$)	10034-99-8	21 CFR §184.1443
Ammonium sulfate [$(NH_4)_2SO_4$]	7783-20-2	21 CFR §184.1143
Potassium dihydrogen phosphate (KH_2PO_4)	7778-77-0	No 21 CFR for the intended use
Disodium hydrogen phosphate (Na_2HPO_4)	7558-79-4	No 21 CFR for the intended use
Calcium chloride ($CaCl_2$)	10043-52-4	21 CFR §184.1193
Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	7446-20-0	21 CFR §182.8997 (zinc sulfate)
Cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	7758-99-8	21 CFR184.1261 (copper sulfate)
Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$)	13446-34-9	21 CFR184.1446 (manganese chloride)
Ferric citrate	2338-05-8	21 CFR184.1298
Methanol	67-56-1	No 21 CFR for the intended use
Ammonium hydroxide	1336-21-6	21 CFR184.1139
Phosphoric acid	7664-38-2	21 CFR182.1073
Sodium chloride	7647-14-5	21 CFR §182.1
Hydrochloric acid	7647-01-0	21 CFR §182.1057

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

2.C. Identifications of PQQ Disodium Salt

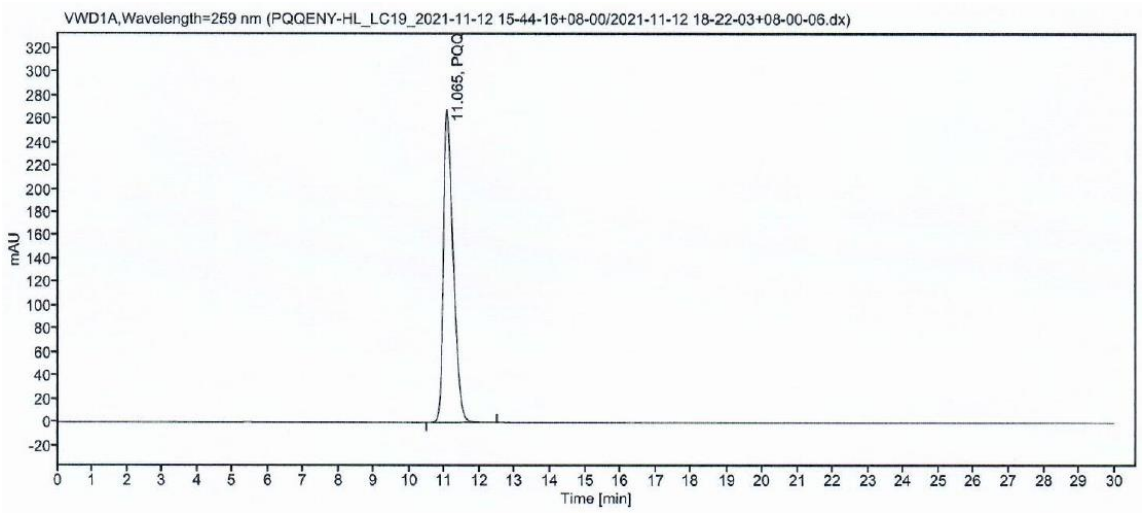
2.C.1 HPLC Analysis for PQQ Disodium Salt

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

Chromatographic Conditions

Instrument: Liquid chromatography (LC);
Chromatographic column: Octadecyl silane bonded silica gel chromatographic column, i.e., KromasilC18, 4.6 mm×150 mm, 5 μm;
Column temperature: 30℃;
Detection wavelength: 259 nm (UV);
Flow rate: 1.0 ml/min;
Mobile phase: Acetonitrile: buffer solution =28: 72 (V/V);
Injection volume: 20 μL;
Running time: 30 min

a) USP Reference Standard (USP catalog No.: 1589040; USP Lot No.: F10550)



b) ZMC's PQQ Disodium Salt (Batch No. 311PQ210501)

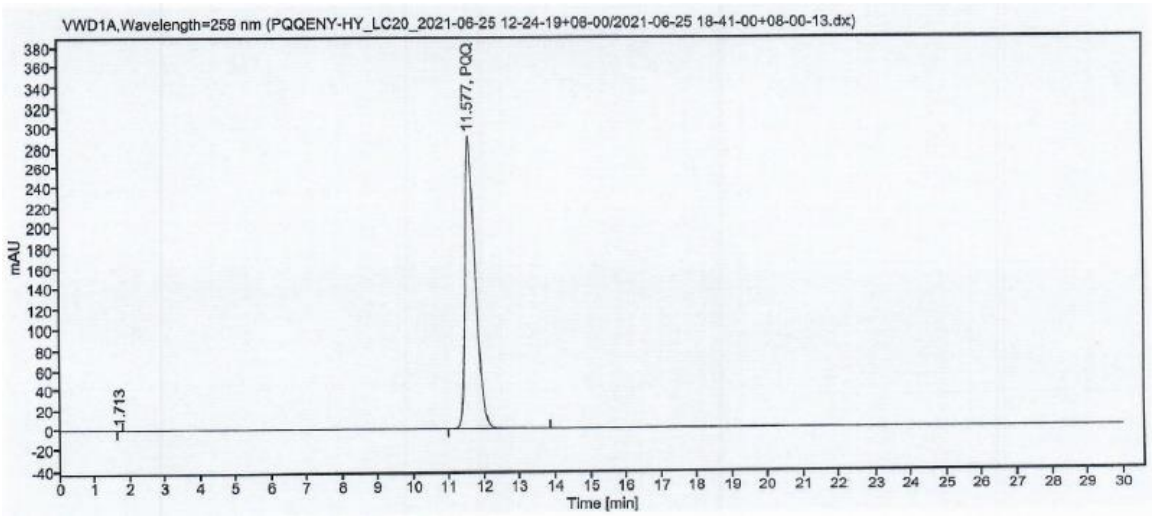


Figure 3. HPLC Chromatograms Comparing Commercial Standard and ZMC's PQQ Disodium Salt

2.C.2. Specifications

Specifications for PQQ disodium salt are presented in Table 4. The ZMC's PQQ disodium salt is ≥98% pure on a dry weight basis, as measured by HPLC. Specifications include the minimum content of PQQ disodium salt (≥98%) and limits for lead (≤0.2 mg/kg), arsenic (≤ 0.2 mg/kg), cadmium (≤0.2 mg/kg), and mercury (≤0.2 mg/kg) as well as microbial contaminants such as total aerobic counts (≤1,000 cfu/g), yeasts and molds (≤100 cfu/g), *Enterobacteria* (<10 cfu/g or <detection limit of the assay), *Escherichia coli* (*E. coli*; absent in 25 g), *Staphylococcus aureus* (absent in 10 g), *Salmonella* (absent in 25 g) and *Listeria* (absent in 25 g).

Analytical data on multiple batches confirmed the purity of the product (≥98%) and its manufacturing consistency by meeting specifications (Table 5; Appendix B).

Table 4. Specifications for ZMC's PQQ Disodium Salt

Items	**Methods**	**Specifications**
Appearance	Visual inspection	Red or reddish-brown powder
Water content, g/100 g	USP<921>	≤ 13.0
PQQ (dry wt. basis), g/100 g	USP<621>	≥ 85
PQQ disodium salt (dry wt. basis), g/100 g	USP<621>	≥ 98.0
Sodium content (dry wt. basis), g/100 g	USP<621>	10.5-12.9
Lead, mg/kg	USP<233>	≤ 0.2
Arsenic, mg/kg		≤ 0.2
Cadmium, mg/kg		≤ 0.2
Mercury, mg/kg		≤ 0.2
Microbial Limit		
Total aerobic counts, cfu/g	USP<2021>	≤ 1,000
Yeasts and molds, cfu/g	USP<2021>	≤ 100
Enterobacteria, cfu/g	USP<2021>	≤ 10
Escherichia coli, in 25g	USP<2022>	Absent in 25 g
Staphylococcus aureus, cfu/10g	USP<2022>	Absent in 10 g
Salmonella, in 25g	USP<2022>	Absent in 25 g
*Listeria**, in 25g	/	Absent in 25 g

Note: *The test of *Listeria* is performed once a year.

Table 5. Analytical Values for ZMC's PQQ Disodium Salt

Test Items	**Specifications**	311PQ210501	311PQ210502	311PQ210601
Appearance	Red or reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder
Water content, g/100 g	≤ 13.0	8.9	9.0	8.8
PQQ (dry wt. basis), g/100 g	≥ 85	88	88	88
PQQ disodium salt (dry wt. basis), g/100 g	≥ 98.0	100.2	100.1	100.1
Sodium content (dry wt. basis), g/100 g	10.5-12.9	12.3	12.2	12.3
Pb (Lead, mg/kg)	≤ 0.2	< 0.2	< 0.2	< 0.2
As (Arsenic, mg/kg)	≤ 0.2	< 0.2	< 0.2	< 0.2
Cd (Cadmium, mg/kg)	≤ 0.2	< 0.2	< 0.2	< 0.2
Hg (Mercury, mg/kg)	≤ 0.2	< 0.2	< 0.2	< 0.2
Microbial Limit				
Total aerobic counts, cfu/g	≤ 1,000	< 10	< 10	< 10
Yeasts and molds, cfu/g	≤ 100	< 10	< 10	< 10
Enterobacteria, cfu/g	< 10	< 10	< 10	< 10
Escherichia coli, in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
Staphylococcus aureus, in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g
Salmonella, in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
Listeria, in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g

2.D. Shelf-life

Test samples were stored at polyethylene bags and tested for storage stability. The data presented in Table 6 supported the stability of PQQ disodium salt for 12 months at 25℃ and at 60% relative humidity.

Table 6. Stability of ZMC's PQQ Disodium Salt

Batch No.	Test Items \ Duration (Month)		0	3	6	9	12
311PQ210501	Appearance	Red or reddish- brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder
	Water (g/100 g)	≤ 13.0	8.9	8.9	9.0	8.9	8.8
	Assay (g/100 g), dry wt. basis basis	≥ 98.0	100.2	101.1	100.2	100.2	100.0
311PQ210502	Appearance	Red or reddish- brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder
	Water (g/100 g)	≤ 13.0	9.0	8.8	9.0	8.9	8.8
	Assay (g/100 g), dry wt. basis basis	≥ 98.0	100.1	100.9	100.4	100.0	99.9
311PQ210601	Appearance	Red or reddish- brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder
	Water (g/100 g)	≤ 13.0	8.8	8.8	9.0	8.8	8.7
	Assay (g/100 g), dry wt. basis basis	≥ 98.0	100.1	100.9	100.4	100.1	99.0

PART 3. DIETARY EXPOSURE

3.A. Estimated Dietary Intakes (EDIs) of PQQ Disodium Salt Under the Intended Use

The intended use and use levels of PQQ disodium salt of the current notice is the same as those employed in GRN 709 (Table 1). Using food intake data reported in the 2015-2018 National Health and Nutrition Examination Survey (NHANES), exposure levels to PQQ disodium salt that will result from the intended uses were estimated.

The results of the EDI assessment are summarized in the two tables below (Tables 7-1 to7-2). Under the intended use, approximately 60.1% of the population is estimated to be the users of PQQ disodium salt. Based on the NHANES 2015-2018 dataset, the mean and 90 percentile all-user intakes of PQQ disodium salt were estimated to be approximately 21 and 46 mg/person/day, respectively. Males older than 19 years of age would have the highest intake among the various age/gender groups, with a 90th percentile value of 52 mg/person/day for all-users. On a body weight basis, children aged 2-5 years had the highest 90th percentile EDI at 0.98 mg/kg bw/day for all-users.

These EDIs 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. 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 within the safe intake levels.

Table 7-1. Maximum EDIs of PQQ Disodium Salt Under the Intended Use*, mg/day

Population	% all-user	N, total population	Per User (mg/day)		Per Capita (mg/day)	
			Mean	90th Percentile	Mean	90th Percentile
2-5 y	48.8	987	7.80	16.6	3.81	11.8
6-12 y	60.7	1,744	10.8	22.0	6.6	17.3
13-18 y males	64.0	720	19.3	41.0	12.4	30.0
13-18 y females	64.8	713	17.6	37.7	11.4	29.0
19+ males	59.9	4,080	25.5	52.3	15.2	43.5
19+ females	60.7	4,461	21.9	45.3	13.3	38.3
2-99 y	60.1	12,705	21.3	45.8	12.8	38.1

*Assuming all the foods will be used at the maximum use levels; NHANES 2015-2018.

Table 7-2. Maximum EDIs of PQQ Disodium Salt Under the Intended Use, mg/kg bw/day

Population	Per User (mg/kg bw/day)		Per Capita (mg/kg bw/day)	
	Mean	90th Percentile	Mean	90th Percentile
2-5 y	0.46	0.98	0.23	0.69
6-12 y	0.31	0.64	0.19	0.50
13-18 y males	0.27	0.58	0.17	0.45
13-18 y females	0.28	0.53	0.18	0.47
19+ males	0.29	0.60	0.17	0.48
19+ females	0.29	0.60	0.18	0.49
2-99 y	0.30	0.62	0.18	0.49

*Assuming all the foods will be used at the maximum use levels; NHANES 2015-2018.

Cumulative EDIs from the maximum use levels adopted from all previous GRAS notices are presented in Appendix C.

3.B. Food Sources of PQQ

PQQ is naturally occurring in small quantities in food products, particularly vegetables, fruits, and fermented soy and dairy products (Kumazawa et al. 1995; Noji et al., 2007). As shown in Table 8, concentrations of PQQ in foods are low, typically in the ppb range. Typical free PQQ contents in selected food products were found between 1 and 61 ng/g (Kumazawaet al., 1995).

Table 8. 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	17.8 ±6.78	Green soybeans	9.26 ± 3.82
Potato	16.6 ± 7.34	Sweet potato	13.3 ± 3.72
Parsley	34.2 ± 11.6	Cabbage	16.3 ± 3.96
Carrot	16.8 ± 2.81	Celery	6.33 ± 2.41
Green pepper	2.12 ± 0.4 to 28.2 ± 13.7	Spinach	7.0 ± 2.17 to 21.9 ± 6.19
Tomato	ND to9.24 ± 1.82	Apple	6.09 ± 1.36
Banana	12.6 ± 3.81	Kiwi fruit	27.4 ± 2.64
Orange	6.83 ± 2.20	Papaya	26.7 ± 8.57
Field mustard	5.54 ± 1.50	Broccoli sprout	1.55 ± 0.37
Japanese radish	0.70 ± 0.42	Rape blossom	5.44 ± 0.8
Green tea	0.16 ±0.05 to 29.6 ± 12.9	Miso (bean paste)	16.7 ± 3.30
Coke	20.1 ± 3.17	Fermented soybeans(natto)	61.0 ± 31.3
Wine	5.79 ± 2.73	Fermented soybeans	1.42 ± 0.32

Oolong (tea)	27.7 ±1.92	Tofu (bean curd)	24.4 ± 12.5
Whiskey	7.93 ±1.84	Skim milk (dry wt. basis)	2.5[1] ± 1.4
Sake	3.65 ± 1.39	Milk	3.4 ± 0.4
Beer	1.66 ± 0.82	Egg yolk[2]	7.0 to 19.3
Bread	9.14 ± 3.64	Egg white[2]	4.1 to 28.8

PQQ = pyrroloquinoline quinone
Adapted from Kumazawa et al. 1995; Noji et al., 2007.
[1] Units for skim milk lyophilizate are ng/g dry weight.
[2] Eggs were obtained from domestic fowl (*Gallus gallus*) and duck (*Cairinamoschata*).

3.C. 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 NHANES databases. Using the dietary content of PQQ available from the literature (Table 8), GRN 709 reported the EDIs of PQQ from the diet. NHANES 2011-2012 dietary data for age 2 years and older were used to estimate PQQ exposure from select dietary sources (Table 9). The dietary sources being analyzed are 36 common foods that contain PQQ as listed in Part 3.B.

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 9. 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.
Adopted from GRN 709.

3.D. EDI of Other Components Under the Intended Use

The sodium content of PQQ disodium salt is approximately 12%. GRN 709 reported the EDIs of sodium under the intended use based on the EDI of PQQ disodium salt (Table 10) and the sodium content of PQQ disodium salt. The estimated intakes of sodium under the intended use are negligible compared to usual intakes of sodium from the diet. Thus, intended use of PQQ disodium salt would likely have no significant impact on sodium intakes in Americans.

Table 10. 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.
Adopted from GRN 709.

Summary of Consumption Data

Based on the NHANES 2015-2018 dataset, the mean and 90th percentile all-user intakes of PQQ disodium salt were estimated to be approximately 21 and 46 mg/person/day, respectively. It is assumed that ZMC's PQQ disodium salt will replace currently marketed PQQ disodium salt or other PQQ sources. Thus, cumulative exposure is not expected to change.

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. 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.

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 due to the unpleasant taste1. Additionally, the cost of the product (approximately $3,000/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.

PART 6. BASIS FOR GRAS DETERMINATION

6.A. Current Regulatory Status

The FDA has previously issued 'no question' letters on GRAS and NDI notices related to PQQ disodium salt produced by a bacterial fermentation technique using *Hyphomicrobium denitrificans* (GRN 641, FDA, 2016a; GRN 694, FDA, 2017a; GRN 701, FDA, 2017b; GRN 709, FDA, 2018; NDIN RPT 417, FDA, 2007) (Table 11). In addition, the FDA has issued a 'no question' letter on synthetic PQQ disodium salt (GRN 625, FDA 2016a).

Table 11. Summary of GRAS Notices of PQQ Disodium Salt

	Intended Use (Ingredient in)	Maximum use level	Mean and 90th percentile EDI in all-users	Manufacturing method
GRN 625 (FDA, 2016b); Nascent Health Sciences (pages 6, 13, 25-26)	Energy, sports, and isotonic drinks; non-milk-based meal replacement beverages; water (bottled, enhanced, fortified); milk-based meal replacement beverages	8 mg/serving	26.5 and 61.4 mg/person/day (0.4 and 0.9 mg/kg bw/d)	Synthetic
GRN 641 (FDA, 2016a); Zheijang Hisun Pharmaceutical Co. (pages 4, 24-25)	Energy, sports, and electrolyte drinks; enhanced and fortified water	up to 5-20 mg/serving	12.8 and 27.8 mg/person/day (0.21 and 0.48 mg/kg bw/d)	Fermentation via *Hypho-microbium denitrificans*
GRN 694 (FDA, 2017a); Fuzhou Contay (pages 4, 14-17)	Energy, sports, and electrolyte drinks; bottled, enhanced, and fortified water; and non-milk-based meal replacement beverages	up to 0.083% in final product (correspond-ing to 8-20 mg/serving at that time)	19.2 and 42.7 mg/person/d[a] (0.29 and 0.62 mg/kg bw/d)[b]	Fermentation via *H.denitrificans*
GRN 701 (FDA, 2017b); Nutraland USA (pages 4, 15-16)	Energy, sports, and electrolyte drinks; bottled, enhanced, and fortified water; and non-milk-based meal replacement beverages	up to 8-20 mg/serving	29.9 and 65.8 mg/person/d (0.40 and 0.87 mg/kg bw/d)	Fermentation via *H. denitrificans*
GRN 709 (FDA, 2018); Shangdong	Energy, sports, and electrolyte drinks; bottled, enhanced, and	up to 8-20 mg/serving	28.2 and 63.1 mg/person/d (0.41 and 0.89	Fermentation via *H. denitrificans*

Jincheng Bio-Pharmaceutical Co. (pages 5, 19-21)	fortified water; and non-milk-based meal replacement beverages		mg/kg bw/d; based on 2011-2014 NHANES)	
Current notice	Energy, sports, and electrolyte drinks; bottled, enhanced, and fortified water; and non-milk-based meal replacement beverages	up to 8-20 mg/ serving*	21.3 and 45.8 mg/person/d (0.30 and 0.62 mg/kg bw/d) (based on 2015-2018 NHANES)	Fermentation via *Methylovorus glucosotrophus*

bw = body weight; d = day; *Serving sizes for many beverage products have been changed from 240 to 360 mL in the past 2 years.

[a, b]Based on the 2011-2014 and 2015-2018 National Health and Nutrition Examination Survey (NHANES) datasets, we obtained significantly higher EDIs than those reported in GRN 694. Details are discussed in Appendix C.

6.B. Review of Safety Data

The PQQ disodium salt in this GRAS notice has similar specifications and purity compared to those of PQQ disodium salt described in the previous FDA GRAS notices (Table 12). Based on a comparison of the specifications for these products, it is concluded that these PQQ disodium salt preparations are substantially equivalent. Thus, it is recognized that the information and data in previous GRAS notices 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 2018. The subject of the present GRAS notice is PQQ disodium salt produced via microbial fermentation using Methylotrophic bacteria, *Methylovorus glucosotrophus* (powder form).

Table 12. Comparison of the PQQ Disodium Salt Preparations

Reference		Purity
GRN 625	Reddish brown crystalline powder	>98% (page 19)
GRN 641	Henna powder	>99% (page13)
GRN 694	Reddish brown crystalline powder	>99% (page 11)
GRN 701*	Reddish brown crystalline powder	>94% (page 69)
GRN 709	Reddish brown crystalline powder	>99%(page 16)
Current notice	Reddish brown crystalline powder	≥98%

*Although the purity specification for PQQ disodium salt in GRN 701 was >85% (page 12), the certificate of analysis showed the purity to be >94% (page 69).

6.B.1. Metabolism of PQQ

Since the FDA's last review of GRN 625 (pages 15-16 or stamped pages 27-28), GRN 641 (pages 27-29), GRN 701 (pages 21-22), and GRN 709 (page 27), no new metabolism study has been published. The following studies that were discussed in previous GRAS notices are briefly summarized as follows.

In a study by Smidt et al. (1991), male Swiss-Webster mice (n = 5 per time point) were orally administered (gavage) a single dose of 1.5 mg/kg body weight (bw) of radiolabeled (^{14}C) PQQ. Based on the amount retained in the tissues, and amount in the urine and carbon dioxide, approximately 62% of the PQQ was estimated to be absorbed through the small intestine and 81% of that was excreted within 24 hours via urine. The data indicate that PQQ is absorbed effectively, with most of it excreted in urine. The radioactive PQQ was detected in the kidneys (10.7%), the carcass (3.7%), 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 hours), 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 hours. At 6 hours, the blood cell fraction constituted about 10% of the absorbed label. This fell to 1.2% at 24 hours. No radioactivity was detected in the expired air.

A human study conducted by Harris et al. (2013) found that levels of PQQ peaked in serum at ~2 h following a single dose of PQQ (0.2 mg PQQ/kg bw). In this experiment, five male and five female subjects (mean age of 28.1 years) received a single dose of 0.2 mg PQQ/kg bw in a drink (corresponding to 14 mg PQQ for a 70-kg adult) and blood and urine samples were collected at 0, 2, 4, 8, 24, or 48 h post-administration. Serum concentration of PQQ peaked at 2 h post-administration at 9 nM (~ 3.4 ng/mL).

In another experiment by Harris et al. (2013), the urinary excretion and serum concentration of PQQ in 10 healthy adults (five men and five women) who received PQQ in a drink at increasing dose levels of 0.075, 0.15, and 0.3 mg/kg bw/day (corresponding to approximately 5.25, 10.5, and 21 mg PQQ/day for a 70 kg individual) over three consecutive 7-

day periods. At each dose (up to 0.3 mg/kg bw/day), ~0.1% of the PQQ ingested was recovered in urine as nonderivatized PQQ. Serum concentrations of nonderivatized PQQ increased in response to dietary intake up to 14 nM at a dose of 0.3 mg/kg bw/day, and the daily excretion of PQQ in urine was directly related to serum concentrations. After a 3-day administration of PQQ, multiple measurements of urinary parameters were made throughout the period of 72 h. PQQ supplementation did not alter urinary excretion of glucose and ketone-related products but decreased urinary excretion of lactate ($P<0.05$) and pyruvic acid by 30-40% and the initial ratio of lactate to pyruvate was reduced from 4.5±0.5 to 3.6±0.35 after 3-day ingestion of PQQ at 0.3 mg/kg. In addition, total urinary excretion of total amino acids decreased by ~15% ($P<0.01$). The amounts of urinary purine-related metabolites such as hypoxanthine or uracil, hippuric acid (a marker for changes in the microbiome), and myo-inositol were not changed following PQQ supplementation. These data suggest that PQQ might influence mitochondrial efficiency, assuming that the relative reduction in urinary concentrations reflects increased oxidation and flux of citric acid cycle intermediates via mitochondria.

Human tissue contains from 1 to 3 ng of non-derivatized PQQ per gram of tissue or milliliter of fluid. In human tissues, the highest levels were found in the spleen, pancreas, lung, and kidney, while no PQQ was detected in the brain or heart (Kumazawa et al., 1992). In the absence of a known mammalian biosynthetic pathway for PQQ, the endogenous tissue levels of PQQ in humans are likely derived from dietary exposure (Kumazawa et al., 1995). 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).

PQQ, alone or with its corresponding imidazolopyrroloquinoline (IPQ) derivatives, condensed products of PQQ with amino acids, have been detected in human milk at concentrations of 140 to 180 ng/mL (Mitchell et al., 1999).

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

6.B.2.1. Mutagenicity and Genotoxicity Studies of ZMC's PQQ Disodium Salt

Bacterial Reverse Mutation Test of ZMC's PQQ Disodium Salt Produced via *Methylovorus glucosotrophus*

The objective of this study was to evaluate PQQ disodium salt for its ability to induce reverse mutations at the histidine locus of four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and at the tryptophan locus of *Escherichia coli* WP2 *uvrA*, in the presence and absence of exogenous metabolic activation (Aroclor 1254 induced rat liver S9) (Seol et al., 2022). The mutagenicity of PQQ disodium salt was evaluated in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2 *uvr*A using the plate incorporation

method. Doses were selected based on a dose range-finding assay, which indicated that doses up to 5,000 μg/plate PQQ disodium salt were not cytotoxic with no precipitation.

The tester strains used in the definitive mutagenicity assay were TA98, TA100, TA1535, and TA1537, and WP2 *uvrA*. The assay was conducted both in the presence and absence of the S9 mix along with concurrent negative/solvent control and positive controls. Based on the result of dose range-finding assay, dose levels tested in the definitive mutagenicity assay with all tester strains were 0, 100, 250, 500, 1,000, 2,500, or 5,000 μg/plate PQQ disodium salt in the absence or presence of metabolic presence of S9, 2-aminoanthracene served as the positive control for *S. typhimurium* TA98, TA100, TA1535, and TA1537, and *E. coli* WP2 *uvr*A. The positive controls in the absence of S9 were 2-nitrofluorene for *S. typhimurium* TA98, sodium azide for *S. typhimurium* TA100 and TA1535, acridine mutagen ICR-191 for *S. typhimurium* TA1537, and methyl methane-sulfonate for *E. coli* WP2 *uvr*A. The plates were incubated for approximately 48 hours at 37±2°C. The result was deemed positive for *S. typhimurium* TA1535 and TA1537 if the increase in mean revertant colonies was equal to or greater than 3-fold of the negative solvent control. For *S. typhimurium* TA98 and TA100 and *E. coli* WP2 *uvr*A, the result was deemed positive if the increase in mean revertant colonies was equal to or greater than 2-fold of the negative solvent control.

From the results in the first definitive mutagenicity assay, there were less than three non-toxic dose levels for evaluating the results of TA1537 with S9 mix, and the revertant value of the negative control was not comparable with historical data for TA98 with S9 mix. TA98 and TA1537 with S9 in the first definitive mutagenicity assay were considered invalid for evaluating the result; they were repeated with concurrent negative/solvent control and positive controls. The dose levels tested in the second definitive mutagenicity assay were 25, 55, 110, 220, 550, 1,650, and 5,000 μg per plate.

The results of the second definitive mutagenicity assay as well as the validated results from the first mutagenicity assay were used to evaluate the mutagenicity of PQQ disodium salt (Tables 13 and 14). All positive controls induced the expected increase (three-fold or greater) in the mean number of revertant colonies, in both the presence and the absence of the S9 mix, relative to the concurrent negative/solvent control, thereby confirming the responsiveness of the strains.

No precipitate was observed in any tester strains at any dose level in the absence and presence of S9. PQQ disodium salt did not induce more than 2-fold increases in *S. typhimurium* TA98 and TA100 and *E. coli* WP2 *uvr*A nor 3-fold increases in *S. typhimurium* TA1535 and TA1537 in the mean number of revertant colonies at any dose levels in the absence and presence of S9 compared to the negative solvent control. In addition, no dose response was observed.
It was concluded that PQQ disodium salt was not mutagenic under the test conditions.

Table 13. First Definitive Mutagenicity Assay Results for ZMC's PQQ Disodium Salt

	Dose (μg/plate)		Mean Revertant Colony Counts Per Plate				
			TA98	TA100	TA1535	TA1537	WP2 *uvr*A
-S9	Sterile water	0	16.67±2.31	124.67±14.01	12.33±3.51	8.00±1.00	23.33±2.52
	PQQ disodium salt	100	24.67±2.52	123.33±4.62	8.67±3.06	9.67±3.06	24.00±2.65
		250	24.67±9.87	120.00±8.89	10.33±4.04	6.00±2.65	16.00±4.00
		500	19.00±7.55	112.00±22.34	11.00±4.36	7.33±4.73	21.33±7.57
		1,000	21.67±1.53	114.33±15.01	8.00±6.08	6.00±0.00	16.67±2.08
		2,500	14.67±6.35	87.00±10.44	9.00±1.73	6.00±4.58	11.67±3.06
		5,000	12.33±1.53	68.00±7.00	10.00±4.36	4.67±1.15	8.33±2.52
	2-NF	10.0	1,825.33±95.69				
	SA	1.0		867.00±12.12	575.33±23.63		
	ICR-191	1.0				334.67±31.09	
	MMS	2.5 μL					755.00±22.61
+S9	Sterile water	0	42.33±10.41	153.67±6.35	12.00±3.00	15.33±0.58	26.00±7.94
	PQQ disodium salt	100	24.33±2.31	129.67±13.61	8.33±2.31	11.67±4.04	26.67±4.16
		250	19.33±3.06	143.67±12.01	10.67±2.31	13.67±4.62	23.33±4.04
		500	24.67±4.51	126.00±10.82	12.00±5.57	7.00±1.00	20.00±2.65
		1,000	21.67±2.89	114.00±7.21	11.00±1.73	5.33±5.13	14.67±1.53
		2,500	20.00±4.36	100.67±9.07	9.33±2.89	6.67±2.52	13.33±7.09
		5,000	11.33±3.79	81.00±6.08	9.00±3.46	6.00±3.46	11.00±4.00
	2-AA	2.0	974.00±43.51	1,217.33±24.44	97.33±7.02	92.33±7.23	256.33±17.01

ICR-191 = acridine mutagen ICR-191; MMS = methyl methane-sulfonate; PQQ = pyrroloquinoline quinone; SA = sodium azide; 2-AA = 2-aminoanthracene; 2-NF = 2-nitrofluorene.

Table 14. Second Definitive Mutagenicity Assay Results for ZMC's PQQ Disodium Salt

	Dose (μg/plate)		Mean Revertant Colony Counts Per Plate	
			Salt *S. typhimurium*TA98	Salt *S. typhimurium*TA1537
+S9	Sterile water	0	33.00±7.21	19.67±1.53
	PQQ disodium salt	25	31.67±7.37	18.33±4.51
		55	24.67±5.13	21.00±2.65
		110	20.67±6.03	21.67±5.13
		220	25.67±4.04	19.67±1.53
		550	23.00±6.08	24.33±3.06
		1,650	22.67±0.58	21.00±1.00
		5,000	14.33±0.58	16.00±3.00
	2-AA	2.0	1,203.67±10.69	132.33±26.10

PQQ = pyrroloquinoline quinone; 2-AA = 2-aminoanthracene.

In Vivo Micronucleus Assay of ZMC's PQQ Disodium Salt Produced via *Methylovorus glucosotrophus*

The objective of this study was to evaluate the clastogenic, and/or disrupting the mitotic apparatus potential of PQQ disodium salt by scoring micronucleated polychromatic erythrocyte cells (MNPCE) in the bone marrow of 78 adult male and female Sprague-Dawley (SD) rats after administrated PQQ disodium salt once by oral gavage (Seol et al., 2022).

Doses for the micronucleus assay were selected based on a previous single oral dose toxicity study in SD rats, that reported no mortality or apparent toxicity for doses up to 2,000 mg/kg bw PQQ disodium salt. Seventy-eight rats received a single dose of vehicle control (purified water), 500, 1,000, or 2,000 mg/kg bw PQQ disodium salt by oral gavage, or 20 mg/kg bw cyclophosphamide monohydrate, the positive control, by intraperitoneal (i.p.) injection.

At 24 and/or 48 hours post-dosing, animals were sacrificed for bone marrow harvest. After 48 hours of dosing, additional rats in the negative control and high-dose groups were sacrificed. The bone marrow from each animal was examined microscopically to determine the proportion of polychromatic erythrocyte (PCE) to total erythrocytes and the number and frequency of MNPCE. The result was deemed positive if the rate of MNPCE increased significantly compared to the negative control for at least one test substance dose group, and dose response is apparent if more than one dose group is used. No bone marrow toxicity was observed for the percentage of PCE in total erythrocytes in all test substance groups (Table 15). No statistically significant micronucleus formation was observed at any dose. The positive control induced statistically significant increases in micronucleus formation in male and female rats compared to the concurrent control group ($P \leq 0.05$), validating the assay system. It was concluded that PQQ disodium salt was not genotoxic under the test conditions.

Table 15. *In Vivo* Rat Micronucleus Results for ZMC's PQQ Disodium Salt

	Concentration of PQQ disodium salt (mg/kg bw)	Sampling time (hours)	PCE Percentage (%)	Total number of MNPCE observed	MNPCE frequency (%)
Male	Control	24	91.6±1.0	26	1.3±0.4
		48	87.7±2.2	20	1.0±0.2
	500	24	88.6±1.3	16	0.8±0.2
	1,000	24	89.0±1.5	17	0.8±0.1
	2,000 mg/kg bw	24	90.5±0.5	4	0.7±0.2
	2,000 mg/kg bw	48	88.6±1.2	16	0.8±0.2
	20 mg/kg bw CP	24	87.6±2.5	678	32.9±3.4[#]
Female	Control	24	78.2±1.4	27	1.3±0.3
		48	74.5±4.5	27	1.3±0.2
	500 mg/kg bw	24	72.6±3.8	27	1.3±0.3
	1,000 mg/kg bw	24	77.2±2.6	30	1.4±0.4
	2,000 mg/kg bw	24	77.7±6.0	28	1.0±0.1
	2,000 mg/kg bw	48	76.0±4.0	32	1.6±0.3
	20 mg/kg bw CP	24	60.3±3.0	288	14.1±1.6[#]

CP = cyclophosphamide monohydrate; MNPCE = micronucleated polychromatic erythrocyte; PCE = polychromatic erythrocyte; PQQ = pyrroloquinoline quinone. #ANOVA, P≤0.05

Overall, ZMC's PQQ disodium salt was not mutagenic or genotoxic under the test conditions. Table 16 summarizes these studies.

Table 16. Mutagenicity and Genotoxicity Studies of ZMC's PQQ Disodium Salt

Test system	PQQ concentration	Test	Outcome	Reference
S. typhimurium TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvr*A	0, 100, 250, 500, 1,000, 2,500, or 5,000 μg/plate ± S9	Ames test (mutagenicity), *in vitro*	Not mutagenic	Seol et al., 2022
S. typhimurium TA98 and TA1537	0, 25, 55, 110, 220, 550, 1,650, or 5,000 μg/plate + S9			
SD rats	0, 500, 1,000, or 2,000 mg/kg bw/day	*In vivo* mouse micronucleus assay	Not genotoxic	Seol et al., 2022

6.B.2.2. Other Mutagenicity and Genotoxicity Studies

It is recognized that the information and data in previous GRAS notices 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 notices, and will not discuss previously reviewed references in detail.

GRN 641 (FDA, 2016a, pages 37-38), GRN 709 (FDA, 2018; pages 27-28), and Nakano et al. (2013) reported that PQQ disodium salt preparations obtained from microbial fermentation by *Hyphomicrobium denitrificans* were not mutagenic or genotoxic (mutagenicity study at up to 5,000 ug/plate with and without S9 activation; *in vitro* chromosomal aberration test with human peripheral lymphocytes, 3,750 ug/mL with and without S9 activation; *in vivo* mouse micronucleus test, up to 2,000 mg/kg bw). Additionally, synthetically manufactured PQQ disodium salt also showed no mutagenicity and genotoxicity (Table 21: 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.

6.B.3. Animal Toxicity Studies

6.B.3.1. Studies of ZMC's PQQ Disodium Salt

Acute Oral Toxicity Study of ZMC's PQQ Disodium Salt Produced via *Methylovorus glucosotrophus* in Rats

The potential toxicity and the maximum tolerated dose (MTD) of PQQ disodium salt were evaluated in rats after a single oral dose (Lee et al., 2022). Overnight fasted Sprague Dawley (SD) rats (~7-10 weeks of age; body weight range of 243.7-283.4 g for males and 165.3-203.5 g for females) received a single dose of 0 (vehicle control, purified water), 300, 1,000, or 2,000 mg/kg bw PQQ disodium salt (n=5/sex/group) and observed for 14 days. Viability (assessed by morbidity and mortality), clinical observations, body weight, food consumption, and gross observation at necropsy were examined during the observation period.

A female rat at 2,000 mg/kg bw was found dead with no gross lesion at necropsy and the cause of death was not determined. Test substance-related clinical signs included abnormal stools and soiled coat for male and females at doses ≥1,000 mg/kg bw. Additionally, females at 300 mg/kg bw were observed to have abnormal stool. These clinical signs disappeared at day 4 and, thus, were not deemed as adverse effects. No test substance-related changes in body weight and food consumption were observed for all males and females at doses ≤1,000 mg/kg bw. In the female 2,000 mg/kg bw group, the slight test substance-related decrease in mean body weight gain was associated with decreased food consumption and, thus, not adverse. No test substance-related gross lesion was observed for any dose regardless of sex. The MTD for PQQ disodium salt was

determined to be 1,000 mg/kg bw/day for males and females. The LD_{50} was observed to be greater than 2,000 mg/kg bw for both male and female rats, the highest dose tested.

Subchronic Toxicity Study of ZMC's PQQ Disodium Salt Produced via *Methylovorus glucosotrophus* in Rats

The potential toxicity of PQQ disodium salt was evaluated in SD rats after 91 days of administration. SD rats (~5-6 weeks old; body weight range of 122.3-158.3 g for males and 115.9-157.6 g for females) received 0 (vehicle control, purified water), 50, 100, or 200 mg/kg bw/day PQQ disodium salt (n=20/sex/group) for 91 days (Lee et al., 2022). Viability, clinical observations, body weight, food consumption, ophthalmology, clinical pathology (hematology, coagulation, serum chemistry, and urinalysis), organ weight, gross pathology, and histopathology were examined during the study.

A total of three unscheduled deaths was found in the female groups but none were considered as test substance-related effects. A female each in the low-dose (50 mg/kg bw/day) and high-dose (200 mg/kg bw/day) PQQ disodium salt groups were found dead on day 14, and a female in the low-dose group was euthanized on day 76. The deaths were considered as sampling injury or stress. Another female in the 50 mg/kg bw/day group was euthanized due to poor clinical condition, decreased appetite, and severe body weight loss compared to other animals in the group. All other animals survived to the scheduled necropsy. Macroscopic and microscopic findings of this animal were consistent with multicentric lymphosarcoma.

Test substance-related effects included green or black stool, which may be caused by the color of the residual test substance in the stool. No test substance-related abnormalities were observed in clinical signs, body weight, food consumption, and ophthalmic parameters. Hematology (Table 17), serum chemistry (Table 18), and urinalyses (Table 19), and absolute and relative organ weights (Tables 20-21) revealed no test substance-related abnormalities. For some test parameters, statistically significant differences were observed although differences were small in magnitude. For example, males at 100 mg/kg bw/day had significantly decreased mean corpuscular hemoglobin concentration (MCHC; 34.4 vs. 34.8 g/dL, $P \leq 0.05$) and significantly increased erythrocyte count distribution width (RDW; 13.6 vs. 13.1%, $P \leq 0.05$) compared to the control. For serum chemistry parameters, females at 50 mg/kg bw/day had significantly increased sodium levels compared to the control (143 vs. 141 mmol/L, $P \leq 0.01$). All of these differences were considered incidental and non-adverse because differences were small in magnitude with no dose response and/or were within the historical references ranges for the facility.

Urinalysis results showed no significant differences in appearance and urinary pH and specific gravity. Although potential nephrotoxicity was reported by another research group (Nakano et al., 2014; also, Part 6. B.3.2 of this notice), this study did not find any abnormalities in urinary parameters.

Histopathology of the male 200 mg/kg bw/day group revealed a possible test substance-related effect, increased incidence or severity of pancreatic changes including minimal or mild islet fibrosis, minimal islet hemorrhage, and/or minimal pigmented macrophages, which were considered non-adverse because these changes were commonly observed in aging rats.

Based on the results, the authors determined the NOAEL of PQQ disodium salt as 200 mg/kg bw/day, the highest level tested, for rats.

Table 17. Hematology and Coagulation Parameters of SD Rats Consuming ZMC's PQQ Disodium Salt for 13 Weeks

	Male (mg/kg bw/day)				Female (mg/kg bw/day)			
Parameters	0	50	100	200	0	50	100	200
WBC ($x10^3/\mu L$)	7.11±1.97	7.27±1.87	7.33±1.74	6.93±1.72	4.31±1.13	4.31±1.43	3.90±1.10	4.57±1.66
RBC ($x10^6/\mu L$)	8.43±0.37	8.45±0.33	8.41±0.50	8.47±0.38	7.75±0.28	7.66±0.28	7.80±0.25	7.75±0.31
Hb (g/dL)	14.9±0.4	14.8±0.4	14.5±0.6	14.7±0.6	14.5±0.6	14.6±0.6	14.5±0.4	14.4±0.6
HCT (%)	42.8±1.0	42.7±1.3	42.2±2.0	42.6±1.8	40.3±1.2	40.5±1.3	40.4±1.3	40.5±1.4
MCV (fL)	50.9±1.9	50.6±1.8	50.2±1.3	50.3±1.7	52.1±1.2	53.0±1.8	51.9±1.4	52.3±1.7
MCH (pg)	17.7±0.7	17.5±0.6	17.3±0.5	17.3±0.6	18.8±0.4	19.1±0.7	18.6±0.5	18.6±0.5
MCHC (g/dL)	34.8±0.4	34.5±0.5	34.4±0.4[a]	34.5±0.3	36.1±0.7	36.0±0.5	35.8±0.6	35.6±0.7
RDW (%)	13.1±0.4	13.3±0.6	13.6±0.6[a]	13.4±0.6	12.0±0.3	12.0±0.3	12.1±0.3	12.2±0.5
RET ($x10^9$/L)	201.7±28.8	198.7±30.1	192.6±28.1	189.4±26.1	153.3±27.5	141.7±27.2	139.1±29.7	144.7±25.5
NEUT ($x10^3/\mu L$)	1.09±0.51	0.95±0.43	1.00±0.31	0.98±0.36	0.51±0.17	0.51±0.23	0.52±0.21	0.51±0.17
LYMP ($x10^3/\mu L$)	5.71±1.68	6.03±1.45	6.00±1.60	5.64±1.50	3.60±1.02	3.58±1.20	3.20±0.93	3.83±1.57
MONO ($x10^3/\mu L$)	0.14±0.05	0.12±0.05	0.14±0.06	0.13±0.08	0.08±0.03	0.09±0.04	0.08±0.03	0.09±0.05
EOS ($x10^3/\mu L$)	0.11±0.03	0.10±0.03	0.11±0.02	0.10±0.02	0.07±0.03	0.07±0.02	0.06±0.02	0.08±0.02
BASO ($x10^3/\mu L$)	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01
PLT ($x10^3/\mu L$)	1,026±84	1,023±74	1,019±152	1,024±115	934±116	933±220	970±103	909±135
MPV (fL)	7.8±0.4	7.9±0.6	8.0±0.5	8.1±0.4	7.8±0.7	8.0±0.6	8.1±0.5	8.0±0.3
PT (sec)	15.1±0.8	15.1±0.9	15.0±0.6	15.1±0.8	13.8±0.6	14.1±0.4	13.8±0.9	13.3±0.8
APTT (sec)	18.2±3.7	19.6±3.0	19.1±3.0	18.5±2.7	13.5±1.8	13.4±1.6	13.8±2.5	13.6±1.6
FIB (g/L)	2.34±0.17	2.32±0.14	2.36±0.09	2.27±0.12	1.88±0.13	1.89±0.18	1.93±0.22	1.99±0.19

Values were mean±SD at day 92. [a]P≤0.05.

APTT = activated partial thromboplastin time; BASO = basophil; EOS = eosinophil; FIB = fibrinogen; Hb = hemoglobin; HCT = hematocrit; LYMPH = lymphocyte; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MONO = monocyte; MPV = mean platelet volume; NEUT = neutrophil; PLT = platelet count; PT = prothrombin time; RBC = erythrocyte count; RDW = erythrocyte count distribution width; RET = reticulocyte; WBC = leukocyte count.

Table 18. Serum Chemistry in SD Rats Consuming ZMC's PQQ Disodium Salt for 13 Weeks

	Male (mg/kg bw/day)				Female (mg/kg bw/day)			
Parameters	0	50	100	200	0	50	100	200
ALT (U/L)	28±5	27±4	26±4	27±3	28±8	34±15	27±8	38±45
AST (U/L)	104±19	96±15	94±14	107±24	102±16	117±25	108±25	113±41
TP (g/L)	62.4±2.2	62.4±3.2	62.3±2.3	62.0±3.2	68.2±4.9	65.1±6.2	68.6±6.2	67.6±5.2
ALB (g/L)	31.9±1.0	31.5±1.3	31.9±1.4	31.5±1.4	37.2±2.8	35.4±3.5	37.2±3.5	36.9±3.1
TBIL (μmol/L)	2.32±0.52	2.30±0.49	2.43±0.58	2.35±0.46	2.82±0.41	2.59±0.61	3.00±0.50	2.57±0.52
ALP (U/L)	79±15	78±14	77±14	76±12	43±9	43±12	41±13	43±16
GLU (mmol/L)	10.86±2.20	11.24±2.39	11.24±2.35	10.62±1.84	9.42±0.98	9.93±1.92	9.36±1.50	9.74±2.19
Urea (mmol/L)	4.95±0.79	4.93±0.74	4.67±0.62	5.04±0.86	5.14±0.69	5.42±0.73	4.81±0.78	5.02±0.84
CRE (μmol/L)	22±4	22±2	22±2	22±3	26±3	26±2	25±3	26±4
Ca (mmol/L)	2.40±0.08	2.38±0.07	2.39±0.07	2.41±0.07	2.46±0.07	2.44±0.10	2.47±0.07	2.47±0.08
P (mmol/L)	2.18±0.20	2.06±0.13	2.11±0.17	2.08±0.14	1.87±0.20	1.87±0.24	1.89±0.22	1.86±0.22
TCHO (mmol/L)	1.72±0.36	1.83±0.48	1.81±0.42	1.72±0.34	1.76±0.39	1.95±0.48	1.93±0.49	1.81±0.43
TG (mmol/L)	0.58±0.23	0.60±0.23	0.59±0.23	0.63±0.20	0.41±0.12	0.43±0.09	0.48±0.18	0.53±0.19
K (mmol/L)	5.0±0.3	4.9±0.2	5.0±0.2	4.9±0.2	4.4±0.2	4.3±0.3	4.4±0.2	4.5±0.4
Na (mmol/L)	143±1	143±1	143±1	143±1	141±2	143±1[b]	141±2	142±2
Cl (mmol/L)	103±1	104±1	104±2	104±1	103±2	105±2	104±2	104±2
GLB (g/L)	30.5±1.6	30.8±2.2	30.4±1.5	30.4±2.1	31.0±2.4	29.7±3.0	31.5±3.0	30.7±2.5
A/G	1.05±0.06	1.02±0.06	1.05±0.06	1.04±0.06	1.20±0.06	1.19±0.07	1.18±0.08	1.20±0.07
CK (U/L)	353±138	309±94	299±101	396±227	325±99	338±127	358±234	334±100

Values were mean±SD at day 92. [a]P≤0.05; [b]P≤0.01.

A/G = albumin/globulin ratio; ALB = albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Ca = calcium; CK = creatine kinase; Cl = chloride; CRE = creatinine; GGT = γ-glutamyl transferase; GLB = globulin; K = potassium; Na = sodium; P = inorganic phosphorus; GLU = glucose; TBIL = total bilirubin; TCHOL = total cholesterol; TG = triglyceride; TP = total protein.

Table 19. Urinalysis of SD Rats Consuming ZMC's PQQ Disodium Salt for 13 weeks

	Male (mg/kg bw/day)				Female (mg/kg bw/day)			
Parameters	0	50	100	200	0	50	100	200
pH	6.8±0.3	6.8±0.3	6.9±0.2	6.9±0.2	6.5±0.3	6.5±0.2	6.4±0.3	6.5±0.3
SG	1.03±0.01	1.02±0.01	1.02±0.01	1.02±0.01	1.03±0.01	1.02±0.02	1.02±0.01	1.02±0.01
Volume (mL)	13±5	15±7	15±7	17±10	9±6	12±7	10±7	10±7

Values were mean±SD at day 92. SG = specific gravity.

Table 20. Absolute Organ Weights of SD Rats Consuming ZMC's PQQ Disodium Salt for 13 Weeks

	Group (mg/kg bw/day)			
Weight, g	0	50	100	200
Males				
Terminal bw	554.9±55.3	542.7±52.5	531.7±42.0	549.9±36.2
Adrenals	0.062±0.012	0.061±0.012	0.058±0.010	0.062±0.007
Brain	2.22±0.08	2.22±0.06	2.22±0.08	2.21±0.08
Epididymides	1.51±0.14	1.48±0.16	1.47±0.16	1.48±0.13
Heart	1.66±0.19	1.64±0.18	1.64±0.15	1.68±0.14
Kidneys	3.40±0.30	3.42±0.31	3.41±0.31	3.41±0.31
Liver	13.92±1.99	13.81±1.92	13.56±1.49	13.91±1.38
Lungs	1.78±0.20	1.76±0.13	1.72±0.14	1.73±0.16
Pituitary	0.0130±0.0026	0.0126±0.0021	0.0122±0.0018	0.0132 ±0.0034
Prostate	1.38±0.21	1.29±0.21	1.29±0.20	1.36±0.24
Spleen	0.867±0.141	0.902±0.159	0.901±0.106	0.841±0.135
Testes	3.60±0.25	3.62±0.27	3.61±0.42	3.65±0.33
Thymus	0.378±0.098	0.351±0.095	0.346±0.076	0.353±0.077
Thyroids	0.039 ±0.007	0.037±0.007	0.035±0.006	0.037±0.006
Females				
Terminal bw	269.2±21.1	271.1±28.8	273.2±27.8	278.4±29.6
Adrenals	0.066±0.011	0.062±0.010	0.064±0.008	0.063±0.011
Brain	1.97±0.07	1.99±0.11	1.97±0.08	1.98±0.08
Heart	0.962±0.082	0.987±0.131	0.964±0.090	0.983±0.099
Kidneys	1.80±0.16	1.85±0.20	1.86±0.15	1.87±0.19
Liver	6.92±0.92	6.79±0.82	6.80±0.93	7.08±0.99
Lungs	1.22±0.09	1.25±0.14	1.23±0.12	1.22±0.10
Ovaries	0.093±0.013	0.096±0.018	0.090±0.015	0.098±0.027
Pituitary	0.016±0.003	0.015±0.002	0.015±0.002	0.016±0.004
Spleen	0.494±0.073	0.511±0.098	0.491±0.088	0.477±0.058
Thymus	0.253±0.044	0.279±0.082	0.250±0.055	0.269±0.060
Thyroids	0.022±0.004	0.024±0.006	0.022±0.004	0.022±0.004
Uterus	0.755±0.269	0.720±0.187	0.816±0.400	0.725±0.294

bw = body weight.

Table 21. Relative Organ Weights of SD Rats Consuming ZMC's PQQ Disodium Salt for 13 Weeks

Parameters	Group (mg/kg bw/day)			
	0	50	100	200
Males				
Adrenals (10^{-3})	0.112±0.016	0.113±0.019	0.109±0.015	0.113±0.014
Brain	0.0040±0.0030	0.0041±0.0040	0.0042±0.0003	0.0040±0.0003
Epididymides (10^{-3})	2.73±0.27	2.75±0.32	2.78±0.26	2.71±0.28
Heart (10^{-3})	2.99±0.18	3.03±0.23	3.08±0.16	3.06±0.20
Kidneys	0.0061±0.0004	0.0063±0.0004	0.0064±0.0005	0.0062±0.0006
Liver	0.0250±0.0018	0.0254±0.0019	0.0255±0.0015	0.0253±0.0018
Lungs (10^{-3})	3.20±0.30	3.26±0.27	3.25±0.20	3.15±0.27
Ovaries (10^{-3})	-	-	-	-
Pituitary (10^{-3})	0.0234±0.0033	0.0233±0.0036	0.0229±0.0028	0.0241±0.0064
Prostate (10^{-3})	2.50±0.39	2.38±0.39	2.45±0.39	2.47±0.41
Spleen (10^{-3})	1.56±0.19	1.67±0.28	1.70±0.21	1.53±0.26
Testes (10^{-3})	6.53±0.67	6.72±0.73	6.82±0.81	6.67±0.69
Thymus (10^{-3})	0.681±0.153	0.644±0.139	0.651±0.128	0.641±0.126
Thyroids (10^{-3})	0.070±0.013	0.068±0.013	0.065±0.011	0.067±0.013
Females				
Adrenals (10^{-3})	0.245±0.036	0.230±0.037	0.235±0.030	0.227±0.035
Brain	0.0074±0.0006	0.0074±0.0006	0.0073±0.0008	0.0072±0.0007
Heart (10^{-3})	3.58±0.25	3.64±0.27	3.54±0.28	3.53±0.21
Kidneys	0.0067±0.0005	0.0068±0.0005	0.0068±0.0005	0.0068±0.0006
Liver	0.026±0.003	0.025±0.001	0.025±0.003	0.025±0.002
Lungs (10^{-3})	4.53±0.32	4.61±0.24	4.53±0.42	4.39±0.39
Ovaries (10^{-3})	0.344±0.039	0.355±0.056	0.330±0.052	0.352±0.073
Pituitary (10^{-3})	0.059±0.011	0.056±0.010	0.057±0.009	0.057±0.014
Spleen (10^{-3})	1.84±0.26	1.88±0.26	1.80±0.26	1.71±0.12
Thymus (10^{-3})	0.942±0.161	1.024±0.252	0.918±0.180	0.963±0.174
Thyroids (10^{-3})	0.084±0.016	0.090±0.021	0.080±0.014	0.078±0.012
Uterus (10^{-3})	2.82±0.99	2.66±0.66	3.03±1.52	2.64±1.18

bw = body weight.

Teratogenicity of ZMC's PQQ Disodium Salt

The purpose of this study was to investigate the effects of PQQ disodium salt on embryo-fetal development in rats when administered orally from Gestation Day 6 (GD 6) to GD 20 and to characterize the dose response relationship to the toxicity (Lee et al., 2022). Mated female SD rats were orally administered 0 (vehicle control), 50, 100, or 200 mg/kg bw/day PQQ disodium salt by gavage from gestation day (GD) 6 to 20. On GD 21, all study animals were necropsied and examined macroscopically. The ovaries and uteri were examined for determination of litter data. The uteri without visible implantation were placed in an ammonium sulfide solution for detection

of early resorptions. All the live fetuses were weighed and examined for external abnormalities; their crown-lump length was measured and their sex was determined. Approximately 1/2 of the live fetuses in each litter were fixed with modified Davidson's fixative for soft tissue examination and the remaining fetuses were stained with Alizarin red solution for subsequent skeletal examination. No mortality was observed in the study. There were no treatment related changes on clinical observations, body weights, and macroscopical observations.

There were no test substance-related changes on the litter data and sex ratio (Table 22) and litter-implantations loss (Table 23). The mean fetal weights were significantly increased in the female low- and high-dose groups but were not considered as test substance-related due to no dose response and no correlation with significantly increased crown-rump length.

There were no test substance-related visceral abnormalities (Table 24) and skeletal abnormalities and skeletal malformations found in the fetuses (Table 25). Based on the results, the NOAEL of PQQ disodium salt was 200 mg/kg bw/day, the highest dose tested, for maternal toxicity and embryo-fetal developmental toxicity.

Table 22. Summary of Litter Data and Sex Ratio of Offspring of Female Rats Consuming ZMC's PQQ Disodium Salt

Parameters	Group (mg/kg bw/day)			
	0	50	100	200
Viable fetuses	15.8 (2.7)	14.2 (3.2)	14.2 (3.2)	14.6 (2.9)
% Male	52.3 (14.9)	51.3 (14.2)	51.3 (14.2)	50.5 (14.2)
Dead fetuses	0.04 (0.20)	0 (0)	0 (0)	0.04 (0.20)
Embryonic resorptions	0.08 (0.28)	0.18 (0.50)	0.21 (0.66)	0.25 (0.44)
Total non-viable	0.13 (0.34)	0.18 (0.50)	0.21 (0.66)	0.29 (0.46)
Fetal weight, male (g)	5.42 (0.27)	5.72 (0.52)	5.68 (0.40)	5.68 (0.29)
Fetal weight, female (g)	5.11 (0.22)	5.43 (0.48) *	5.37 (0.30)	5.40 (0.28) *
Fetal crown-rump length, male (mm)	43.23 (1.34)	43.99 (1.75)	43.85 (1.23)	44.04 (0.91)
Fetal crown-rump length (female) (mm)	42.05 (1.18)	42.82 (1.82)	42.88 (1.35)	43.04 (0.92)

Data presented as mean (SD); *p<0.05 compared to control.

Table 23. Summary of Litter Implantation Loss

Parameters	Control	Low-dose	Mid-dose	High-dose
Corpora Lutea	16.1 (2.8)	14.2 (3.6)	14.6 (3.0)	15.4 (3.3)
Implantations	15.9 (2.8)	14.0 (3.5)	14.4 (3.0)	14.9 (3.0)
Implantation Loss %, pre-	0.9 (2.6)	1.4 (2.6)	1.3 (4.1)	2.7 (5.9)
Implantation Loss %, post-	2.6 (1.9)	1.7 (4.8)	1.7 (4.8)	1.8 (2.9)

Data presented as mean (SD); The numbers in parentheses represent percentage of implantation loss which is calculated as (No. of Corpora Lutea – No. of Implantations) / No. of Corpora Lutea × 100; Post-implantation Loss (%) = (No. of implantations – total no. of live fetuses) / No. of implantations × 100.

Table 24. Summary of Fetal Visceral Examinations

Parameter	Control	Low-dose	Mid-dose	High-dose
Fetal Visceral Examinations				
No. fetuses (litters) examined	184 (24)	147 (22)	162 (24)	170 (24)
No. fetuses (litters) with malformations	1 (1)	2 (2)	0 (0)	0 (0)
% Fetuses (litters) with malformations	0.54 (4.17)	1.36 (9.09)	0 (0)	0 (0)
No. fetuses (litters) with variations	15 (10)	8 (7)	14 (8)	13 (11)
% Fetuses (litters) with variations	8.15 (41.67)	5.44 (31.82)	8.64 (33.33)	7.65 (45.83)
Fetal Visceral Abnormalities				
Heart, malpositioned	1 (1)	0 (0)	0 (0)	0 (0)
Heart, misshapen	1 (1)	0 (0)	0 (0)	0 (0)
Heart, 3-chambered	1 (1)	0 (0)	0 (0)	0 (0)
General, situs inversus	0 (0)	1 (1)	0 (0)	0 (0)
Kidney (small)	0 (0)	1 (1)	0 (0)	0 (0)
Lung (fused)	1 (1)	0 (0)	0 (0)	0 (0)
Renal pelvis (dilated)	1 (1)	3 (2)	2 (1)	2 (2)
Ureter (convoluted)	14 (10)	8 (7)	14 (8)	12 (10)
Ureter (dilated)	0 (0)	1 (1)	2 (1)	0 (0)

Table 25. Summary of Fetal Skeletal Examinations

Parameter	Control	Low-dose	Mid-dose	High dose
Fetal Skeletal Examinations				
No. fetuses (litters) examined	195 (24)	156 (22)	178 (24)	181 (24)
No. fetuses (litters) with malformations	0 (0)	0 (0)	0 (0)	0 (0)
% fetuses (litters) with malformations	0 (0)	0 (0)	0 (0)	0 (0)
No. fetuses (litters) with variations	26 (16)	19 (11)	13 (7)	13 (9)
% fetuses (litters) with variations	13.33% (66.67%)	12.18% (50.00%)	7.30% (29.17%)	7.18% (37.50%)
Fetal Skeletal Abnormalities				
Hyoid, incomplete ossification	11 (9)	2 (2*)	4 (4)	0 (0**)
Lumbar centrum, bipartite ossification	1 (1)	0 (0)	0 (0)	0 (0)
Rib, nodulated	1 (1)	0 (0)	1 (1)	0 (0)
Rib, short	0 (0)	0 (0)	1 (1)	1 (1)
Rib, supernumerary, short	11 (8)	16 (9)	6 (3)	9 (6)
Sternebra, incomplete ossification	1 (1)	0 (0)	0 (0)	1 (1)

Sernebra, unossified	0 (0)	0 (0)	1 (1)	0 (0)
Thoracic centrum, bipartite ossification	0 (0)	0 (0)	0 (0)	2 (2)
Thoracic centrum, dumbbell-shaped	1 (1)	1 (1)	0 (0)	0 (0)

*p<0.05 vs. control, Fisher's Exact Test; **p<0.01 vs. control, Fisher's Exact Test.

Table 26 summarizes the oral toxicity studies of ZMC's PQQ disodium salt in rats. Taken together, it is reasonable to conclude that the NOAEL of ZMC's PQQ disodium salt is 200 mg/kg bw/day.

Table 26. Summary of Oral Toxicity Studies of ZMC's PQQ Disodium Salt in Rats

Animal	Dose, PQQ disodium salt	Duration	Measurements	NOAEL or LD_{50}	Reference
Acute toxicity study					
SD rats	0, 300, 1,000, or 2,000 mg/kg bw/day	Single dose	Mortality, clinical signs, bw, gross lesions	MTD, 1,000 mg/kg bw/day; LD_{50} ≥ 2,000 mg/kg bw/day	Seol et al., 2022
Subchronic toxicity study					
SD rats	0, 50, 100, or 200 mg/kg bw/day	13 weeks	Mortality, bw, food consumption, ophthalmic examination, hematology, clinical chemistry, coagulation, urinalysis, clinical signs, organ weights and pathology	NOAEL, 200 mg/kg bw/day;	Seol et al., 2022
Teratogenicity study					
Crl:CD®[SD] SPF rats	0, 50, 100, or 200 mg/kg bw/day	Gestation days 6-20 (terato-genicity)	Mortality, clinical signs, bw, food consumption, litter data, external abnormalities, skeletal examination	Both maternal and embryo-fetal toxicity NOAEL, 200 mg/kg bw/day	Lee et al., 2022

MTD= maximum tolerated dose; NOAEL=no-observed-adverse-effect level.

6.B.3.2. Studies on Other Sources of PQQ Disodium Salt

Because the specifications of the proposed GRAS substance, ZMC's PQQ disodium salt, are similar to those that have received FDA no question letters, it is recognized that the information and data described in previous GRAS notices 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 notices, and will not discuss previously reviewed references in detail.

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. However, Liang et al. (2015) did not evaluate urinary parameters. The findings of the animal toxicity studies collectively support the safety of PQQ disodium salt supplementation at daily doses of 100 mg/kg bw/day or higher (Lianget al., 2015; Nakano et al., 2014; Table 27).

A reproductive toxicity study (sperm shape abnormality assay) in mice indicated that there were 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 (or ~0.3 mg/kg bw/day) did not impact reproductive performance.

Nephrotoxicity Associated with High Levels of PQQ

High doses of PQQ disodium salt produced via *Hyphomicrobium denitrificans*may induce nephrotoxicity in rats (Nakano et al., 2014). In a dose-range finding study, Crl:CD(SD) rats received 0, 3, 12, 48, 192, or 768 mg/kg bw/day PQQ (>99.1% pure) by gavage for 14 days. No mortality was observed in any groups. There was no statistically significant difference in body weights among the groups. During the 14-day oral administration of PQQ to rats, nephrotoxicity was seen in the high dose (768 mg/kg bw/day) group (Nakano et al., 2014; source, Mitsubishi Gas Chemical Co). Crystals in urine were found in some females at 3, 12, 192, and 768 mg/kg bw/day and a male at 192 mg/kg bw/day. Urinary protein levels were increased in a female at 12 mg/kg bw/day, a female at 192 mg/kg bw/day, and 2 females and 2 males at 768 mg/kg bw/day. The high dose group also had significant increased urinary sodium levels in both males and females. The female high dose group, but not male high dose group, also had increased relative kidney weight (approximately 14%, $p<0.05$), along with histopathological changes (focal basophilic changes and atrophy of the renal tubules) of minimal to moderate severity as well as green-colored cecal contents. No other toxicologically relevant histopathological changes were reported. The data suggest nephrotoxicity of PQQ disodium salt at 768 mg/kg bw/day.

In the follow up 28-day study by Nakano et al. (2014), female SD rats received 0, 200, or 700 mg/kg bw/day PQQ by gavage for 4-weeks followed by a 4-week recovery period. No mortality and no significant differences in body weight, water intake, and clinical biochemistry parameters were observed between the control and the treatment groups (data not shown). The high-dose group had blackish stools due to the dark color of the test substance in the dosing formulation. Urinalysis revealed an increased incidence of crystals in urinary sediment at daily doses of 200 and 700 mg/kg bw during the administration period. Urinary crystal was observed only in one animal in the low-dose group at the end of the recovery period. Moreover, the animals had protein present in their urine with increased incidence with increasing dose, but was alleviated

in the recovery period. Urinary protein was detected in 1/12 control, 5/12 low-dose, and 6/12 high-dose rats at week 4 with a low or 'slight' grade. It was still observed in 1/6 low-dose and 2/6 high-dose rats at the end of four-week recovery period. However, these effects were not accompanied by any other significant changes in clinical chemistry parameters related to kidney function, or in the results of the gross and histopathological examinations, and were resolved during the 4-week recovery period.

In the 90-day oral toxicity study involving lower doses of PQQ (>99.1% pure) (0, 3, 20, or 100 mg/kg bw/day by gavage; Nakano et al., 2014), no treatment-related abnormalities were reported in hematology, clinical biochemistry, urinalysis, or gross necropsy and histopathology in Crl:CD(SD) rats (10/sex/group), with the exception of green-colored feces, which were observed in male and female rats in the highest dose group beginning on days 7-11 and thereafter. This was due to the excretion of unabsorbed test substance. No test substance-related adverse effects were observed for body weight, food consumption, ophthalmology, organ weights, and hematology. Increased protein levels > 100 mg/dL were found in 1 high-dose male and increased protein levels between 30-100 mg/dL in 1 high-dose male, 2 mid-dose males, 3 low-dose males, 2 control males, and 2 high-dose females. Crystals in urinary sediment were observed in 1 control male, 1 low-dose male, 3 mid-dose males, 2 high-dose males, and 2 high-dose females. The urinary findings were not accompanied by significant changes in other parameters including blood chemistry, gross findings, or histopathological examination. Thus, these urinary findings were not considered of toxicological concern. The authors stated that 'While protein and crystals were observed in the urine of the female animals in the 28-day study, these effects were resolved during the recovery period and did not occur in the 90-day subchronic study at doses up to 100 mg/kg bw/d' (page 121). The authors concluded that the NOAEL for PQQ disodium salt was determined to be 100 mg/kg bw/day.

In another 90-day study conducted in Sprague-Dawley rats (10/sex/group), synthetic PQQ disodium salt was administered by gavage at doses of 0 (control), 100, 200, or 400 mg/kg bw/day (Liang et al., 2015). PQQ disodium salt was well-tolerated and no treatment-related abnormalities were observed in body weight, food consumption, clinical chemistry and hematology, absolute and relative organ weights, and histopathological changes, although one rat in the high-dose group was reported to have deposition of calcium salts in the renal tubule (additional details not specified). The authors concluded that a NOAEL of PQQ disodium salt was 400 mg/kg bw/day, the highest dose tested. However, urinary parameters were not reported by the authors.

Effects of PQQ Administered by Intraperitoneal Administration

In addition, GRNs 625 (page 43) and 641 (expert panel report pages 5-6) discussed a study by Watanabe et al., (1989) that was published in Japanese. The intraperitoneal administration of PQQ to rats for 4 days at a dose of 11.5 mg/kg bw/day also produced clear functional and morphologic changes of the kidneys (*i.e.*, vacuolar degeneration, atrophy, and necrosis of the proximal tubular epithelium in the renal cortex, dilation and regeneration of the tubules). The most

prominent finding was necrotic and degenerative changes of the proximal tubular epithelium as well as hematuria and an elevation of serum creatinine concentration. Urinalysis revealed increased excretion of protein, glucose, ketone body, and occult blood, although the statistical significance of these changes was not addressed. The PQQ group had significantly higher blood urea nitrogen and serum concentrations of creatinine and glutamate pyruvate transaminase and glutamate oxaloacetate transaminase activities levels, while having significantly lower serum triglycerides concentrations. Gross examination revealed swelling of the kidneys, which was accompanied by increased absolute and relative kidney weights, the latter of which was significant.

This nephrotoxicity was confirmed in a study by Zhu et al. (2006) who compared the cardioprotective effects of PQQ with metoprolol. The authors noted that high-dose PQQ (20 mg/kg, i.v.) produced renal and hepatic toxicity and that PQQ (3 mg/kg) given at the onset of reperfusion had no evident renal or hepatic toxicity. However, details were not provided.

Route differences may have significant impact on the safety profile. In addition, these studies tested effects of bolus doses (after i.v. administration of 20 mg/kg or i.p. administration of 11.5 mg/kg), which would not occur following oral ingestion. Thus, the data presented in these papers may not be relevant when evaluating the safety of orally consumed PQQ disodium salt, especially because the substance is incorporated in foods.

Conclusions from Animal Toxicity Studies

Overall, it is reasonable to conclude that the NOAEL of ZMC's PQQ disodium salt is 200 mg/kg bw/day.

After applying a safety margin of 100, it can be concluded that doses of up to 2 mg/kg bw/day or 140 mg/person/day would be safe in adults weighing 70 kg (please note that an average American adult weighs approximately 73 kg).

Table 27. Oral Toxicity Studies of Other Sources of PQQ Disodium Salt in Animals

Animal	Dose, PQQ disodium salt	Duration	Measured Outcome	NOAEL or LD_{50}	Reference
Subacute toxicity studies					
72 male and female Sprague-Dawley rats	0, 3, 12, 48, 192, or 768 mg/kg bw/d	14 days	Body weight, food consumption, urinalysis, hematology, mortality, serum clinical biochemistry, organ wt, and histopathology	NOAEL: 192 mg/kg bw/d; nephrotoxicity at 700 mg/kg bw/d	Nakano et al., 2014; RPT 417(FDA, 2007) bacterial fermentation with *Hypho-microbiumde nitrificans*)
36 female Sprague-Dawley rats	0, 200, or 700 mg/kg bw/d	28 days		NOAEL: <200 mg/kg bw/d; nephrotoxicity at 700 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 set by the authors, 400 mg/kg bw/d, the highest level tested; no urinary parameters were tested	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 days (13 weeks)		NOAEL, 100 mg/kg bw/d, the highest level tested	Nakano et al., 2014

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

The literature search was conducted for animal studies that examined the effects of orally administered PQQ disodium salt on various outcomes. Since the FDA review in 2018, a few animal efficacy studies of PQQ disodium salt were published (Table 34: Liu et al., 2020; Long et al., 2022; Ming et al., 2021; Qiu et al., 2021; Wang et al., 2020; Yin et al., 2019; Zhang et al., 2019) to confirm the safety of PQQ disodium salt. 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 28).

Measured outcomes reported in these efficacy studies include the following parameters:

- semen quality; antioxidant indices of seminal plasma in aging layer breeder roosters (Long et al., 2022),
- metabolic dysfunction-associated fatty liver disease in laying hens (Qiu et al., 2021),
- growth performance, diarrhea incidence, hematology, serum biochemistry and antioxidant enzymes, and histopathological assessment of some tissues (Ming et al., 2021),
- biochemical, and inflammatory parameters; indicators of tissue damage in Kunming mice (Liu et al., 2020),
- performance and intestinal development in pigs (Yin et al., 2019),
- intestinal health in offspring in cross-bred multiparity gestation sows (Wang et al., 2020), and
- reproductive performance and intestinal barrier functions of gestating and lactating female SD rats and their offspring (Zhang et al., 2019).

Administration of 75.0 mg PQQ/kg diet for 4 weeks in pigs and 20 mg/kg feed during gestation and lactation in sows did not result in any adverse effects on measured outcomes. In addition, no adverse effects of PQQ disodium salt were reported on measured outcomes in these studies.

A few animal efficacy studies were reported in previous GRAS notices (GRN 709, pages 31-35). These animal efficacy studies showed that PQQ disodium salt at the level of up to 2 mg/kg diet/day for 8 weeks in mice (Stites et al., 2006) did not cause any adverse effects on measured outcomes and was well tolerated. No studies showed adverse effects of PQQ or PQQ disodium salt on measured outcomes.

Table 28. Summary of Animal Efficacy Studies of PQQ Disodium Salt Published Since 2017

Animal (n)	Dose	Duration	Measured Outcome	Safety-Related Results	Author
63-wk-old breeder roosters (96)	0, 0.5, 1, 2 mg/kg diet	6 wk	Semen quality; antioxidant indices of seminal plasma (total superoxide dismutase, glutathione peroxidase, malondialdehyde; hydroxyl radical scavenging ability, and/or superoxide scavenging capacity)	No treatment-related adverse effects on measured outcomes.	Long et al., 2022
288 29-week-old laying hens with metabolic dysfunction-associated fatty liver disease	Normal diet; high-energy low-protein diet; high-energy low-protein diet with 0.08 and 0.16 mg/kg	4 wk	Performance; egg quality; serum indices of lipid metabolism and anti-oxidative capacity;histological analysis of the liver	No treatment-related adverse effects on measured outcomes.	Qiu et al., 2021
108 white weaned pigs (~28 day old)	0, 7.5, or 75.0 mg/kg diet	4 wk	Growth performance, diarrhea incidence, hematology, serum biochemistry and antioxidant enzymes; histopathological assessment of heart, liver, spleen, lung and kidney; general health	No treatment-related adverse effects on measured outcomes.	Ming et al., 2021
40 Kunming mice (8 weeks old)	0, 5, 10, or 20 mg/kg/day	2 wk, 6 d/wk	Biochemical, and inflammatory parameters; indicators of tissue damage (serum creatine kinase and lactate dehydrogenase); mitochondrial morphology and membrane potential oxygen consumption rate	No treatment-related adverse effects on measured outcomes.	Liu et al., 2021
216 pigs (Duroc x Landrace x Yorkshire) weaned at 28 d	0, 1.5, 3.0, 4.5, 6.0, or 7.5 mg/kg feed	4 wk	Growth performance, diarrhea incidence, intestinal morphology, intestinal mucosal redox status and cytokines (antioxidant enzymes; IL-1β, IL-2, TNF-α, and INF-γ) in the small intestinal mucosa; the expression of jejunal tight junction proteins (ZO-1 and occluding)	No treatment-related adverse effects on measured outcomes.	Yin et al., 2019

40 cross-bred multiparity gestation sows with an average parity of 4.3	0 or 20 mg/kg feed	During gestation and lactation	Intestinal health in offspring (gene expression of antioxidant status and inflammatory cytokines in the jejunum; gene expression of tight junction proteins and glucose transporter; Intestinal morphology and brush border enzymes activities)	No treatment-related adverse effects on measured outcomes.	Wang et al., 2020
120 pregnant female rats	5 groups: 0, 0.2, 0.4, 0.8, or 1.6 mg/kg feed(n=24)	From gestation day (GD) 0 to postnatal day (PD) 21 (until newborn rats were weaned); 8/group were sacrificed on GD 20.	Reproductive performance (the number of implanted embryos per litter during gestation and lactation at GD 20; the number of viable fetuses per litter, the weight of uterine horns with fetuses at day 1, etc.); intestinal barrier functions (placental antioxidant and glucose transporter gene expression); plasma concentrations of hormones (follicle-stimulating hormone, luteinizing hormone, prolactin, progesterone and estradiol at GD 20); antioxidant enzyme (CAT, SOD and GPx) status in small intestine at GD20;intestinal morphology and microflora of weaned rats; gene expression of tight junction proteins and concentrations of cytokines in jejunal of weaned rats	No treatment-related adverse effects on measured outcomes.	Zhang et al., 2019

CAT=catalase; GPx2=glutathione peroxidase; IL= interleukin; INF-γinterferon; SOD=superoxide dismutase; Slc2a1= solute carrier family 2 member 1; Slc2a3= solute carrier family 2 member 3; TNFα=tumor necrosis factor WD=Western diet; ZO-1= zonula occludens-1.

6.B.5. Human Clinical Studies

Since the FDA's last review of GRN 625 (pages 43-44), GRN 641 (pages 39-40), GRN 701 (pages 30-32), and GRN 709 (pages 36-37), two human clinical studies have been published (Hwang et al., 2020; Shiojima et al., 2021). These studies did not find any adverse effects of PQQ disodium salt when 23 male subjects consumed 20 mg per day for 6 weeks (Hwang et al., 2020) or when 64 healthy subjects ingested a daily dose of 21.5 mg for 12 weeks (Shiojima et al., 2021). In the study by Shiojima et al., 2021, 7 mild adverse events (fatigue: 1 case, dizziness: 1 case, urticaria: 1 case, headache: 1 case, loose stool: 1 case, diarrhea: 2 cases) were reported in 3 subjects in the placebo group and nine mild adverse events (stomach discomfort: 3 cases, palpitation: 1 case, urticaria: 1 case, headache: 1 case, lower back pain: 1 case, knee pain: 1 case, leg cramp: 1 case) were reported in 6 subjects in the test group. However, no serious adverse events were reported. The authors concluded that PQQ disodium salt was safe.

GRNs 625, 641, 694, 701, and 709 also reported that no human clinical studies reported adverse effects of PQQ disodium salt (Itoh et al., 2016; Nakano et al., 2012, 2015a, 2015b, 2016; Rucker et al., 2009). Daily doses up to 60 mg per person were well tolerated and did not result 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 29. Studies employing less than 3 weeks of intervention are not included in this review.

Table 29. Human Clinical Studies of PQQ Disodium Salt

Subjects	Dose	Duration	Measured Outcome	Safety-Related Results	Reference
Studies published since FDA's 2018 review					
64 healthy elderly (mean age 71-72 y)	21.5 mg PQQ disodium salt	12 wk	Cognitive performance (memory, attention, judgment, and cognitive flexibility); clinical biochemistry, hematology; urinalysis; adverse events; compliance	7 mild AEs in 3 control subjects and 9 mild AEs in 6 subjects in the test group; the authors concluded that PQQ was safe.	Shiojima et al., 2021
23 males (18-30 y)	20 mg	6 wk	Aerobic exercise performance; PGC-1α; a biochemical marker for mitochondrial biogenesis; body composition	No treatment-related adverse effects on measured outcomes.	Hwang et al., 2020
Studies reviewed in the GRNs 625 (pages 43-44), 641 (pages 39-40), 701 (pages 30-32), and 709 (pages 36-37)					

20 healthy adults	0, 20, or 60 mg/d	4 wk	Liver toxicity, renal function indicator (urinary N-acetyl-β-(D)-glucosaminidase activity), serum biochemistry (glucose, TG, lipoprotein fractions), liver function indicator enzyme (AST); adverse effects	No adverse effects of PQQ were noted.	Rucker et al., 2009 (Cited in Tsuji et al., 1998)
20 elderly healthy subjects (50-70 y)	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; adverse events	No treatment-related adverse effects on measured outcomes.	Nakano et al., 2016
41 elderly healthy subjects (mean, 58.4 - 58.6 y)	20 mg	12 wk	Cognitive functions (selective attention by the Stroop and reverse Stroop test, and visual spatial cognitive function by the Touch M test); cerebral blood flow	No treatment-related adverse effects on measured outcomes.	Itoh et al., 2016
29 healthy adults (40-57 y)	20 mg	6 and 12 wk	Serum lipid profile; % body fat; body mass index	No treatment-related adverse effects on measured outcomes.	Nakano et al., 2015a
22 healthy women with mildly dry skin (20-49 y)	20 mg	8 wk	Skin health (skin moisture, viscoelasticity, and trans epidermal water loss)	No treatment-related adverse effects on measured outcomes.	Nakano et al., 2015b
17 healthy workers with a diagnosed sleep disorder or complaint and fatigue (20-60 y)	20 mg	8 wk	Changes in stress, fatigue, quality of life measures; sleep-related parameters; the increase of salivary cortisol level from soon after awakening to 30 min after awakening; adverse events	No treatment-related adverse effects on measured outcomes.	Nakano et al., 2012

AEs=adverse events; AST=aspartate transaminase; Hb= hemoglobin; PGC-1α=peroxisome proliferator-activated receptor γ coactivator-1α; TG=triglyceride.

6.B.6. Safety of Production Microorganism

The strain producing PQQ disodium was identified as *Methylovorus glucosotrophus*. Molecular identification *via* Average Nucleus Identity (ANI) genomic sequence analysis demonstrates that ZMC's production microorganism, *Methylovorus glucosotrophus*, has a 97.02% sequence similarity with the type strain, *Methylovorus glucosotrophus* DSM6874.

*Methylovorus glucosotrophus*is a facultatively methylotrophic, non-spore forming, gram-negative, straight or slightly curved rod-shaped bacteria (Govorukhina and Trotsenko 1991). Further morphological and biochemical analyses demonstrate that *Methylovorus glucosotrophus*is a gram-negative bacterium that forms pink, creamy or milky colonies. *Methylovorus glucosotrophus* is not a genetically modified organism. *Methylovorus glucosotrophus* is used for the production of PQQ disodium salt and is maintained in-house by ZMC and is subject to strict quality control for compliance with established internal specifications. Table 30 presents the taxonomic classification of *Methylovorus glucosotrophus*.

Table 30. Taxonomic Classification of *Methylovorus glucosotrophus*

Class	Scientific Classification
Kingdom	Prokaryota
Division	Bacteria
Subdivision	Proteobacteria
Class	Betaproteobacteria
Order	Nitrosomonadales
Family	Methalophilaceae
Genus	Methylovorus
Species	*Methylovorus glucosotrophus*

The strain (lot number, MP15-07-10) was subjected to whole genome sequencing and bioinformatics analysis to determine the safety of the ZMC's *Methylovorus glucosotrophus* strain. The whole genomic DNA of the samples was extracted and tested, and then gene assembly was performed to obtain the genomic sequence. Based on whole genomic sequence and bioinformatics analysis of the production microorganism, antibiotic resistance genes and virulence genes were screened using the Comprehensive Antibiotic Resistance Database (CARD), ResFinder database, Virulence Factors of Pathogenic Bacteria (VFDB), VirulenceFinder database, and PathogenFinder database. No genes related to antibiotic resistance, pathogenicity, or virulence were detected. The WGS analysis report is presented in Appendix A.

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. ZMC's PQQ disodium salt (powder form) is manufactured under the principles of cGMP using common food industry materials and processes. ZMC 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 of PQQ disodium salt (GRN 625, 641, 694, 701, and 709), the FDA did not question the safety of PQQ disodium salt for the specified food uses.

4. ZMC's PQQ disodium salt will be used as a food ingredient in selected beverages (energy, sports, and electrolyte drinks; bottled, enhanced and fortified water beverages; and non-milk-based meal replacement beverages) at maximum use levels of 8 to 20 mg/serving (reference amounts customarily consumed, 21CFR 101.12). Among consumers in the total population, the mean and 90^{th} percentile all-user intakes of PQQ were determined to be 21.3 and 45.8 mg/person/day, respectively. It is assumed that ZMC's PQQ disodium salt will replace currently marketed PQQ disodium salt or other PQQ sources. Thus, cumulative exposure is not expected to change.

5. 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 of ZMC's PQQ disodium salt was determined to be 200 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 2 mg/kg bw/day or 140mg/person/day would be safe in adults weighing 70 kg. The EDIs under the intended use are within the safe intake levels in humans.

6. 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

6.D.1. Common Knowledge Element of the GRAS Determination

Several sources of PQQ disodium salt have been evaluated by the FDA and other global regulatory agencies over the past few years for proposed incorporation of PQQ disodium salt in foods for human consumption. Relevant US GRAS notifications include GRNs 625, 641, 694, 701, and 709 (FDA, 2016a, 2016b, 2017a, 2017b, 2018). All of the GRAS 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.

6.D.2. Technical Element of the GRAS Determination (Safety Determination)

Numerous animal studies have reported the 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. The intended uses of PQQ disodium salt have been determined to be safe though scientific procedures as set forth in 21 Code of Federal Regulations 170.3(b), thus, satisfying the "technical" element of the GRAS determination. Thus, it was concluded that these uses of PQQ disodium salt is GRAS based on scientific procedures and that other experts qualified to assess the safety of foods and dietary ingredients would concur with these conclusions. Therefore, not only is the proposed use of PQQ disodium salt safe within the terms of the Food, Drug, and Cosmetic (FD&C) Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also GRAS according to Title 21 Code of Federal Regulations.

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, ZMC's PQQ disodium salt, is very similar 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-pathogenic, non-toxigenic bacteria, *Methylovorus glucosotrophus*, and its purity is over 98% on a dry weight basis. 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. The specifications and the purity of PQQ disodium salt produced by

Methylovorus glucosotrophus and other bacteria such as *Hyphomicrobium denitrificans* are comparable.

Literature search did not identify safety/toxicity concerns related to PQQ disodium salt *Hyphomicrobium denitrificans* at doses up to 100 mg/kg bw/day. 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 100 mg/kg bw/day, although PQQ may be nephrotoxic at higher levels. However, recently published papers reported the NOAEL of 200 mg/kg bw/day, the highest level tested, as ZMC's PQQ disodium salt produced by *Methylovorus glucosotrophus* did not show any treatment-related abnormalities in any measured outcomes including urinalysis and histopathological examination outcomes of kidneys. Thus, it is reasonable to conclude that the NOAEL of ZMC's PQQ disodium salt is 200 mg/kg bw/day.

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.

ZMC 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.

ZMC 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.

ZMC 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

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

No applicable.

Appendix A: Whole Genome Sequencing Analysis of *Methylovorus glucosotrophus*

Summary

The ZMC strain (*Methylovorus glucosotrophus*, batch number: MP15-07-10) was subjected to whole genome sequencing and bioinformatics analysis to determine the safety of the strain. The genomic DNA of the strain was extracted and sequenced, and then the genome assembly was performed to obtain a whole genomic sequence. Queries of the genome sequences in the Comprehensive Antibiotic Resistance Database (CARD), ResFinder, Virulence Factors of Pathogenic Bacteria (VFDB), VirulenceFinder, and PathogenFinder databases resulted in detecting no potential drug resistance or virulence related genes in the genome of the strain, suggesting that the strain is non-toxigenic and non-pathogenic.

1. Purpose

1. Whole genome sequencing and bioinformatics analysis of the *Methylovorus glucosotrophus* strain (Lot number MP15-07-10) from Zhejiang Medicine Co., Ltd. (ZMC) was conducted to identify any drug resistance genes, virulence factors, and pathogenic factors for safety evaluation of ZMC's *Methylovorus glucosotrophus*.

2. Procedure

2.1 Genome sequencing analysis procedure

The genomic DNA was extracted from the *Methylovorus glucosotrophus* strain (Lot number MP15-07-10). A sequencing library was constructed by 1) first breaking the isolated genomic DNA into approximately 500 bp in size, 2) repairing sticky ends to blunt ends, then adding a base "A" at the 3' end so that the DNA fragments could be ligated to a joint with a "T" base at the 3' end, 3) recovering the ligated product by electrophoresis, 4) amplifying the DNA fragment with the joint at both ends by PCR, and 5) finally using the sequencing library for cluster next generation sequencing.

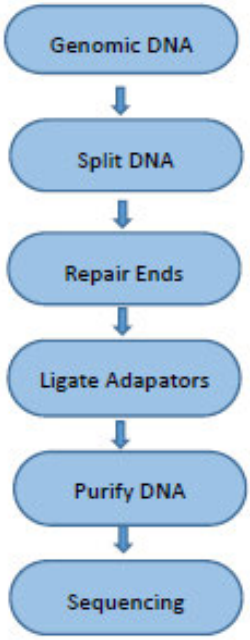


Figure 1: Library construction and sequencing process of the small genomic fragment

2.2 Bioinformatics analysis

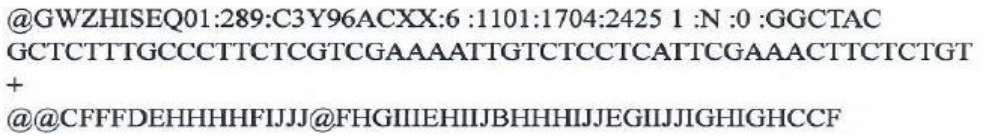
The obtained sequence reads were assembled to generate a whole genome sequence. Bioinformatic analysis was conducted to predict protein coding genes, rRNAs, tRNAs, and other ncRNAs from the whole genome sequence. Then a functional annotation analysis was conducted to predict the functions of the identified genes according to the databases of Kyoto encyclopedia of genes and genomes (KEGG), Cluster of Orthologous Groups of proteins (COG), Gene Ontology (GO), etc. We also performed an advanced functional annotation to identify any drug resistance genes, virulence factors, and pathogenic factors using the Comprehensive Antibiotic Resistance Database (CARD), ResFinder, Virulence Factors of Pathogenic Bacteria (VFDB), VirulenceFinder and PathogenFinder databases.

3. Genome sequencing results

3.1 Sequencing data processing

During the sequencing, Illumina's built-in software determined whether a read was to be retained or discarded based on the quality of the first 25 bases of each sequenced fragment, generating raw reads (Pass Filter Data). The raw read data were processed (Base Calling) using the software Bcl2fastq (v2.17.1.14) and initially analyzed for quality. The results were stored in FASTQ file format, which contains the sequencing information (second line of FASTQ format) and its corresponding sequencing quality information (fourth line of FASTQ format).

Each read in the FASTQ format file is described by four lines, as follows.



Line 1 and 3 are the sequence name (some FASTQ [*.fq] files omit the sequence name after "+" in the third line to save storage space) generated by the sequencer; Line 2 is the sequence; Line 4 is the sequencing quality of the sequence, each character corresponds to each base in Line 2, and each character in Line 4 corresponds to the ASCII value minus 33. Starting with Illumina GA Pipeline v1.8 (currently v1.9), the range of base quality values is 0 to 41.

The quality of sequenced bases is affected by sequencing machines, sequencing reagents, and samples, and the error rate is usually higher for the first few bases at the 5' end of the sequence and increases at the 3' end as the length of the sequenced fragment is extended, as determined by the characteristics of high-throughput sequencing technologies (*Erlich and Mitra, 2008; Jiang et al., 2011*). The sequencing error rate distribution was used to detect any unusual base positions with high error rates within the sequencing length range. For example, the sequencing error rate was significantly higher at the middle positions than at other positions. In general, the sequencing error rate for each base position should be less than 0.5%.

3.1.1 Statistics of sequencing data

The sequencing file of the Paired-End Sequencing consists of two FASTQ (*.fq) files, one is used for saving the fragment Read1 and another is for the fragment Read2.

The sequencing error rate is one of the factors affecting the quality of base sequencing. Depending on the technical characteristics of the Illumina sequencing platform, the error rate is higher for the first few base pairs (bp) of sequenced fragments (Reads) and at the ends of fragments. Sequencing error rates also increase with the length of the reads increasing, which is caused by chemical consumption during sequencing and is a common feature of Illumina's high-throughput sequencing platforms.

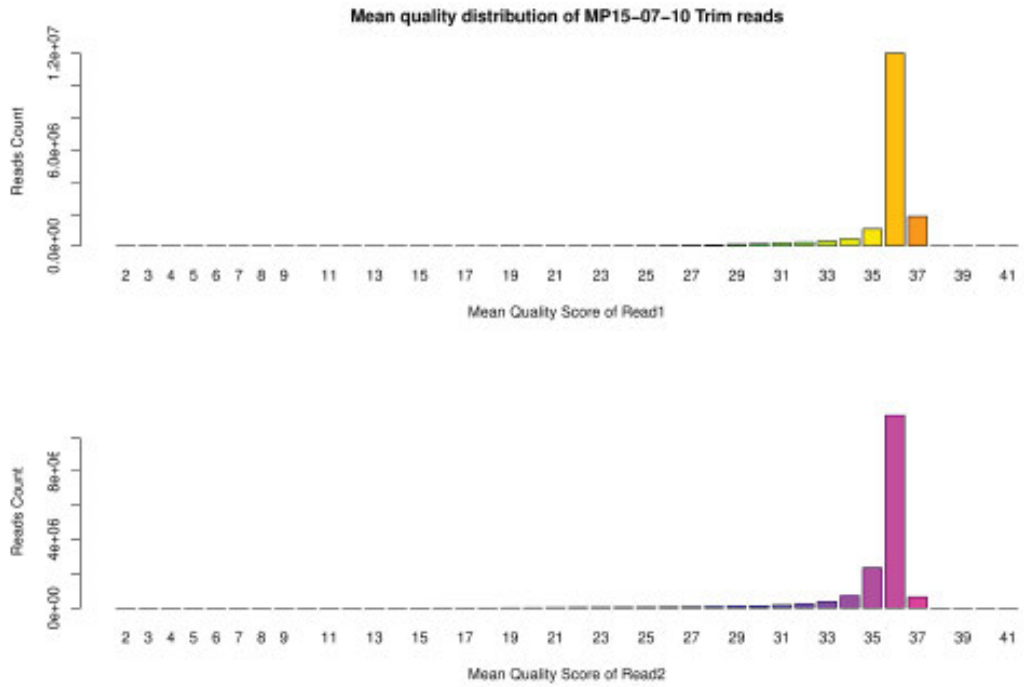


Figure 2: Mean quality distribution of Reads

Note: the abscissa indicates the mean bases quality of Reads; the ordinate indicates the Reads counts of the sequencing data.

The GC content distribution check is used to detect the presence or absence of AT and GC separation. Given the random interruption of the sequence and the principle of equal G/C and A/T content, respectively, the GC and AT contents should theoretically be equal on each sequencing cycle and essentially constant and horizontal throughout the sequencing process (if the sample source is an amplification product with obvious base preferences, the four bases of ATCG content will fluctuate considerably).

3.1.2 Quality optimization of sequencing data

During sequencing, some reads were detected with adapter sequences, or the bases at the 3' end of the reads were of low quality due to excessive sequencing lengths, which may negatively affect the results of subsequent analyses. Therefore, the raw data should be pre-processed, and the low-quality data should be filtered to remove contaminants and adapters.

The adapters and low-quality reads were removed from the Pass Filter Data using the sequenced data quality statistic software cut adapt II(v1.9.1) to obtain the clean data for the subsequent information analysis.

Finally, the clean data are shown in Table 1.

Table 1. Statistic data after the pre-processing of sequence reads

Sample	Length (bp)	#Reads	#Bases	Q20 (%)	Q30 (%)	GC (%)	N (ppm)
MP 15-07-10	149.44	32846170	4908542818	98.01	94.44	53.64	10.39

Remark:

(1) Sample: sequencing sample name;
(2) Length (bp): mean length of reads
(3) #Reads: the number of sequenced reads
(4) Bases (bp): the total number of bases
(5) Q20, Q30: the percentage of bases of which the Phred value is larger than 20 and 30 respectively
(6) GC%: the percentage of the total number of base G and C in total bases
(7) N (ppm): the number of N per million bases

3.2 Genome assembly

Using the pre-processed sequence reads, k-mer analysis was performed by Velvet (version 1.2.10) software, and the overlap relationships among k-mer data were used to construct a de Brujin map. Due to sequencing errors, low sequencing depth regions, and the presence of duplicate sequences, a complete de Brujin plot is generally not obtained, and some bifurcation points cannot be further joined, which eventually leads to segmented contig sequences splicing.

Then, the SSPACE (version 3.0) software is used to match all the reads sequenced from the library back to the contig sequences, and the contig sequences were further assembled to obtain the scaffold sequences using the pairwise relationships between the paired-end reads and the insert size.

Scaffold sequences were obtained from all the sequenced reads using the software GapFiller (version 1.10). The gaps in the scaffold sequences were completed using the compared reads for extending the scaffold sequences, generating long scaffold sequences with a low N percentage of unknown bases.

Finally, the GapFiller (version 1.10) software was used to match all the sequence reads to the scaffold sequences, and the gaps in the scaffold sequences were filled in using the matched reads. The scaffold sequences were updated with a lower percentage of unknown bases N and a longer sequence length. The scaffold sequences are shown in Table 2.

Table 2. Sequence Assembly results

Sample	MP15-07-10
#Total sequences	17
Total bases (bp)	2854860
Min length (bp)	281

Max length (bp)	819106
Average length (bp)	167932.9
N50 (bp)	347551
(G+C)s%	55.43
Ns%	0

Remark:

(1) Sample: sequencing sample name
(2) #Total sequences: total number of sequences
(3) Total bases(bp): total sequence length
(4) Min length(bp): minimum sequence length
(5) Max length(bp): maximum sequence length
(6) Average length(bp): average sequence length
(7) N50(bp): The length of the sequence when it reaches 50% of the genome length, summed from longest to shortest.
(8) (G+C)s%: the content of G and C
(9) Ns%: percentage of the uncertain bases in the total genome length

3.3 Prediction of coding genes

Predict the genome by the software prodigal (version 3.02)

Table 3. Prediction of Coding Gene

Sample	Gene num	Total bases (bp)	Min length (bp)	Max length (bp)	Average length (bp)	N50 (bp)	(G+C)s%	Ns%
MP15-07-10	2672	2611290	90	8724	977.28	1215	56.17	0.00

Remark:

(1) Sample: sequencing sample name;
(2) Gene num: number of predicted gene;
(3) Total bases(bp): total length of predicted coding-gene;
(4) Min length(bp): minimum length of predicted coding-gene
(5) Max length(bp): maximum length of predicted coding-gene
(6) Average length(bp): average length of predicted coding-gene
(7) N50(bp): Length of the sequence when it reaches 50% of the genome length by adding up the length of the sequences from longest to shortest
(8) (G+C)s%: the percentage of GC bases in total gene.
(9) Ns%: the content of N bases

3.4 Non-coding RNA prediction

Non-coding RNAs are RNAs that do not encode proteins, including rRNA, tRNA, snRNA, snoRNA and microRNA, as well as some RNAs of unknown function. The common feature of these RNAs is that they are transcribed from the genome, but not translated into protein, and can perform their respective biological functions at the RNA level.

Non-coding RNAs can be divided into 3 categories in terms of length: <50nt, including microRNA, siRNA, piRNA; 50nt~500nt, including rRNA, tRNA, snRNA, snoRNA, SLRNA, SRPRNA, etc.; >500nt, including the long non-coding RNA of mRNA-like, long non-coding RNA without the polyA tail, etc.

Prediction of the tRNA and rRNA regions was conducted using the software tRNAscan and barrnap, respectively. Sequence comparison was performed with the Rfam (version 12.0) database to obtain the other non-coding RNA.

Table 4. Prediction of Non-coding RNA

Sample	Total ncRNA	rRNA	tRNA	Other ncRNA
MP15-07-10	67	5	45	17

Remark:

(1) Total ncRNA: the total number of non-coding RNA
(2) rRNA: the number of rRNA
(3) tRNA: the number of tRNA
(4) Other ncRNA: the number of other non-coding RNA

3.5 Gene function annotation

The main method of gene function annotation is to sequence match the predicted protein sequence of the coding gene with the proteins contained in the databases. If the protein sequence of a gene has significant sequence similarity with the protein sequence in the database, then it can be inferred that the gene has the same or similar function as the protein in the database.

Based on the predicted protein sequence of the coding gene, the BLAST software (version 2.2.31+) was used to match the protein sequence in the database. The E-value of the sequence matching was set to 1*e-5, and the best match was selected as the annotation result of the gene.

3.5.1 KEGG database annotation

The KEGG is a database for the systematic analysis of gene function and genomic information, integrating genomic, biochemical and phylogenetic data and mapping biological pathways according to different biological processes. It helps researchers to study gene and expression information as a holistic network.

By annotating genes on the KEGG Biopathway Database, it is possible to see which biological pathways these genes are involved in and what important biological functions they perform.

The KEGG database divides the biological metabolic pathway into 6 categories: 1) Cellular Processes, 2) Environmental Information Processing, 3) Genetic Information Processing, 4) Human Diseases, 5) Metabolism, and 6) Organismal Systems. Each category of the pathway is systematically divided into secondary classifications. The number of genes in each metabolic pathway of secondary classifications is counted and shown in Figure 3.

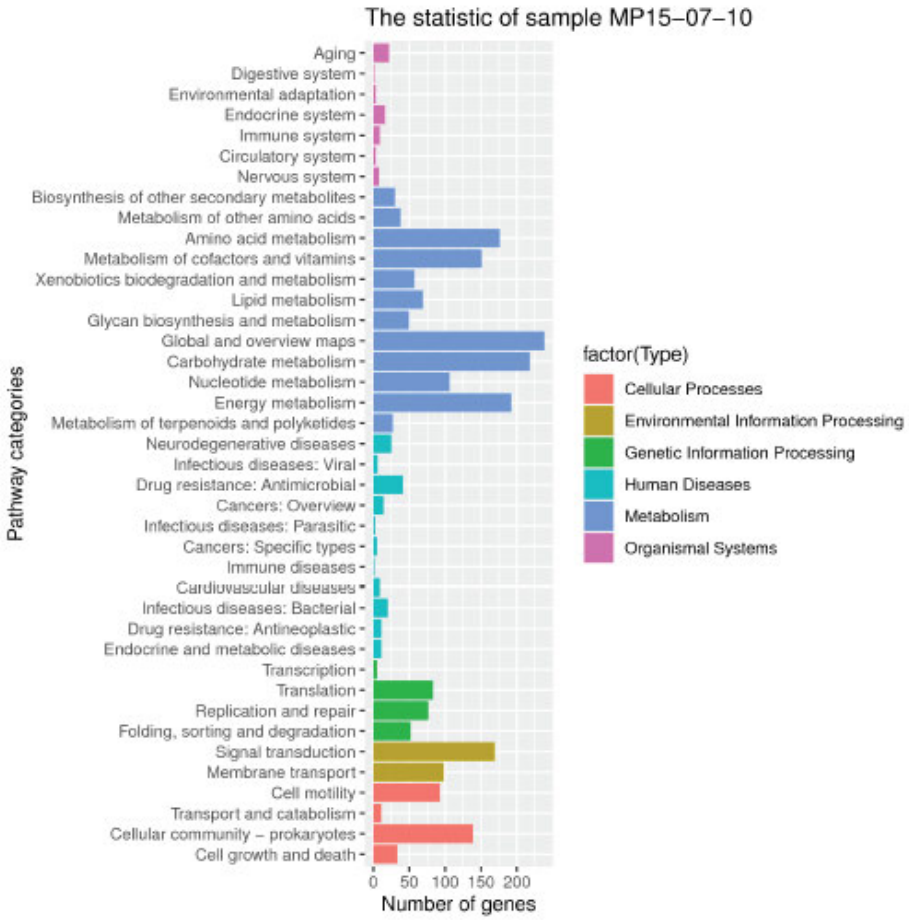


Figure 3. KEGG pathway map of Strain MP15-07-10

3.5.2 COG database annotation

The COG, a protein database created and maintained by NCBI, was constructed by classifying the phylogenetic relationships of the proteins encoded in the complete genomes of bacteria, algae, and eukaryotes. By comparison, it is possible to annotate a protein sequence to a particular COG, with each cluster of COGs consisting of direct homologous sequences, allowing the function of that sequence to be inferred.

The COG function classification of strain (MP15-07-10) is shown in Figure 4.

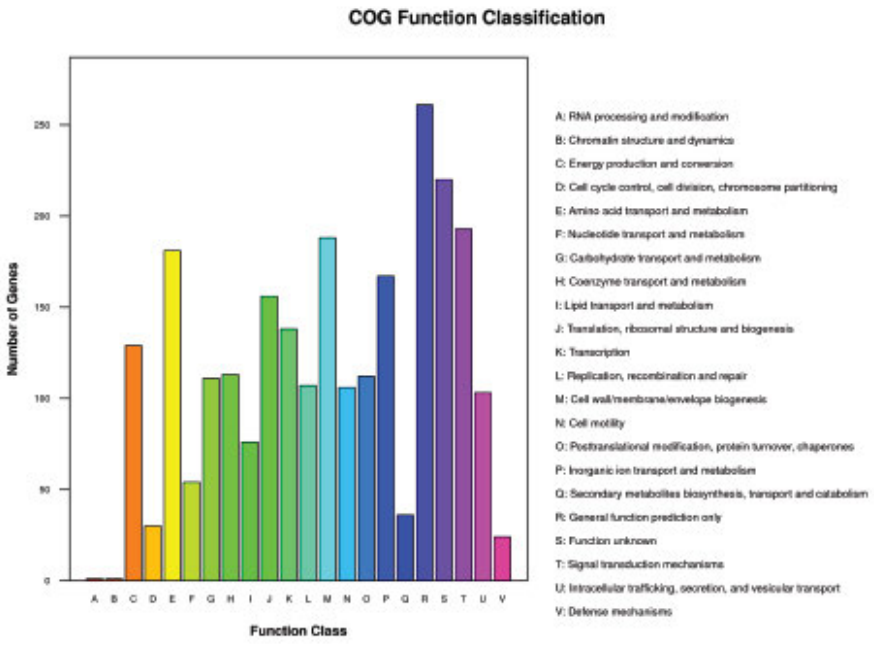


Figure 4. COG functional classification of Strain (MP15-07-10)

3.5.3 GO database annotation

The GO database is a database created by the Gene Onotology Consortium to create a biolinguistic vocabulary standard for qualifying and describing gene and protein function across a wide range of species, which can be updated as research progresses.

GO defines a standard language of tertiary structures (ontologies): (1) Molecular Function (M) of the gene product; (2) Biological Process (P); and (3) Cellular Component (C). The distribution of genes in different GO terms can be visualized at the secondary level. The histogram of the strain distribution in GO Term is shown in Figure 5.

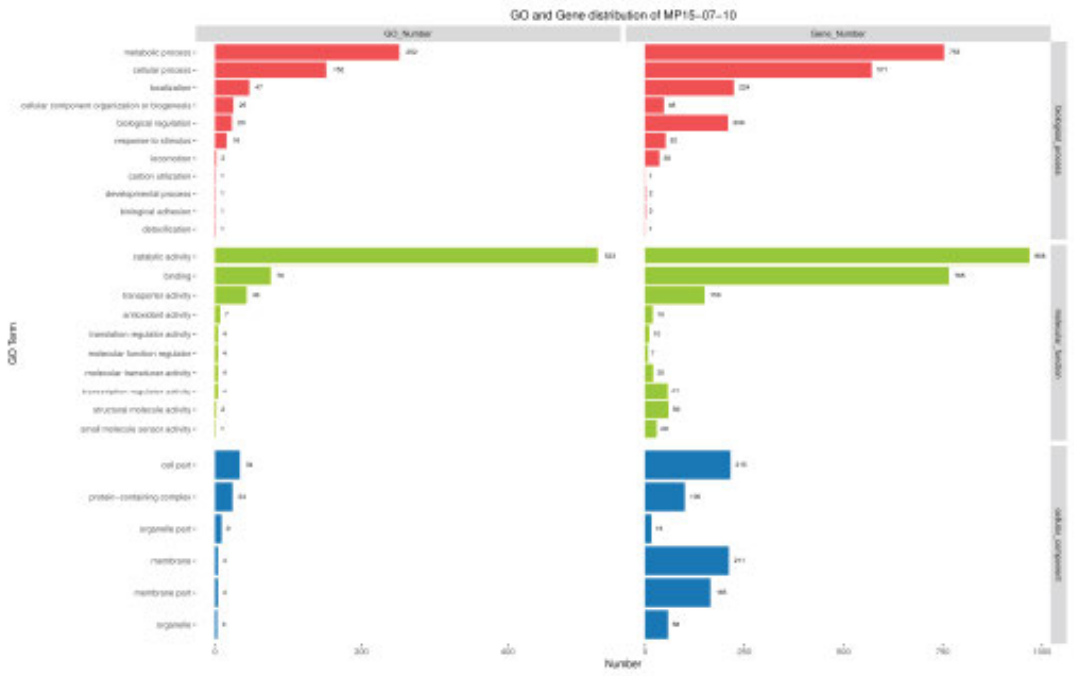


Figure 5. Histogram of gene distribution in GO Term

Remark: the ordinate indicates the GO term; the abscissa indicates the number of the gene. Different colors are used to distinguish the Biological Processes, Cellular Component, and Molecular Function.

3.6 Advanced function annotation

The main method of the advanced function annotation was to compare the protein sequences of predicted coding genes with the protein database with special functions (translocator, virulence factor, and antibiotics resistance). If the protein sequence of a gene has a significant similarity to the protein sequences in the database, it can be inferred that the gene has the same function as the protein in the database.

The protein sequences of the predicted coding genes to the proteins contained in each database were compared using the software BLAST (version 2.2.31+). The E-value of the sequence comparison was set to be l*e-5, and the optimal matching result with the following criteria was used to annotate the function of the gene.

Criteria: Sequence similarity ≥85% and sequence coverage ≥60%.

3.6.1 CARD database annotation

The CARD (Comprehensive Antibiotic Resistance Database, https://card.mcmaster.ca/) provides data, models and algorithms on the molecular basis of antibiotic resistance, containing selected reference sequences and SNPS derived from the antibiotic resistance ontology (ARO). It currently

contains 2653 reference sequences, 1216 SNPs, and 2615 testing models of drug resistance genes. The prediction and research for the antibiotic resistance can be conducted based on these data and models. When the predicted genes in the genome of the ZMC's strain were submitted to the CARD, no genes associated with potential drug resistance were detected.

3.6.2 ResFinder database

The ResFinder database (https://cge.cbs.dtu.dk/services/ResFinder/) was created and is maintained by the Technical University of Denmark. It currently contains 2817 drug resistance gene sequences of 49 kinds of antibiotics in 15 major categories, including the resistance genes associated with aminoglycoside, beta lactam, colistin, fosfomycin, fuscidic acid, glycopeptides, macrolides, nitroimidazoles, oxazolidones, phenylpropanols, rifamycin, sulfamides, tetracyclines, trimethoprim and other antibiotic resistance. When the predicted genes in the genome of ZMC's strain were submitted to the ResFinder database, no genes associated with potential drug resistance were detected.

3.6.3 VFDB database annotation

The VFDB database (http://www.mgc.ac.cn/VFs/), named Virulence Factors of Pathogenic Bacteria (VFDB), is used to study pathogenic factors of pathogenic bacteria, chlamydia and mycoplasma. It currently contains 74 pathogenic genera, 951 strains of bacteria, 1079 virulence factors and 32451 toxigenic genes. When the predicted genes in the genome of ZMC's strain were submitted to the VFDB database, no genes associated with potential virulence were detected.

3.6.4 VirulenceFinder database

The VirulenceFinder (https://cge.cbs.dtu.dk/services/VirulenceFinder/) is a part of CGE (Center for Genomic Epidemiology), which was created and is maintained by the Technical University of Denmark. It currently contains virulence genes in 4 categories of microorganisms of *Listeria, Streptococcus aureus, Escherichia coli, Enterococcus.* When the predicted genes in the genome of ZMC's strain were submitted to the VirulenceFinder database, no genes associated with potential virulence were detected.

3.6.5 Pathogenicity analysis

PathogenFinder database (https://cge.cbs.dtu.dk/services/ResFinder/) was created and is maintained by the Technical University of Denmark. It is used to predict the pathogenicity of bacteria by analyzing the protein sequence, genome sequence, and original sequence of bacterial strains. The method generated relies on the proteome without regard to its annotation function nor its known involvement in pathogenicity, which is applicable to all taxa of bacteria and the entire training set. Pathogenic or non-pathogenic microorganisms can be predicted by identifying protein families that are frequently present in pathogenic or non-pathogenic microorganisms. When the predicted genes in the genome of ZMC's strain were submitted to the PathogenFinder database, the results showed that the sample was predicted to be a non-potential human pathogens strain.

3. Conclusion

Based on the whole gene sequencing, bioinformatics analysis, and queries of the predicted genes in the genome in multiple databases of the genes associated with antibiotics resistance, virulence factors, and pathogenicity, no genes showing sequence similarities with the known genes associated with antibiotic resistance, virulence factors, or pathogenic factors were detected in the *Methylovorus glucosotrophus* of Zhejiang Medicine Co., Ltd. The ZMC strain is determined to be non-pathogenic and non-toxigenic.

References

Erlich, Y. andMitra, P.P. Alta-Cyclic: a self-optimizing base caller for next-generation sequencing. Nature Methods. 2008;5: 679-682.

Jiang, L., Schlesinger, F., Davis, C.A., Zhang, Y., Li, R., Salit, M., Gingeras, T.R., and Oliver, B. Synthetic spike-in standards for RNA-seq experiments. Genome Research. 2011; 21(9):1543-51.

Appendix B. Certificate of Analysis of PQQ Disodium Salt

浙江医药 ZMC

Report Date: 2022-09-06

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C1060103
Specification	Chemical intermediate	Batch No.	311PQ210501
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2021/05/24	Quantity	20.50kg
Expiry Date	2023/05/23	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	8.9%
4 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	88%
5 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.2%
6 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.3%
7 Lead, mg/kg	USP<233>	≤0.2	<0.2
8 Arsenic, mg/kg		≤0.2	<0.2
9 Cadmium, mg/kg		≤0.2	<0.2
10 Mercury, mg/kg		≤0.2	<0.2
11 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "[1]" is performed once a year Only for registration

Printer: 2022.09.06 Reviewer: 2022.09.06 Approver: 2022.09.06

Mfg :Zhejiang Medicine Co., Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675

Add.:98 East Xinchang Dadao Road , Xinchang , Zhejiang, 312500 P.R.China
Email: QC@xcpharma.com

浙江医药 ZMC

Report Date: 2022-09-06

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C1060104
Specification	Chemical intermediate	Batch No.	311PQ210502
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2021/06/01	Quantity	17.40kg
Expiry Date	2023/05/31	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	9.0%
4 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	88%
5 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.1%
6 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.2%
7 Lead, mg/kg	USP<233>	≤0.2	<0.2
8 Arsenic, mg/kg		≤0.2	<0.2
9 Cadmium, mg/kg		≤0.2	<0.2
10 Mercury, mg/kg		≤0.2	<0.2
11 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "[1]" is performed once a year Only for registration

Printer: 2022.09.06 Reviewer: 2022.09.06 Approver: 2022.09.06

Mfg :Zhejiang Medicine Co., Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675
Add.:98 East Xinchang Dadao Road , Xinchang , Zhejiang, 312500 P.R.China
Email: QC@xcpharma.com

浙江医药 ZMC

Report Date: 2022-09-06

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C1060105
Specification	Chemical intermediate	Batch No.	311PQ210601
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2021/06/13	Quantity	21.98kg
Expiry Date	2023/06/12	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	8.8%
4 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	88%
5 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.1%
6 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.3%
7 Lead, mg/kg	USP<233>	≤0.2	<0.2
8 Arsenic, mg/kg		≤0.2	<0.2
9 Cadmium, mg/kg		≤0.2	<0.2
10 Mercury, mg/kg		≤0.2	<0.2
11 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "[1]" is performed once a year Only for registration

Printer: 2022.09.06 Reviewer: 2022.09.06 Approver: 2022.09.06

Mfg.:Zhejiang Medicine Co., Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675
Add.:98 East Xinchang Dadao Road, Xinchang, Zhejiang, 312500 P.R.China
Email: QC@xcpharma.com

Appendix C. Cumulative EDIs Under the Intended Use of All GRAS Notices

In this Section, cumulative EDIs were calculated. If the different use levels were proposed in previous GRAS notices for the same intended use food category, the highest level was selected for each category. Although GRN 694 described the use levels in percentage of foods (page 4), EDI calculations were based on mg PQQ disodium salt per serving (page 14). Thus, cumulative use levels and EDI calculations were based on the use levels per serving as follows.

Table C1. Comparison of the Intended Use Levels

Food-Uses	Proposed Maximum Use Level (mg/serving)					
	GRN 625	GRN 641	GRN 694	GRN 701	GRN 709 and current notice	Cumulative use levels
Energy Drinks	8	5	8	8	12	12
Sports and Electrolyte Drinks	8	5	8	8	8	8
Enhanced and Fortified Water Beverages	8	20	20	20	20	20
Bottled water	8	0	20	8	8	20
Non-Milk Based Meal Replacement Beverages	8	0	8	8	8	8

*Table 1 of GRN 625 (page 6) and Table 1.3-1 of GRN 694 (page 4) have typos by 1 decimal point: Based on the FDA's response letter, correct use levels were recalculated to be 0.00333%, instead of 0.0333% which were presented in the original submissions of GRN 625 and GRN 694.

Using food intake data reported in the 2015-2018 National Health and Nutrition Examination Survey (NHANES), cumulative exposure levels to PQQ disodium salt that will result from the intended uses were estimated. Cumulative EDIs from the maximum use levels adopted from all previous GRAS notices are presented in Tables C2 andC3. Based on the NHANES 2015-2018 dataset, the mean and 90th percentile all-user intakes of PQQ disodium salt were estimated to be 47.4 and 106.8 mg/person/day, respectively. On a body weight basis, mean and 90th percentile EDIs were 0.66 and 1.43 mg/kg bw/day, respectively.

These cumulative intended use levels and cumulative EDIs are comparable to those described in GRN 694 (Tables C.4 to C.7). As shown in Table C1., the only difference in use levels was in energy drinks: 12 mg/serving for cumulative use and 8 mg/serving for GRN 694.The bottled water contributed the most to EDIs. Intended use levels of bottled water, which contributed the most to EDIs, were 20 mg/serving in GRN 694 as opposed to 8 mg/serving in other GRAS notices.

Under the intended use of GRN 694, the mean and 90th percentile all-user intakes of PQQ disodium salt were estimated to be approximately 47.2 and 106.6 mg/person/day, respectively,

when the NHANES 2015-2018 dataset was used. Corresponding EDIs were 42.4 and 99.2 mg/person/day, respectively, based on the NHANES 2011-2014 dataset. Respectively, on a body weight basis, the mean and 90th percentile EDIs were 0.66 and 1.43 mg/kg bw/day in 2015-2018 and 0.61 and 1.37 mg/kg bw/day in 2011-2014.

Overall, cumulative EDIs are comparable to those under the intended use of GRN 694 when the same NHANES dataset (2015-2018) was used.

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. 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, cumulative use will result in EDIs within the safe intake levels.

Table C.2. Maximum EDIs of PQQ Disodium Salt under the Cumulative Use*, mg/day

Population	% all-user	N, total population	Per User (mg/person/day)		Per Capita (mg/person/day)	
			Mean	90th Percentile	Mean	90th Percentile
2-5 y	48.8	987	17.6	36.7	8.6	27.7
6-12 y	60.7	1744	23.9	50.8	14.5	40.2
13-18 y males	64.0	720	42.3	84.5	27.1	66.4
13-18 y females	64.8	713	41.0	93.1	26.6	69.7
19+ males	59.9	4,080	54.9	120.1	32.9	97.8
19+ females	60.7	4,461	50.2	110.3	30.5	89.4
2-99 y	60.1	12,705	47.4	106.8	28.5	84.4

*Assuming all the foods will be used at the maximum use levels; NHANES 2015-2018.

Table C.3. Maximum EDIs of PQQ Disodium Salt under the Cumulative Use*, mg/kg bw/day

Population	Per User (mg/kg bw/day)		Per Capita (mg/kg bw/day)	
	Mean	90th Percentile	Mean	90th Percentile
2-5 y	1.04	2.23	0.51	1.63
6-12 y	0.69	1.51	0.42	1.08
13-18 y males	0.60	1.21	0.38	0.94
13-18 y females	0.64	1.32	0.41	1.11
19+ males	0.62	1.39	0.37	1.08
19+ females	0.67	1.41	0.40	1.13
2-99 y	0.66	1.43	0.40	1.12

*Assuming all the foods will be used at the maximum use levels; NHANES 2015-2018.

Table C.4. Maximum EDIs of PQQ Disodium Salt under the Intended Use of GRN 694*, mg/day

Population	% all-user	N, total population	Per User (mg/person/day)		Per Capita (mg/person/day)	
			Mean	90th Percentile	Mean	90th Percentile
2-5 y	48.8	987	17.6	36.7	8.6	27.7
6-12 y	60.7	1744	23.9	50.8	14.5	40.2
13-18 y males	64.0	720	42.2	84.5	27.0	66.4
13-18 y females	64.8	713	40.9	93.1	26.5	69.7
19+ males	59.9	4,080	54.5	120.0	32.6	97.3
19+ females	60.7	4,461	50.1	110.3	30.5	89.2
2-99 y	60.1	12,705	47.2	106.6	28.4	84.3

*Assuming all the foods will be used at the maximum use levels; NHANES 2015-2018.

Table C.5. Maximum EDIs of PQQ Disodium Salt under the Intended Use of GRN 694, mg/kg bw/day

Population	Per User (mg/kg bw/day)		Per Capita (mg/kg bw/day)	
	Mean	90th Percentile	Mean	90th Percentile
2-5 y	1.04	2.23	0.51	1.63
6-12 y	0.69	1.51	0.42	1.08
13-18 y males	0.59	1.21	0.38	0.94
13-18 y females	0.64	1.32	0.41	1.11
19+ males	0.62	1.39	0.37	1.07
19+ females	0.66	1.40	0.40	1.13
2-99 y	0.66	1.43	0.40	1.12

*Assuming all the foods will be used at the maximum use levels; NHANES 2015-2018.

Table C.6. Maximum EDIs of PQQ Disodium Salt under the Intended Use of GRN 694*, mg/day

Population	% all-user	N, total population	Per User (mg/person/day)		Per Capita (mg/person/day)	
			Mean	90th Percentile	Mean	90th Percentile
2-5 y	45.8	1262	16.5	34.7	7.6	24.8
6-12 y	54.7	2206	21.6	49.2	11.8	34.9
13-18 y males	58.5	822	39.6	100.4	23.2	69.5
13-18 y females	65.4	838	34.2	74.5	22.4	59.2
19+ males	51.5	4294	48.2	111.5	24.8	82.7
19+ females	54.9	4739	46.3	103.4	25.4	80.1
2-99 y	53.7	14161	42.4	99.1	22.7	70.6

*Assuming all the foods will be used at the maximum use levels; NHANES 2011-2014.

Table C.7. Maximum EDIs of PQQ Disodium Salt under the Intended Use of GRN 694, mg/kg bw/day

Population	Per User (mg/kg bw/day)		Per Capita (mg/kg bw/day)	
	Mean	90th Percentile	Mean	90th Percentile
2-5 y	0.97	2.14	0.45	1.49
6-12 y	0.61	1.38	0.34	1.00
13-18 y males	0.56	1.28	0.33	0.96
13-18 y females	0.57	1.21	0.37	0.97
19+ males	0.56	1.30	0.29	0.93
19+ females	0.63	1.37	0.34	1.10
2-99 y	0.61	1.37	0.33	1.02

*Assuming all the foods will be used at the maximum use levels; NHANES 2011-2014.

Appendix D. Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Pyrroloquinoline Quinone (PQQ) Disodium Salt for Use as an Ingredient in Selected Foods

Summary of Opinions of the Expert Panel

An independent panel of experts (the Expert Panel), qualified by scientific training and experience to evaluate the safety of food and food ingredients, was requested by Zhejiang Medicine Co., Ltd. (ZMC) to evaluate the safety and Generally Recognized as Safe (GRAS) status of the use of Pyrroloquinoline Quinone (PQQ) Disodium Salt as a food ingredient.

The Panel independently and collectively evaluated all the available information submitted by ZMC which included comprehensive data and information pertaining to the method of manufacture, product specifications and analytical data, stability, the conditions of intended use of PQQ disodium salt in specified food and beverage products, dietary consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature through July 2022. The Expert Panel unanimously concluded that the intended uses described herein for ZMC's PQQ disodium salt, meeting appropriate food-grade specifications as described in the supporting dossier (Determination of The Generally Recognized As Safe [GRAS] Status of Pyrroloquinoline Quinone (PQQ) Disodium Salt as a Food Ingredient) and manufactured according to current Good Manufacturing Practices (cGMP), are GRAS based on scientific procedures. For purposes of the Expert Panel's evaluation, "safe" or "safety" indicates that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i). A summary of the basis for the Expert Panel's conclusion is provided below.

Description

The common names of the subject of this GRAS assessment are pyrroloquinoline quinone disodium salt or pyrroloquinoline quinone and are often abbreviated as PQQ disodium salt, PQQ sodium salt, or PQQ. PQQ disodium salt's chemical formula is $C_{14}H_4N_2Na_2O_8$; the molecular weight is 374.17 Da with CAS No. of 122628-50-6.

Manufacturing Process

The principle of ZMC's PQQ disodium salt production method (via bacterial fermentation) is similar to those described by other investigators and other companies, such as Mitsubishi Gas Chemical Company, Hisun, Nutraland USA, and Shangdong Jincheng Bio-Pharmaceutical Co., whose production methods for PQQ disodium salt received no objection letters from the FDA (GRN 641, FDA, 2016a; GRN 694, FDA, 2017a; GRN 701, FDA, 2017b; GRN 709, FDA, 2018; FDA, 2007, NDIN RPT 417). The difference is that the above mentioned companies described methods using the *Hyphomicrobium denitrificans* strains in a fermentation process to produce PQQ disodium salt. In this GRAS determination, a non-pathogenic, non-toxigenic strain of Methylotrophic bacteria, *Methylovorus glucosotrophus*, is used to produce PQQ disodium salt.

The difference is that the above mentioned companies described a method using the *Hyphomicrobium denitrificans* strains in a fermentation process to produce PQQ disodium salt. In this GRAS determination, a non-pathogenic, non-toxigenic strain of Methylotrophic bacteria, *Methylovorus glucosotrophus*, is used to produce PQQ disodium salt.

The first step for the production of PQQ disodium salt is fermentation using *Methylovorus glucosotrophus*. The major components of the fermentation medium are mineral, nitrogen, and carbon sources such as magnesium sulfate, ammonium sulfate, potassium dihydrogen phosphate, disodium hydrogen phosphate, calcium chloride, zinc sulfate heptahydrate, cupric sulfate pentahydrate, manganese chloride tetrahydrate, ferric citrate, methanol, and ammonium hydroxide. During the fermentation process with a non-pathogenic and non-toxigenic *Methylovorus glucosotrophus* strain, PQQ disodium salt is biosynthesized inside the cells and exported into the culture broth.

Upon completion of fermentation, the culture supernatant containing PQQ disodium salt is subjected to filtration, chromatography, nano filtration (optional), and crystallization to get the first crude PQQ disodium salt. The first crude PQQ disodium salt is dissolved in deionized water and is subjected to filtration, chromatography, and crystallization to get the second crude PQQ disodium salt. The second crude PQQ disodium salt is dissolved in deionized water and is subjected to the third crystallization, filtration, drying, and milling before being packaged.

All raw materials used in the manufacturing process are suitable food-grade and are used in accordance with applicable U.S. Federal Regulations. All processing aids are suitable for use in food manufacturing and are compliant with applicable U.S. Federal Regulations. ZMC's fermentation process does not use antibiotics or inhibitors and the manufacturing process does not use strong organic solvents or other toxic substances.

The original strain (*Methylovorus glucosotrophus* strain) for producing PQQ was isolated from soil samples collected in Beijing, Hebei, HeiLongjiang, LiaoNing, and ShanDong Provinces, P. R. China. The analysis of whole genomic sequence identified ZMC's strain as a non-pathogenic and non-toxigenic bacterial species.

Identification, Specifications, and Analytical Values

The product is ≥98% pure on a dry weight basis, as measured by HPLC. Specifications include the minimum content of PQQ disodium salt (≥98% on a dry weight basis) and limits for lead (≤ 0.2 mg/kg), arsenic (≤ 0.2 mg/kg), cadmium (≤ 0.2 mg/kg), and mercury (≤ 0.2 mg/kg) as well as microbial contaminants such as total aerobic counts (≤ 1,000 cfu/g), yeasts and molds (≤ 100 cfu/g), *Enterobacteria* (<10 cfu/g or <the detection limit of the assay), *Escherichia coli* (absent in 25 g), *Staphylococcus aureus* (absent in 10 g), *Salmonella* (absent in 25 g), and *Listeria* (absent in 25 g).

Analytical data on multiple batches confirmed the purity of the product (>98% on a dry weight basis) and its manufacturing consistency by meeting specifications as demonstrated in the certificates of analysis. ZMC's PQQ disodium salt is very similar to the PQQ disodium salt ingredients produced by other manufacturers that received FDA's no question letters. ZMC's PQQ disodium salt is chemically identical to a reference standard as confirmed by high performance liquid chromatography (HPLC).

Stability

Based on ZMC's stability test results, PQQ disodium salt is stable at 25°C and at 60% relative humidity for 12 months.

Intended Use and Exposure Estimates

ZMC intends to use PQQ disodium salt as a food ingredient in selected beverages (energy, sports, and electrolyte drinks, enhanced and fortified water beverages, bottled water, and non-milk-based meal replacement beverages) at maximum use levels of up to 8 to 20 mg/serving.

The intended use and use levels of PQQ disodium salt of the current notice are the same as those employed in GRN 709. Using food intake data reported in the 2015-2018 National Health and Nutrition Examination Survey (NHANES), exposure levels to PQQ disodium salt that will result from the intended uses were estimated. Based on the NHANES 2015-2018 dataset, the mean and 90th percentile all-user intakes of PQQ disodium salt were estimated to be approximately 21 and 46 mg/person/day, respectively. Males older than 19 years of age would have the highest intake among the various age/gender groups, with a 90^{th} percentile value of 52 mg/person/day for all-users. On a body weight basis, children aged 2-5 years had the highest 90^{th} percentile EDI at 0.98 mg/kg bw/day for all-users.

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. 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. ZMC's PQQ disodium salt will be used as a replacement for the currently marketed PQQ disodium salt, thus, cumulative exposures are not expected to change.

Regulatory Status

The FDA has previously issued 'no question' letters on GRAS and NDI notices related to PQQ disodium salt produced by a bacterial fermentation technique using *Hyphomicrobium denitrificans* (GRN 641, FDA, 2016a; GRN 694, FDA, 2017a; GRN 701, FDA, 2017b; GRN 709, FDA, 2018; NDIN RPT 417, FDA 2007). In addition, the FDA has issued a 'no question' letter on synthetic PQQ disodium salt (GRN 625, FDA 2016b).

Narratives

Metabolism

In a study by Smidt et al. (1991), male Swiss-Webster mice (n = 5 per time point) were orally administered (gavage) a single dose of 1.5 mg/kg bw of radiolabeled (^{14}C) PQQ. Based on the amount retained in the tissues, and amount in the urine and carbon dioxide, approximately 62% of the PQQ was estimated to be absorbed through the small intestine and 81% of that was excreted within 24 hours via urine. This shows that PQQ is absorbed effectively, with most of it is excreted in urine. The radioactive PQQ was detected in the kidneys (10.7%), the carcass (3.7%), 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 hours), 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 hours. At 6 hours, the blood cell fraction constituted about 10% of the absorbed label. This fell to 1.2% at 24 hours. No radioactivity was detected in the expired air.

A human study conducted by Harris et al. (2013) found that levels of PQQ peaked in serum at ~2 h following a single dose of PQQ (0.2 mg PQQ/kg bw) after receiving a single dose of 0.2 mg PQQ/kg bw in a drink (corresponding to 14 mg PQQ for a 70-kg adult) in 10 adults. Serum concentration of PQQ peaked at 2 h post-administration at 9 nM (~ 3.4 ng/mL).

In another experiment by Harris et al. (2013), the urinary excretion and serum concentration of PQQ in 10 healthy adults (five men and five women) who received PQQ in a drink at increasing dose levels of 0.075, 0.15, and 0.3 mg/kg bw per day (corresponding to approximately 5.25, 10.5, and 21 mg PQQ/day for a 70 kg individual) were measured over three consecutive 7-day periods. At each dose (up to 0.3 mg/kg bw/day), ~0.1% of the PQQ ingested was recovered in urine as nonderivatized PQQ. Serum concentrations of nonderivatized PQQ increased in response to dietary intake up to 14 nM at a dose of 0.3 mg/kg bw per day, and the daily excretion of PQQ in urine was directly related to serum concentrations.

PQQ, alone or with its corresponding imidazolopyrroloquinoline (IPQ) derivatives, condensed products of PQQ with amino acids, have been detected in human milk at concentrations of 140 to 180 ng/mL (Mitchell et al., 1999).

Pre-clinical Studies

PQQ disodium salt has been evaluated in *in vitro* and *in vivo* genotoxicity studies, acute and subacute oral toxicity studies in rodents, and subchronic toxicity and reproductive toxicity studies in rodents. Test substances included ZMC's PQQ disodium salt produced by fermentation with *Methylovorus glucosotrophus or* Hyphomicrobium sp. and synthetic PQQ disodium salt. PQQ disodium salt was reported as non-mutagenic and non-clastogenic in all studies conducted (Nakano et al., 2013; Seol et al., 2022).

ZMC's PQQ Disodium Salt

In general, animal studies have demonstrated that PQQ disodium salt is of low oral toxicity. The NOAEL found from subchronic toxicity and teratogenicity studies of ZMC's PQQ disodium was set at 200 mg/kg bw/day (Lee et al., 2022; Seol et al., 2022). Although potential nephrotoxicity was reported by another research group (Nakano et al., 2014), the study by Seol et al. (2022) did not find any abnormalities in urinary parameters. Thus, the NOAEL of 200 mg/kg bw/day was chosen when evaluating the safety of ZMC's PQQ disodium salt in this GRAS determination.

Other Sources of PQQ Disodium Salt

Because the specifications of the proposed GRAS substance, ZMC's PQQ disodium salt, are almost identical to other PQQ disodium salt products that have received FDA no question letters, the information and data described in previous GRAS notices 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 notices and will not discuss previously reviewed references in detail.

The results from subchronic toxicity studies by Nakano et al. (2014; *Hyphomicrobium denitrificans* source) and Liang et al. (2015; synthetic) 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. However, Liang et al. (2015) did not evaluate urinary parameters. The findings of the animal toxicity studies collectively support the safety of PQQ disodium salt supplementation (*Hyphomicrobium denitrificans* source) at daily doses of 100 mg/kg bw/day or higher (FDA 2016b, pages 29-34 and 42-43; FDA 2016a, pages 30-33; Nakano et al., 2014).

A reproductive toxicity study (sperm shape abnormality assay) in mice indicated that there were 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 (or ~0.3 mg/kg bw/day) did not impact reproductive performance.

Nephrotoxicity Associated with High Levels of PQQ

High doses of PQQ disodium salt may induce nephrotoxicity in rats (Nakano et al., 2014). In a dose-range finding study, Crl:CD(SD) rats received 0, 3, 12, 48, 192, or 768 mg/kg bw/day PQQ disodium salt (>99.1% pure), produced via *Hyphomicrobium denitrificans*, by gavage for 14 days. No mortality was observed in any groups. There was no statistically significant difference in bw among the groups. During the 14-day oral administration of PQQ disodium salt to rats, nephrotoxicity was seen in the high dose (768 mg/kg bw/day) group (Nakano et al., 2014; source, Mitsubishi Gas Chemical Co). Crystals in urine were found in some females at 3, 12, 192, and 768 mg/kg bw/day and a male at 192 mg/kg bw/day. Urinary protein levels were increased in a female at 12 mg/kg bw/day, a female at 192 mg/kg bw/day, and 2 females and 2 males at 768 mg/kg bw/day. The high dose group also had significant increased urinary sodium levels in both males

and females. The female high dose group, but not male high dose group, also had increased relative kidney weight (approximately 14%, $p<0.05$), along with histopathological changes (focal basophilic changes and atrophy of the renal tubules) of minimal to moderate severity as well as green-colored cecal contents. No other toxicologically relevant histopathological changes were reported. The data suggest nephrotoxicity of PQQ disodium salt at 768 mg/kg bw/day.

In the follow up 28-day study by Nakano et al. (2014), female SD rats received 0, 200, or 700 mg/kg bw/day PQQ disodium salt by gavage for 4-weeks followed by a 4-week recovery period. No mortality and no significant differences in body weight, water intake, and clinical biochemistry parameters were observed between the control and the treatment groups (data not shown). The high-dose group had blackish stools due to the dark color of the test substance in the dosing formulation. Urinalysis revealed an increased incidence of crystals in urinary sediment at daily doses of 200 and 700 mg/kg bw during the administration period. Urinary crystal was observed only in one animal in the low-dose group at the end of the recovery period. Moreover, the animals had protein present in their urine with increased incidence with increasing dose, but was alleviated in the recovery period. Urinary protein was detected in 1/12 control, 5/12 low-dose, and 6/12 high-dose rats at week 4 with a low or 'slight' grade. It was still observed in 1/6 low-dose and 2/6 high-dose rats at the end of four-week recovery period. However, these effects were not accompanied by any other significant changes in clinical chemistry parameters related to kidney function, or in the results of the gross and histopathological examinations and were resolved during the 4-week recovery period.

In the 90-day oral toxicity study involving lower doses of PQQ disodium salt (>99.1% pure) (0, 3, 20, or 100 mg/kg bw/day by gavage; Nakano et al., 2014), no treatment-related abnormalities were reported in hematology, clinical biochemistry, urinalysis, or gross necropsy and histopathology inCrl:CD(SD) rats (10/sex/group), with the exception of green-colored feces, which were observed in male and female rats in the highest dose group beginning on days 7-11 and thereafter. This was due to the excretion of unabsorbed test substance. No test substance-related adverse effects were observed for body weight, food consumption, ophthalmology, organ weights, and hematology. Increased protein levels > 100 mg/dL were found in 1 high-dose male and increased protein levels between 30-100 mg/dL in 1 high-dose male, 2 mid-dose males, 3 low-dose males, 2 control males, and 2 high-dose females. Crystals in urinary sediment were observed in 1 control male, 1 low-dose male, 3 mid-dose males, 2 high-dose males, and 2 high-dose females. The urinary findings were not accompanied by significant changes in other parameters including clinical chemistry, gross findings, or histopathological examination. Thus, these urinary findings were not considered of toxicological concern. The authors concluded that the NOAEL for PQQ disodium salt was determined to be 100 mg/kg bw/day, the highest level tested.

In another 90-day study conducted in SD rats (10/sex/group), synthetic PQQ disodium salt was administered by gavage at doses of 0 (control), 100, 200, or 400 mg/kg bw/day (Liang et al., 2015). PQQ disodium salt was well-tolerated and no treatment-related abnormalities were

observed in body weight, food consumption, clinical chemistry and hematology, absolute and relative organ weights, and histopathological changes although one rat in the high-dose group was reported to have deposition of calcium salts in the renal tubule (additional details not specified). The authors concluded that a NOAEL of PQQ disodium salt was 400 mg/kg bw/day, the highest dose tested. However, urinary parameters were not reported by the authors.

Effects of PQQ Administered by Intraperitoneal Administration

In addition, GRNs 625, 641, and 709 discussed a study by Watanabe et al. (1989) that was published in Japanese. The intraperitoneal administration of PQQ to rats for 4 days at a dose of 11.5 mg/kg bw/day also produced clear functional and morphologic changes of the kidneys (*i.e.*, vacuolar degeneration, atrophy, and necrosis of the proximal tubular epithelium in the renal cortex, dilation and regeneration of the tubules). The most prominent finding was necrotic and degenerative changes of the proximal tubular epithelium as well as hematuria and an elevation of serum creatinine concentration. Urinalysis revealed increased excretion of protein, glucose, ketone body, and occult blood, although the statistical significance of these changes was not addressed. The PQQ group had significantly higher blood urea nitrogen and serum concentrations of creatinine and glutamate pyruvate transaminase and glutamate oxaloacetate transaminase activities levels, while having significantly lower serum triglycerides concentrations. Gross examination revealed swelling of the kidneys, which was accompanied by increased absolute and relative kidney weights, the latter of which was significant.

This nephrotoxicity was confirmed in a study by Zhu et al. (2006) who compared the cardioprotective effects of PQQ with metoprolol. The authors noted that high-dose PQQ (20 mg/kg, i.v.) produced renal and hepatic toxicity and that PQQ (3 mg/kg) given at the onset of reperfusion had no evident renal or hepatic toxicity. However, details were not provided.

Route differences may have significant impact on the safety profile. In addition, these studies tested effects of bolus doses (after intravenous administration of 20 mg/kg or intraperitoneal administration of 11.5 mg/kg), which would not occur following oral ingestion. Thus, the data presented in these papers may not be relevant when evaluating the safety of orally consumed PQQ disodium salt, especially because the substance is incorporated in foods.

Taken together, it is reasonable to conclude that the NOAEL of ZMC's PQQ disodium salt is 200 mg/kg bw/day.

Human Clinical Studies

Since the FDA's last review of GRN 625 (pages 43-44), GRN 641 (pages 39-40), GRN 701 (pages 30-32), and GRN 709 (pages 36-37), two human clinical studies have been published (Hwang et al., 2020; Shiojima et al., 2021). These studies did not find any adverse effects of PQQ disodium salt when 23 male subjects consumed 20 mg per day for 6weeks (Hwang et al., 2020) or when 64 healthy subjects ingested a daily dose of 21.5 mg for 12 weeks (Shiojima et al., 2021).

GRNs 625, 641, 701, and 709 also reported that no human clinical studies reported adverse effects of PQQ disodium salt (Itoh et al., 2016; Nakano et al., 2012, 2015a, 2015b, 2016;Rucker et al., 2009). Daily doses up to 60 mg per person were well tolerated and did not result 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. Studies employing less than 3 weeks of intervention are not included in this review.

Safety of Production Microorganism

Methylovorus glucosotrophus is a facultatively methylotrophic, non-spore forming, gram-negative, straight or slightly curved rod-shaped bacteria (Govorukhina and Trotsenko, 1991). Further morphological and biochemical analyses demonstrate that *Methylovorus glucosotrophus* is a gram-negative bacterium that forms pink, creamy, or milky colonies. *Methylovorus glucosotrophus* is not a genetically modified organism. *Methylovorus glucosotrophus* is used for the production of PQQ disodium salt and is maintained in-house by ZMC and is subject to strict quality control for compliance with established internal specifications. The strain was subjected to whole genome sequencing and bioinformatics analysis to determine the safety of the strain. The whole genomic DNA of the samples was firstly extracted and tested, and then gene assembly was performed to obtain the genomic sequence. Based on whole genomic sequence and bioinformatics analysis of the production microorganism, antibiotic resistance genes and virulence genes were screened using the Comprehensive Antibiotic Resistance Database (CARD), ResFinder database, Virulence Factors of Pathogenic Bacteria (VFDB), VirulenceFinder database, and PathogenFinder database. No genes related to antibiotic resistance, pathogenicity, or virulence were detected, indicating that the ZMC's production microorganism, *Methylovorus glucosotrophus,* is non-pathogenic and non-toxigenic.

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, 641, 701, and 709 (FDA, 2016a, 2016b, 2017b, 2018). All of the GRAS notices provided information/clinical study data that supported the safety of the proposed PQQ disodium salt ingredients for use in human foods. 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 through scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. The PQQ disodium salt that is the subject of this GRAS determination is produced by a *Methylovorus glucosotrophus* strain, a non-pathogenic and non-toxigenic strain, and its purity is over 98%. The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in food manufacturing processes.

Subchronic oral toxicity and teratogenicity studies conducted on PQQ disodium salt ingredient manufactured by ZMC using *Methylovorus glucosotrophus* reported the NOAEL value as 200 mg/kg bw/day in rats (Lee et al., 2022; Seol et al., 2022). Subchronic oral toxicity studies of other sources of PQQ disodium salt (either produced via *Hyphomicrobium denitrificans* or chemical synthesis) reported the NOAEL values of 100 to 400 mg/kg bw/day in rats. Although potential nephrotoxicity was reported by a research group for PQQ disodium salt produced by *Hyphomicrobium denitrificans*(Nakano et al., 2014), the studies of ZMC's PQQ disodium salt by Seol et al. (2022) did not find any abnormalities in urinary parameters at doses of up to 200 mg/kg bw/day. Thus, it is reasonable to conclude that the NOAEL of ZMC's PQQ disodium salt is 200 mg/kg bw/day, the highest level tested.

The literature also contains a wealth of publicly available studies on the safety of PQQ disodium salt in humans.

Conclusion

We, the undersigned members of the Expert Panel, have individually and collectively critically evaluated the materials summarized above on the safety of ZMC's PQQ disodium salt and other information deemed appropriate and unanimously conclude that ZMC's PQQ disodium salt, manufactured as described in the dossier and consistent with cGMP, and meeting appropriate food grade specifications, is GRAS based on scientific procedures for use as an ingredient in beverage products at 8-20 mg/serving at levels specified in the accompanying dossier. It is our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions.

[redacted] 9/02/22

Susan Cho, Ph.D. Date

AceOne RS, Inc. (formerly NutraSource, Inc.), Clarksville, Maryland, U.S.A.

[redacted] 8/27/22

George C. Fahey, Jr, Ph.D. Date

Professor Emeritus, University of Illinois, Urbana, Illinois, U.S.A.

[redacted] 8/29/22

Michael Falk, Ph.D. Date

LSRO Solutions LLC, Rockville, Maryland, U.S.A.

[redacted] 9/1/22

Yong-Su Jin, Ph.D. Date

Professor, University of Illinois, Urbana, Illinois, U.S.A.

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Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001118	Oct 11, 2022
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Wan Zhang	Senior Scientist

Organization *(if applicable)*: Zhejiang Medicine Co., Ltd.

Mailing Address *(number and street)*: 98 East XinchangDadao Road

City	State or Province	Zip Code/Postal Code	Country
Xinchang	Zhejiang Province	312500	China

Telephone Number	Fax Number	E-Mail Address
+86-575-8602-4939		zhangwan@zmc-china.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Susan S. Cho	Chief Science Officer

Organization *(if applicable)*: AceOne RS, Inc.

Mailing Address *(number and street)*: 5903 Hampton Forest Way

City	State or Province	Zip Code/Postal Code	Country
Fairfax	Virginia	22030	United States of America

Telephone Number	Fax Number	E-Mail Address
3018756454	7039980103	scho@aceoners.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Pyrroloquinoline Quinone Disodium Salt

2. Submission Format: *(Check appropriate box(es))*

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 000709

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☒ e) Other or Additional *(describe or enter information as above)* GRN 000641, 000694, 000701, and 000625; NDIN RPT 417

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Intended use and use levels, in terms of per serving size, of ZMC's PQQ disodium salt is the same as those employed in GRN 709. ZMC proposes to use PQQ disodium salt as a food ingredient in selected beverages (such as energy, sports, and electrolyte drinks, enhanced and fortified water beverages, bottled water, and non-milk-based meal replacement beverages) at 8-20 mg/serving. ZMC does not intend to use PQQ disodium salt as a component of infant formula or in foods under the United States Department of Agriculture (USDA)'s jurisdiction such as meat, poultry, and egg products. The population expected to consume the substance consists of members of the general population who consume at least one of the products described above.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Zhejiang Medicine Co., Ltd.
(name of notifier)

has concluded that the intended use*(s)* of pyrroloquinoline quinone (PQQ) disodium salt
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Zhejiang Medicine Co., Ltd. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

98 East XinchangDadao Road, Xinchang, Zhejiang Province, 312500 P. R. China
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	Wan Zhang, Senior Scientist	09/14/2022

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	PQQGRASfinal9-14-22SubmittedtoFDA.pdf	Administrative

May 31, 2023

To: Dr. Kaiping Deng, Ph.D.
Division of Food Ingredients
Center for Food Safety and Applied Nutrition

Subject: Amendment to GRN 001118, Pyrroloquinoline Quinone (PQQ) Disodium Salt

From: Susan Cho at AceOne RS, Inc., Agent for Zhejiang Medicine Co., Ltd. (ZMC)

Dear Dr. Deng,

In responses to FDA questions, **Zhejiang Medicine Co., Ltd.** (ZMC) has prepared its responses as follows:

FDA Question 1.
In Table 5 (p. 19) of the notice, you provide the results of the analyses of three batches of PQQ disodium salt. Please clarify whether these batches were manufactured non-consecutively. If the three batches were consecutive, please provide additional data so that results from the analyses of three non-consecutive batches have been provided.

ZMC's Response:

The three batches (311PQ210501, 311PQ210502, and 311PQ210601) provided in the original GRAS notification were manufactured consecutively.

The results of three additional, non-continuous batches (311PQ210702, 311PQ221101, and 311PQ221103) are listed below in Table 1, and the Certificates of Analysis (CoAs) are presented in Annex 1.

Table 1: Test Results of Three Non-continuous Batches

Test Items	**Specifications**	311PQ210702	311PQ221101	311PQ221103
Appearance	Red or reddish -brown powder	Reddish -brown powder	Reddish -brown powder	Reddish -brown powder
Identification	Conforms to the reference solution	Conform	Conform	Conform
Water content, g/100 g	≤ 13.0	8.7%	8.9%	8.9%
PQQ (dry wt. basis), g/100 g	≥ 85	88%	88%	89%
PQQ disodium salt (dry wt. basis), g/100 g	≥98.0	100.0%	100.1%	100.5%

Sodium content (dry wt. basis), g/100 g	10.5-12.9	12.3%	12.3%	12.1%
Lead, mg/kg)	≤ 0.2	0.0503	< 0.0016	0.0170
Arsenic, mg/kg	≤ 0.1	< 0.0334	< 0.0334	< 0.0334
Cadmium, mg/kg	≤ 0.1	0.0206	< 0.0004	0.0042
Mercury, mg/kg	≤ 0.1	< 0.0090	< 0.0090	< 0.0090
Microbial Limit				
Total aerobic counts, cfu/g	≤ 1,000	< 10	< 10	< 10
Yeasts and molds count, cfu/g	≤ 100	< 10	< 10	< 10
Enterobacteria, cfu/g	< 10	< 10	< 10	< 10
Escherichia coli, in 25g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
Staphylococcus aureus, in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g
Salmonella, in 25g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
Listeria, in 25g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g

FDA Question 2.
In Table 4 (p. 18) of the notice, the method of analysis cited for the determination of PQQ, PQQ disodium salt, and sodium content is USP chapter 621 (chromatography). The monograph for PQQ disodium salt listed in the USP-NF includes multiple analytical methods, including identification by infrared spectroscopy or nuclear magnetic resonance spectroscopy, content of PQQ disodium salt by liquid chromatography and content of sodium by ion chromatography. Please clarify the identities of the methods used for these specification parameters.

ZMC's Response:

The method used for the determination of PQQ, PQQ disodium salt, and sodium content was USP chapter 621 (High-performance liquid chromatography (HPLC) for PQQ and PQQ disodium salt and ion chromatography for content of sodium).

The detailed methods of analysis are presented below:

1) PQQ and PQQ disodium salt

Chromatographic Condition

Mode: LC

Detector: UV 259 nm

Column: Octadecyl silane bonded silica gel chromatographic column (i.e., Kromasil C18, 4.6mm × 15cm, 5μm)

Column temperature: 30℃

Flow rate: 1.0 mL/min

Injection volume: 20 μL

Run time: 30 min

Buffer: Prepare a solution containing 10 mM dibasic potassium phosphate and 15 mM tetrabutylammonium bromide in water. Adjust the solution with phosphoric acid to a pH of 7.4.

Mobile phase: Acetonitrile and buffer solution (28:72)

Diluent: Acetonitrile and water (1:3)

Glycine standard solution: 0.1 mg/mL of USP Glycine RS in water

Standard solution: 0.1 mg/mL of PQQ disodium RS in diluent

System suitability solution: Transfer 1 mL of the Standard solution and 0.1 mL of the Glycine standard solution to an HPLC vial, cap the vial, and heat at 60° in a water bath for 30 min. Cool in an ice bath to room temperature and analyze immediately.

Sample solution: 0.1 mg/mL of PQQ disodium in diluent

Suitability requirements

Resolution: NLT 2.5 between PQQ and imidazole pyrroloquinoline, System suitability solution

Tailing factor: NMT 2.0, Standard solution

Relative standard deviation: NMT 2.0%, Standard solution

Procedure

Samples: Standard solution and Sample solution

Calculate the percentage of PQQ disodium ($C_{14}H_4N_2Na_2O_8$):

$$\text{Result} = (r_u/r_s) \times (Cs/Cu) \times 100$$

r_u= Peak response of PQQ from the Sample solution

r_s= Peak response of PQQ from the Standard solution

Cs= Concentration of USP PQQ disodium RS in the Standard solution (mg/mL)

Cu= Concentration of PQQ disodium in the Sample solution (mg/mL)

Calculate the percentage of PQQ ($C_{14}H_6N_2O_8$):

$$\text{Result} = (r_u/r_s) \times (Cs/Cu) \times 330.17/374.17 \times 100$$

330.17= Relative molecular mass of pyrroloquinoline quinone ($C_{14}H_6N_2O_8$)

374.17= Relative molecular mass of pyrroloquinoline quinone disodium ($C_{14}H_4N_2Na_2O_8$)

2) Content of sodium

Chromatographic Condition

Mode: Ion chromatography

Detector: Conductivity

Columns

Guard: 4mm × 5cm; 7.0- to 9.0-µm packing L106 (i.e., Thermo Dionex Ionpac™ CG12A, 4mm × 5cm)

Analytical: 4mm × 25cm; 7.0- to 9.0-µm packing L106 (i.e., Thermo Dionex Ionpac™ CG12A, 4mm × 25cm)

Cation suppressor: Use a self-regenerating cation suppressor. Set the current to 70 milliamperes or according to manufacturer's recommendations to achieve optimal signal-to-noise ratio.

Column temperature: 35℃

Flow rate: 1.0 mL/min

Injection volume: 25 µL

Run time: 20 min

Mobile phase: 20 mM methanesulfonic acid in water

Standard solutions: Prepare solutions containing 4, 8, 12, 16, 20, and 24 µg/mL of sodium in water from commercially available sodium Standard solution for ion chromatography.

Sample solution: 0.2 mg/mL of PQQ disodium in water

System suitability

Sample: Standard solution (16 µg/mL)

Suitability requirements

Column efficiency: NLT 5000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Correlation coefficient: NLT 0.99 for the regression line as determined in analysis

Procedure

Samples: Standard solution and Sample solution

Plot the sodium peak responses against the sodium concentrations, in mg/mL, in the six Standard solutions, and establish a calibration curve via least-squares regression. Using the sodium peak response from the Sample solution, calculate the concentration, C, in mg/mL, of sodium in the Sample solution.

Calculate the percentage of sodium in the portion of PQQ disodium taken:

$$\text{Result} = (C/C_u) \times 100$$

C = Concentration of sodium in the Sample solution (mg/mL) determined from the calibration curve

C_u = Concentration of PQQ disodium in the Sample solution (mg/mL)

FDA Question 3.
The specification limits for lead, arsenic, cadmium, and mercury are listed as ≤ 0.2 mg/kg and the results of the analyses of three batches of PQQ disodium salt for these metals are reported as < 0.2 mg/kg in all cases. Please provide the limit of quantitation for the method used in the analyses of these heavy metals. We note that specifications help to ensure that the ingredient is being manufactured in accordance with good manufacturing practices, and we would like to remind you of FDA's recent "Closer to Zero" initiative that focuses on reducing dietary exposure to lead, arsenic, cadmium and mercury from food. Further, we request that specifications for heavy metals be as low as possible and consistent with the methods used and the results obtained from the batch analyses.

ZMC's Response:

We analyzed heavy metals present in PQQ disodium salt according to the ICH Guideline Q3D on elemental impurities. A detailed analytical method validation report for heavy metals in PQQ disodium salt is presented in Annex 2. We lowered the limits of heavy metals to ≤ 0.1 mg/kg for arsenic, cadmium, and mercury. We tested the previous batches of PQQ disodium salt, and the test results are shown below:

	As (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	Pb (mg/kg)
LOQ	0.0334	0.0004	0.0090	0.0016
311PQ210501	<0.0334	0.0080	<0.0090	0.0953
311PQ210502	<0.0334	0.0088	<0.0090	0.0691
311PQ210601	<0.0334	0.0054	<0.0090	0.0334
311PQ221101	<0.0334	<0.0004	<0.0090	<0.0016
311PQ210702	<0.0334	0.0206	<0.0090	0.0503
311PQ221103	<0.0334	0.0042	<0.0090	0.0170
311PQ211001	<0.0334	0.0122	<0.0090	0.1388

LOQ=Limit of quantitation.

FDA Question 4.
You state that the original strain (Methylovorus glucosotrophus) for producing PQQ disodium salt was isolated from soil samples in China and provided the lot number of the strain submitted for genome sequencing. If the strain is deposited in
a publicly accessible culture collection, please provide the depository information. Please note that an available depository name should be used for a GRAS notice to ensure the strain is available and can be verified.

ZMC's Response:

The original *Methylovorus glucosotrophus* strain has been deposited in China General Microbiological Culture Collection Center (CGMCC) with the following code: CGMCC NO.4096. The preservation credential is presented in Annex 3.

FDA Question 5.

In Table 30 (p. 55) of the notice, you describe the taxonomic classification of Methylovorus glucosotrophus. Please provide a reference or guideline which the taxonomical analysis followed.

ZMC's Response:

We used the following website for taxonomical analysis:
'*Methylovorus glucosotrophus* | Type strain | DSM 6874, BKM 1745, VKM B-1745 | BacDiveID:7244 (dsmz.de)'. This site does not show Subdivision anymore.

Table 2: Taxonomic Classification of *Methylovorus glucosotrophus*

Class	Scientific Classification
Kingdom	Prokaryota
Division	Bacteria
Subdivision	Proteobacteria
Class	Betaproteobacteria
Order	Nitrosomonadales
Family	Methalophilaceae
Genus	Methylovorus
Species	*Methylovorus glucosotrophus*

The website, 'ITIS - Report: *Methylovorus glucosotrophus*', lists Phylum as Proteobacteria and Order as Methalophilales instead of Nitrosomonadales.

FDA Question 6.

You describe the purity of PQQ disodium salt in the final product as >98%. Please provide information to clarify whether the Methylovorus glucosotrophus strain produces undesirable secondary metabolites that are co-purified with the PQQ disodium salt.

ZMC's Response-

Undesirable secondary metabolite, imidazole pyrroloquinoline (IPQ), is possibly produced during the fermentation process of PQQ. As shown in Table 3, IPQ is removed during the purification process. The maximum limits of IPQ in the 1st crude, 2nd crude, and the finished ingredient are less than 2%, 0.1%, and 0.1%, respectively. The analysis of three non-consecutive lots revealed no detectable amounts of IPQ in the finished ingredient. The analytical method for the related

substances and content of the fermentation broth, 1st crude, 2nd crude, and finished product are in accordance with the current USP monograph. The test results of IPQ are shown in Table 3.

Table 3: Test Results for IPQ During the Production of PQQ Disodium Salt

Samples	Limit	Test results		
		311PQ210702	311PQ221001	311PQ221102
Fermentation broth	/	0.65%	0.24%	0.97%
1st crude	2%	0.03%	0.06%	0.21%
2nd crude	0.1%	n.d.	0.01%	0.03%
Finished product	0.1%	n.d.	n.d.	n.d.
Remark: "n.d." refers to not detected.				

FDA Question 7.

For the administrative record, please briefly specify how the purity of the Methylovorus glucosotrophus inoculum is ensured.

ZMC's Response:

We established specifications for the master cell bank (MCB) and working cell bank (WCB) and periodically carry out strain identification of the MCB to ensure no mutations occur to *Methylovorus glucosotrophus*. In addition, stability studies are periodically performed for the MCB and WCB. We also established specifications for the inoculum, including appearance, pH, optical density (OD), and microscopic observation. It can be considered that *Methylovorus glucosotrophus* grows well if the appearance is white or light pink, has a pH 4.5~6.5, and exhibits an OD $\geq$ 0.5. Microscopic observation can directly show whether the strain is contaminated with foreign bacteria. Only qualified inoculum can be used for the production of PQQ disodium salt.

We are monitoring the quality of MCBs, WCBs, and inoculums used for the production of PQQ disodium salt and the stability of MCBs and WCBs to make sure that all meet our internal standards.

FDA Question 8.

Please clarify that the fermentation process is continuously monitored for contaminants.

ZMC's Response:

ZMC continuously monitors contaminants during the PQQ fermentation process via microscopic examination to ensure that no foreign bacteria are present in the PQQ inoculum before seed cultivation. After completing seed cultivation and before transferring to fermentation culture, microscopic examination is performed. During the fermentation stage, samples are taken for microscopic examination on the second morning. Afterwards, samples are taken every 12 hours for microscopic examination. After fermentation is complete, microscopic examination is carried out again. In addition, the appearance, the pH, the OD, and microscopic observation of the fermentation broth are observed and executed. If these indicators exceed the established range, the bacterial body

may be contaminated. If the results of above items exceed the specified limit, this would indicate that the mycelium may be contaminated, which can promptly reflect whether the inoculum is contaminated.

FDA Question 9.

Please provide the details/parameters of your updated literature search/review, including the date range covered in your review.

ZMC's Response:

The literature search was performed using PubMed. The key words pyrroloquinoline quinone, or PQQ, were searched. Relevant animal toxicity and efficacy studies as well as human clinical studies published between January 2018 and July 2022 were manually searched.

FDA Question 10.

The EFSA publication on the safety of PQQ disodium salt was not discussed in your GRAS notice. The scientific panel concluded a PQQ disodium salt no observed adverse effect level (NOAEL) of 100 mg/kg body weight (bw)/d based on a 90-day study, which included a thorough urinalysis component.

ZMC's Response:

We acknowledge that the EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) set the NOAEL of PQQ sodium salt as 100 mg/kg bw/day based on a 90-day toxicity study corresponding to a urinalysis reporting crystal formation at higher doses.

The Expert Panel was aware of the EFSA NDA report, which set the NOAEL of PQQ disodium salt as 100 mg/kg bw/day. However, the Expert Panel believes the NOAEL of 200 mg/kg bw/day is appropriate for ZMC Medicine's PQQ disodium salt because WuXi AppTec (Suzhou) Co., Ltd., a reputable Good Laboratory Practice (GLP)-certified laboratory in China (or Seol et al., 2022), reported no abnormalities found from the urinalysis at this level. It is not likely that such a reputable GLP-certified laboratory would have overlooked urinary crystal formation. Considering the study results by Nakano et al. (2014), the urinary crystal issue should have been specifically discussed in this GLP-certified laboratory report.

Thus, Dr. Fahey at University of Illinois-Urbana, Champaign and Dr. Michael Falk at LSRO Solutions, and Susan Cho re-discussed the issue on May 23, 2023. However, the Panel is willing to lower the NOAEL to 100 mg/kg bw/day recognizing that the GLP-certified laboratory did not specifically mention that 'no crystal was found' during the urinalysis.

FDA Question 11.
For supporting your safety conclusion, you list five GRAS notices (000625, 000641, 000694, 000701, and 000709) related to PQQ disodium salt. As each GRAS notice stands on its own, for the administrative record, please provide a brief paragraph summarizing the information pertaining to safety for each of these GRAS notices.

ZMC's Response:

The following summarizes information pertaining to safety for each of the five previous GRAS notices.

GRN 000625 (stamped pages 28-44), filed by Nascent Health Sciences, is the first GRAS notice related to PQQ disodium salt. The subject of GRN 000625 was produced by a chemical synthesis.

A. The safety of its synthetic PQQ disodium salt (>98% purity) was summarized as follows:

1) PQQ disodium salt was not mutagenic nor genotoxic in the bacterial reverse mutation test, sperm malformation assay in mice, and in vivo mouse micronucleus assay.
2) The LD_{50} was considered to be 5,010 mg/kg bw (3,440 to 7,300 mg/kg/bw) in females and 3,690 mg/kg bw (2,710 to 5,010 mg/kg/bw) in males.
3) Subchronic toxicity study (Liang et al., 2014): Eighty healthy Sprague-Dawley rats (10/sex/group) received 0, 100, 200, and 400 mg/kg bw of PQQ disodium salt daily by gavage for 90 days. No treatment-related abnormalities were observed in any parameters, such as mortality, body weight, food intake, weight gain, food utilization rate, hematology, biochemistry, organ weight, and histopathological examination parameters. The NOAEL of PQQ disodium salt was considered to be 400 mg/kg bw/day in rats. However, this study did not perform urinalysis.
4) Teratogenicity study (unpublished report): Pregnant Wistar rats (SPF grade) randomly received 0, 78, 310, and 1250 mg/kg/bw of PQQ disodium salt by gavage from the 7th to the 16th day of gestation (n=24/group). Pregnant rats were euthanized on the 20th day. PQQ disodium salt had no significant effects on embryo survival and development, fetal gross malformations, and fetal bone and organ development. Pregnant rats receiving the highest dose had transient, significantly reduced body weights on days 9 and 12 compared to the rats receiving the negative control. However, they were not considered to be toxicologically significant as the body weight reductions were transient. The authors concluded that PQQ disodium salt was not teratogenic.

B. Safety of Other Sources of PQQ disodium salt
In addition, GRN 000625 reviewed the safety of other sources of PQQ disodium salt, in particular, BioPQQ™, PQQ disodium salt (99.1% purity) produced by bacterial fermentation via *Hyphomicrobium denitrificans* (Mitsubishi Gas Chemical Co. Inc.). It was evaluated in in vitro (Nakano *et al.,* 2013) and in vivo (Nakano et al., 2014) assays.

1) PQQ disodium salt was not mutagenic nor genotoxic (Nakano et al., 2013)
2) The acute oral LD_{50} value for males was reported to range from 1,000-2,000 mg/kg/bw. The corresponding LD_{50} in females was 500-1,000 mg/kg/bw (Nakano *et al*., 2014).
3) In a 28-day, repeat-dose, follow-up study in female SD rats (12/group), rats received one of the following three treatments: 0, 200, and 700 mg/kg bw/d for 28 days. At the end of the

administration period, six animals from each group were exsanguinated and necropsied. The remaining six animals in each group were monitored during a 28-day recovery period. Slight-to-moderate elevations in urinary protein and crystals were reported in the 200 and 700 mg/kg/bw groups; however, these changes were not dose-responsive and resolved by the end of the 4-week recovery period. No other corresponding changes in clinical chemistry or histopathology suggestive of kidney toxicity were observed (Nakano et al., 2014).

4) In the 90-day study, rats received one of the following four treatments for 90 days: 0, 3, 20, or 100 mg PQQ/kg bw/day by oral gavage (N=10/sex/group). No toxicologically significant effects were reported with respect to hematology, clinical biochemistry, urinalysis, gross necropsy, or histopathology. Based on the results of these three studies, the NOAEL for PQQ was determined to be 100 mg/kg bw/day (Nakano et al., 2014).

This GRAS notice also summarized human clinical studies reporting no adverse effects at daily doses up to 20-60 mg/person. Repeated consumption of 20 mg PQQ per day for 8 to 12 weeks was well-tolerated in healthy adult subjects (8-week study, Nakano et al., 2012; 12-week study, Nakano et al., 2009). Additionally, a double-blind study evaluating the administration of PQQ to human subjects (information not provided) for 4 weeks at doses of 60 mg/day did not induce adverse effects (unpublished data reviewed in Nakano et al., 2014).

GRN 000641, (pages 22-24; 30-40), filed by Zhejiang Hisun Pharmaceutical Co., Ltd. (Hisun), summarized the following studies:

1) PQQ, produced by *Hyphomicrobium denitrificans* ATCC 51888, was found to be negative in the reverse mutation (Ames) assay, the in vivo micronucleus assay, and in chromosomal aberration tests (Zhejiang Hisun Pharmaceutical Co., Ltd., 2012; Nakano et al., 2013).
2) NOAEL determinations of 100 mg/kg/bw and 400 mg/kg/bw as reported by Nakano et al. (2014) and Liang et al. (2014) were considered appropriate when evaluating the safety of Hisun's PQQ disodium salt. These determinations are further supported by the fact that the chemical composition of Hisun's PQQ ingredient are substantially equivalent to those studied by Nakano et al. (2014) and Liang et al. (2014).
3) Repeated consumption of 20 mg PQQ per day for 8 to 24 weeks was well-tolerated in healthy adult subjects (Nakano et al., 2009, 2012; Koikeda et al., 2011). Additionally, a double-blind study evaluating the administration of PQQ to 10 healthy subjects for 4 weeks at doses of 20 or 60 mg/day did not induce any toxicologically relevant changes in standard clinical tests or markers of liver toxicity (as reviewed in Rucker et al., 2009).

GRN 000694 (pages 17-22), filed by Fuzhou Contay Biotechnology Co., Ltd., summarized the following studies:

1) The study by Nakano et al. (2013) reported that PQQ disodium salt, produced by *Hyphomicrobium denitrificans*, was not genotoxic nor mutagenic.
2) A 13-week toxicity study in Sprague-Dawley rats reported the NOAEL of PQQ disodium salt as 400 mg/kg bw/day (Liang *et* al., 2015). However, this study found no unscheduled deaths and no treatment-related abnormalities in food consumption, body weight gain, and histopathological examination parameters.

3) At 1,000 and 2,000 mg/kg dose levels in an acute oral toxicity study, adverse effects were observed. At 2,000 mg/kg/bw, 17 rats died in a 7-day period, and hypothermia was observed in 3 female rats and 3 male rats at the same dose level. At the 1,000 and 2,000 mg/kg/bw dose levels, decreases in body weight gain, enlarged kidneys and livers, soft feces and diarrhea, and decreases in defecation were observed in all animals (Nakano et al., 2014).
4) In a 14-day, repeated-dose study, urinalysis revealed increased sodium levels in the high-dose group as well as green colored content in the cecum of 3 animals of each sex. An increase in relative kidney weight was observed in females of the high-dose group. Histopathological examination revealed focal basophilic changes and atrophy of the renal tubules in females of the high-dose group (Nakano et al., 2014).
5) The NOAEL in the 90-day study by Nakano et al. (2014) was considered to be 100 mg/kg bw/day based on the urinalysis (crystal formation).
6) GRN 000694 references GRN 625 and 641, which discusses consumption of PQQ at doses up to 60 mg/kg/day for 4 weeks or 20 mg/kg bw/day for 24 weeks (Nakano et al., 2009, 2013; Rucker et al., 2009; Koikeda et al., 2011).

GRN 000701 (page 22-32), filed by Nutraland USA, summarized the following studies:

1) PQQ disodium salt ingredients, produced by *Hyphomicrobium denitrificans*, were not genotoxic nor mutagenic.
2) Unpublished acute and reproductive toxicity studies: Orally administered PQQ disodium salt caused dose-dependent mortalities with a median lethal dose (LD50) of 3.86 g/kg/bw and a 95% confidence interval of 3.49~4.25 g/kg/bw.
3) Unpublished sperm abnormality test in mice: No treatment-related sperm abnormalities were exhibited at any dose level of PQQ disodium salt at 500–2,000 mg/kg/bw in mice.
4) 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, which are 100 mg/kg bw/day and 400 mg/kg bw/day, respectively. The study by Liang et al. (2015) did not include results from a thorough urinalysis.
5) Animal efficacy studies showed that PQQ at the level of up to 30 mg/kg bw/day (Hamagishi et al., 1990) did not cause any adverse effects on measured outcomes.
6) In human studies, no adverse effects of PQQ were noted at daily doses of up to 20-60 mg/person/day (Itoh et al., 2016; Nakano et al., 2015a, 2015b; Rucker et al., 2019).

GRN 000709 (pages 27-37), filed by Shangdong Jincheng, summarized the following studies:

1) PQQ disodium salt preparations obtained from microbial fermentation by *Hyphomicrobium denitrificans* were not mutagenic nor genotoxic.
2) Orally administered PQQ disodium salt caused dose-dependent mortalities with the median lethal dose (LD50) of 3.47 g/kg/bw with a 95% confidence interval of 3.12-3.84 g/kg/bw.
3) 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. The study by Liang et al. (2015) did not include results from a thorough urinalysis. In the amendment, GRN 000709 states that the NOAEL was set at 100 mg/kg bw/day in rats after considering crystal formation in urine at the 100 mg/kg bw/day level in rats (Nakano et al., 2014).
4) In human studies, no adverse effects of PQQ were noted at daily doses of up to 20-60 mg/person/day (Itoh et al., 2016; Nakano et al., 2015a, 2015b; Rucker et al., 2019).

Overall, GRN 000694 and 000709 specifically state that the NOAEL of PQQ disodium salt was set at 100 mg/kg bw/day based on the study by Nakano et al. (2014), which reported urinary crystal formation at higher dose levels in rats.

Reviewer Comments:
The challenge with the Seol et al. (2022) publication and why it could not be considered pivotal safety data in support of a GRAS conclusion:
There are several issues with the Seol et al. 2022 publication of the subchronic toxicity study, discussed in the GRAS notice to support the safety conclusion of the proposed use of PQQ disodium salt. First, it did not address or thoroughly investigate the issue of crystal formation in the urinary sediment as a result of PQQ disodium salt exposure. This issue was originally reported by Nakano et al. (2014).

Secondly, the Seol et al. study is published in a journal whose academic reputation, peer-review process, readership, and impact factor (as a toxicology or nutrition journal) are not known. This journal is neither indexed in PubMed nor is it easily available/accessible. Currently, there are many open access journals available online. Many such journals and their publishers have been flagged by various sources as non-reliable and/or predatory/pay-to-publish. All this information is now publicly available. Therefore, FDA is not able to independently verify the quality and reliability of journals (and their publishers) that are not in PubMed. Subject matter experts tend to rely more or exclusively on journals/publications that are available in PubMed.

ZMC's Response:

Seol et al. (2022) submitted the manuscript with the same content to *Regulatory Toxicology and Pharmacology*. The journal declined the review for publication because:

1) Similar papers reporting a 90-day toxicity study of PQQ disodium salt have already been published in another journals (Liang et al., 2015; Nakano et al., 2014).

2) The dose levels tested in ZMC's study were already covered in the study by Laing et al. (2015), which confirmed no toxicity of PQQ disodium salt (ZMC, up to 200 mg/kg bw/day; Laing et al., up to 400 mg/kg bw/day). Although it was important for ZMC to confirm no toxicity at up to 200 mg/kg bw/day, the findings from this study did not have a significant impact in the research community as the study did not report any new findings not covered by Liang et al. (2015), and the highest test dose level in the ZMC study was lower than that employed in the published study by Liang et al. (2015).

The journal did not address the validity of the research or the conclusions of the Seol et al (2022) study, therefore the journal's decision to decline to publish is neutral on the conclusions of the study. We will not dispute FDA's position on ability to verify journal in which this study was published. This essentially relegates the study to the status of unpublished research.

The conclusion of GRAS status by the expert panel is based on the substantial equivalence of the ZMC PQQ ingredient to the PQQ ingredients in the previously submitted GRAS notifications and

on the wealth of scientific evidence offered in support of those notifications as well as the corroborative support provided by the Seol et al (2022) study on the ZMC PQQ ingredient.

While we agree that the Seol et al (2022) publication could have addressed the issue of urinary crystal formation at higher PQQ doses, there is no evidence that PQQ induces urinary crystal formation at 100 mg/kg bw/day or lower. Therefore, the NOAEL of 100 mg/kg bw/day remains supportable and the conclusions of GRAS status under the conditions of use is still as valid as it was when the previous GRAS notifications were submitted (The conclusions of the GRAS status and the response to FDA questions were discussed among the Expert Panel members on May 28-30, 2023).

We hope we properly responded to FDA questions. If you need further clarification, please contact me.

Sincerely,

Susan Cho, Ph.D.
AceOne RS, Inc.
Agent for ZMC
scho@aceoners.com or susanscho1@yahoo.com
+1-301-875-6454

Annex 1. CoAs

Commented [Z1]: The CoAs are updated for the results of heavy metals

Batch No. 311PQ210702

浙江医药 ZMC

Report Date: 2023-05-29

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C1090072
Specification	Chemical intermediate	Batch No.	311PQ210702
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2021/07/19	Quantity	19.26kg
Expiry Date	2023/07/18	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	8.7%
4 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	88%
5 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.0%
6 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.3%
7 Lead, mg/kg	USP<233>	≤0.2	0.0503
8 Arsenic, mg/kg		≤0.1	<0.0334
9 Cadmium, mg/kg		≤0.1	0.0206
10 Mercury, mg/kg		≤0.1	<0.0090
11 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "1" is performed once a year Only for registration

Printer: 2023.05.29 Reviewer: 2023.05.29 Approver: 2023.05.29

Mfg:Zhejiang Medicine Co., Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675

Add:98 East Xinchang Daolao Road , Xinchang , Zhejiang, 312500 P.R.China
Email: QC@zcpharma.com 02

Batch No. 311PQ221101

浙江医药 ZMC

Report Date: 2023-05-29

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C2120010
Specification	Chemical intermediate	Batch No.	311PQ221101
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2022/11/11	Quantity	20.62kg
Expiry Date	2024/11/10	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	8.9%
4 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	88%
5 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.1%
6 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.3%
7 Lead, mg/kg	USP<233>	≤0.2	<0.0016
8 Arsenic, mg/kg		≤0.1	<0.0334
9 Cadmium, mg/kg		≤0.1	<0.0004
10 Mercury, mg/kg		≤0.1	<0.0090
11 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "[1]" is performed once a year Only for registration

Printer: 2023.05.29 Reviewer: 2023.05.29 Approver: 2023.05.31

Mfg.:Zhejiang Medicine Co., Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675
Add.:98 East Xinchang Dadao Road , Xinchang , Zhejiang, 312500 P.R.China
Email: QC@xcpharma.com
02

Batch No. 311PQ221103

浙江医药 ZMC

Report Date: 2023-05-29

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C2120016
Specification	Chemical intermediate	Batch No.	311PQ221103
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2022/11/25	Quantity	25.33kg
Expiry Date	2024/11/24	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	8.9%
4 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	89%
5 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.5%
6 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.1%
7 Lead, mg/kg	USP<233>	≤0.2	0.0170
8 Arsenic, mg/kg		≤0.1	<0.0334
9 Cadmium, mg/kg		≤0.1	0.0042
10 Mercury, mg/kg		≤0.1	<0.0090
11 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "[1]" is performed once a year Only for registration

Printer: 2023.05.29 Reviewer: 2023.05.29 Approver: 2023.05.29

Mfg.:Zhejiang Medicine Co., Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675
Add.:98 East Xinchang Dadao Road , Xinchang , Zhejiang, 312500 P.R.China
Email: QC@xcpharma.com
02

Annex 2. Analytical Method Validation Report for Heavy Metals in PQQ Disodium Salt

1. Purpose

This method validation of arsenic, cadmium, mercury, and lead for PQQ disodium salt was carried out according to ICH Q3D, USP 232, and USP 233.

The validation items included limit of detection (LOD), limit of quantitation (LOQ), linearity, precision, intermediate precision, accuracy, and stability. These items help ensure the accuracy and reliability of this methodology for the testing of heavy metals found in PQQ disodium salt.

2. Items and Requirements

Items	Requirement	Result	Conclusion
1. LOD & LOQ	Inject blank for several times and calculate the LOD&LOQ.	LOQ of ^{75}As is 0.0334 ppm LOD of ^{75}As is 0.0100 ppm. LOQ of ^{111}Cd is 0.0004 ppm; LOD of ^{111}Cd is 0.0001 ppm. LOQ of ^{202}Hg is 0.0090 ppm; LOD of ^{202}Hg is 0.0027 ppm. ^{2}LOQ of ^{208}Pb is 0.0016 ppm. LOD of ^{208}Pb is 0.0005 ppm.	Conform
2. Linearity and range	Correlation coefficient between 0ng/ml and 2.0ng/ml should be not less than 0.99.	Correlation coefficient of ^{75}As is 0.9996. Correlation coefficient of ^{111}Cd is 0.9999. Correlation coefficient of ^{202}Hg is 0.9996. Correlation coefficient of ^{208}Pb is 1.0000.	Conform
3. Precision	Prepare 6 spiked test solutions in parallel. The RSD of content results should be not greater than 20%.	RSD of ^{75}As is 3%. RSD of ^{111}Cd is 2%. RSD of ^{202}Hg is 5%. RSD of ^{208}Pb is 2%.	Conform
4.Intermediate precision	Another analyst repeat the 6 spiked test solutions in parallel. Inject precision solutions and record the concentration results. The RSD of content results should be not greater than 20%. RD of two analysts' results	RSD of ^{75}As is 4%. RSD of ^{111}Cd is 3%. RSD of ^{202}Hg is 4%. RSD of ^{208}Pb is 11%. RD of ^{75}As is 6%. RD of ^{111}Cd is 3%. RD of ^{202}Hg is 6%. RD of ^{208}Pb is 5%.	Conform

Commented [Z2]: Typo

	should be not more than 25%.		
5. Accuracy	The recovery of arsenic, cadmium, mercury and lead at three concentration levels of 0.25ng/ml, 0.5ng/ml and 0.75ng/ml should be between 70% and 150%.	The recovery of arsenic, cadmium, mercury and lead at three concentration levels of 0.25ng/ml, 0.5ng/ml and 0.75ng/ml is between 70% and 150%.	Conform
6. Solution stability	Place the linearity solution and precision solution at room temperature for one day, compare the results with that at 0 hour. The difference of tested concentration for linearity solution 1 should be not more than LOQ. The deviation of tested concentration for linearity solution 2~6 should be not more than 30%. The deviation of tested concentration for precision solution should be not more than 30%.	Place the linearity solution and precision solution at room temperature for 22h and inject these solutions. The maximum deviation of ^{75}As for linearity solution is 5%. The maximum deviation of ^{111}Cd for linearity solution is 4%. The maximum deviation of ^{202}Hg for linearity solution is 2%. The maximum deviation of ^{208}Pb for linearity solution is 3%. The maximum deviation of ^{75}As for precision solution is 7%. The maximum deviation of ^{111}Cd for precision solution is 1%. The maximum deviation of ^{202}Hg for precision solution is 1%. The maximum deviation of ^{208}Pb for precision solution is 1%.	Conform

3. Methods

3.1 Samples and Reagents

Reagents	Manufacturer	Concentration	Expiry date	Batch No.
Nitric acid	Thermo	70%	2023.11.21	1121110
Hydrochloric acid	Adamas	37%	2023.11.16	P2295845
Multi-Element Standard solution 2A (containing As, Cd, Pb)	Aglent	10μg/mL	2023.06.30	51-268CRY2
Standard solution -Hg	Aglent	10μg/mL	2023.06.30	13-70HGY2A

Internal Standard solution	Aglent	100μg/mL	2023.07.31	58-027CRY2
Pyrroloquinoline Quinone Disodium Salt	Xinchang Pharma	/	/	311PQ230302

3.2 Instruments

Instrument	Model/Serial Number	Equipment No.	Calibration validity
Inductively coupled plasma mass spectrometer	7700X	ICP01	2023.06.14
Microwave digestion apparatus	Multiwave PRO	DIG03	2023.06.14

3.3 Instrument Parameters

The settings can be adjusted appropriately according to the needs of optimizing sensitivity, stability, and background level.

Parameters	Setting
Integration time	0.3 sec per point
Peak mode	Full Quant (3)
Repeat times	3
Test mode	He mode
Speed of peristaltic pump	0.1rps
Internal standard element	^{45}Sc , ^{72}Ge , ^{159}Tb , ^{175}Lu , ^{209}Bi
Element to be tested	^{75}As , ^{111}Cd , ^{202}Hg , ^{208}Pb

3.4 Solution Preparation

3.4.1 Digestion procedure

Add 8ml of nitric acid to the digestion tube, heat to 110°C, and keep pre-reaction for 30 min. Subsequently, carry out microwave digestion at 180°C for 1 hour. Finally, cool down the solution and catch acid until the volume is between 1~2ml.

3.4.2 Solution preparation

Diluent: Measure 30ml HNO_3 and 10ml HCl in ultrapure water; add ultrapure water to dilute to 1000ml; and mix well.

Internal Standard solution: Pipette 250μl Internal Standard solution; dilute to 25ml with diluent; and mix well.

Standard curve stock solution A: Pipette 0.1ml of Multi-Element Calibration Standard 2A (10mg/L) to a 10ml volumetric flask; dilute to 25ml with diluent; and mix well.

Standard curve stock solution B: Pipette 0.1ml of Standard solution -Hg(10mg/L) to a 10ml volumetric flask; dilute to 10ml with diluent; and mix well.

Standard curve solution: Accurately pipette 0µl, 10µl, 50µl, 100µl, 150µl, and 200µl of Standard curve stock solution A and 0µl, 10µl, 50µl, 100µl, 150µl, and 200µl of Standard curve stock solution B to a 10ml volumetric flask, and then add 100µl of internal Standard solution. Dilute to the mark with diluent and mix well. (The linear concentrations of arsenic, cadmium, mercury, and lead are 0, 0.1ng/ml, 0.5ng/ml, 1.0ng/ml, 1.5ng/ml, and 2.0ng/ml in sequence corresponding to 0, 0.02ppm, 0.1ppm, 0.2ppm, 0.3ppm, and 0.4ppm of sample).

Blank Sample solution: Conduct digestion procedure and transfer to a 50ml volumetric flask; add 500µl of internal Standard solution; and dilute to the mark with diluent and mix well.

Sample solution: Place 250mg of sample into a digestion tank and conduct digestion procedure. Transfer the rest to a 50ml volumetric flask. Add 500µl of internal Standard solution; dilute to the mark with diluent; and mix well. (If the concentration of the target element in the Sample solution exceeds the concentration range of the standard curve, change the dilution ratio of the Sample solution and the quality control Sample solution so that the concentration of the target element is within the linear range.)

Quality control Sample solution: Weigh 250mg of sample; add 250µl of Standard curve stock solution A and 250µl of Standard curve stock solution B; and conduct digestion procedure. Transfer to a 50ml volumetric flask. Add 500µl of internal Standard solution; dilute to the mark with diluent; and mix well. (The concentrations of spiked arsenic, cadmium, mercury, and lead are 0.5ng/ml and equal to 0.1ppm of sample).

3.5 System Suitability Requirements

3.5.1 The linear correlation coefficient of the standard curve of the element to be tested should be NLT 0.99.

3.5.2 Calibration Control

At the end of the sequence, the calibration control solution should be tested. The relative deviation between the concentration of each element and the theoretical concentration should be NMT ±20%.

3.5.3 Calculation

The recovery of each target element in the quality control Sample solution should be within 70% and 150%. Recovery is calculated by the following formula:

$$R = \frac{A_{found}}{A_{smp} + \frac{A_{add}}{W_{smp}}} \times 100\%$$

In which:

R: Recovery (%)

A_{found}: Content of target element in quality control sample (μg/g)

A_{smp}: Content of target element in unspiked sample (μg/g)

A_{add}: Mass of target element added to quality control sample (μg)

W_{smp}: Weight of quality control sample (g)

The content of the target element in the sample is calculated as follows:

$$X = \frac{(C-B) \times V}{W}$$

In which:

X: Content of target element in sample (μg/g)

C: Concentration of target element in sample solution (ng/ml)

B: Concentration of target element in sample blank solution (ng/ml)

V: Dilution volume of sample solution (ml)

W: Weight of sample (mg)

3.6 Specification

Item	Specification (ppm)
Lead	≤0.2
Arsenic	≤0.1
Cadmium	≤0.1
Mercury	≤0.1

4. Deviation

Validation was carried out according to the protocol, and no deviation occurred.

5. Analytical Method Validation Procedures

5.1 Solution Preparation

Stock	Diluent	Dilute 30ml of HNO_3 and 10ml of HCl with ultrapure

solution		water to 1,000ml, and mix well.
	Standard curve stock solution A	Pipette 0.1ml of Multi-Element Calibration Standard 2A (10mg/L) to a 10ml volumetric flask; dilute to 25ml with diluent; and mix well.
	Standard curve stock solution B	Pipette 0.1ml of Standard solution-Hg (10mg/L) to a 10ml volumetric flask; dilute to 10ml with diluent; and mix well.
	Internal Standard solution	Pipette 250µl of Internal Standard solution (100mg/L); dilute to 25ml; and mix well.
LOD & LOQ	Blank	Add 500µl of internal Standard solution to a 50ml volumetric flask; dilute to the mark with diluent; and mix well.
Linearity and range	Linear solution 1	Add 100µl of internal Standard solution to a 10ml volumetric flask; dilute to the mark with diluent; and mix well.
	Linear solution 2	Accurately pipette 10µl of Standard curve stock solution A and 10µl of Standard curve stock solution B to a 10ml volumetric flask; add 100µl of internal Standard solution; dilute to the mark with diluent; and mix well. (The linear concentrations of arsenic, cadmium, mercury, and lead are 0.1ng/ml).
	Linear solution 3	Accurately pipette 50µl of Standard curve stock solution A and Standard curve stock solution B to a 10ml volumetric flask; add 100µl of internal Standard solution; dilute to the mark with diluent; and mix well. (The linear concentrations of arsenic, cadmium, mercury, and lead are 0.5ng/ml).
	Linear solution 4	Accurately pipette 100µl of Standard curve stock solution A and Standard curve stock solution B to a 10ml volumetric flask; add 100µl of internal Standard solution; dilute to the mark with diluent; and mix well. (The linear concentrations of arsenic, cadmium, mercury, and lead are 1.0ng/ml).
	Linear solution 5	Accurately pipette 150µl of Standard curve stock solution A and Standard curve stock solution B to a 10ml volumetric flask; add 100µl of internal Standard solution; dilute to the mark with diluent; and mix well. (The linear concentrations of arsenic, cadmium, mercury, and lead are

		1.5ng/ml).
	Linear solution 6	Accurately pipette 200μl of Standard curve stock solution A and Standard curve stock solution B to a 10ml volumetric flask; add 100μl of internal Standard solution; dilute to the mark with diluent; and mix well. (The linear concentrations of arsenic, cadmium, mercury, and lead are 2.0ng/ml).
Precision	Blank sample solution	Conduct digestion procedure and transfer to a 50ml volumetric flask; add 500μl of internal Standard solution; dilute to the mark with diluent; and mix well.
	Spiked sample solution	Place 250mg of sample into a digestion tank; add 250μl of Standard curve stock solution A and Standard curve stock solution B; and conduct digestion procedure. Transfer the rest to a 50ml volumetric flask. Add 500μl of internal Standard solution; dilute to the mark with diluent; and mix well. Prepare six copies in parallel.
Intermediate precision	Blank sample solution	Conduct digestion procedure and transfer to a 50ml volumetric flask; add 500μl of internal Standard solution; dilute to the mark with diluent; and mix well.
	Spiked sample solution	Place 250mg of sample into a digestion tank; add 250μl of Standard curve stock solution A and Standard curve stock solution B; and conduct digestion procedure. Transfer the rest to a 50ml volumetric flask. Add 500μl of internal Standard solution; dilute to the mark with diluent; and mix well. Prepare six copies in parallel.
Accuracy	Blank sample solution	Conduct digestion procedure and transfer to a 50ml volumetric flask; add 500μl of internal Standard solution; dilute to the mark with diluent; and mix well.
	Sample solution	Place 250mg of sample into a digestion tank and conduct digestion procedure. Transfer the rest to a 50ml volumetric flask. Add 500μl of internal Standard solution; dilute to the mark with diluent; and mix well.
	Accuracy solution 1	Place 250mg of sample into a digestion tank; add 125μl of Standard curve stock solution A and Standard curve stock solution B; and conduct digestion procedure. Transfer the rest to a 50ml volumetric flask. Add 500μl of internal Standard solution; dilute to the mark with diluent; and mix well. Prepare three copies in parallel.

	Accuracy solution 2	This solution is same as spiked sample solution of precision solution preparation.
	Accuracy solution 3	Place 250mg of sample into a digestion tank; add 375µl of Standard curve stock solution A and Standard curve stock solution B; and conduct digestion procedure. Transfer the rest to a 50ml volumetric flask. Add 500µl of internal Standard solution; dilute to the mark with diluent; and mix well. Prepare three copies in parallel.
Stability	Stability solution	Place linear solution and spiked sample solution for one day and inject these solutions.

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5.2 LOD&LOQ

Inject blank solution 10 times. LOD is 3 times of the results' SD. LOQ is 10 times of the results' SD. See table 5.2-1 for details.

Table 5.2-1: Test Results of LOD&LOQ

Concentration of each element in blank (ng/ml)				
SN	^{75}As	^{111}Cd	^{202}Hg	^{208}Pb
1	-0.0632	-0.0003	0.0142	-0.0035
2	-0.0768	0.0002	0.0070	-0.0036
3	-0.0692	-0.0001	0.0050	-0.0019
4	-0.0974	-0.0001	0.0037	-0.0042
5	-0.0816	-0.0005	0.0040	-0.0027
6	-0.0370	0.0000	0.0007	-0.0042
7	-0.0643	-0.0001	0.0026	-0.0027
8	-0.0565	-0.0003	0.0005	-0.0036
9	-0.0721	-0.0004	-0.0003	-0.0021
10	-0.0851	-0.0002	-0.0009	-0.0024
Average	-0.0703	-0.0002	0.0037	-0.0030
SD	0.0167	0.0002	0.0045	0.0008
LOD	0.0501	0.0006	0.0135	0.0024
LOD (ppm)	0.0100	0.0001	0.0027	0.0005
LOQ	0.1670	0.0020	0.0450	0.0080
LOQ (ppm)	0.0334	0.0004	0.0090	0.0016

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5.3 Linearity and Range

Inject linear solution 1-6 and record the concentration results. The correlation coefficient should not be less than 0.99. See Table 5.3-1~5.3-4 for details.

Table 5.3-1: Linearity Data Sheet of ^{75}As (Range: 0~2.0ng/ml)

SN	1	2	3	4	5	6
Content related to sample (μg/g)	0	0.02	0.1	0.2	0.3	0.4
Theoretical concentration (ng/ml)	0	0.1	0.5	1.0	1.5	2.0
Concentration (ng/ml)	0.000	0.070	0.521	1.000	1.525	1.977
Linear equation	y=0.0340*x+0.0086					
Correlation coefficient	0.9996					

Table 5.3-2: Linearity Data Sheet of ^{111}Cd (Range: 0~2.0ng/ml)

SN	1	2	3	4	5	6
Content related to sample (μg/g)	0	0.02	0.1	0.2	0.3	0.4
Theoretical concentration (ng/ml)	0	0.1	0.5	1.0	1.5	2.0
Concentration (ng/ml)	0.000	0.101	0.503	0.998	1.515	1.989
Linear equation	y=0.0095*x+0.0000045					
Correlation coefficient	0.9999					

Table 5.3-3: Linearity Data Sheet of ^{202}Hg (Range: 0~2.0ng/ml)

SN	1	2	3	4	5	6
Content related to sample (μg/g)	0	0.02	0.1	0.2	0.3	0.4
Theoretical concentration (ng/ml)	0	0.1	0.5	1.0	1.5	2.0
Concentration (ng/ml)	0.000	0.099	0.500	0.996	1.460	2.032
Linear equation	y=0.0130*x+0.000028					
Correlation coefficient	0.9996					

Table 5.3-4: Linearity Data Sheet of ^{208}Pb (Range: 0~2.0ng/ml)

SN	1	2	3	4	5	6
Content related to sample (μg/g)	0	0.02	0.1	0.2	0.3	0.4
Theoretical concentration (ng/ml)	0	0.1	0.5	1.0	1.5	2.0
Concentration (ng/ml)	0.000	0.093	0.515	1.000	1.495	2.000
Linear equation	y=0.0532*x+0.00036					
Correlation coefficient	1.0000					

5.4 Precision

Prepare six spiked test solutions in parallel. Inject precision solutions and record the concentration results. The RSD of tested results should not be greater than 20%. See Table 5.4-1~5.4.4 for details.

Table 5.4-1: Precision Data Sheet of ^{75}As

/	Concentration of sample blank solution (ng/ml)	Spiked sample solution (ng/ml)	Content of element (μg/g)
1	-0.135	0.546	0.135
2		0.506	0.128
3		0.506	0.128
4		0.509	0.128
5		0.499	0.127
6		0.541	0.134
Average	/	/	0.130
RSD	/	/	3%

Table 5.4-2: Precision Data Sheet of ^{111}Cd

/	Concentration of sample blank solution (ng/ml)	Spiked sample solution (ng/ml)	Content of element (μg/g)
1	0.000	0.496	0.099
2		0.507	0.101

3		0.513	0.102
4		0.525	0.105
5		0.503	0.100
6		0.507	0.101
Average	/	/	0.101
RSD	/	/	2%

Table 5.4-3: Precision Data Sheet of ^{202}Hg

/	Concentration of sample blank solution (ng/ml)	Spiked sample solution (ng/ml)	Content of element (μg/g)
1	0.015	0.460	0.088
2		0.476	0.092
3		0.461	0.089
4		0.460	0.089
5		0.456	0.088
6		0.508	0.098
Average	/	/	0.091
RSD	/	/	5%

Table 5.4-4: Precision Data Sheet of ^{208}Pb

/	Concentration of sample blank solution (ng/ml)	Spiked sample solution (ng/ml)	Content of element (μg/g)
1	0.009	0.598	0.117
2		0.608	0.119
3		0.616	0.121
4		0.607	0.119
5		0.609	0.120
6		0.610	0.119
Average	/	/	0.119
RSD	/	/	2%

5.5 Intermediate Precision

Prepare 6 spiked test solutions in parallel. Inject precision solutions and record the concentration results. Calculate the content of each element.

The RSD of content results should not be greater than 20%.

RD of content results between two analysts should not be more than 25%. See Table 5.5-1~5.5-5 for details.

Table 5.5-1: Intermediate Precision Data Sheet of ^{75}As

/	Concentration of sample blank solution (ng/ml)	Spiked sample solution (ng/ml)	Content of element (μg/g)
1	-0.131	0.469	0.116
2		0.454	0.117
3		0.499	0.124
4		0.465	0.116
5		0.437	0.111
6		0.457	0.115
Average	/	/	0.117
RSD	/	/	4%

Table 5.5-2: Intermediate Precision Data Sheet of ^{111}Cd

/	Concentration of sample blank solution (ng/ml)	Spiked sample solution (ng/ml)	Content of element (μg/g)
1	0.001	0.492	0.095
2		0.492	0.098
3		0.486	0.096
4		0.481	0.094
5		0.503	0.098
6		0.468	0.091
Average	/	/	0.095
RSD	/	/	3%

Table 5.5-3: Intermediate Precision Data Sheet of ^{202}Hg

/	Concentration of sample blank solution (ng/ml)	Spiked sample solution (ng/ml)	Content of element (μg/g)
1	0.055	0.469	0.080
2		0.468	0.083
3		0.464	0.081
4		0.449	0.077
5		0.471	0.081
6		0.468	0.081
Average	/	/	0.082
RSD	/	/	4%

Table 5.5-4: Intermediate Precision Data Sheet of ^{208}Pb

/	Concentration of sample blank solution (ng/ml)	Spiked sample solution (ng/ml)	Content of element (μg/g)
1	0.027	0.704	0.131
2		0.672	0.129
3		0.635	0.120
4		0.826	0.156
5		0.616	0.115
6		0.729	0.137
Average	/	/	0.131
RSD	/	/	11%

Table 5.5-5: Comparison of Two Analysts

Element	Analyst 1 (ug/g)	Analyst 2 (ug/g)	RD (%)
^{75}As	0.130	0.117	6
^{111}Cd	0.101	0.095	3
^{202}Hg	0.091	0.082	6
^{208Pb}	0.119	0.131	5

5.6 Accuracy

The recovery of arsenic, cadmium, mercury, and lead at three concentration levels of 0.25ng/ml, 0.5ng/ml, and 0.75ng/ml should be between 70% and 150%. See Table 5.6-1~5.6-4 for details.

Table 5.6-1: Recovery Data Sheet of ^{75}As

/	Content of unspiked solution(μg/g)	Concentration in sample blank solution (ng/ml)	Concentration in spiked sample solution (ng/ml)	Content of spiked solution (μg/g)	Added amount (μg/g)	Recovery (%)	RSD (%)
Accuracy solution 1	0.009	-0.135	0.194	0.066	0.05	112	6
			0.169	0.061		103	
			0.203	0.068		115	
Accuracy solution 2			0.546	0.135	0.10	125	4
			0.506	0.128		118	
			0.506	0.128		118	
Accuracy solution 3			0.839	0.195	0.15	123	5
			0.775	0.181		114	
			0.840	0.194		123	

Table 5.6-2: Recovery Data Sheet of ^{111}Cd

/	Content of unspiked solution(μg/g)	Concentration in sample blank solution (ng/ml)	Concentration in spiked sample solution (ng/ml)	Content of spiked solution (μg/g)	Added amount (μg/g)	Recovery(%)	RSD (%)
Accuracy solution 1	0.002	0.000	0.238	0.048	0.05	91	3
			0.248	0.050		95	
			0.247	0.049		94	
Accuracy solution 2			0.496	0.099	0.10	97	2
			0.507	0.101		99	
			0.513	0.102		100	
Accuracy solution 3			0.738	0.148	0.15	97	2
			0.751	0.150		99	
			0.753	0.150		99	

Table 5.6-3: Recovery Data Sheet of ^{202}Hg

/	Content of unspiked solution(μg/g)	Concentration in sample blank solution (ng/ml)	Concentration in spiked sample solution (ng/ml)	Content of spiked solution (μg/g)	Added amount (μg/g)	Recovery (%)	RSD (%)
Accuracy solution 1	0.001	0.015	0.244	0.046	0.05	91	2
			0.248	0.047		92	
			0.252	0.047		94	
Accuracy solution 2			0.460	0.088	0.10	88	3
			0.476	0.092		92	
			0.461	0.089		89	
Accuracy solution 3			0.690	0.135	0.15	90	2
			0.687	0.134		89	
			0.702	0.137		91	

Table 5.6-4: Recovery Data Sheet of ^{208}Pb

/	Content of unspiked solution(μg/g)	Concentration in sample blank solution (ng/ml)	Concentration in spiked sample solution (ng/ml)	Content of spiked solution (μg/g)	Added amount (μg/g)	Recovery (%)	RSD (%)
Accuracy solution 1	0.017	0.009	0.351	0.068	0.05	103	1
			0.349	0.068		102	
			0.351	0.068		103	
Accuracy solution 2			0.598	0.117	0.10	101	2
			0.608	0.119		103	
			0.616	0.121		104	
Accuracy solution 3			0.847	0.168	0.15	101	1
			0.857	0.169		102	
			0.867	0.171		103	

5.7 Solution Stability

Place the linearity solution and precision solution at room temperature for one day; compare the results with those at 0 hour. The difference of the tested concentration of the linearity solution 1 should be not more than LOQ. The deviation of the tested concentration of the linearity solutions

2~6 should not be more than 30%. The deviation of the tested concentration of the precision solution should not be more than 30%. See Table 5.7-1~5.7-4 for details.

Commented [Z5]: Typo

Table 5.7-1: Stability Data Sheet of ^{75}As

Time	0h	22h	Difference or deviation
Linear solution 1	0.000	0.000	0
Linear solution 2	0.070	0.077	5%
Linear solution 3	0.521	0.485	4%
Linear solution 4	1.000	1.006	1%
Linear solution 5	1.525	1.507	1%
Linear solution 6	1.977	1.997	1%
Content (μg/g)	0.135	0.118	7%

Table 5.7-2: Stability Data Sheet of ^{111}Cd

Time	0h	22h	Difference or deviation
Linear solution 1	0.000	0.000	0
Linear solution 2	0.101	0.109	4%
Linear solution 3	0.503	0.505	1%
Linear solution 4	0.998	1.009	1%
Linear solution 5	1.515	1.508	1%
Linear solution 6	1.989	1.988	0
Content (μg/g)	0.099	0.100	1%

Table 5.7-3: Stability Data Sheet of ^{202}Hg

Time	0h	22h	Difference or deviation
Linear solution 1	0.000	0.000	0
Linear solution 2	0.099	0.099	0
Linear solution 3	0.500	0.494	1%
Linear solution 4	0.996	0.997	0%

Linear solution 5	1.460	1.507	2%
Linear solution 6	2.032	1.998	1%
Content (μg/g)	0.088	0.087	1%

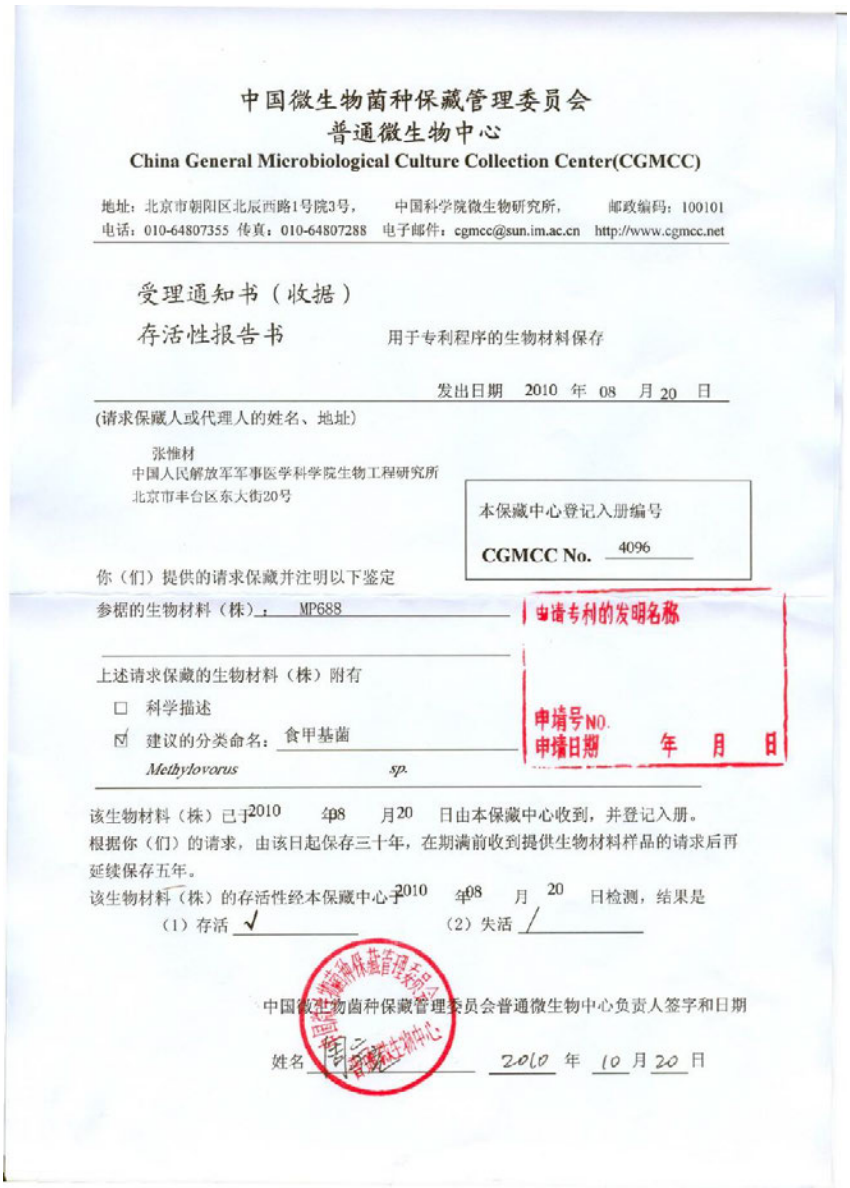
Table 5.7-4: Stability Data Sheet of ^{208}Pb

Time	0h	22h	Difference or deviation
Linear solution 1	0.000	0.000	0
Linear solution 2	0.093	0.098	3%
Linear solution 3	0.515	0.503	2%
Linear solution 4	1.000	1.006	1%
Linear solution 5	1.495	1.503	1%
Linear solution 6	2.000	1.994	1%
Content (μg/g)	0.117	0.118	1%

6. Conclusion

The method exhibits good precision, linearity, and accuracy.
LOQ of ^{75}As is 0.0334 ppm LOD of ^{75}As is 0.0100 ppm.
LOQ of ^{111}Cd is 0.0004 ppm; LOD of ^{111}Cd is 0.0001 ppm.
LOQ of ^{202}Hg is 0.0090 ppm; LOD of ^{202}Hg is 0.0027 ppm.
LOQ of ^{208}Pb is 0.0016ppm. LOD of ^{208}Pb is 0.0005ppm.
The stability time is 22h.

Annex 3. Strain Preservation Credential



中国微生物菌种保藏管理委员会
普通微生物中心
China General Microbiological Culture Collection Center(CGMCC)

地址：北京市朝阳区北辰西路1号院3号， 中国科学院微生物研究所， 邮政编码：100101
电话：010-64807355 传真：010-64807288 电子邮件：cgmcc@sun.im.ac.cn http://www.cgmcc.net

受理通知书（收据）
存活性报告书 用于专利程序的生物材料保存

发出日期 2010 年 08 月 20 日

(请求保藏人或代理人的姓名、地址)

张惟材
中国人民解放军军事医学科学院生物工程研究所
北京市丰台区东大街20号

本保藏中心登记入册编号
CGMCC No. 4096

你（们）提供的请求保藏并注明以下鉴定
参据的生物材料（株）：MP688 申请专利的发明名称

上述请求保藏的生物材料（株）附有
□ 科学描述
☑ 建议的分类命名：食甲基菌 申请号NO.
Methylovorus sp. 申请日期 年 月 日

该生物材料（株）已于2010 年08 月20 日由本保藏中心收到，并登记入册。
根据你（们）的请求，由该日起保存三十年，在期满前收到提供生物材料样品的请求后再延续保存五年。
该生物材料（株）的存活性经本保藏中心于2010 年08 月 20 日检测，结果是
（1）存活 √ （2）失活 /

中国微生物菌种保藏管理委员会普通微生物中心负责人签字和日期
姓名 2010 年 10 月 20 日

China General Microbiological Culture Collection Center (CGMCC)

Address: No.3, House 1, West Beichen Road, Chaoyang District, Beijing
Institute of Microbiology, Chinese Academy of Sciences Zip code: 100101
Tel: 010-64807355 Fax: 010-64807288 Email: cgmcc@sun.im.ac.cn http:// www.cgmcc.net

Notice of Acceptance (Receipt)

Survivability Report for the preservation of biological material for patented proced

Issued by August 20, 2010

(Name and address of the person or agent requesting the preservation)

Zhang Weicai
Institute of Biological Engineering and Research
No.20 East Street, Fengtai District, Beijing

Registration Number:
CGMCC No. 4096

You have provided a request for preservation with the following identification strains: MP688.

Invention Name of Patent application

Application No.:
Date: (YY/MM/DD)

The above preserved strains have

□ Scientific description
■ Proposed classification: *Methylovorus sp.*.

The strains were received on August 20, 2010, by CGMCC and registered.

As per your request, the strain will be preserved for 30 years upon the reception date. During the preservation, if you buy your own strain from CGMCC, the rest strains can be preserved for extra 5 years.

Commented [Z6]: The wording is revised

This strain's survivability was detected on August 20, 2010, by CGMCC, and the result is:

(1) Surviving √ (2) Inactivated /.

Signature & Date of Head of CGMCC

Name: Zhou Yuguang October 20, 2010

June 16, 2023

To: Dr. Kaiping Deng, Ph.D.
Center for Food Safety and Applied Nutrition

Subject: Second Amendment to GRN 001118, Pyrroloquinoline Quinone (PQQ) Disodium Salt

From: Susan Cho at AceOne RS, Inc., Agent for Zhejiang Medicine Co., Ltd. (ZMC)

Dear Dr. Deng,

In responses to FDA questions, Zhejiang Medicine Co., Ltd. (ZMC) has prepared its responses as follows:

FDA Question 1.
In your response to Question 3, the specification limits for arsenic, mercury and cadmium were lowered to ≤0.1 mg/kg and the limit for lead is maintained at ≤0.2 mg/kg, as described in the notice. You provided the results of the analyses of seven batches of pyrroloquinoline quinone (PQQ) disodium salt for heavy metals that demonstrated lead levels were in the range of below the limit of quantitation (0.0016 mg/kg) to as high as 0.1388 mg/kg (batch 311PQ211001). We note that we would not expect a fermentation-derived ingredient produced using good manufacturing practices to have heavy metals present. Please discuss the potential source(s) of lead in the manufacture of PQQ disodium salt and any mitigation efforts employed by the notifier to minimize the presence of heavy metals. We encourage you to lower the specification for lead to match those of the other heavy metals.

ZMC's Response:

Lead is not intentionally added during the manufacturing of PQQ disodium salt, but trace amounts of this metal element may be introduced by raw materials and equipment used. The lead levels of 38 batches of our PQQ disodium salt produced in the past 1.5 years are presented in Table 1. As shown in Table 1, the lead levels of the most recent batches are below 0.10 mg/kg. Based on the recent sample data, we propose to tighten the limit of lead to 0.10 mg/kg.

Table 1: Lead Levels in ZMC's PQQ disodium salt

2022			
Batch No.	Pb (mg/kg)	Batch No.	Pb (mg/kg)
311PQ220101	0.0466	311PQ220601	0.0138
311PQ220102	0.0291	311PQ220602	0.0152
311PQ220103	0.0436	311PQ220603	0.0086
311PQ220201	0.0128	311PQ220701	0.0166
311PQ220202	0.0176	311PQ220702	＜0.0016
311PQ220301	0.0118	311PQ220901	0.0098

311PQ220302	0.0179	311PQ220902	0.0160
311PQ220401	0.0156	311PQ221001	0.0120
311PQ220402	0.0162	311PQ221101	＜0.0016
311PQ220403	0.0151	311PQ221102	0.0146
311PQ220501	0.0088	311PQ221103	0.0170
311PQ220502	0.0130	311PQ221201	0.0038
311PQ220503	0.0437	311PQ221202	0.0154
2023			
Batch No.	Pb (mg/kg)	Batch No.	Pb (mg/kg)
311PQ230101	0.0197	311PQ230402	0.0122
311PQ230102	0.0104	311PQ230403	0.0173
311PQ230103	0.0125	311PQ230404	0.0310
311PQ230301	0.0088	311PQ230501	0.0303
311PQ230302	0.0096	311PQ230502	0.0316
311PQ230401	0.0090	311PQ230503	0.0243

FDA Question 2.

In your response to Question 6, you described maximum limits for the presence of imidazolopyrroloquinoline (IPQ) in the crude and final PQQ disodium salt products and provided the results of the analyses of three non-consecutive batches that indicated IPQ was not detected in the finished ingredient and within the maximum limit of 0.1%. You noted that the analytical method used and the limits for IPQ are in accordance with the current USP monograph for PQQ disodium salt. The current USP/NF (2023) monograph does include a suitability requirement for the assay method to differentiate between IPQ and PQQ and includes acceptance criteria for organic impurities of ≤1.0% of total organic impurities and ≤0.1% of any individual organic impurity. Please clarify whether the notifier intended to include the maximum limit of ≤0.1% for IPQ or other individual organic impurities as part of the specifications for PQQ disodium salt.

ZMC's Response:

According to the current USP monograph for PQQ disodium salt, ZMC has added the related parameters to the specifications: ≤1.0% for total impurities and ≤0.1% for any individual impurity. The IPQ is controlled as any individual impurity, which is not more than 0.1%. The revised certificates of Analysis are presented in Annex 1.

We would appreciate your kind attention to this matter.

Sincerely,

Susan Cho, Ph.D.
AceOne RS, Inc.
scho@aceoners.com or susanscho1@yahoo.com
+1-301-875-6454

Annex 1: Certificates of Analysis

Batch No. 311PQ210702

浙江医药 ZMC

Report Date: 2023-06-15

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C1090072
Specification	Chemical intermediate	Batch No.	311PQ210702
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2021/07/19	Quantity	19.26kg
Expiry Date	2023/07/18	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	8.7%
4 Related substances			
Any individual impurity	USP<621>	≤0.1%	<0.05%
Total impurities	USP<621>	≤1.0%	<0.05%
5 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	88%
6 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.0%
7 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.3%
8 Lead, mg/kg	USP<233>	≤0.1	0.0503
9 Arsenic, mg/kg		≤0.1	<0.0334
10 Cadmium, mg/kg		≤0.1	0.0206
11 Mercury, mg/kg		≤0.1	<0.0090
12 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "[1]" is performed once a year Only for registration

Printer: 2023.06.15 Reviewer: 2023.06.15 Approver: 2023.06.15

Mfg: Zhejiang Medicine Co., Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675
Add.:98 East Xinchang Dadao Road, Xinchang, Zhejiang, 312500 P.R China
Email: QC@xcpharma.com

03

Batch No. 311PQ221101

浙江医药 ZMC

Report Date: 2023-06-15

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C2120010
Specification	Chemical intermediate	Batch No.	311PQ221101
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2022/11/11	Quantity	20.62kg
Expiry Date	2024/11/10	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	8.9%
4 Related substances			
Any individual impurity	USP<621>	≤0.1%	<0.05%
Total impurities	USP<621>	≤1.0%	<0.05%
5 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	88%
6 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.1%
7 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.3%
8 Lead, mg/kg	USP<233>	≤0.1	<0.0016
9 Arsenic, mg/kg		≤0.1	<0.0334
10 Cadmium, mg/kg		≤0.1	<0.0004
11 Mercury, mg/kg		≤0.1	<0.0090
12 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "[1]" is performed once a year Only for registration

Printer: 2023.06.15 Reviewer: 2023.06.15 Approver: 2023.06.15

Mfg :Zhejiang Medicine Co , Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675
Add.:98 East Xinchang Dadao Road , Xinchang , Zhejiang, 312500 P.R.China
Email: QC@xcpharma.com
03

Batch No. 311PQ221103

浙江医药 ZMC

Report Date: 2023-06-15

Certificate of Analysis

Product Name	Pyrroloquinoline Quinone Disodium	Test No.	C2120016
Specification	Chemical intermediate	Batch No.	311PQ221103
Sample Source	Plant 311	Package	PE bags+Aluminum Bag
Mfg. Date	2022/11/25	Quantity	25.33kg
Expiry Date	2024/11/24	Test Standard	/

ITEMS	METHODS	SPECIFICATIONS	RESULTS
1 Appearance	Visual inspection	Red or reddish-brown powder	Reddish-brown powder
2 Identification	USP<621>	Conforms to the reference solution	Conform
3 Water Content, g/100g	USP<921>	≤13.0%	8.9%
4 Related substances			
Any individual impurity	USP<621>	≤0.1%	<0.05%
Total impurities	USP<621>	≤1.0%	<0.05%
5 PQQ(dry wt. basis), g/100g	USP<621>	≥85%	89%
6 PQQ disodium salt (dry wt. basis), g/100g	USP<621>	≥98.0%	100.5%
7 Sodium Content(dry wt. basis), g/100g	USP<621>	10.5%~12.9%	12.1%
8 Lead, mg/kg	USP<233>	≤0.1	0.0170
9 Arsenic, mg/kg		≤0.1	<0.0334
10 Cadmium, mg/kg		≤0.1	0.0042
11 Mercury, mg/kg		≤0.1	<0.0090
12 Microbial limit			
Total aerobic microbial count, cfu/g	USP<2021>	≤1000	<10
Total yeasts and moulds count, cfu/g	USP<2021>	≤100	<10
Enterobacterial, cfu/g	USP<2021>	<10	<10
Escherichia coli, in 25g	USP<2022>	Absent in 25g	Absent
Staphylococcus aureus, in 10g	USP<2022>	Absent in 10g	Absent
Salmonella, in 25g	USP<2022>	Absent in 25g	Absent
Listeria[1], in 25g	/	Absent in 25g	Absent

Conclusion:Conform

Remark: The remark "[1]" is performed once a year Only for registration

Printer: 2023.06.15 Reviewer: 2023.06.15 Approver: 2023.06.15

Mfg :Zhejiang Medicine Co , Ltd. Xinchang Pharmaceutical Factory
Tel,Fax:+86-575-86021395/86024675
Add.:98 East Xinchang Dadao Road , Xinchang , Zhejiang, 312500 P.R.China
Email: QC@xcpharma com